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Removal of cadmium and lead from dilute aqueous solutions by *Rhodotorula rubra*

E. Salinas [a], M. Elorza de Orellano [b], I. Rezza [b], L. Martinez [c], E. Marchesvky [c], M. Sanz de Tosetti [d,*]

[a] *Area de Biologia, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina*
[b] *Area de Quimica Biológica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina*
[c] *Area de Química Analítica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina*
[d] *Area de Tecnología Química y Biotecnología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina*

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Abstract

Removal of cadmium (Cd) and lead (Pb) from dilute aqueous solution (5–40 mg/l) by the yeast *Rhodotorula rubra* was examined. The influence of pH and temperature of the solution and the state of the cells (viable and nonviable biomass) on heavy metal removal were studied. The uptake of Cd and Pb was significantly affected by the initial pH of the solution. At low pH the removal of Cd and Pb decreased while the removal of the metals increased with increasing pH. The optimum initial pH values were 4–4.5 and 5.5–6 for uptake of Pb and Cd, respectively. The effect of temperature was different on each metal. For Pb uptake the increase in temperature (25°C to 37°C) was adverse while Cd uptake increased with temperature. A Langmuir sorption model was used to evaluate the sorption behaviour of the yeast and Langmuir parameters were obtained. Metal uptakes at equilibrium residual concentrations of 10 mg/l (q_{10}) were also calculated for comparison with other biosorbents. The q_{10} value for Cd or Pb uptake by *R. rubra* biomass was higher than the q_{10} value reported for *Saccharomyces cereviseae* or fungal biomass. Desorption was carried out with either 0.1 M EDTA or 0.1 M HCl. The maximum amount of Cd was desorbed in 10 ml 0.1 M EDTA, while desorption efficiency of 0.1 M HCl was lower. In Pb desorption tests there was no difference between the two elutants.

Keywords: Cadmium; Lead; Yeasts; *Rhodotorula rubra*

1. Introduction

Physico-chemical processes usually achieve the removal of heavy metals from a metal-bearing wastewater before discharging the effluent into a natural water body. Conventional treatment technologies such as precipitation and coagulation become less effective and more expensive when situations involving high volumes and low metal concentrations (typically less than 50 mg/l) are encountered. Conversely, membrane processes have been limited in their use due to their high costs, process complexity and low efficiency of heavy metal removal. (Kapoor and Viraraghavan, 1995). Alternatively, the potential use of microorganisms in the treatment of metal-bearing wastewater has been studied intensively. Many organisms, including bacteria, fungi, and algae are able to remove metals from solutions (Gadd, 1990; Kapoor and Viraraghavan, 1995).

Yeasts, among which *Saccharomyces cerevisiae* has been the most studied, possess a documented potential for taking up a range of metal cations. Mechanisms of adsorption (Avery and Tobin, 1993), the influences of chemical and physical properties of metals (Brady et al., 1994), cellular physiology (Blackwell et al., 1995), and environmental conditions, e.g., pH and temperature (Brady and Duncan, 1994) on metal uptake by *Saccharomyces cerevisiae* cells have been described. However *S. cereviseae* biomass has been referred to as a mediocre biosorbent (Volesky and Holan, 1995a).

The potential of other yeasts for heavy metal uptake has been less studied. For instance, some soil

* Corresponding author. Fax: +54-2652-430-224.
E-mail address: msanz@unsl.edu.ar (M. Sanz de Tosetti)

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Table 1
Admissible Cd and Pb levels in Argentina according Dangerous Wastes Act (National Act number 24 051)

	Metal level μg / l	
	Cd	Pb
Drinking water	1	50
Dam/river water	0.2	1
Irrigation water	5	200
Watering place	20	200

yeasts including *Rhodotorula* sp, are resistant to heavy metals (Falih, 1998a,b). Particularly, *Rhodotorula rubra* has been isolated from mine effluents (Linardi et al., 1995) and minerals (Rezza et al., 1997), but the available information about the removal of heavy metals from solutions by this yeast is limited. Norris and Kelly (1979) and Kurek et al. (1982) reported that *R. mucilaginosa* was able to accumulate cadmium and copper. More recently, Falih (1998a) reported that Cd and Pb were accumulated in biomass of *Rhodotorula minuta* in amounts between 4 and 5 mg/g dry weight at a metal initial concentration of 400 mg/l in the medium.

