



## Removal of heavy metals using a brewer's yeast strain of *Saccharomyces cerevisiae*: Chemical speciation as a tool in the prediction and improving of treatment efficiency of real electroplating effluents

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### ABSTRACT

In the present work, the influence of the competitive effect of inorganic ligands (carbonates, chlorides, fluorides, phosphates, nitrates and sulphates), which can be present in real multi-metal electroplating effluents, on the biosorption of chromium, copper, nickel and zinc ions by yeast cells of *Saccharomyces cerevisiae* was rationally examined. Additionally, chemical speciation studies allowed optimizing the amount of yeast biomass to be used in the treatment of effluents contaminated with nickel.

The applicability of chemical simulation studies was tested using two simulated effluents and validated using one real electroplating effluent, all containing high concentrations of nickel (about  $303 \mu\text{mol l}^{-1}$ ). For nickel removal, heat-killed biomass of a brewing flocculent strain of *S. cerevisiae* was used, in a batch mode. After the implementation of the bioremediation process ( $12 \text{ g dry weight l}^{-1}$  of yeast cells), the concentration of nickel in the real effluent ( $34 \mu\text{mol l}^{-1}$ ) reached the quality criteria for industrial effluents discharge, after the second or third batch according to the U.S.-Environmental Protection Agency and Portuguese law, respectively. This corresponded to a removal of nickel of 89%.

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### 1. Introduction

The threat of heavy metal pollution to public health and wildlife lead to an enlarged interest in the development of effective technologies for heavy metals immobilization in a non-bioavailable form or their re-speciation into less toxic forms. The current technologies (precipitation, ion exchange, reverse osmosis or evaporation) have been shown inadequate or expensive; conversely, the use of a biological-based technology seems to be a very attractive way of metal ions removal [1].

Flocculent yeast cells of *Saccharomyces cerevisiae* constitute a new approach in the heavy metals bioremediation [2]. Recently, it was shown that brewing cells of *S. cerevisiae*, heat-killed at  $45^\circ\text{C}$ , retain their flocculation ability in wastewaters with different heavy metals composition [3] and present a greater metals

uptake capacities than live cells [4], being very promissory for use in the treatment of heavy metals-loaded effluents. Due to the auto-aggregation properties of this biomass, the solid-liquid separation process after effluent treatment is strongly facilitated, overcoming the need of cell immobilization (like gel entrapment) which, on large engineering scale, can be prohibitively expensive. Flocculent cells can be used in stirred tank reactors, operating in a batch or continuous mode without need of energy input for solid/liquid (biomass/effluent treated) separation stage (like it is required in centrifugation, filtration or flotation), prior to subsequent regeneration/metal recovery or disposal stage [2,3]. Moreover, the natural aggregation properties of flocculent yeast cells allows the use, in a batch mode, of the sedimentation tanks already available in the wastewater treatment plants of the electroplating industries without the need of any further capital investment.

Yeast metal accumulation is affected by several factors such as pH, redox potential ( $E_h$ ), presence of anions, cations and soluble organic compounds. Some of these factors can act at different levels simultaneously. For instance, pH of the solution affect the percentage of ionized groups of yeast cell wall; at low pH, the increase of protonation of yeast cell wall ligands decrease metals adsorption [5–11]. Simultaneously, pH affect metal speciation (the different physicochemical forms of the metal in solution, which together make up its total concentration) [12] and consequently the avail-

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able metal (i.e., the free and labile metal) to be sequestered by the microorganisms. On the other hand, the increase of pH can result in the precipitation of metals hydroxides, reducing metal accumulation by the biomass [13]. The presence of anions (carbonates, chlorides, fluorides, phosphates and sulphates) in solution can complex metal ions and thus reduce or inhibit their adsorption to *S. cerevisiae* [14–16]. Heavy metals can also be complexed by organic compounds (ligands present in the effluents or/and cellular compounds); these soluble ligands can compete with the cells for the metals, reducing the efficiency of heavy metals removal [17–19]. Redox potential can also affect the speciation of a given element; for example, chromium exists as Cr(VI) or Cr(III) according to the  $E_h$  of the solution. The presence in solution of other cations besides the metals of interest can reduce heavy metals accumulation by biomass, by competition for binding sites on yeast cell wall [6,20].

In recent years, the importance of chemical speciation in the treatment of wastewaters contaminated with heavy metals has been emphasized [21–23]. Romera et al. [24] compared the biosorption of different metal ions (Cd, Cu, Ni, Pb and Zn) by the marine brown alga *Fucus spiralis*; the predicted (theoretical) sorption values calculated using the speciation program PHREEQCI, were compared with experimental values. The influence of inorganic species distribution of Hg(II), Pt and Pd, as a function of the pH of the solution and of chloride concentration, on the sorption capacity by the macroalga *Cystoseira baccata* [25] and by sulfate-reducing bacteria [26] was also examined. However, all these works were carried out in simple water synthetic solutions. Real effluents are more complex matrices because they usually have multi-elements and inorganic ligands in competition; these aspects are particularly critical when nickel is one of the metal ions to be removed due to the low affinity of nickel to biomass [4,27].

