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Lanthanum Biosorption by Different *Saccharomyces cerevisiae* Strains

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Biosorption can be a promising technology in rare earth metal separation and recovery due to the low costs of waste biomasses (used as biosorbents) and the high selectivity exploiting specific interaction between metals and biological active sites. In this work, *Saccharomyces cerevisiae* biomass was used to recover lanthanum. Biosorption properties of two *S. cerevisiae* strains, wild type and rim20Δ mutant, have been tested. Potentiometric titrations were carried out for rim20Δ mutant strain and compared with wild type. Nature of the main active sites and their concentration were determined by implementing mechanistic models. Carboxylic, amino and phosphoric sites are the main groups present. Higher concentration of negatively charged sites was found in rim20Δ (0.0024 mol/g) than in wild type (0.0022 mol/g). The rate of lanthanum biosorption process is very fast requiring only 10-20 minutes to reach equilibrium condition for both strains. Then biosorption equilibrium tests were done for both biomasses by testing two equilibrium pH (4.0 and 6.0). Maximum uptake capacities (q_{max}) were: 70 mg/g and 40 mg/g at pH 4.0 for rim20Δ and wild type, respectively, and 67 mg/g and 80 mg/g at pH 6.0 for wild type and rim20Δ, respectively. These data evidenced that: rim20Δ mutant had a higher maximum biosorption capacity with respect to wild type counterpart, and that pH had a relevant effect on lanthanum removal.
S. cerevisiae yeast denoted good lanthanum biosorption properties and, between tested strains, rim20Δ was found to be the most promising for such aim.

1. Introduction

Actually rare earth metals (REM) are fundamental for the production of several new technologies. Lanthanum, cerium, neodymium and yttrium constitute the most part of the global market of REM. Nevertheless, geopolitical issues (95% of world supply for REM is located in China) and fast depletion of primary sources (Das and Das, 2013) risk to compromise and limit future applications. An alternative way is promoting REM recovery from Waste Electrical and Electronic Equipment (WEEE). It is calculated that each EU citizen produces about 17 kg of WEEE/year and these wastes contain rare earth concentrations higher than primary sources (Iannicelli Zubiani, et al. 2015). Following this aim different innovative hydrometallurgical processes were developed, based leaching, precipitation, ion exchange and solvent extraction (SX) operations to separate and purify REM contained in WEEE. However REM recovery technologies (and especially SX) present high costs and sometimes also low selectivity (Lucas et al., 2014).
Biosorption can be a promising alternative technology for REM separation and recovery due to the low costs of waste biomasses (used as biosorbents) and the high selectivity exploiting specific interaction between ionic species in solutions (REM) and biological active sites (Wang and Chen, 2009). Several biomasses can be used for such purpose. In this study *Saccharomyces cerevisiae* yeast was chosen due to its low cost as waste of different industries. In addition it can be easy cultivated and it is a model organism in biology, easy to study and to improve. *S.cerevisiae* is characterized by a cell wall, which is mainly constituted of proteins and polysaccharides. These macromolecules contain different functional groups, which can be involved in biosorption. As reported by Di Caprio et al. (2014), the main functional groups responsible for copper removal

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by *S. cerevisiae* are carboxylic, amino and phosphoric groups according to an ion exchange mechanism. Biosorption onto yeast can be affected by different operating parameters, extensively investigated in previous works (Wang and Chen, 2006). Most influential parameters are pH, temperature and presence of competing ions in solution. Although *S. cerevisiae* was extensively studied in many biosorption applications, no one reported sorption data concerning lanthanum removal using such biomass.
In this work different *S. cerevisiae* strains were evaluated for their lanthanum biosorption properties. Wild type strain was compared with rim20Δ mutant. Previous works reported that rim20Δ mutant has higher surface concentration of negatively charged groups on the cell wall with respect to the wild type, when stained with alcian blue (Conde et al., 2003). According to such properties this mutant is a good candidate for biosorption of lanthanum, generally present as cationic species in aqueous conditions. rim20Δ mutant is lacking of the rim20p protein, which is required for the response to external pH variation. It has been reported that this mutant is characterized by a higher concentration of mannosylphosphates than wild type, giving the higher negative superficial charge.
In this study these two strains were characterized to determine their negative charge concentration by potentiometric titrations. Then biosorption properties were tested in lanthanum removal equilibrium tests at pH 4.0 and 6.0. Maximum uptake capacity (q_{max}) was then estimated for all tested conditions and strains.

