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Cr(III) removal and recovery from *Saccharomyces cerevisiae*

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Abstract

Heavy metal recovery from biosorbents is of major importance in the assessment of competitiveness of biosorption processes. Several desorption agents (H_2SO_4, HNO_3, HCl, CH_3COOH and EDTA) were tested for the selection of the optimal elution conditions for Cr(III) recovery from *Saccharomyces cerevisiae* cells.

Sorption time was optimised as it plays an important role in the sorption–desorption process, being shown that a 30 min sorption period is the best option to ensure metal removal from solution and good recovery from biosorbent. The optimal contact time with desorption agents was also studied, as long exposures to these ones may cause cell damage, affecting biosorbent metal uptake capacity in subsequent sorption cycles.

Each eluant was analysed in terms of its desorption capacity and its effect on the biomass metal uptake capacity in multiple sorption–desorption cycles. Considering the effectiveness of chromium desorption from loaded biomass, it was possible to conclude that H_2SO_4 (pH $\approx$ 1) was the most effective eluant tested, accomplishing the highest Cr(III) recovery from *S. cerevisiae* in three consecutive sorption/desorption cycles.

Regarding the damage caused by acid treatment on *S. cerevisiae* cells, assessed by the reduction on metal uptake capacity after elution, it was possible to observe that sulphuric acid was the most harmful eluant causing long term negative effects in metal uptake. By the time the experiments were interrupted (nearly 26 h of continuous cycles) biomass uptake capacity was reduced to about 77% of the value reached before acid treatment.

Keywords: Biosorption; Desorption; Heavy metals; *Saccharomyces cerevisiae*

1. Introduction

Wastewater contaminated with heavy metals is a serious environmental problem. Biosorption, using biosorbents from industrial or natural sources, may provide an efficient and competitive solution to treat this wastewater, particularly for high volumes of dilute solutions [1,2]. *Saccharomyces cerevisiae* residual cells from brewing industries have the potential for high metal uptake, selectivity and recovery [3]. Furthermore, it is an available, low-price biosorbent.

The economical and ecological feasibility of biosorption processes depend on the biosorbent metal uptake capacity to reach metal concentration legal limits for wastewater discharge and the ability of eluants to release sequestered metal in subsequent recovery [4–6]. Recovery allows metal recycling, leading to energy savings and materials conservation [7]. Finally, biosorbent regeneration for use in multiple adsorption–desorption cycles [6], contributes to process cost effectiveness.

The efficiency of metal recovery depends on choice of eluant and elution conditions [8], as various eluants presenting different desorption mechanisms may be used [4]. Lowering pH (e.g. with mineral acids) causes metal desorption [9], resulting from competition between protons and metal ions for binding sites [4,5]. Mineral acids such as HCl, H_2SO_4 and HNO_3 and the organic acid CH_3COOH are efficient desorption agents [1,5,6,10], although high concentrations may

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damage biosorbents, limiting their use in subsequent adsorption cycles. Carbonates which form complexes with metal ions are efficient eluants. However, biomass can be damaged due to high equilibrium pH [11]. The strong chelating agent EDTA is another eluant commonly used [4].

The eluants used for metal recovery should be: (a) non-toxic, (b) cause no damage to biosorbent to allow reuse and (c) achieve maximum recovery at lowest possible concentrations [3]. This can be expressed by the solid-to-liquid ratio (S/L), the mass of loaded biosorbent per eluant volume, an important parameter to be optimised [4].

The purpose of this work is to study optimal conditions for Cr(III) desorption from the flocculating yeast *S. cerevisiae* from beer production such as eluant type and concentration, sorption and desorption contact time. Cr(III) is an important end product in the reduction by metabisulphite of the hexavalent chromium from electroplating and similar industries. It is far more prevalent than its hexavalent counterpart and causes a great deal of environmental stress.

2. Materials and methods

2.1. Microorganism

S. cerevisiae was obtained from the Portuguese brewing company – UNICER. Biomass was washed three times in distilled water followed by centrifugation (3 min, 3000 rpm). Approximately 50 g of wet yeast previously washed were suspended in 100 mL of distilled water. This final yeast suspension was mixed with metal solutions in the following proportion: 10 mL yeast suspention/100 mL metal solution, in order to achieve a biomass concentration around 4 g/L (dry weight).

2.2. Metal solutions

Solutions were prepared by dissolving $CrCl_3{\cdot}6H_2O$ in distilled water. Cr(III) solution was adjusted to pH 5.0 with NaOH 0.1 M, prior to mixing with the yeast suspension.

2.3. Eluants

HNO_3, H_2SO_4, HCl and CH_3COOH solutions were used at concentrations of 0.1, 0.5 and 1.0 M; EDTA solutions were used at 0.01, 0.05 and 0.1 M, due to the low solubility of this compound.