Cadmium and lead are included among the major pollutants because of their high toxicity. In Argentina, the levels of Cd and Pb in waters (Table 1) are regulated by the National Act number 24 051 on Dangerous Wastes. Therefore, the objectives of this work were to determine the ability of a strain of *Rhodotorula rubra* for Cd and Pb removal from dilute aqueous solutions and to examine the influence of solution physicochemical parameters and different physiological states of the cells on the heavy metal uptake.

2. Methods

2.1. Microorganism, medium and growth conditions

Rhodotorula rubra was isolated from Spodumene in our laboratory as previously described (Rezza et al., 1997). Cultures were grown in a liquid medium comprising: glucose, 5 g/l; yeast extract, 0.05 g/l; $(NH_4)_2SO_4$, 0.5 g/l; $FeSO_4$, 0.005 g/l; $MgCl_2 \cdot 6H_2O$, 0.2 g/l; KH_2PO_4, 0.2 g/l. Cultures were incubated at 30°C on an orbital shaker (200-rpm).

Viable biomass was obtained in the following way: cells from stationary phase (24 h), were harvested by centrifugation ($1200 \times g$, 10 min) at room temperature and washed twice with distilled deionized water.

Nonviable biomass was obtained from culturable cells by heating at 90°C in a waterbath for 1 h. To verify the effectivity of heat treatment, cells were stained with methylene blue and were plated on plates containing growth medium with 2% agar. Results showed that cells did not retain viability.

2.2. Chemicals

From the nitrate salts of either Cd(II) or Pb(II), solutions containing from 5 to 40 mg/l of metals were prepared.

2.3. Metal accumulation experiments

Aliquots (50 ml) of each metal solution were pìpetted into 250-ml Erlenmeyer flasks. Before adding viable or nonviable biomass (0.5 g wet weight), the pH of each solution was adjusted to pH 4 using 0.5 M HNO_3. In all cases, the flasks were incubated at a constant temperature (25°C) on an orbital shaker at 200 rpm for 1 h. After incubation, cells were separated by centrifugation at $12\,000 \times g$ for 5 min, and the supernatant liquid was used for metal analysis. Metal accumulation in the biomass was determined by the difference in metal content between flasks containing no biomass (control) and those containing biomass (tests).

2.4. Analysis of heavy metal ions

Metal analysis was carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES, BAIRD ICP 2070, Bedford, MA, USA).

2.5. pH and temperature

In order to evaluate the effect of pH and temperature on metal uptake, the pH of the solution was adjusted to be in the range between 2 and 6 before mixing viable biomass. The pH was adjusted to the required value with 0.1 M HNO_3 or 0.1 M NaOH. The initial concentration of metal was 5 mg/l. Experiments were performed at 25°C and 37°C.

2.6. Adsorption isotherms

In order to obtain the sorption kinetics data, the metal uptake value (q) was calculated using the following equation:

$$q\ (\text{mg metal/g of biomass}) = V(C_i - C_f)/1000\ m,$$

where V is the volume of metal solution (ml), C_i and C_f are the initial and final concentration of metal respectively, and m is the mass of the yeast.

The Langmuir sorption model was chosen for estimation of the maximum metal uptake (q_{max}):

$$q = q_{max} b C_f/(1 + bC_i),$$

where b is the Langmuir constant and q_{max} the maximum metal uptake.

2.7. Desorption

Following the metal sorption experiments, biomass loaded with Cd or Pb was separated by centrifugation, washed, and returned to 100 ml Erlenmeyer flasks with 10 ml of elutant solution (0.1 M EDTA or 0.1 M HCl). The desorption was carried out on a rotatory shaker (25°C, 200 rpm) for 1 h. The concentration of metal released into the solution was also determined by ICP-AES.

2.8. Statistical analysis

All experiments were performed in triplicate. Statistical analyses were done with MicroCal Origin 5.0 (MicroCal Software, 1997). All data were subjected to the analysis of variance (one way ANOVA). Since the efficiency of desorption data were evaluated as percentages of desorption, an angular transformation was performed for statistical analysis.