In this work, the influence of the matrix of real effluents on the removal of heavy metals by bioremediation with heat-killed biomass of *S. cerevisiae* was evaluated by analysing metals chemical speciation and validated for nickel through the bioremediation of a real electroplating effluent and two synthetic effluents. This work also explores the importance of metal chemical speciation in order to predict and optimize the treatment of real effluents, where multi-metal and multi-ligands can be present.

## 2. Materials and methods

### 2.1. Strain, media and culture conditions

In this work, the flocculent brewing strain of *S. cerevisiae* National Collection of Yeast Culture (NCYC) 1364 was used. The strain was routinely maintained at 4 °C, on YEPD agar slants (10 g yeast extract l<sup>-1</sup>, 20 g peptone l<sup>-1</sup>, 20 g glucose l<sup>-1</sup> and 20 g agar l<sup>-1</sup>).

Pre-cultures were prepared in 40 ml of YEPD broth (3 g yeast extract l<sup>-1</sup>, 5 g peptone l<sup>-1</sup> and 20 g glucose l<sup>-1</sup>) in 100 ml Erlenmeyer flasks. Cells were incubated at 25 °C on an orbital shaker Sanyo Gallenkamp IOC 400 (West Sussex, UK), at 150 rpm, during 24 h.

Cultures in YEPD broth with 50 g glucose l<sup>-1</sup> were prepared by inoculating 1 l of culture medium in 2 l Erlenmeyer flasks with 4% (v/v) from pre-cultures. Cells were incubated in the same conditions of the pre-culture for 48 h.

### 2.2. Preparation of cell suspensions

After growth, cells were harvested by centrifugation (2000 g, 5 min) and washed two times with 30 mmol l<sup>-1</sup> ethylenediaminetetraacetic acid (EDTA) solution (Merck) and two times with deionized water. Cells were heat-inactivated by drying live

biomass, at 45 °C, until constant weight. In a previous work, it was found that with this treatment all the biomass is dead [4]. After being heat-killed, cells were washed five times with an excess of deionized water.

### 2.3. Determination of biomass

Cells concentration was determined spectrophotometrically (Unicam, Helios  $\gamma$ ) at 600 nm after appropriate dilution of the samples in EDTA solution (30 mmol l<sup>-1</sup>) to prevent cell aggregation. Calibration curves (absorbance versus either number of cells or dry weight) were previously made.

### 2.4. Industrial effluent characterization

In this study, an effluent from an electroplating industry from the Metropolitan Area of Oporto, Portugal, was used. To characterize this effluent, pH value, redox potential, total heavy metals (Cu, Ni, Zn and Cr), total inorganic ligands (chlorides, fluorides, phosphates, nitrates and sulphates) and total organic and inorganic carbon concentrations were determined. Inorganic ligands were analysed according to Standard Methods [28]. All determinations were done at least in duplicate. Thus, fluorides were determined by direct potentiometry using a fluoride-ion-selective electrode (ISE) (Orion 96-09, Boston, USA), assembled to a Kent 7045 pH meter. Chlorides were determined by the argentometric method. Nitrates were determined by direct potentiometry using a nitrate-ion-selective electrode (ISE) (Crison, Barcelona, Spain) assembled to a Crison MicropH 2002 pH meter. Sulphates were determined by a turbidimetric method. Concentration of inorganic phosphate was determined by the molybdate method.

Total carbon (TC) and inorganic carbon (IC) was determined using the analyser TOC-5000A (Shimadzu). Total organic carbon (TOC) was calculated by the difference of TC and IC.

Total heavy metals concentration was determined by Atomic Absorption Spectroscopy (AAS) with flame atomization in a Perkin Elmer AAnalyst 400 spectrometer (Norwalk, CT, USA). Cr(VI) was determined by a colorimetric method (with diphenylcarbazide). The difference between total and hexavalent chromium was taken as trivalent chromium concentration.

### 2.5. Preparation of the synthetic effluents

Two synthetic effluents (named, synthetic 1 and synthetic 2), with the same amount of heavy metals present in industrial effluent, prepared from metal salt stock solutions of Cu(NO<sub>3</sub>)<sub>2</sub>, NiCl<sub>2</sub>, ZnCl<sub>2</sub> and Cr(NO<sub>3</sub>)<sub>3</sub>, were produced in MES pH buffer (10 mmol l<sup>-1</sup>), at pH 6.0. In the case of synthetic effluent 1, the same amount of inorganic ligands (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>) of the industrial effluent was also added. MES is a suitable pH buffer for heavy metal studies because it does not complex with several metal ions, such as cadmium, copper, lead, and zinc [29,30].

### 2.6. Computer chemical simulations

Chemical speciation calculations were carried out using the MINEQL+ software (version 4.5) [31]. For each case, total heavy metals and ligands (inorganic ligands quantified in the real effluent and inactivated biomass) concentrations, as well as all the affinity constants between heavy metals and ligands [4,6,32] and solubility product constants [32] were introduced in the MINEQL+.