2. Materials and Methods

2.1 Yeast production
Yeast cells were grown in YPD medium (1% peptone, 1% yeast extract, 2% glucose, DIFCO) for 72 h at 303 K. Then cells were centrifuged and rinsed twice with distilled water to remove residual traces of the culture medium. The obtained pellet was dried in an oven at 338 K until a constant weight was attained.

2.2 Potentiometric titrations
Potentiometric titrations were carried out by following the method reported by Di Caprio et al. (2014).

2.3 Kinetic tests
For realizing kinetic tests 0.25 g of dried yeast biomass was suspended in 150 mL flask with 80 mL of lanthanum solution (La^{3+} 100 mg/L, $NaNO_3$ 0.01 M, fixed pH). Tests were carried out for both strains rim20Δ and wild type at pH 4.0 ± 0.2 and 6.0 ± 0.2. At 10, 15, 20, 70 and 90 minutes samples were collected and centrifuged at 8000 rpm. Supernatant was stored at 277 K.

2.4 Lanthanum analysis
For all biosorption tests, La^{3+} concentration in the collected samples was analysed by atomic absorption spectroscopy (Analytic Jena Contra 300).

2.5 Adsorption equilibrium tests
Equilibrium isotherms were determined for rim20Δ and wild type strains both at pH 4.0 ± 0.2 and 6.0 ± 0.2. For each isotherm different initial concentrations in the range of 100-400 mg/L of La^{3+} were prepared and used. Dried yeast biomass was suspended in 150 mL flask with 80 mL of lanthanum solution. For solutions with a concentration between 200 and 400 mg/L, 0.16 g of biomass was weighted. Instead for the tests with 100 mg/L of initial lanthanum concentration, 0.05 g of biomass was weighted. Lanthanum solutions were prepared using $La(NO_3)_3 \cdot 6H_2O$ salt. After 10, 30 and 60 minutes pH was measured and corrected at desired value with NaOH 1 M or HCl 1 M. After 60 minutes, 5 mL samples were collected, centrifuged at 8,000 rpm, and supernatant stored at 277 K.

3. Results and discussion

3.1 Potentiometric titrations
In potentiometric titrations, pH was determined as a function of titrant addition (mol of NaOH added to the biomass suspension). Imposing electro-neutrality condition in the system, the net surface charge concentration onto the biomass (Q, mol/g) was estimated for each titrant addition (i.e. for each point of the titration curve). Net surface charge concentration was calculated by using the following equation

$$Q = \frac{([Na^+] + [H^+] - [OH^-] - [Cl^-] - [NO_3^-])V}{m} \quad (1)$$

where V (mL) is volume of the suspension, m (g) the amount of biomass in the volume and Q the net charge of the biomass.

In Figure 1 potentiometric titrations of both strains were reported: only a subsample of the experimental data were reported in this graph in order to distinguish experimental data and modelling prediction. Both rim20Δ

and wt biomasses are characterized by net positive surface charge at low pH values, and by a predominance of negative charged sites at high pH values (Figure 1). Accordingly if ionic exchange is the main mechanism involved in lanthanum biosorption, it will be influenced by pH variation, and in particular lanthanum-site interactions will be favoured as pH increases. The point of zero charge (PZC) were 5.78 ± 0.06 and 6.3 ± 0.6 for rim20Δ mutant and wild type, respectively. Titration data showed that the mutant was characterized by a higher concentration of negative charges. The previous results obtained by using alcian blue method (Conde et al., 2003) are therefore confirmed also by potentiometric titrations.
Mechanistic models developed by Di Caprio et al. (2014) were used in order to describe experimental data, characterize acid-base properties and quantify concentration of charged sites. In particular, the model 2s-A and 3s-A were tested assuming specific schemes of protonation reactions for the active sites on the biomass surface. Model 3s-A assumed the presence of three sites, first and second sites are negative charged in deprotonated form and neutral in protonated form, while the third site is positive when protonated and neutral when deprotonated. Model 2s-A is a reduction of 3s-A model, obtained removing the second site.
Negative charge surface concentration given by 3s-A model is reported in Eq. (2).

