Using a flocculent brewer's yeast strain of *Saccharomyces cerevisiae* in the removal of heavy metals

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Heavy metals pollution is a growing problem mainly caused by industrialization, being of particular concern in developing countries. Metal-bearing effluents required a pre-treatment step before being discharged in a water body or directed to a municipal sewage treatment plant. Conventional technologies (precipitation-filtration, ion exchange and membrane technologies) are not adequate (the metal removal is incomplete) or often economically prohibitive and/or impracticable for the treatment of large volumes of wastewater containing relative low metal concentrations.
An effective and economic alternative to traditional treatments in detoxification of metal-bearing wastewaters is the use of biological processes. Among the different kinds of biomass, yeast cells of *Saccharomyces cerevisiae* can constitute a good alternative to wastewater treatment since has the ability to accumulate a broad range of heavy metals under a wide range of external conditions (Blackwell *et al.* 1995; Ferraz and Teixeira 1999; Wang and Chen 2006). A common problem of biomass application is usually associated to its difficulty of separation from the reaction mixture. To solve this problem, immobilized microbial biomass has been used (Tsezos 1990). Nevertheless, immobilization techniques are very expensive when used in large scale. A novel approach may involve the use of flocculent yeasts. Recently, it was shown that a flocculent strain of *S. cerevisiae* accumulated more Cu^{2+} than the isogenic (except for the marker genes and the gene *FLO1*) non-flocculent strain (Soares *et al.* 2002).
In this work, the effect of cell concentration, the presence of different heavy metals and the killing temperature on the yeast settling profile were studied. Our results shown that yeast strain used was able to sediment in the presence of Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Cr^{3+}, which evidences that the flocculation can be used as a cheap and natural separation process for an enlarged range of industrial effluents. For a biomass concentration higher than 0.5 g/l, more than 95 % of the cells were settled after 5 minutes; this fact shows that the auto-aggregation of yeast biomass is a rapid and efficient separation process. Cells inactivated at 45 ºC maintain the sedimentation characteristics.
Additionally, a detailed comparison (using kinetic and equilibrium uptakes studies) of the ability of live and dead (killed at 45ºC) flocculent cells of *S. cerevisiae* to accumulate nickel, copper and zinc was carried out. Equilibrium studies have shown that inactivated biomass displayed a greater Zn^{2+} and Ni^{2+} accumulation (163 Zn^{2+} and 134 Ni^{2+} μmol g^{-1} dry weight biomass), than live yeasts (12 Zn^{2+} and 8 Ni^{2+} μmol g^{-1} dry weight biomass). For Cu^{2+}, live and dead cells displayed similar accumulation: 122 and 156 μmol g^{-1} dry weight biomass, respectively.
Fluorescence and scanning electron microscopy and infrared spectroscopy studies have shown that no appreciable structural or molecular changes occurred in the cells during the killing process. The increased metal uptake capacities observed in dead

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cells can be most likely explained by the loss of cell membrane integrity, which allows the exposition of further metal-binding sites inside the cells. Infrared analyses suggest the involvement of carboxyl, amino, hydroxyl and amides groups in yeast metals uptake.
In conclusion, flocculent cells, inactivated at 45 ºC, retain the ability to sediment rapidly in the presence of different heavy metals. This type of biomass shows a higher heavy metal removal ability, being more suitable for further bioremediation works. Together, the results evidences that the use of this type of biomass to remove heavy metals from industrial effluents is particularly promissory as it is a residue from brewing industry available in large quantities at low cost. Furthermore, the costs associated with cell separation after effluent treatment are minimized due to the natural aggregation characteristics of the strain. In addition, it is possible the use of different configurations of suspended biomass reactors without the risk of biomass washout.

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