## 2.7. Bioremediation of effluents using inactivated biomass in a batch reaction system

The efficiency of using inactivated yeast biomass in the removal of heavy metals from electroplating effluents was tested using three effluents: one real and two synthetics.

Industrial effluent was adjusted to pH 6.0 with NaOH. Then, the effluent was allowed to stand undisturbed for one night and was filtered through a 0.45  $\mu\text{m}$  pore size membrane.

Washed cells, with a concentration near 12 g dry weight  $\text{l}^{-1}$ , were added to the effluents and shaken in 500 ml plastic flasks, at 150 rpm, at 25 °C. After 30 min of contact between the biomass and the effluent, cells were removed and the supernatant added to new yeast biomass, in a subsequent batch. Kinetic studies performed in a previous work [4] have shown that a contact time of 30 min was enough to reach the equilibrium between dead cells and metals in solution.

Control experiments were done incubating the effluents in the absence of biomass, in the same conditions described above; these control experiments showed that no appreciable amount of nickel (less than 1%) was adsorbed to the plastic flasks. Heavy metals remained in solution were determined as described above.

## 2.8. Reproducibility of the results

All experiments were performed in duplicate and repeated, at least, three times ( $n=6$ ).

## 3. Results and discussion

### 3.1. Physicochemical characterization of industrial effluent

The real effluent was collected from a Portuguese electroplating industry, which is devoted to the production of bath accessories. First, physicochemical characterization of the effluent was performed. For this purpose, total concentrations of metals, inorganic ligands, total carbon and total organic carbon were determined (Table 1); the permissible discharge levels for various heavy metals and inorganic ligands regulated by the Portuguese law [33] were included. European Union (EU) has some directives concerning the discharge of dangerous chemicals, which should be followed by the countries. However, all these documents are general guidelines without any limit discharge criteria. Thus, each country of the EU has its own laws, which take into account the communitarian directives. For comparative purposes and in order to give a wider regulatory perspective, the U.S.-Environmental Protection Agency [34] criteria were also included.

The analysis of Table 1 evidences that nickel concentrations is above US-EPA and Portuguese limit discharge of effluents and thus should be removed before the effluent will be discharged. The presence of metals in the effluent is mainly due to periodical discharge of rinsing waters of electrolytic lines. The values of redox potential (238 mV) and pH (2.0) indicate that chromium should be present as chromium (III) [35], which is in the agreement with the obtained results (Table 1). Thus, the concentration of all other metals besides nickel (chromium, copper and zinc), as well as inorganic ligands, are below the legal limit of discharge.

Additionally, Table 1 also point outs that molar concentrations of chlorides and sulphates (2.55  $\text{mmol l}^{-1}$  and 3.89  $\text{mmol l}^{-1}$ , respectively), even if below the legal limits, are about 10 times higher than those of nickel concentration (0.303  $\text{mmol l}^{-1}$ ). The presence of these ligands in the electroplating effluents comes from the pickling and rinsing waters and, in some industries, from the addition, in the treatment tank, of an acid (chloridric acid) solution of bisulphite, which is used for reduction of chromium (VI) to chromium

**Table 1**

Physicochemical characterization of a raw industrial effluent from an electroplating unit. The level of contaminants are compared to the U.S.-Environmental Protection Agency (US-EPA) [34] and the Portuguese [33] wastewater limit discharge criteria.

	Wastewater limit discharge criteria		Industrial effluent
	US-EPA	Portuguese law	
pH (units)		6.0–9.0	2.0
$E_h$ (mV)			238
TC ( $\text{mg l}^{-1}$ )			11.5
TOC ( $\text{mg l}^{-1}$ )			10.7
IC ( $\text{mg l}^{-1}$ )			0.8
$\text{Cl}^-$ ( $\text{mg l}^{-1}$ )			90.5
$\text{F}^-$ ( $\text{mg l}^{-1}$ )			12
$\text{NO}_3^-$ ( $\text{mg l}^{-1}$ )		50	7.8
$\text{SO}_4^{2-}$ ( $\text{mg l}^{-1}$ )		2000	374
$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )			0.08
$\text{Cu}^{2+}$			
( $\text{mg l}^{-1}$ )	3.38	1.0	0.5
( $\mu\text{mol l}^{-1}$ )	53.1	16	8
$\text{Ni}^{2+}$			
( $\text{mg l}^{-1}$ )	3.98	2.0	17.8
( $\mu\text{mol l}^{-1}$ )	67.8	34	303
$\text{Zn}^{2+}$			
( $\text{mg l}^{-1}$ )	2.61	NS	0.8
( $\mu\text{mol l}^{-1}$ )	39.9	NS	12
Total Cr			
( $\text{mg l}^{-1}$ )	2.77	2.0	1.2
( $\mu\text{mol l}^{-1}$ )	53.3	38	23
$\text{Cr}^{6+}$			
( $\text{mg l}^{-1}$ )		0.1	<0.06
( $\mu\text{mol l}^{-1}$ )		2	<1

TC: total carbon. TOC: total organic carbon. IC: inorganic carbon.

(III) [36]. Table 1 also shows that the amount of organic matter (quantified by the total organic carbon) and phosphates is negligible, whereas the concentrations of nitrates, fluoride and carbonates (expressed as IC) are of the same order of magnitude of nickel concentration.