3. Results and discussion

Based on preliminary assays in which viable *Rhodotorula rubra* cells were able to take up Cd (II) and Pb (II) ions from solutions of the nitrate salts, we studied the effect of several factors on the uptake of heavy metals by this microorganism. These included the specific properties of the organism (depending on the physiological state of the cells) and solution physicochemical parameters such as temperature and pH.

3.1. Effect of pH and temperature on heavy metal uptake

The results obtained, as depicted in Figs. 1 and 2 clearly showed that the uptake of Cd and Pb by viable cells was significantly ($P = 0.05$) affected by the initial pH of the solution. At low pH, removal of Pb and Cd decreased, while removal of both metals increased as pH increased. The optimum initial pH values were 4–4.5 and 5.5–6 for uptake of Pb and Cd, respectively. It was observed that the final pH at equilibrium was 3–3.5 when initial pH was in a range between 4 and 6. This fact in addition to the decrease in metal uptake at low pH suggest that cations and protons would compete for the same sites.

The effect of changes in the temperature on the metal uptake is also shown in Figs. 1 and 2. The effect was different for each metal. While for Pb uptake the increase in temperature produced an adverse effect, Cd uptake increased with increasing temperature. This response suggested a different interaction between the ligands on the cell wall and the metals: Physical adsorption reactions are normally exothermic, thus the extent of adsorption generally increases with decreasing

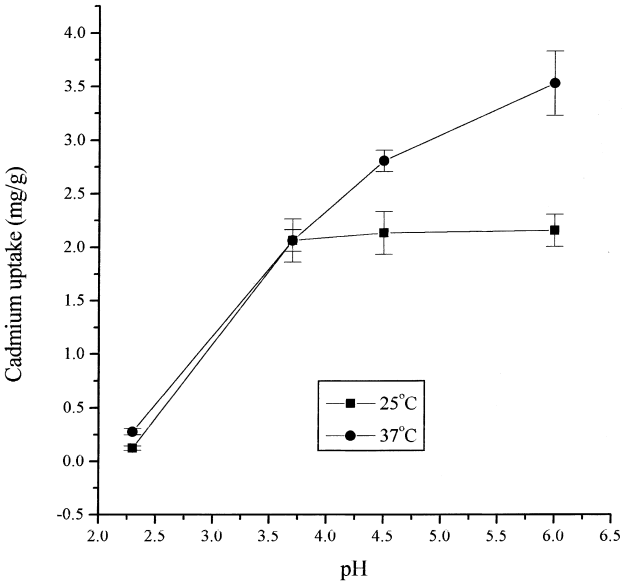

Fig. 1. Effect of pH and temperature on cadmium uptake from dilute solution (5 mg/l) by *Rhodotorula rubra* viable biomass. All values are means of triplicates ±SD.

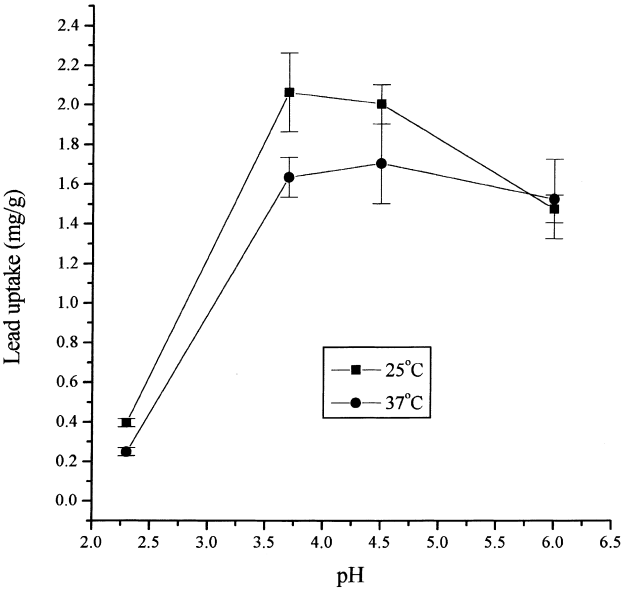

Fig. 2. Effect of pH and temperature on lead uptake from dilute solution (5 mg/l) by *Rhodotorula rubra* viable biomass. All values are means of triplicates ±SD.

temperature. If chemical adsorption mechanisms play a dominant role in the whole adsorption process, adsorption is expected to increase by increasing the temperature (Sag and Kutsal, 1995). For Cd uptake, the effect of the temperature was more noticeable at pH levels between 4.5 and 6. Probably, at this pH range the state of the ligands on the cell wall allowed covalent bondings. (Avery and Tobin, 1993). Despite the fact that

optimum pH for uptake of Cd was higher, we continued the assays at pH 4 because Cd containing industrial wastewaters usually have an acidic pH (between 1–4). Also, for practical purposes the lower temperature was chosen.