2.4. Sorption time

Two hundred and fifty milliliter of Cr(III) solutions were prepared in 500 mL flasks, with initial concentration of 10, 25 and 50 mg Cr(III)/L. Metal solutions were mixed with 25 mL of yeast suspension and incubated at 30 °C with orbital shaking (150 rpm). After 0.25, 0.5, 2 and 24 h exposure, 50 mL samples were collected and biomass, loaded with metal, was separated by centrifugation (3 min, 3000 rpm) and mixed with eluant. The eluant volume used was half the volume of treated metal solution, corresponding to an S/L ratio of 8 g/L. After 30 min of elution, 10 mL samples were centrifuged to evaluate chromium recovery. Chromium (III) uptake and recovery were calculated according to Eqs. (1) and (2), respectively:

$$q = \frac{(C_i - C_f)V}{m} \text{ (mg Cr(III)/g)} \quad (1)$$

$$\text{Cr(III) recovery} = \frac{C_{f_{el}} V_{el}/m}{q} \times 100\,(\%) \quad (2)$$

where q is the metal uptake (mg/g); C_i the Cr(III) initial concentration (mg/L); C_f the Cr(III) final concentration (mg/L); V the solution volume (L); m the biosorbent dry weight (g); $C_{f_{el}}$ the Cr(III) final concentration in eluant (mg/L); V_{el} the eluant volume (L).

2.5. Eluant-biomass contact time selection

Hundred milliliter of metal solutions were prepared in 250 mL flasks with initial concentrations of 10, 25 and 50 mg Cr(III)/L. Chromium solutions were mixed with 10 mL yeast suspension and incubated at 30 °C with orbital shaking (150 rpm). After 30 min exposure, metal laden biomass was separated by centrifugation (3 min, 3000 rpm) and mixed with the eluant (S/L ratio of 8 g/L). Samples were collected after 5, 15, 30 min and 1 h contact with the eluant to evaluate metal recovery.

2.6. Sorption–desorption cycles

Cr(III) solutions (C_i = 25 mg Cr(III)/L) were prepared in 500 mL flasks. Solutions were inoculated with yeast suspension and incubated at 30 °C with orbital shaking (150 rpm). After 30 min, part of this solution was centrifuged (3 min, 3000 rpm) and the metal loaded yeast collected mixed with eluant (HCl, H_2SO_4, HNO_3 or CH_3COOH 0.1 M) in a 2:1 volume proportion. The remaining solution was further incubated in order to follow Cr(III) uptake for the following 24 h. At the end of the exposure period to eluant (30 min), the solution was centrifuged (3 min, 3000 rpm) and biomass regenerated by washing successively with water and NaOH 0.1 M, until initial pH of approximately 5.5 was obtained [1]. Regenerated yeast was used to initiate another cycle. The described procedures were repeated twice.

2.7. Biomass dry weight

Gelman membranes (pore size 0.45 mm) were washed with 20 mL of distilled water, dried at 105 °C and weighted. For each assay, 10 mL of metal solution with biomass suspension was filtered and dried at 105 °C until constant weight was reached.

2.8. Analytical methods

Chromium concentration in samples was determined by atomic absorption spectroscopy after biomass removal by filtration. Samples were preserved by acidification at pH = 2 with concentrated HNO_3 and kept at 4 °C.

3. Results and discussion

3.1. Sorption time selection

The optimisation of Cr(III) desorption from loaded *S. cerevisiae* began with the characterization of the effect of contact time between biomass and the metal solution on recovery efficiency. Sorption times under study were: 15 and 30 min, 2 and 24 h. Metal initial concentrations were: C_i = 10, 25 and 50 mg Cr(III)/L.

Fig. 1 presents chromium uptake by *S. cerevisiae* for a 24 h period. Typical biosorption kinetics [12] with an initial rapid metal uptake (attributed to adsorption) followed by slow uptake (associated to metabolic dependent mechanisms) were observed. This kinetic model has been accepted for various biosorbents such as bacteria and fungi (including yeasts) under similar operation conditions to the ones described in the present study [13]. The explanation based on metabolic activity for the second kinetic period with the slow uptake of metal is a plausible one considering that Cr, when in a low concentrations range, and even in its most noxious valency, may readily participate in cell metabolism [13]. In fact this participation is enhanced by certain starvation conditions for the biomass, acting as a driving force for Cr penetration into the cells [12].

A similar trend was observed for the three initial chromium concentrations with respect to the sorption time on metal recovery.

Higher chromium recovery was achieved when biomass submitted to elution was laden previously for 15 min with the metal solution (Fig. 2). Nevertheless, for short contact periods between biomass and metal solution, Cr(III) uptake is low (Fig. 1). Increasing contact time, though bringing benefits to metal uptake, can reduce recovery levels. Recovery drops to values below 10% after 24 h of elution except with sulphuric acid 0.5 and 1.0 M, and nitric acid 1.0 M.

This trend emphasizes that sorption times have important effects on recovery efficiency, which decreases significantly with increasing biosorbent contact time with the metal solution. This is a consequence of intracellular Cr(III) uptake, as mentioned previously. Metal accumulation inside the cell may result from bioaccumulation, a slow metabolic dependent removal mechanism, or by simple metal diffusion [14]. Intracellular uptake limits metal recovery when long sorption periods are permitted, thus they should be avoided when metal recovery is designed.