### 3.2. Optimization of the bioremediation process

The efficiency of bioremediation process of metal ions, from effluents, is dependent both on the chemical speciation of the metal ions present in the effluent and the accumulation parameters of biomass used:  $Q_{\text{max}}$  and  $\log K$ ;  $Q_{\text{max}}$  represents the maximum possible amount of metal ion adsorbed per unit weight of biomass and  $\log K$  represents the equilibrium constant related to the affinity of the binding sites for the metals at a defined pH value. The most important factors which define metals speciation in the effluent are: pH, redox potential, concentration of metal ions and complexing agents (organic and inorganic ones), as well as their affinity for metal ions. This last parameter is of paramount importance, as it will be discussed below.

The values of  $Q_{\text{max}}$ , described in the literature, vary widely among the different types of biomass (bacteria, filamentous fungi, yeasts or algae) in evaluation and also within the same group of organisms [37]. This variability can be attributed to the different characteristics of the biomass, but also due to the different experimental conditions used, namely biomass concentration, pH of the solution and contact time between biomass and metals. As previously described, the heat-treated cells of *S. cerevisiae* used in the present work present intermediary values of metals accumulation (Cu, Ni and Zn), when compared with the values shown in others works, which have used *S. cerevisiae* cells [4].

For bioremediation treatment, pH 6.0 was chosen as being a good commitment solution between the efficiency of biomass to biosorb metal ions and the amount of hydroxide necessary to raise the pH of the effluent. Extremes of pH often decrease the accumulation of heavy metals by the biomass. Yeast cells of *S. cerevisiae* are

**Table 2**

Equilibrium affinity constants, as log  $K$ , among the different ligands and copper, nickel and zinc, at pH 6.0, and chromium at pH 5.0<sup>a</sup>.

Ligand	log $K$				Reference
	Cr(OH) <sup>2+</sup>	Cu <sup>2+</sup>	Ni <sup>2+</sup>	Zn <sup>2+</sup>	
Biomass	3.56				[6]
Cl <sup>-</sup>	3.77 <sup>a</sup>	3.99	3.34	3.59	[4]
F <sup>-</sup>	8.86 <sup>a</sup>	0.20 <sup>b</sup>	0.41 <sup>b</sup>	0.40 <sup>b</sup>	
NO <sub>3</sub> <sup>-</sup>	2.30 <sup>a</sup>	1.80 <sup>b</sup>	1.40 <sup>b</sup>	1.30 <sup>b</sup>	
HCO <sub>3</sub> <sup>-</sup>	–	0.50 <sup>b</sup>	0.40 <sup>b</sup>	0.40 <sup>b</sup>	[32]
		–3.56 <sup>c</sup>	–	–	
SO <sub>4</sub> <sup>2-</sup>	2.38 <sup>d</sup>	1.80 <sup>b</sup>	2.10 <sup>b</sup>	1.50 <sup>b</sup>	
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	6.42 <sup>a</sup>	2.36 <sup>b</sup>	2.30 <sup>b</sup>	2.34 <sup>b</sup>	
		–	–	–	

<sup>a</sup> Conditional affinity constants were calculated assuming the following equilibria, wherein M is metal and L is ligand:

<sup>a</sup> Cr(OH)<sup>2+</sup> + L + H<sup>+</sup> ⇌ CrL + H<sub>2</sub>O.

<sup>b</sup> M + L ⇌ ML.

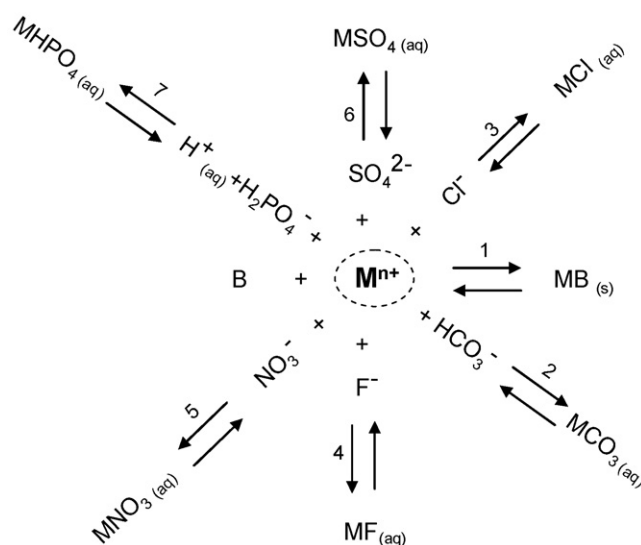
<sup>c</sup> Cu<sup>2+</sup> + HCO<sub>3</sub><sup>-</sup> ⇌ CuCO<sub>3</sub> + H<sup>+</sup>.