$$Q = -\frac{S_{1\,tot}}{1+(K_{H1}[H^+])^{m_1}} - \frac{S_{2\,tot}}{1+(K_{H2}[H^+])^{m_2}} + \frac{S_{3tot}\left(K_{H3}[H^+]\right)^{m_3}}{1+(K_{H3}[H^+])^{m_3}} \quad (2)$$

where the concentration of the three active sites is indicated by the terms S_1, S_2 and S_3, while $K_{H,1}$, $K_{H,2}$ and $K_{H,3}$ are the equilibrium constants relative to the protonation reactions of the corresponding sites. They indicate average K_H of the active sites estimated. It is important to remark that $\log K_{H,j}$ is equal to $-\log K_{a,j}$, (pK_a), where $K_{a,j}$ is the acid dissociation constant of the corresponding site. The terms m_1,m_2 and m_3, are related to the heterogeneity of the sites, these terms are near to 1 when the heterogeneity is low and on the contrary, when it is high, they are near to 0 (Pagnanelli, 2011).
For rim20Δ mutant the best results were obtained by using 3s-A model (i.e. model giving the lowest confidence intervals of the estimated parameters), while best model for wild type is 2s-A model.
In Table 1 the estimated parameters obtained by non-linear regression of experimental data of potentiometric titrations by using these two models were reported.

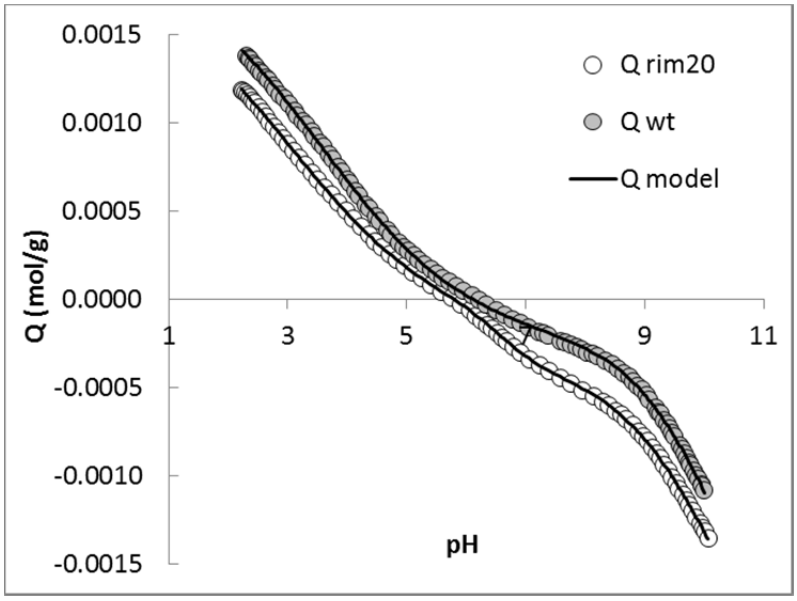

Figure 1: Potentiometric titrations for rim20Δ mutant and wild type strain of S. cerevisiae. With a black line the best models which describe experimental data are reported.

The total concentration of negative charged sites was 0.0024 ± 0.0001 mol/g for rim20Δ mutant and 0.0022 ± 0.0001 mol/g for wild type strain. Three active sites for *rim20Δ* had $\log K_H$ of 3.05, 6.60 and 10.12 for S_1,S_2 and S_3, respectively. Sites S_1 and S_3 probably correspond to phosphodiester and carboxylic groups (S_1) and amino groups (S_3). The S_2 site had a $\log K_H$ probably corresponding to a phosphoric groups. This kind of groups is relevant only for representing rim20Δ titration, even though with one order magnitude concentration lower than the other two kinds of sites. These results confirmed previous works (Di Caprio et al. 2014) and are in

agreement with other findings (Conde et al., 2003) indicating mannosylphosphate groups as responsible of the high negative charge in rim20Δ.