The above results indicated that a compromise situation was desirable, making it possible to remove most of the metal from solution while keeping recovery at acceptable values. The data shown in Figs. 1 and 2 indicate that the selected sorption time for all the subsequent assays was 30 min. After this sorption period, metal uptake nearly reached 80% of total uptake possible for a 24 h sorption period. It was still possible to recover more than 40% of bound Cr(III) with most eluants tested.

Comparing data from the three graphs of Fig. 2, data obtained for three different initial metal concentrations, it is possible to observe that for the shortest sorption periods in study, initial Cr(III) concentration seems to affect metal recovery, specially when more diluted eluants are used. This effect is clearly noticeable for H_2SO_4, HCl (0.5 and 0.1 M) and CH_3COOH (0.1 M), where an increase in chromium recovery with increasing initial metal concentration was observed. A relation between metal initial concentration and metal recovery was not observed with EDTA.

This initial metal concentration effect may be explained by multilayer sorption onto the biomass surface, making it easier to desorb external metal layers and to recover

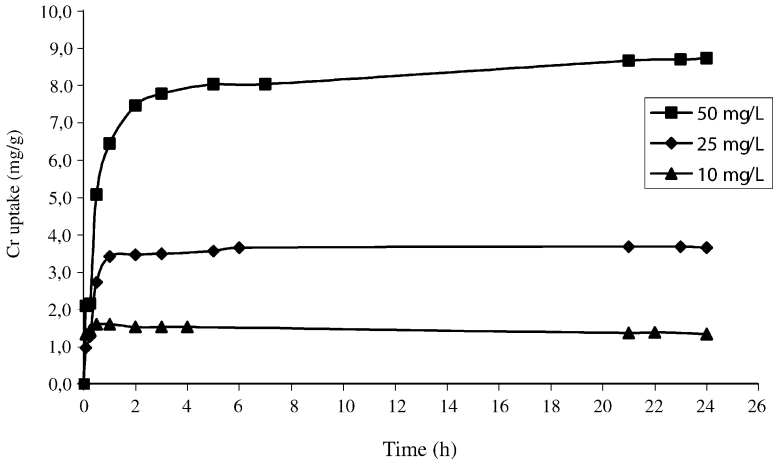


Fig. 1. Chromium uptake at different initial chromium concentrations.

Cr(III) at higher metal concentrations [5], or by differences in binding sites affinities. Kratochvil et al. [15] discussing a similar trend with Cr(VI) desorption from *Sargassum* seaweed, suggested that for low metal concentrations metal binds preferentially to sites with higher affinity, following binding to lower affinity sites, when initial metal concentration is increased. Therefore, chromium bound to lower affinity sites is easier to recover by elution.

However, increasing eluant concentration and using a strong chelating agent diminishes the effect of initial metal concentration, as presented in this report.

Considering longer exposure periods to metal solution, the effect of initial chromium concentration on recovery tends to be less evident, inverting, in some particular cases, the previous tendency, e.g. with H_2SO_4 1.0 M the recovery values after 24 h were 40.9, 14.9 and 16.8% for C_i = 10, 25 and 50 mg Cr(III)/L, respectively. Eventually, higher concentration gradients cause higher intracellular metal accumulation making recovery more difficult after long sorption periods.

An increase in recovery efficiency with increasing acid concentration was also observed, which suggests an ion exchange process [16]. The change observed is highest between