<sup>d</sup> Cr(OH)<sup>2+</sup> + SO<sub>4</sub><sup>2-</sup> ⇌ CrOHSO<sub>4</sub>.

able to remove heavy metals from wastewaters between pH 5.0 and 9.0 [5], being pH close to 5–6 as the optimal for Cu<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> and Cr<sup>3+</sup> biosorption by yeast cells [5,6,8,10,38]. At pH 6.0, the main chemical groups of biomass surface, that are able to participate in the removal of metal cations (carboxyl, phosphate, sulfhydryl, hydroxyl and nitrogen-containing groups), are already totally (carboxylic acids) or partially (phosphate and amine groups) deprotonated [32]. As a consequence, good  $Q_{max}$  are obtained. On the other hand, pH 6 is the minimum pH value limit for discharging the wastewaters according to the Portuguese law (Table 1) and thus the pH of the effluents should be obligatorily raised up to 6 before being discharged. Additionally, if effluents also contain large amounts of other metal ions [e.g. copper and chromium (III) ions], they will start to precipitate at this pH value, as metal hydroxides [32], and thus more biomass will be available for removing nickel. However, the use of high pH values originates larger hydroxide consumptions and precipitation of other metals (e.g. nickel and zinc) hydroxides starts to occur [32].

The major driving force for the success of an efficient bioremediation treatment of an effluent containing metal ions is the relative affinity of each metal for biomass versus the several ligands (inorganic and organic ligands) present in the effluent, which can compete with yeast cells. Thus, in order to optimize the conditions of the bioremediation process, metal affinity constants among metal ions and inorganic ligands and yeast cells of *S. cerevisiae* were taking into account (Table 2). For all metals, unless chromium, affinity constants of the aquocomplex metal ion, M<sup>2+</sup> (aq) were considered. In the case of chromium, this metal ion is very acidic and starts to hydrolysis at about pH 2; in an aqueous solution, Cr(OH)<sup>2+</sup> (aq) is the major species present in solution before precipitation of Cr(OH)<sub>3</sub> occurs and this is the reason why we have included the affinity constants for this component in Table 2. A diagram exemplifying the competitive effect of ligands, present in the effluent, with yeast cells biomass is shown in Fig. 1.

The analysis of Table 2 shows that, for copper, nickel and zinc, the affinity constants between metal ions and yeast biomass are much higher (two decades or more) than with some inorganic ligands. The relative magnitude of the affinity constants indicate that carbonates, chlorides, fluorides and nitrates do not compete with biomass for these metal ions even if present in large excess. In practice, MHCO<sub>3</sub>, MCl, MF and MNO<sub>3</sub> species, corresponding to reactions 2, 3, 4 and 5, respectively (Fig. 1), are not formed in appreciable extension; thus, accumulation of copper and/or nickel and/or zinc ions by yeast cells (reaction 1) in effluents containing carbonates and/or chlorides and/or fluorides and/or nitrates, would not be influenced by the presence of these ligands. Table 2 also shows



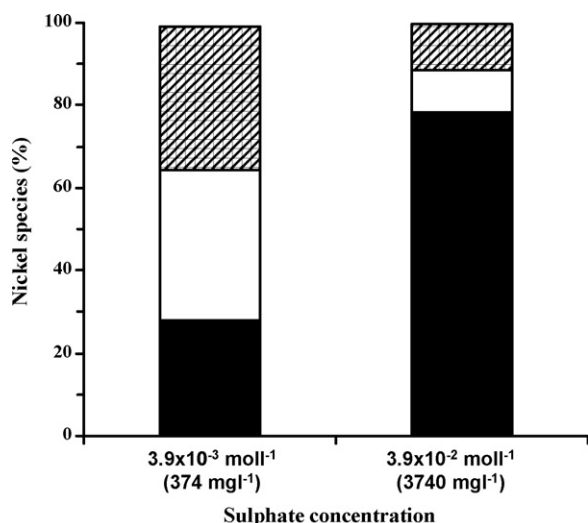
**Fig. 1.** Diagrammatic representation of metal–ligands interactions. Metal ions: M<sup>n+</sup>. Biomass (yeast cells): B. For simplicity of representation, the charges of the complexes were omitted. Yeast cells are able to remove heavy metals from industrial effluents (reaction 1). The presence of ligands in the effluents (reactions 2–7) can reduce the efficiency of the process by competing with biomass for metal removal.

that the affinity constants between copper or zinc ions and yeast biomass are much higher (two decades or more) than with sulphates. On the other hand, the relative magnitude of the affinity constants between nickel ions and sulphates or biomass only differs one decade. These values anticipate that effluents containing molar concentrations of sulphates much higher than that of nickel ions can compete with yeast cells and thus will reduce the bioremediation efficiency (reaction 6). However, this effect can be more or less pronounced depending on the amount of biomass used. Theoretical simulations of the bioremediation process using 1.5 g l<sup>-1</sup> of biomass, of an effluent with the same composition of the effluent described in Table 1, and the same effluent but with a concentration of sulphates 10 times higher (3740 mg l<sup>-1</sup>), predict a reduction of 24% of the bioremediation efficiency, i.e., the % of nickel associated with the biomass (Fig. 2); for the lower concentration of sulphates (374 mg l<sup>-1</sup>), the fraction of nickel associated with biomass is 35%, reducing to 11% in the presence of a concentration of sulphates ten times higher (Fig. 2). High concentrations of sulphates can occur in these type of effluents when reduction of chromium (VI) to chromium (III) is performed with a sulphuric acid solution of bisulphite. Taking into account the negative impact of sulphates in the bioremediation of nickel (Fig. 2), the use of sulphuric acid should be substituted by another mineral acid, for example nitric or chloridric acid, when acidification is necessary for previous reduction of chromium (VI) to chromium (III). Besides, addition of bisulphite in the treatment tank can be reduced by controlling the redox potential and the pH; usually a redox potential of 270 mV and a pH of 2.5 are enough to reduce efficiently chromium (VI) to chromium (III) [35].