Table 1: Parameters estimated by non-linear regression of potentiometric data using mechanistic models 2s-A and 3s-A for wild type and rim20Δ, respectively; intervals of confidence calculated with α=0.05.

	Rim20Δ (model: 3s-A)	Wild type (model: 2s-A)
$[S_1]$ (mol/g)	0.0021 ± 0.0001	0.0022 ± 0.0001
$[S_2]$ (mol/g)	0.00025 ± 0.00004	-
$[S_3]$ (mol/g)	0.00192 ± 0.00008	0.0019 ± 0.0001
$logK_{H,1}$ ($pK_{a,1}$)	3.05 ± 0.06	3.6 ± 0.1
$logK_{H,2}$ ($pK_{a,2}$)	6.60 ± 0.03	-
$logK_{H,3}$ ($pK_{a,3}$)	10.12 ± 0.06	10.2 ± 0.1
m_1	0.34 ± 0.02	0.35 ± 0.03
m_2	1.2 ± 0.1	-
m_3	0.55 ±0.02	0.59 ± 0.03

3.2 Determination of lanthanum equilibrium isotherms

The effect of operating conditions on La biosorption onto *S. cerevisiae* strains was assessed by performing equilibrium tests for different initial La concentration in solution and at different equilibrium pH. In particular the effect of two pH values (4.0 ± 0.2 and 6.0 ± 0.2) was tested for both strains.

Equilibrium time was preliminary determined by kinetic tests. These preliminary tests showed that 20 minutes are sufficient for having no more relevant variation of lanthanum residual concentration in solution for both strains and pH tested (data not shown here).

Results of equilibrium tests were reported in Table 2. Experimental data show that there is not a dependence of the amount of adsorbed lanthanum (q_e) with respect to corresponding tested equilibrium concentrations in solution (C_e). This indicates that both strains experienced saturation conditions at the two levels of pH in the investigated range of concentrations. This means high efficiency in lanthanum removal at low initial lanthanum concentrations. For each isotherm the measured q_e were therefore considered as independent estimations of the maximum adsorption capacity (q_{max}). The average value of the q_e experimentally determined was used to estimate q_{max}. Data were then statistically analyzed by using an analysis of variance (ANOVA) by considering a 2^2 design with α=0.05. Through this analysis both pH and strains used have shown a statistically positive significant effect on maximum sorption capacity. This is shown in Figure 2, in which the effects calculated for each investigated factor are compared with respect to the least significant difference (LSD).

Table 2: Amount of equilibrium lanthanum adsorbed for the corresponding concentrations in solution are reported for both strains at pH 4 and 6.

		pH 4			pH 6	
Strain	C_e (mg/L)	q_e (mg/g)	average (q_{max})	C_e (mg/L)	q_e(mg/g)	average (q_{max})
Wild type	112	54	40 ± 10	75	68	67 ± 6
	171	56		151	60	
	260	26		216	69	
	320	47		279	64	
	68	39		47	75	
Rim20Δ	60	73	70 ± 10	28	94	80 ± 10
	133	68		116	87	
	202	58		176	74	
	266	72		237	67	
	30	98		40	96	

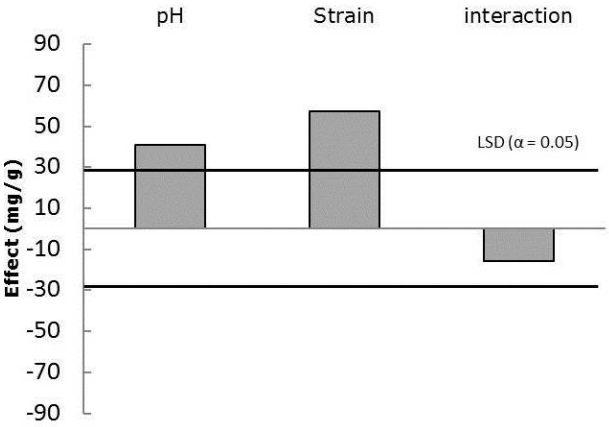

Figure 2: Effects of the investigated factors on the maximum lanthanum adsorption capacity (q_{max}). With straight lines value of the least significant difference (LSD) is indicated.