3.2. Uptake of metals by viable and nonviable biomass

Cd and Pb biosorption isotherms for *Rhodotorula rubra* are depicted in Figs. 3 and 4. Nonviable biomass demonstrated higher metal uptake than viable biomass (means were significantly different at $P = 0.05$). A Langmuir sorption model was used to evaluate the sorption behaviour of viable and nonviable cells and the Langmuir parameters are summarized in Table 2. Metal uptakes at equilibrium residual concentrations of 10 mg/l (q_{10}) were selected for comparison with other biosorbents. The q_{10} was calculated from the Langmuir model. The Langmuir constants (b, l/mg) related to the affinity of the sorbent material for the metal sorbate are also shown in Table 2.

When Cd uptake by *R. rubra* was compared to the metal uptake by *Saccharomyces cereviseae* reported by other authors, it could be seen that the red yeast was a better biosorbent. A $q_{10} = 9.8$ mg/g for *R. rubra* nonviable biomass and $q_{10} = 4.05$ mg/g for *R. rubra* viable biomass were calculated. The q_{10} reported for Cd biosorption by *S. cereviseae* (viable biomass), under similar experimental conditions, was 3 mg/g (Volesky et al., 1993). The q_{10} value for Cd uptake by *R. rubra* nonviable biomass is also higher than the q_{10} value reported for fungal biomass ($q10 = 8$ mg/g for *Penicillium chrysogenum*) and wood sorbents ($q10 = 7$ mg/g for carboxylated sawdust) (Volesky and Holan, 1995b).

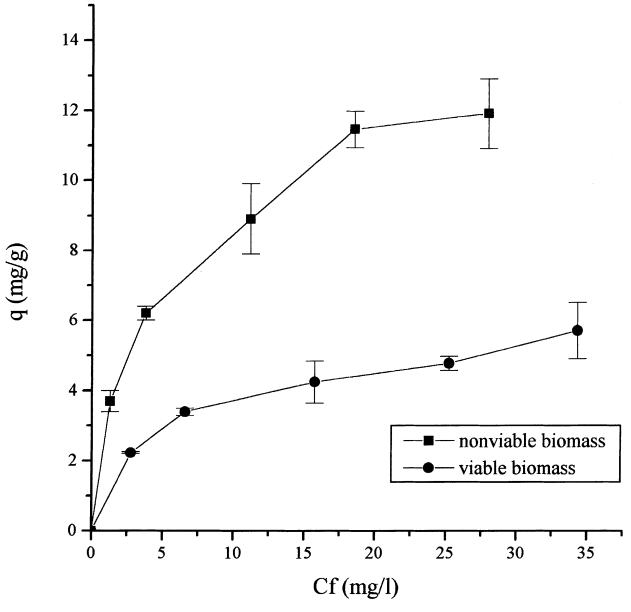

Fig. 3. Cd-sorption isotherms for *Rhodotorula rubra* biomass (pH 4, 25°C). All values are means of triplicates ±SD.