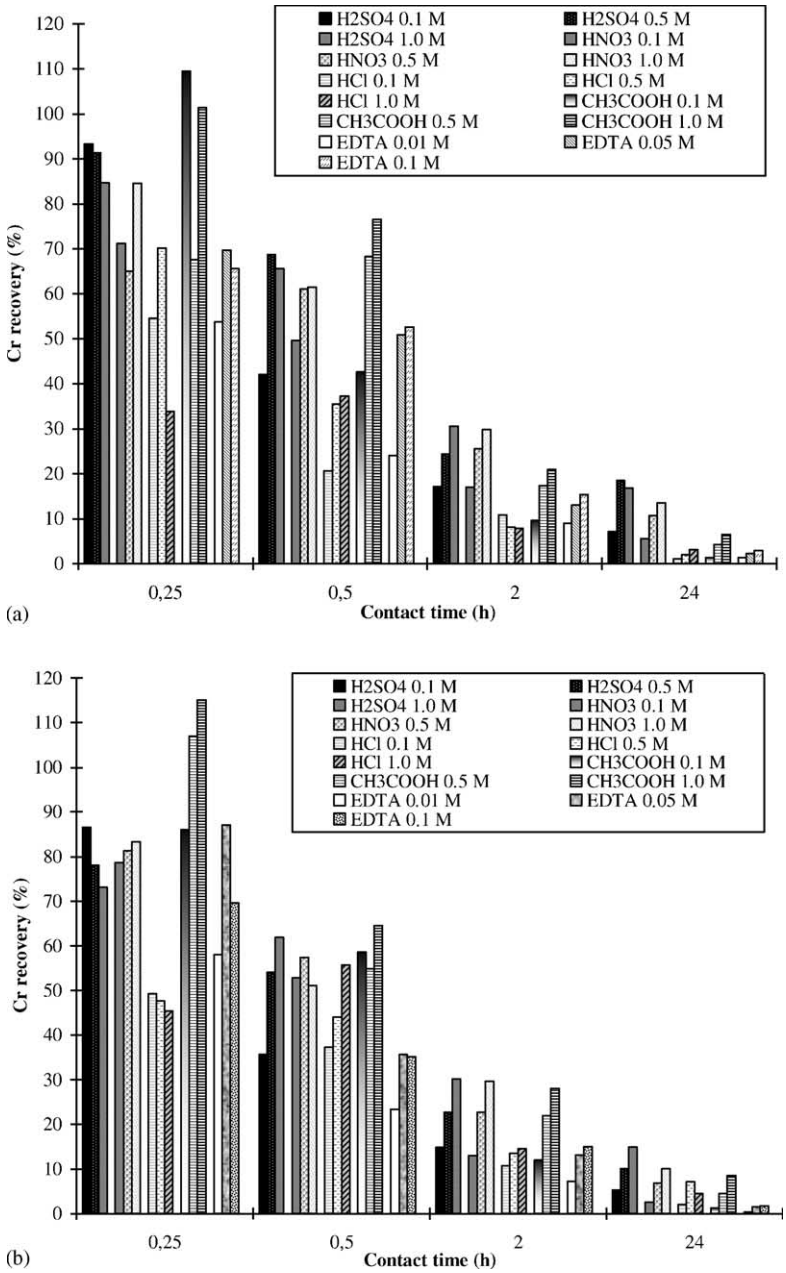


Fig. 2. Dependence of Cr recovery on sorption period, for different eluants and concentrations. (a) C_i = 50 mg Cr(III)/L; (b) C_i = 25 mg Cr(III)/L; (c) C_i = 10 mg Cr(III)/L.

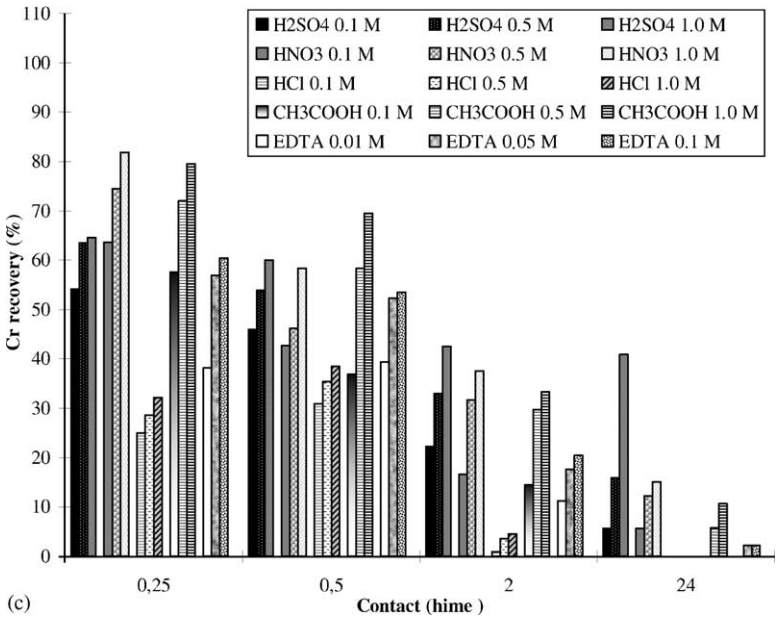


Fig. 2. (*Continued*).

0.1 and 0.5 M, but not significant for most eluants tested. This suggests saturation for proton exchange, indicating that increasing proton concentration does not increase metal recovery. Acid concentration of 0.5 M is sufficient to reach almost maximum recovery.

Considering that acids can damage biosorbents, making unviable their reuse in multiple sorption/desorption cycles and that, according to Wilde and Benemann [17], the ideal pH for elution corresponds to a value below which there is no biosorption – this pH threshold was found to be 2 in a previous study [18], the acid concentrations used in following assays were 0.1 M.

3.2. Biomass-eluant contact time selection

Chromium recovery from biomass increases with increasing exposure time to eluants, Fig. 3. This increase is as evident using EDTA as using CH_3COOH. The increase is not so pronounced for the strong mineral acids tested: HNO_3, H_2SO_4 and HCl. This last one demonstrates a maximum recovery capacity just after 5 min of contact and further elution time did not improve the recovery efficiency.

The experimental data also indicate that Cr(III) recovery obtained with increasing contact time follows an identical trend independently of the eluant concentration used and initial metal concentration (Fig. 3).

The HCl and EDTA solutions gave the lowest metal recovery of all the assays preformed, although the data at 0.1 M are similar. Regarding the chemical properties of EDTA, that is its capacity to form metal complexes, this behaviour seems to indicate a moderate tendency of chromium to complex with EDTA when compared to cell binding. This property is reinforced by the slower desorption kinetics observed. The longer contact time required for this eluant to reach equilibrium denotes a low affinity for EDTA-Cr(III) complex formation.

Although EDTA solutions were prepared at more dilute concentrations than the other acids in the study, therefore the reported results concerning the eluants efficiency being justifiable, it is important to remind that EDTA solutions are harder to prepare as EDTA is a high molecular weight reagent with a low solubility in water. Furthermore, according to Swalaha (1993) referred by [5], the use of EDTA, when compared to other eluants, is not economical. Therefore EDTA was not used in the later assays (sorption/desorption cycles).