The analysis of the relative magnitude of the affinity constants among chromium (III) and biomass or the various inorganic ligands (Table 2), anticipate that, effluents containing fluorides or phosphates complex chromium (reactions 4 and 7, respectively), and thus, the biosorption process under these conditions can be reduced (Fig. 1).

Copper, chromium, nickel and zinc are the metal ions most widely found in the electroplating effluents and the affinity between biomass used in this work and these metal ions follows the following order: Cu ≫ Cr ≅ Zn > Ni (values of log  $K$  are presented in Table 2) [4,6]. The treatment of effluents containing multi-elements



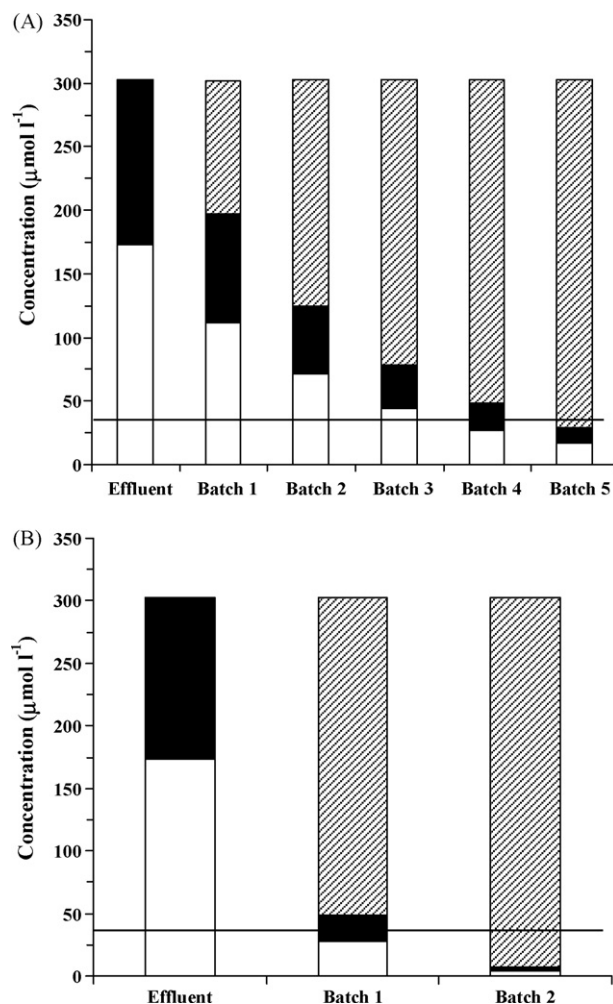


**Fig. 2.** Simulation of the influence of sulphate concentration on the nickel speciation in the presence of  $1.5 \text{ g l}^{-1}$  of inactivated biomass of *S. cerevisiae* NCYC 1364. Free nickel ions (white part of the bar); nickel associated with biomass (dashed part of the bar); nickel associated with sulphate (black part of the bar). The different species of nickel were calculated using the chemical equilibrium computer program (MINEQL+); for more details about calculations see Section 2.6.

is more complex because metal ions compete for binding sites; as a consequence, displacement of one metal species by another, which has higher affinity for biomass binding sites, can occur [39]. Thus, for an efficient bioremediation process, the amount of biomass ([Biomass]) when compared with the total amount of metals ( $\sum[M]$ ) should be in excess in order to have sufficient available biomass sites for all metals accumulation, including those with lower affinity for biomass. However, as lower as the affinity constants between biomass and metal ions are, less amount of metal ions will be biosorbed when equilibrium is attained. Since biomass presents much lower affinity to nickel than copper (five times less) and chromium or zinc (two times less) (Table 2), this means that the efficiency of bioremediation of effluents containing nickel, among the other three metal ions (chromium, copper and zinc), will be conditioned by nickel affinity.

If we want to remove efficiently metals from real multi-metal systems, which contain nickel among other metal ions, nickel biosorption is one of the real challenges that should be firstly studied. Due to this, we decided to study in detail the biosorption of real electroplating effluent containing this metal.