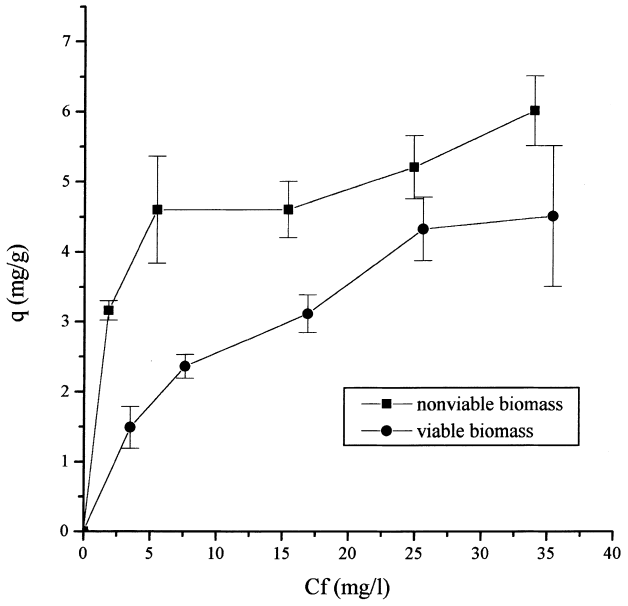

Fig. 4. Pb-sorption isotherms for *Rhodotorula rubra* biomass (pH 4, 25°C). All values are means of triplicates ±SD.

With respect to Pb, we did not find reports of q_{10} values for Pb uptake by yeasts. However, Falih (1998a) reported the accumulation of Pb in *R. minuta* cells reaching concentrations of 2 mg/g dry weight from Pb initial concentrations of 100 mg/l, after 4 weeks of incubation. In the present investigation, the q_{10} for Pb uptake by *R. rubra* (8.28 mg/g) was similar to or higher than the q_{10} of *Rhizopus arrhizus* (8 mg/g) or carboxylated sawdust (6 mg/g), respectively (Volesky and Holan, 1995b).

The results obtained could be due to the composition of the cell wall. *R. rubra* is a basidiomycetous yeast that is characterized by a cell wall with a different composition and structure than the cell walls of ascomycetous yeasts such as *S. cereviseae.* The wall of basidiomycetous is composed of mannan and chitin with traces of glucan while the ascomycetous yeast cell wall is composed of glucan and mannan with traces of chitin, (Spencer and Spencer, 1997). Chitin and chitosan are acknowledged sorbents (Muzzarelli et al., 1980). Coordination of the metal to the amine N of chitin and adsorption in cell wall chitin has been reported to be occurring in uranium adsorption by *R. arrhizus* (Tzesos, 1983).

Another characteristic of red yeast cell wall is the presence of a fucogalactomannan type polymer containing fucose and galactose. This polysaccharide has a highly branched structure but has not been detected in the cell wall of *Saccharomyces* (Lee et al., 1981). This structure could contribute to the higher adsorption capacity of *Rhodotorula rubra*. Nevertheless, the role

Table 2
Langmuir parameters for Cd and Pb uptake by viable and nonviable biomass of the yeast *Rhodotorula rubra*. (pH 4, 25°C). Data were obtained by using MicroCal Origin 5.0 (MicroCal Software, 1997)

		q_{10} (mg/g)[a]	q_{max} (mg/g)	b (l/mg)	r^2
Cd	Viable cells	4.02	5.8	0.21	0.99
	Nonviable cells	9.90	13.3	0.29	0.99
Pb	Viable cells	4.16	5.8	0.25	0.99
	Nonviable cells	8.28	9.09	1.03	0.99

[a] q_{10}: metal uptake at the residual concentration of 10 mg/l; r: correlation coefficient.

played by structural polysaccharides is not fully understood and needs to be studied in greater detail.

Finally, it must also be considered that *R. rubra*, as well as the other members of its genus, produces slime and capsule. α-D and-β-D-mannan, phosphomannan and fucogalactan (fucose: D-galactose: O-acetyl, 1 : 1 : 0.6) are produced by *Rhodotorula* sp (Slodki, 1979). We have previously demonstrated that capsular exopolymers of *R. rubra* played an important role in removing and sequestering metals from minerals (Rezza et al., 1997) and probably capsular exopolymers are also contributing to heavy metal removal.

Results of desorption tests, for viable and nonviable biomass loaded by Cd or Pb, are shown in Tables 3 and 4. The maximum amount of Cd was desorbed in 10 ml 0.1 M EDTA, while the desorption efficiency of 0.1 M HCl was lower. In Pb desorption tests, desorption efficiency of 0.1 M EDTA was similar to the Cd desorption with the same elutant but the desorption efficiency of 0.1 M HCl was higher. This difference in desorption efficiency of Cd and Pb with 0.1 M HCl could be due to different stabilities of the chlorocomplex of Cd (II) and Pb (II) (Inczédy, 1976), as much as to the insolubility of $PbCl_2$.