The use of strong acids to recover Cr(III) bound to *S. cerevisiae* allows shorter elution periods due to easier proton release and promptly reached equilibrium. To ensure high recoveries and as little damage to biomass as possible, the contact time with eluants used in subsequent assays was 30 min. This period accomplished a reasonable compromise between those factors.

3.3. Sorption–desorption cycles

It is possible to notice in Fig. 4 that H_2SO_4 0.1 M is the solution that accomplishes the higher metal recovery in the three sorption/desorption cycles (52, 43 and 35%), followed by HNO_3 (40, 42 and 30%). HCl and CH_3COOH resulted in lower recoveries (34, 29 and 22%; 28, 39 and 24%, respectively).

It may be expected that chromium recovery with HCl would be similar to that obtained with the other strong acids. However, the eluant-biomass solution pH denotes equivalent hydrogen ion concentration for proton exchange for the three mineral acids. On the other hand, acetic acid exhibited higher pH and consequently less accessibility to protons exchange (Table 1).

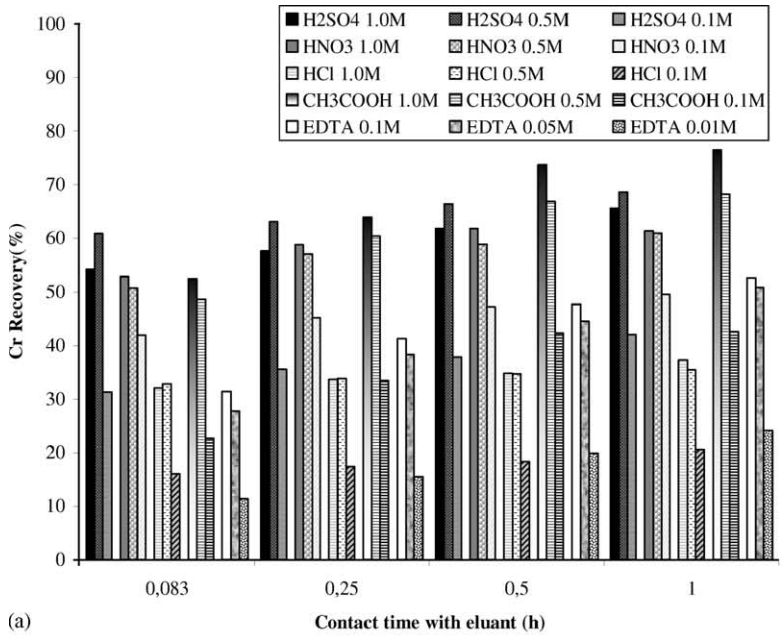
Table 1
Eluant-biomass solution pH (average value for three cycles, measured in the beginning of desorption) and biomass loss (average value)

	H_2SO_4	HNO_3	HCl	CH_3COOH
pH	1.09	1.02	1.06	3.31
Biomass loss (% dry weight)				
Second cycle	17	18	16	27
Third cycle	17	16	17	11

The higher chromium recovery for CH_3COOH in the second cycle can be explained by the elution of part of the metal that was not desorbed during the first cycle.

Since elution does not completely remove chromium bounded to *S. cerevisiae*, and this biomass, when placed in contact with fresh metal solutions, continues to remove metal to residual concentrations equivalent to the achieved in the first cycle, it is possible to assume that biomass did not reached its full saturation during the cycles performed.

Biomass acid treatment has a negative effect in the first period of chromium uptake (Figs. 5–8). The lower metal uptake may have occurred due to changes in cell structure and modification of binding sites chemistry. The first period of chromium uptake, characterized in regular assays by rapid sorption rates, attributed to metal uptake by metabolic inde-



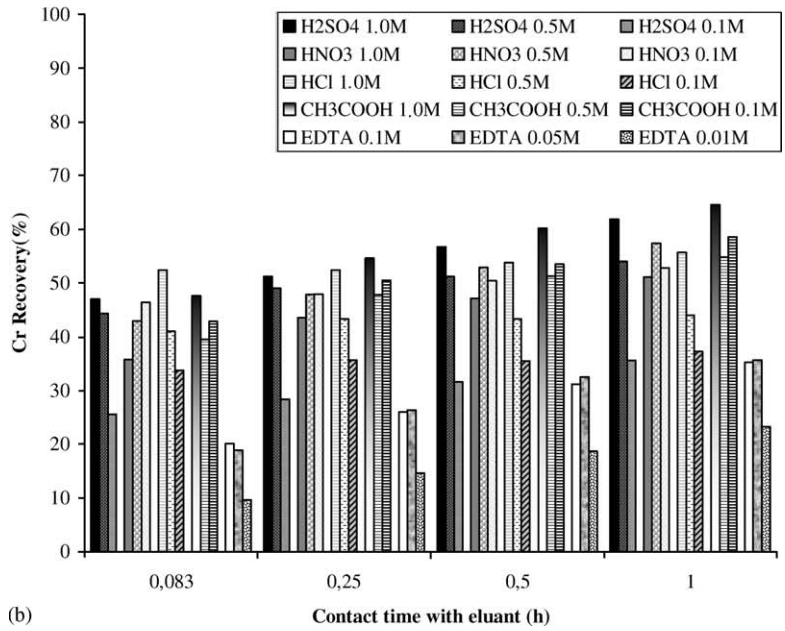


Fig. 3. Dependence of Cr recovery on elution periods, for different eluants and concentrations. (a) C_i = 50 mg Cr(III)/L; (b) C_i = 25 mg Cr(III)/L; (c) C_i = 10 mg Cr(III)/L.