In order to optimize the amount of yeast cells required to treat the effluent described in Table 1, in a batch mode, with no more than three batches, theoretical calculations were done using MINEQL+ software [31]. Thus, for predicting the metal accumulated by the yeast cells, an iterative process of chemical simulations was performed; for this purpose, the program was run with a fixed concentration of biomass in the range between  $1.5$  and  $12 \text{ g l}^{-1}$ , at pH 6.0, assuming the chemical composition of the effluent (Table 1). The analysis of Fig. 3A predicts that the application of  $1.5 \text{ g l}^{-1}$  of biomass will remove about a third of nickel concentration in the first batch. After the fifth batch, the amount of nickel (free nickel ion plus  $\text{NiSO}_4$ ) present in the effluent will be below the limit of discharge criteria according to the Portuguese law. On the other hand, chemical simulations performed with  $12 \text{ g l}^{-1}$  of yeast cells, in each batch, predicted the removal of about 84% of nickel in the first batch; according to these calculations, after the second batch, the concentration of nickel (free nickel ion plus  $\text{NiSO}_4$ ) in the effluent will be below the limit of discharge criteria (Fig. 3B). Amounts of  $1.5$  and  $12 \text{ g l}^{-1}$  of biomass correspond to molar concentrations of  $5.4 \times 10^{-4}$  and  $4.5 \times 10^{-3} \text{ mol l}^{-1}$  [4], respectively. For these exper-



**Fig. 3.** Simulation of the influence of yeast biomass concentration in the nickel removal. (A)  $1.5 \text{ g dry weight l}^{-1}$  of yeast biomass; (B)  $12 \text{ g dry weight l}^{-1}$  of yeast biomass. Nickel ions (white part of the bar); nickel associated with inactivated yeast of *S. cerevisiae* NCYC 1364 (dashed part of the bar); nickel associated with sulphate (black part of the bar). Calculations of the nickel concentration species was done as described in Fig. 2. Solid line represents the Portuguese wastewater limit discharge criteria for nickel:  $2.0 \text{ mg l}^{-1}$  ( $34 \mu\text{mol l}^{-1}$ ).

imental conditions, the molar ratios between the concentration of biomass and nickel are 1.8 and 14.9, respectively. These results evidence that a large excess of biomass in relation to the total molar amount of metals present in the effluents should be used to overcome the lower affinity of biomass to nickel and sulphates competition.

### 3.3. Bioremediation of real and synthetic effluents using a batch reaction system

In this work, inactivated (at  $45^\circ\text{C}$ ) yeast cells of a brewing strain of *S. cerevisiae* were used in the treatment of a real electroplating effluent containing nickel above the legal limit of discharge criteria; in order to compare the impact of inorganic ligands, present in the raw effluent, on the bioremediation process, two synthetic effluents (named, synthetic effluent 1 and synthetic effluent 2) with the same amount of metals as the industrial effluent were prepared. In the case of synthetic effluent 1, the same amount of inorganic ligands ( $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ ) of the industrial effluent was also added.

Taking into account the information given above,  $12 \text{ g l}^{-1}$  of yeast cells were used to remove nickel from the effluents, in a batch mode. A series of batches, using new biomass in each

**Table 3**  
Bioremediation of industrial and synthetic effluents using *S. cerevisiae* NCYC 1364, in a batch mode reaction. The level of the metal reached is compared to the U.S.-Environmental Protection Agency (US-EPA) [34] and the Portuguese [33] wastewater limit discharge criteria.

		Metal concentration ( $\text{mg l}^{-1}$ ) <sup>#</sup>		Original effluent	After 1st batch	After 2nd batch	After 3rd batch
Effluent		Limit discharge criteria					
		US-EPA	Portuguese law				
Nickel	Industrial	3.98	2.0	$17.8 \pm 0.1$	$5.4 \pm 0.5$	$2.7 \pm 0.7$	$2 \pm 1$
	Synthetic 1			$19 \pm 1$	$6.7 \pm 0.3$	$3.2 \pm 0.4$	$2.2 \pm 0.3$
	Synthetic 2			$19 \pm 1$	$5.3 \pm 0.4$	$2.6 \pm 0.4$	$1.6 \pm 0.3$

Synthetic effluent 1 and 2 had the same amount of heavy metals present in industrial effluent and were prepared in MES pH buffer, at pH 6.0. In the case of synthetic effluent 1, the same amount of inorganic ligands ( $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ ) of the industrial effluent was also added.

<sup>#</sup> Mean and standard deviation of three independent experiments performed in duplicate ( $n=6$ ).

batch, was implemented in the effluents (real and synthetics). Final concentrations of nickel and cumulative percentages of nickel removal obtained, after each batch in all effluents, are present in Table 3 and Fig. 4, respectively. Theoretical simulation of cumulative percentages of nickel removal were also calculated and included in Fig. 4; for this purpose, chemical speciation of nickel at pH 6.0 was calculated, assuming the total concentration of nickel, biomass and inorganic ligands present in the effluent, as well as the affinity constants between nickel and the ligands (biomass and inorganic ligands) using the MINEQL+ software [31].