As proved by previous works (Di Caprio et al., 2014, Wang and Chen, 2006) pH has a relevant effect on biosorption of metals onto yeast biomass. This is mainly due to competition between metals and protons for the active sites on the biomass: when pH increases the concentration of negative charges on the biomass surface increases too (Figure 1). This effect leads to an increment of negatively charged active sites available for lanthanum biosorption when pH changes from 4.0 to 6.0, and accordingly to an increment of lanthanum uptake capacity. The best performance of rim20Δ mutant with respect to wild type strain can be explained considering data reported in Figure 1 and in Table 1. For a given pH rim20Δ has a higher concentration of negatively charged sites. La biosorption capacity found in this work is higher than those reported in previous studies for other biomasses. For example La sorption capacity of 22.94 mg/g was found for *Pinus brutia* leaf powder (Kütahyali et al., 2010), and 28.65 mg/g for *Platanus orientalis* leaf powder (Sert et al., 2008). Similar capacity was found for *Sargassum fluitans* ranging between 40 and 70 mg/g for the same pH of the present work (Palmieri et al. 2002). In contrast dramatically higher capacity was found by Kazy et al., (2006) for *Pseudomonas sp.* corresponding to about 1,900 mg/g.

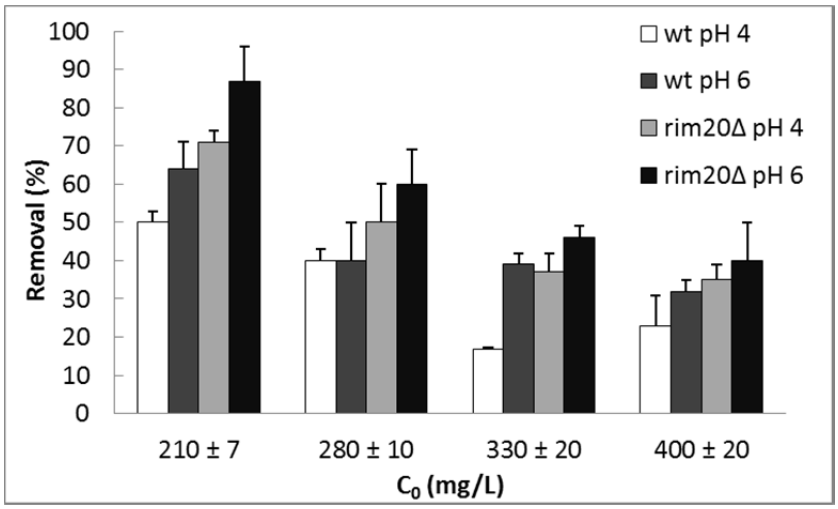

Figure 3: Removal efficiency of the equilibrium tests carried out for rim20Δ and wild type at pH 4.0 and 6.0.

In Figure 3 the removal efficiencies evaluated for the equilibrium tests carried out in this work were reported. Tests done with an initial lanthanum concentration of 100 mg/L were not included because using a different biomass concentration. The removal efficiency decreased when initial lanthanum concentration was increased. The efficiency was 50-90 % for an initial concentration of 200 mg/L and became 20-35% for 400 mg/L.

4. Conclusions

S. cerevisiae yeast was found to be a promising biomass to recover lanthanum in recycling processes for RAEE. In particular most promising properties are the ability to remove high amount of lanthanum also at low concentrations and the loading capacity (q_{max}) higher than different other tested biomasses. The higher biosorption capacity of the rim20Δ mutant with respect to wild type indicated that relevant improvement can be reached by using mutant strains following the way of maximizing negative surface charge as target in strain selection.

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