REMOVAL OF HEAVY METALS FROM INDUSTRIAL EFFLUENTS USING *SACCHAROMYCES CEREVISIAE*

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INTRODUCTION

Heavy metal pollution is a growing problem mainly caused by industrialization. One example of this is the wastewater contamination by heavy metals. These effluents require a pretreatment step before being discharged in surface waters or sending to a municipal sewage treatment plant. Conventional technologies, as chemical and physical treatment, for removal of heavy metals from effluents appear to be inadequate or expensive. Therefore, biological processes are an alternative because they are eco-friendly and they have a great ability to remove heavy metals from solutions. One limitation in the industrial application of microbial biomass for bioremediation is linked with their small size and low density, which can limit the choice of suitable reactors and make it difficult to separate biomass from treated effluent. A practical alternative may be to use flocculating yeasts. *Saccharomyces cerevisiae* is a yeast example with this property. It has been shown that the use of brewing flocculent yeast cells seems to be a new approach for heavy metals bioremediation. This work is a review of many studies that show that this yeast could be a good alternative for heavy metal removal from industrial effluents.

OBJECTIVES

The objectives of the different works were:

1. Find out if flocculation can be a good process of cell separation after effluent treatment.
2. Compare the metal uptake between flocculent and non-flocculent cells to assess which option is the best.
3. Compare the ability of live and dead flocculent cells to accumulate heavy metals to find out which kind of biomass is more efficient.
4. Analyse whether some ions, which are present in real effluents, could affect the flocculation. In this case, $Pb^{2+}$and Ca^{2+}were the elements studied.
5. Assess the efficiency of heat-killed cells of a brewing strain of *Saccharomyces cerevisiae* to remove copper, nickel and chromium from a real electroplating effluent using a batch mode.

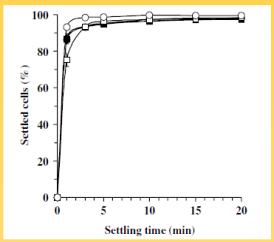

Fig. 1. Time course of sedimentation of *Saccharomyces cerevisiae* NCYC 1364 in the presence of different cations. 5 g dry weight/l was suspended in MES pH buffer (10 mM, pH 6.0), containing 0.2 mM of each cation: (black squares) Cu^{2+}+Ni^{2+}; (black circles) Cu^{2+} + Zn^{2+}; (black triangles) Cu^{2+} + Cd^{2+}; (white squares) Cu^{2+} + $Cr3+$; (white circles) Ca^{2+} (control ion).

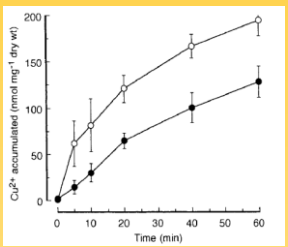

Fig. 2. Accumulation of Cu^{2+} by *Saccharomyces cerevisiae* flocculent strain S646-1B (white circles) and non-flocculent strain S646-8D (black circles). Cells were suspended in 10 mM MES pH buffer, at pH 6, in a final concentration near 0.4 mg ml^{-1}. The initial metal concentration was 0.2 mM.

METHODS AND RESULTS

To test the flocculation ability as a separation process, different synthetic effluents were prepared containing different pairs of metals. Then, the flocculent NCYC 1364 *Saccharomyces cerevisiae* strain was added. Fig . 1 shows that the yeast settled fully in any heavy metals combination. These results show that flocculation can be used as a cell separation process. This fact allows the uses of different configurations of suspended biomass reactors without the risk of biomass washout.

To compare the metal uptake between a flocculent (S646-1B) and non-flocculent (S646-8D) strain, cell suspensions of these strains were mixed with the metal Cu^{2+}. Fig. 2 shows that after 60 min of contact of the yeast with the metal, the flocculent strain accumulated more Cu^{2+}than the non-flocculent strain.

For the objective of comparing metal uptake between live and dead cells, the brewing live flocculent strain NCYC 1364 and the same one inactivated at 45ºC were added in a solution of Zn^{2}, $Ni^{2+}$and Cu^{2+}. Fig 3. shows that heat-inactivated biomass displayed a greater of $Ni^{2+}$and Zn^{2+}accumulation than live yeasts. In the case of Cu^{2+}, live and dead cells showed a similar removal. This can be explained by the high copper toxicity.