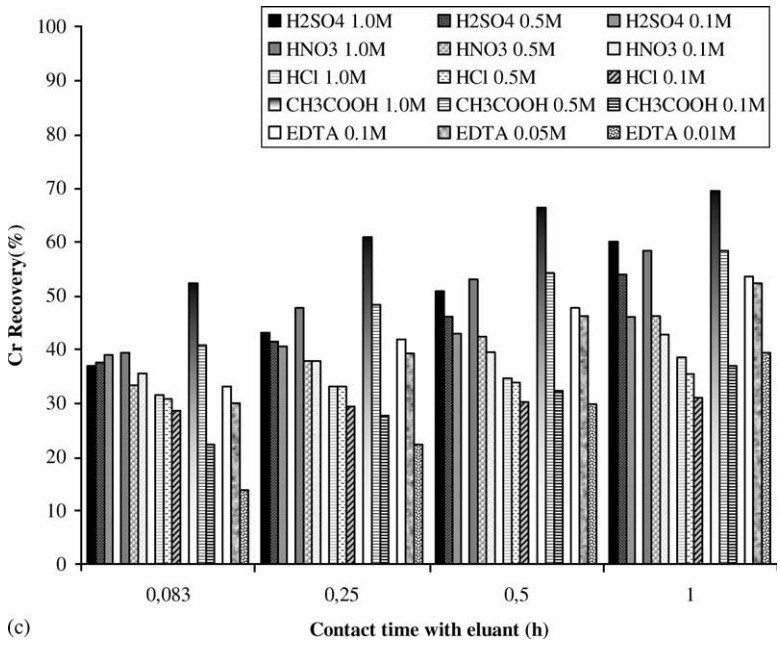


Fig. 3. (*Continued*).

pendent mechanisms, may be affected by the inefficient acid elution, reducing the availability of binding sites in subsequent cycles.

However, when following biosorption through a longer period, it is possible to reach uptake values equivalent to those reached in the first cycle, with the exception of the system treated with H_2SO_4 (after 26 h, second and third cycle reached 77% of the metal uptake achieved in the first cycle).

On the other hand, after desorption with CH_3COOH and HCl, metal uptake increases 7 and 4% when compared to the first cycle. This observation, despite occurring after approximately 22 h, is very important when considering biosorbent reuse in several sorption/desorption cycles. Nev-

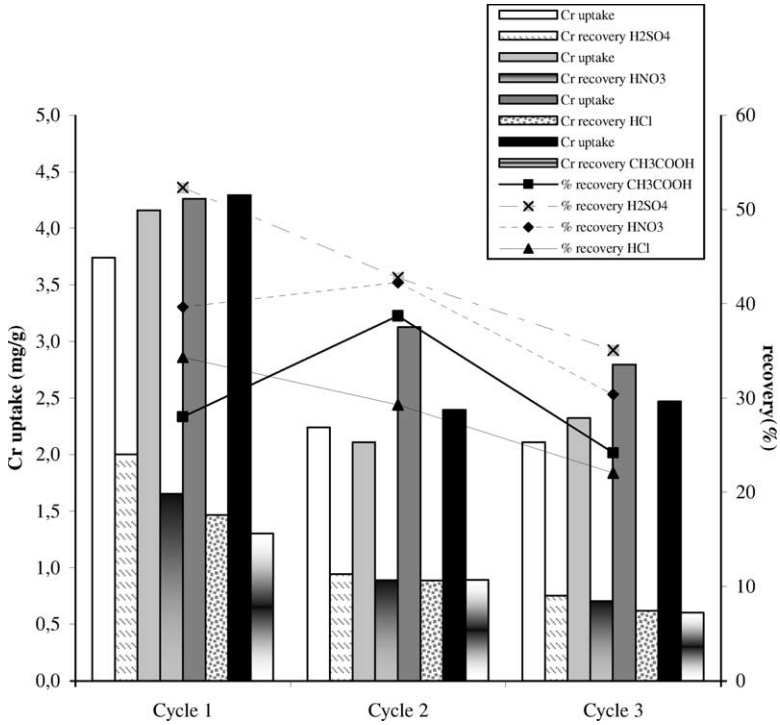


Fig. 4. Cr(III) uptake and recovery in three successive sorption/desorption cycles using different eluants.

