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BIOREMEDIATION OF HEAVY METALS BY A BREWER'S YEAST

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ABSTRACT

In this work, the suitability of live and inactivated cells of a brewing yeast strain of *Saccharomyces cerevisiae* NCYC 1364 in the removal of Cu^{2+}, Ni^{2+} and Zn^{2+} from solution was compared. Kinetics studies showed that 30 minutes of the contact of live or dead cells with all the metals were enough to occur the maximum removal, except for live cells with Cu^{2+}, where the equilibrium was attained after 60 minutes.

Dead cells showed a removal of 60% of Zn^{2+} and 30% of Ni^{2+}, while live cells showed a removal of 22 and 17%, respectively. In the case of Cu^{2+}, live and dead cells showed a similar removal (50%). Since dead biomass showed higher heavy metals removal, they are more appropriate to be used in further works of bioremediation.

KEY WORDS

Copper, zinc; nickel; *Saccharomyces cerevisiae*; bioremediation

INTRODUCTION

Large amount of heavy metals are released into the environment due to the technological activities of humans. The impact of these metals in aquatic systems and their accretion throughout the food chain can cause a serious threat to animals and humans, originating a world-wide environmental problem. The currently technologies (such as, precipitation, ion exchange and reverse osmosis) for removal of heavy metals from industrial effluents appear to be inadequate or expensive; alkaline precipitation often create secondary problems with metal-bearing sludges (Volesky, 1990; 2001).

The potential of metal concentration by certain types of biomass provides the basis for the development of a new approach to remove heavy metals when they occur at low concentration (Volesky, 1990; 2001; Vieira and Volesky, 2000). Some types of industrial fermentation waste biomass are excellent metal sorbers. Yeast cells are an inexpensive source of biomass (they are wastes from brewing industry), with an ability to accumulate a broad range of heavy metals under a wide range of external conditions (Blackwell et al. 1995), being a wastewater treatment alternative, where cost effectiveness is the main attraction. Additionally, microbial biomass is capable of accumulating metal ions, from aqueous solutions, even when the cells have been killed.

The aim of this work was to compare the ability of live and inactivated cells of brewing yeast

to accumulate Cu^{2+}, Ni^{2+} and Zn^{2+}. The obtained results are presented and discussed in terms of the applicability of brewing biomass to metal removal from industrial effluents containing low concentrations of heavy metals, but in excess of the stipulated water quality criteria.

MATERIALS AND METHODS

Strain and culture conditions

The ale-brewing strain of *Saccharomyces cerevisae* NCYC 1364 was used in this work. The strain was grown in YEPD broth (10g/l yeast extract, 20g/l peptone, 20 g/l glucose). The pre-cultures were incubated during 24 hours and cultures during 48h (in YEPD with 50g/l glucose), at 25°C, on an orbital shaker at 150 rpm.

Preparation of cell suspensions

Cells were removed from the medium by centrifugation (2000 x *g*, 5 min), washed twice with 30 mM EDTA, once with deionised water and once with MES [2-(N-morpholino)ethanesulfonic acid] pH buffer, 10 mM, at pH 6.0. Cell concentration was determined spectrophotometrically at 600 nm, after appropriate dilution of the samples. Calibration curves (absorbance vs either number of cells or dry weight) were made previously.

Inactivated cells were obtained by drying of live biomass at 45°C, until constant weight.

Uptake of heavy metals

For kinetics studies, cell suspensions with a concentration near of 4g dry weight/l were shaken in 500 ml plastic flasks at 150 rpm, in an orbital shaker, at 25°C and an appropriate volume of a metal solution (from a stock solution of 1000 mg per litre or 2000 mg per litre) was added. A total volume of 100 ml was used. At defined intervals of time, indicated in the figures, samples (10 ml) were taken and filtered through a 0.45 µm-pore size filter. The filtrates were stored in a refrigerator, at 4°C, until metals determination

In all cases, control experiments (metal solutions in the absence of biomass) were done.

Heavy metals determination

Heavy metals were determined by Atomic Absorption Spectroscopy with flame atomization, after appropriate dilution of the samples.

RESULTS AND DISCUSSION

The kinetics of Cu^{2+}, Ni^{2+} and Zn^{2+} removal by live and dead cells of *S. cerevisiae* NCYC 1364 are depicted in figure 1. After 10-15 minutes of contact of live and dead biomass with Cu^{2+}, 40% of the metal was removed (Fig. 1A). Increasing the contact time lead to a slight increase of metal removal, being 50 % after 1h. In the case of dead cells, equilibrium was attained after 30 minutes, while with live cells it was observed after 60 minutes. Extending the time of incubation of the biomass (live or dead) with metals, until 24 h, did not increase the metal removal % (data not shown).