After the first batch, the percentages of nickel removal were 70%, 65% and 72% (Fig. 4), for real and synthetic effluents with and without ligands, respectively. In the second batch, the replacement of biomass lowered the amount of nickel in solution to 2.7, 3.2 and  $2.6 \text{ mg l}^{-1}$  in real and synthetic effluents, respectively (Table 3). After the third batch, the concentration of nickel lowered to 2, 2.2 and  $1.6 \text{ mg l}^{-1}$  in real and synthetic effluents (Table 3), which corresponded to 89%, 88% and 92% of total cumulative removals, respectively (Fig. 4). This means that, after the implementation of the bioremediation process described in the present work, the concentration of nickel in the effluents reached the quality criteria for industrial effluents discharge ( $3.98$  and  $2.0 \text{ mg l}^{-1}$ ), after the second

or third batch according to U.S.-Environmental Protection Agency [34] and Portuguese law [33] respectively.

The comparative analysis of these results shows that equivalent nickel removal values were obtained from all effluents. Together, these results are in agreement with our prevision that the amount of inorganic ligands, as well as the small amount of organic matter, present in the real effluent did not influence the nickel removal.

Experimental values of nickel removal from effluents were lower than the theoretical simulated values (Fig. 4). The overestimation of the amount of nickel accumulated by the biomass can be a consequence of the stability constants between nickel and inorganic ligands used in the theoretical simulation calculations were not determined in the experimental conditions of the effluents (ionic strength and temperature) and/or due to the release of some ligands by the biomass, which will complex nickel and thus reduce the efficiency of bioremediation process [18,19].

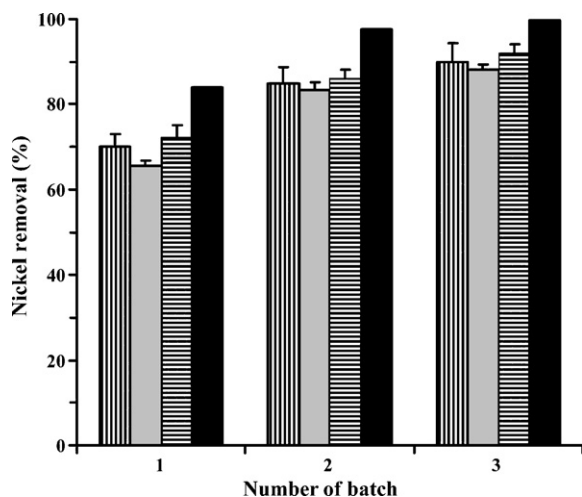
These results evidence that the use of a large excess of yeast biomass comparatively to nickel allowed an efficient removal of nickel from real and synthetic effluents, reducing the amount of metal in solution to the limit of discharge of wastewater in natural waters.

#### 4. Conclusions

This work examines the influence of the competitive effect of inorganic ligands (carbonates, chlorides, fluorides, phosphates, nitrates and sulphates), which can be present in real electroplating effluents, on the biosorption of chromium, copper, nickel and zinc ions by yeast cells of *S. cerevisiae*. The presence of high concentrations of carbonates, chlorides, fluorides, and nitrates do not compete with biomass for copper, nickel and zinc ions. On the other hand, the presence of phosphates or fluorides can reduce the efficiency of chromium biosorption. The presence of sulphates, at molar concentrations higher than that of biomass, can compete with biomass for nickel. To avoid high concentrations of sulphates in the electroplating effluents, and thus the problem of sulphates competition, we suggest the substitution of sulphuric by nitric acid (or chloridric acid) in the bisulphite solution for reduction of chromium (VI) to chromium (III) in the wastewater treatment plants of the electroplating industries.

Theoretical simulations, validated experimentally, have shown that for the bioremediation treatment of effluents containing nickel at pH 6.0, using a serial batch reactor, the amount of biomass ([Biomass]), when compared with the total amount of metals ( $\sum [M]$ ) should be in excess. We have demonstrated that the molar ratio of  $[\text{Biomass}]/(\sum [M])$  to be implemented can be computationally predicted, with an acceptable precision, if the concentrations of nickel and sulphates present in the effluent, as well as the affinity parameters ( $Q_{\text{max}}$  and  $\log K$ ) of the biosorbent, are known.

Finally, brewing flocculent cells of *S. cerevisiae* seems to be a promissory biomass alternative for the removal of nickel from industrial electroplating effluents. After the third batch, the amount



**Fig. 4.** Comparison of the theoretical and real nickel removal by *S. cerevisiae* NCYC 1364 through a series of sequential batches in bench scale experiments. Bar with vertical lines: industrial effluent; grey bar: synthetic effluent 1 (with the same amount of inorganic ligands of industrial effluent); bar with horizontal lines: synthetic effluent 2 (without addition of inorganic ligands); black bar: theoretical value. Theoretical calculations were performed as described in Fig. 2. Experimental assays were carried out with  $12 \text{ g dry weight l}^{-1}$  of biomass added to the effluents reported in Table 3. The effluents plus the biomass were placed in plastic flasks and shaken at  $150 \text{ rpm}$ , at  $25^\circ \text{C}$ . After 30 min of contact of the biomass with the effluents, cells were removed and the supernatant added to a fresh yeast biomass, in a subsequent batch. Values are the mean and standard deviation of two replicates of three independent experiments ( $n=6$ ).

of nickel in the effluent was reduced to values below the limit of discharge of wastewater in natural waters. This corresponded to a removal of nickel of 89%.

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