To study the $Pb^{2+}$and Ca^{2+}effect upon the flocculation process, different brewing strains of *Saccharomyces cerevisiae* were used. The results showed that in presence of Ca^{2+}, the yeasts were more capable to flocculate. This is because Ca^{2+} induces de correct conformation of the lectins in the flocculation process. However, in presence of Pb^{2+}, the yeast cells couldn't flocculate. So, Pb^{2+} is a strong inhibitor of the flocculation but , it isn't irreversible because some results showed that the effect of the Pb^{2+}can be replaced almost completely with a concentration of calcium 50 times higher than Pb^{2+}.

Finally, to test the effect of *Saccharomyces cerevisiae* on real effluents, cells from the strain NCYC 1364 were heat-inactivated at 45ºC. These were added to an electroplating effluent which was collected from a Portuguese electroplating unit. As it can be seen in Table 1 the levels of Ni^{2+}, $Zn^{2+}$and Cr^{6+} were higher than the minimum concentration allowed in the US-EPA and the Portuguese law. Using heat-inactivated cells of *Saccharomyces cerevisiae,* after the first batch, at an initial pH 2.3, 98% of $Cr^{6+}$was removed. Then, the amount of Ni^{2}, Cu^{2}, $Zn^{2+}$and total Cr in solution met the quality criteria of discharge of wastewater in natural waters, according to US-EPA and Portuguese law, after the fourth batch at pH6. This probes that heat-inactivated flocculent cells of *Saccharomyces cerevisiae* are a good alternative for industrial effluents bioremediation.

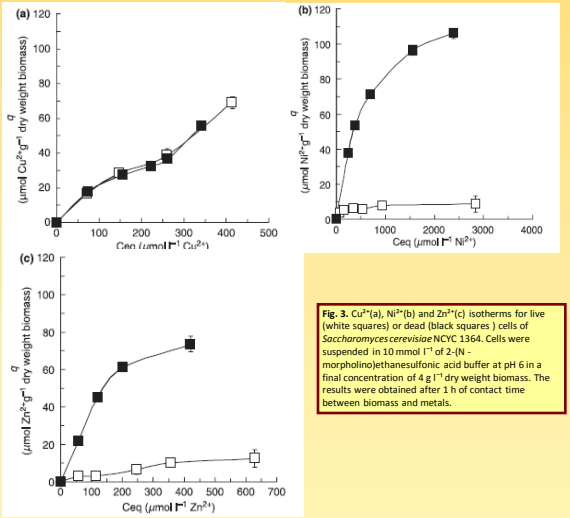

Fig. 3. Cu^{2+}(a), Ni^{2+}(b) and Zn^{2+}(c) isotherms for live (white squares) or dead (black squares) cells of *Saccharomyces cerevisiae* NCYC 1364. Cells were suspended in 10 mmol l^{-1} of 2-(N - morpholino)ethanesulfonic acid buffer at pH 6 in a final concentration of 4 g l^{-1} dry weight biomass. The results were obtained after 1 h of contact time between biomass and metals.

Metal concentration (mg l^{-1})[a]	Limit discharge criteria		Industrial effluent[b]	After 1st batch[c]	After 2nd batch	After 3rd batch	After 4th batch
	US-EPA	Portuguese law					
Ni(II)	3.98	2.0	23.0±2.0	22.0±2.0	11.0±1.0	5.0±1.0	3.0±1.0
Zn(II)	2.61	NS	1.4±0.2	ND	ND	ND	ND
Cu(II)	3.38	1.0	2.57±0.04	2.32±0.03	0.57±0.08	0.25±0.04	0.19±0.04
Total Cr	2.77	2.0	24.0±3.0	9.0±2.0	3.7±0.8	2.4±0.6	1.7±0.4
Cr(VI)	NS	0.1	18.0±1.0	0.3±0.2	0.5±0.5	0.1±0.1	<0.06

Table 1: Bioremediation of an electroplating effluent using *Saccharomyces cerevisiae* NCYC 1364, in a batch mode reaction.

CONCLUSIONS

In conclusion, the use of flocculent brewing yeast of *Saccharomyces cerevisiae* is a natural, easy and cheap method of cell separation from treated effluents, which overcomes the need for cell immobilization (a very expensive technique). Moreover, yeast flocculation also facilitates further recycling of the biomass and the recovery of the metals and also increase the efficiency of metal uptake by the yeasts. In addition, yeast heat-inactivated cells at 45ºC evidenced higher metals uptake capacities than live cells. This increase of metal uptake capacity is most likely explained by the loss of cell membrane integrity, which allows the exposition of further metal-binding sites present inside the cells. All these facts applied to the treatment of real industrial effluents, showed that this yeast may be the solution of the bioremediation of heavy metals from wastewater. Finally, some ions like $Ca^{2+}$and Pb^{2+}can affect the flocculent ability of these yeasts cells, so it's important to take care about it.

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