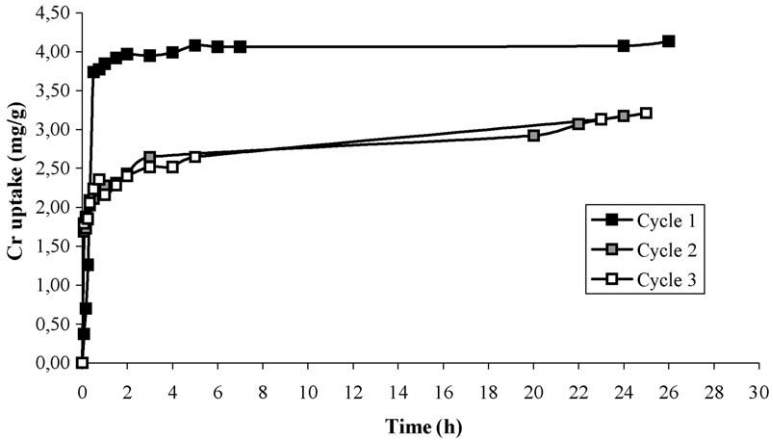


Fig. 5. Cr(III) uptake evolution in three sorption cycles after 30 min elution with H_2SO_4. Elution took place after 30 min of sorption.

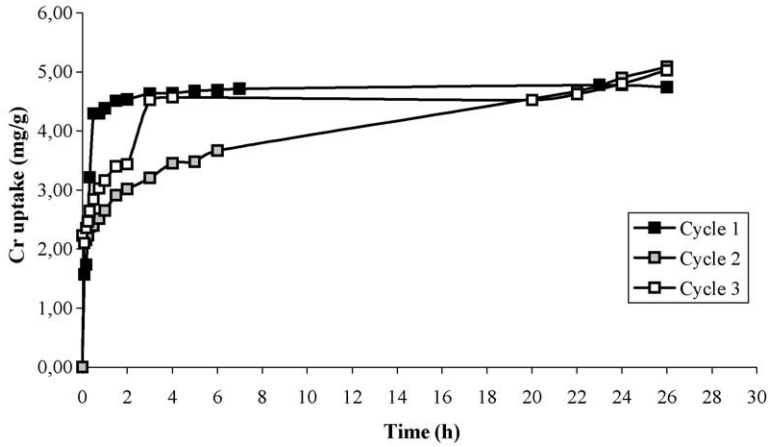


Fig. 6. Cr(III) uptake evolution in three sorption cycles after 30 min elution with CH_3COOH. Elution took place after 30 min of sorption.

ertheless, this situation can make biomass reuse impossible for more than two consecutive cycles, considering previously discussed data that indicate very low metal recovery after 24 h of biosorption. In these assays biomass collection for elution, followed by the initiation of another cycle, occurred after 30 min of contact with metal solution; it was decided to follow Cr(III) uptake during a minimum of 24 h to better evaluate long term effects of elution on biomass.

Regarding the possibility of biomass losing its viability after acid treatment, consequently diminishing intracellular

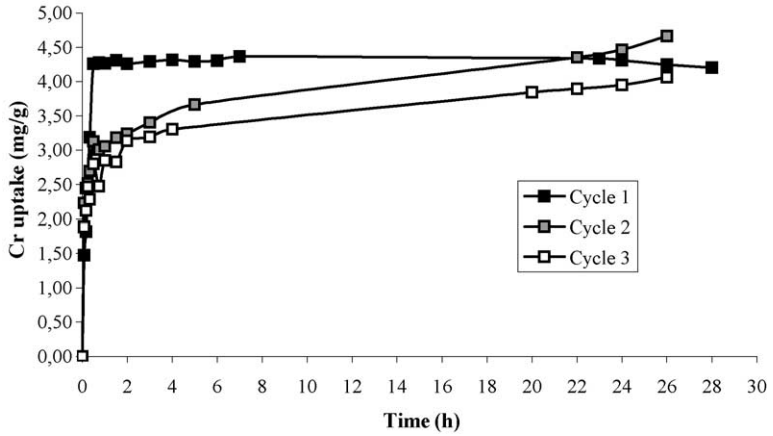


Fig. 7. Cr(III) uptake evolution in three sorption cycles after 30 min elution with HCl. Elution took place after 30 min of sorption.

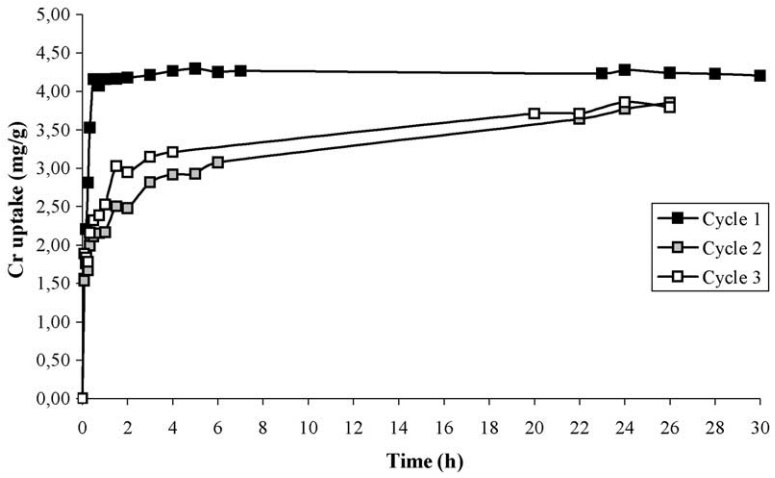


Fig. 8. Cr(III) uptake evolution in three sorption cycles after 30 min elution with HNO_3. Elution took place after 30 min of sorption.

uptake, it is likely that in the second sorption/desorption cycle, when evaluating chromium recovery after 24 h of sorption, the values obtained would be higher than that reported in Section 3.1. This possibility may be tested in subsequent work.