In the case of Ni^{2+} and Zn^{2+}, for live and dead biomass, the maximal removal was observed after 10 minutes. Similarly to Cu^{2+}, extending the incubation time to 24h did not increase the metal removal (data not shown). Ni^{2+} and Zn^{2+} removal by dead biomass is two and three times higher, respectively, comparing with live biomass (Figure 1B and 1C).

Yeast metal uptake occurs, typically, in two steps: the first (surfacial accumulation), is fast (in the first minutes of contact with metal), metabolism independent and happens in live and

dead cells (Blackwell et al. 1995). On the contrary, the second step (bioaccumulation), is generally considered metabolism dependent (occurring, only, in live cells) and is attributed to the intracellular metal uptake across the cell membrane (Blackwell et al. 1995). No bioaccumulation was observed for Ni^{2+} and Zn^{2+}, although both metals are actively transported in *S. cerevisiae* (Fuhrmann and Rothstein 1968), and no appreciable toxic effect could be found for this metal concentration (Mowll and Gadd 1983; Soares et al. 2003). The absence of bioaccumulation step in live cells can be explained by the fact that yeast cells were not incubated in the presence of an external energy source, like glucose, and, probably, the internal cell reserves were not sufficient to accumulate a detectable amount of Ni^{2+} and Zn^{2+} (Figure 1B and 1C).

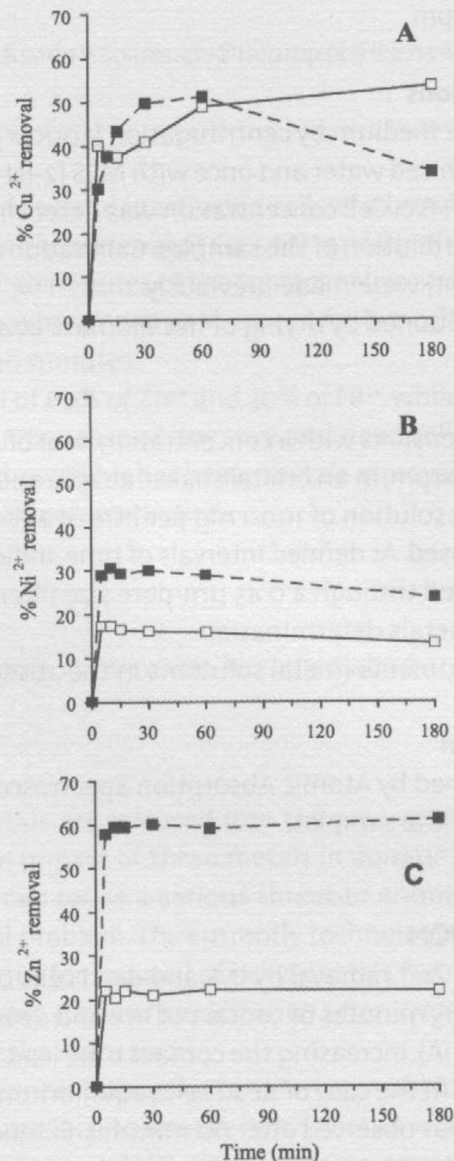

Figure 1
Evolution of Cu^{2+} (A), Ni^{2+} (B) and Zn^{2+} (C) removal for live (□) or dead (■) cells of *S. cerevisiae* NCYC 1364. Cells were suspended in 10 mM MES buffer, pH 6.0, in a final concentration of 4 g dry weight/l; the initial metal concentration was 5 mg/l.

The increase of Ni^{2+} and Zn^{2+} removal by dead cells (Figure 1B and 1C) could be attributed to the exposition of further metal-binding sites inside the cell, as it was reported in other works where, similarly, the integrity of plasma membrane was destroyed by the action of detergents and an enhance in yeast metal uptake was observed (Gadd 1990). In the case of Cu^{2+}, live and dead cells showed similar removal; this can be explained by the high Cu^{2+} toxicity, which provokes lesions of yeast cell membrane (Soares et al. 2003). In *S. cerevisiae*, 5 mg/l of Cu^{2+} induced

lethality at about 50 % after 10 minutes of contact and at about 90 % after 60 minutes (Soares et al. 2003). Thus, live cells were progressively converted into dead cells and the Cu^{2+} accumulation by the initial live cells became similar to inactivated cells (Figure 1A).

CONCLUSIONS

For live and dead biomass of *S. cerevisiae* NCYC 1364, the equilibrium was attained after 30 minutes, for all metals studied, except for live cells with Cu^{2+}, where the equilibrium was observed after 1h. Dead cells showed higher heavy metals removal (except for Cu^{2+}, where live and dead biomass showed similar removal ability) being more suitable in the bioremediation process.

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