The Cd recovered from viable cells was approximately 50% of the metal recovered from nonviable cells (means were significantly different at $P = 0.05$), while differences in Pb recovered from viable and nonviable cells were not significant ($P = 0.05$). Thus, in the case of Cd, a metal accumulation mechanism involving transport of the metal through the cell membrane into the cytoplasm could be possible.

To distinguish between the Cd sorbed by the biomass and Cd actually taken up (bioaccumulation), metal uptake assays were performed in the presence of sodium azide (1 mM), a respiratory inhibitor. Results are shown in Fig. 5. The presence of azide inhibited the metal uptake. This response suggested that Cd is taken up in viable cells by means of two mechanisms: (1) a passive adsorption of the metal on the external cell surface; and (2) a transport of the metal through the cell membrane into the cytoplasm that involves a metabolic process. A similar study was carried out for Pb but the presence of azide did not affect the Pb uptake by viable cells.

The yeast appeared to be capable of removing heavy metals from dilute aqueous solution in a batch system. A commercial concern is the removal of metal from aqueous waste streams with immobilised cells. At pre-

Table 3
Desorption tests for Cd loaded biomass[a]

		Desorption efficiency %	
		Desorbing agnt	
		EDTA 0.1M	HCl 0.1 M
Cadmium	Nonviable cells 6 mg metal /g d.w	$76^a \pm 6$	$36^c \pm 4.5$
	Viable cells 6 mg metal/ g d.w	$35^b \pm 2.6$	$17^d \pm 0.17$

[a] Means with the same letter within a column are not significantly different at $P = 0.05$.

Table 4
Desorption tests for Pb loaded biomass[a]

		Desorption efficiency %	
		Desorbing agent	
		EDTA 0.1 M	HCl 0.1 M
Lead	Nonviable cells 6 mg metal/g d.w	80 ± 2.12	75 ± 2.2
	Viable cells 5 mg metal/ d.w	75 ± 2	68 ± 6.6

[a] Means are not significantly different at $P = 0.05$.

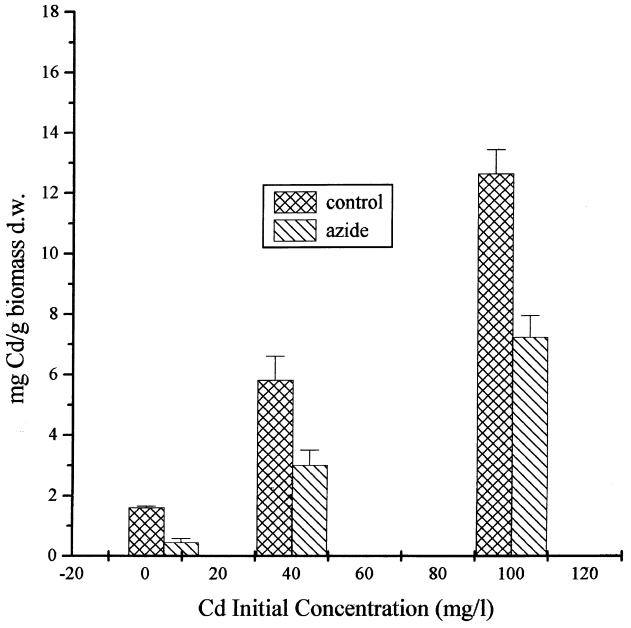

Fig. 5. Effect of sodium azide (1 mM) on the uptake of Cd by viable yeast cells. All values are means of triplicates ±SD.

sent we are studying the possibility of *R. rubra* biofilm formation on sand. This yeast has been isolated from aquatic environments, including effluents (Linardi et al., 1995). Moreover, oligotrophic environments enhance the production of exopolymers produced by *R. rubra* and trigger biofilm development on surfaces where organic nutrients tend to associate (Rezza et al., 1997; Costerton et al., 1995). As biofilm on sand, this red yeast would be an interesting alternative to the "finishing" treatment of wastewater after the application of physicochemical methods. As nonviable biomass, *R. rubra* would also be a good biosorbent for treating heavy metal-containing effluents with low metal concentrations. This yeast is easy to grow and has minimal nutritional requirements, so that the biomass can be obtained using unsophisticated fermentation techniques and inexpensive growth media.

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