It is well documented that medium pH largely determines biosorption, affecting ion speciation in solution and binding sites chemistry [8,17]. The system in the study has an optimum sorption pH of 5.0 [18]. The treatment performed after acid washing, consisting of three fold biomass washing with water and pH adjustment with NaOH 0.1 M, until biomass suspension reached its initial pH [1], largely contributed to the good biomass performance in the second and third cycle, allowing an optimum pH at the beginning of each cycle.

After washing with water, collected supernatant was analysed by AAS and no chromium was detected, leaving out the possibility of losing metal in this intermediate treatment.

The repeated use of biosorbent in sorption/desorption cycles leads to biomass losses with an average value for the second and third cycle of 17.5% (Table 1). The observed agreement between those values suggests that biomass losses are due to the experimental handling, inherent to all assays, and to biomass destruction as a result of acid treatment. This situation can be minimised by immobilisation [19], an aspect beyond the scope of the present work.

It is important to focus that attained recoveries are not as good as expected but they can be improved by lowering medium pH (using higher acid concentrations) and/or augmenting desorption time as previous results indicate. This improvement may be particularly important considering HCl and CH_3COOH as these acids did not cause biomass to lose uptake capacity after 24 h sorption period.

3.4. Comparison to other systems

Many authors refer to complete or almost complete recovery of metals from biosorbents using mostly mineral acid treatments: Cu^{2+} total recovery from immobilised *Chlorella vulgaris* with HCl 0.1 M [9]; Cu^{2+} recovery from *S. cerevisiae* – 85% with H_2SO_4 1 M, 80% with HCl 1 M and 80.5% with HNO_3 1 M [3]; Cd^{2+} total recovery from *Sargassum fluitans* with H_2SO_4, HCl and HNO_3 0.1 M [4]; Ag^+ total recovery from *Aspergillus niger* with HNO_3 0.1 N [10]; Cd^{2+} recovery from *Sargassum baccularia* – 80% with HCl (pH = 2) and almost complete with EDTA 3.24 mM [20].

However, bibliographic data for chromium desorption in similar conditions indicate much lower recoveries as can be seen in Table 2.

The above values indicate that chromium is, in general, more difficult to recover than other metals, eventually because it follows a different biosorption mechanism [21,22]. Comparing the results obtained in the present study with the presented in Table 2, it is possible to observe that the latest set are more discouraging: recoveries obtained with the system in study are higher using more dilute acid solutions.

Table 2
Recovery data for chromium

Biosorbent	Metal	Eluant	Recovery (%)	Authors
Mucor meihi	Cr^{3+}	H_2SO_4 (study in function of pH)	pH = 1.0; 10%, pH ≈ 0; 30%	Tobin and Roux [23]
Waste activated sludge	Cr^{3+} (among others)	CH_3COOH 2.5%, HCl 0.2 N	10–50%[a], 45–70[a]	Bux et al. [5]
S. cerevisiae	Cu^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Cr^{2+}	HCl 0.1 M; HCl 1.0 M	>90% for all metals except Cr^{2+}; 34% for Cr^{2+} in first cycle; 0% in second cycle	Wilhelmi and Duncan [1]

[a] C_i = 30 and 60 mg/L.

4. Conclusions

The present work clearly shows that sorption time has a great influence in chromium recovery from metal loaded *S. cerevisiae* residual from a brewing industry. Considering this aspect it is important to clearly establish the main purposes of system operation, given that the operation until biomass saturation is reached may turn unviable metal recovery and biosorbent reuse in multiple sorption/desorption cycles.

The optimisation of operational conditions for metal recovery such as eluant concentration and laden biomass contact time with eluant, was focused on maximisation of Cr(III) desorption from *S. cerevisiae* minimising biomass damages, so dilute acid solutions were used (0.1 M) and the chosen contact time with these solutions was 30 min.

The best desorption results – 52, 43 and 35% in three consecutive cycles – were obtained with sulphuric acid 0.1 M, yet causing a considerable drop in *S. cerevisiae* metal uptake capacity. Using hydrochloric acid as desorption agent, metal recovery was reduced to 34, 29 and 22% in the three cycles performed but in longer exposures to metal solutions it was possible to reach initial Cr(III) uptake levels.

Comparing the results accomplished in the present study with other published data it is possible to conclude that chromium desorption has low efficiency values when compared to other metals, being possible to assume that Cr(III) recovery from *S. cerevisiae* in the conditions in study is more favourable than other reported data. Still, these results are far from ideal, being necessary to make more detailed studies to clarify the chromium biosorption mechanism in order to reach maximum recovery.

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