Bioprocess Engineering 21 (1999) 431–437 © Springer-Verlag 1999

The use of flocculating brewer's yeast for Cr(III) and Pb(II) removal from residual wastewaters

A.I. Ferraz, J.A. Teixeira

Abstract The use of inexpensive biosorbents to sequester heavy metals from aqueous solutions, is one of the most promising technologies being developed to remove these toxic contaminants from wastewaters. Considering this challenge, the viability of Cr(III) and Pb(II) removal from aqueous solutions using a flocculating brewer's yeast residual biomass from a Portuguese brewing industry was studied. The influence of physicochemical factors such as medium pH, biomass concentration and the presence of a co-ion was characterised. Metal uptake kinetics and equilibrium were also analysed, considering different incubation temperatures. For both metals, uptake increased with medium pH, being maximal at 5.0. Optimal biomass concentration for the biosorption process was determined to be 4.5 g dry weight/l. In chromium and lead mixture solutions, competition for yeast binding sites was observed between the two metals, this competition being pH dependent. Yeast biomass showed higher selectivity and uptake capacity to lead. Chromium uptake kinetic was characterised as having a rapid initial step, followed by a slower one. Langmuir model describes well chromium uptake equilibrium. Lead uptake kinetics suggested the presence of mechanisms other than biosorption, possibly including its precipitation.

1 Introduction

The continuous use of heavy metals in industrial applications with the production of contaminated wastewaters is a serious environmental problem. The importance of developing efficient and inexpensive treatments to these residual waters is clear considering that heavy metals represent a threat to public health due to their accumulation through the food chain (Holan and Volesky, 1994) and are non-renewable natural resources some of them with commercial value.

Received: 10 December 1998

A.I. Ferraz, J.A. Teixeira (✉)
Centro de Engenharia Biológica-IBQF,
Universidade do Minho 4700 Braga, Portugal

The authors acknowledge the financial support provided by the Instituto de Biotecnologia e Química Fina (IBQF), the Sub-Programa Ciência e Tecnologia do 2º Quadro Comunitário de Apoio through J.N.I.C.T (Junta Nacional de Investigação Científica e Tecnológica), and the project PEAM/SEL/516/95.

An alternative to traditional treatments, such as chemical precipitation with lime and resin ionic exchange, often inadequate when applied to large volumes of dilute solutions (Volesky, 1987), is the use of biological processes. Many microorganisms are able to accumulate and concentrate heavy metals from aqueous solutions, by several mechanisms (Rus et al., 1995). These include biosorption, a metabolism independent mechanism usually with high kinetics allowing metals recovery by elution, bioaccumulation a metabolism dependent mechanism only carried out by living cells, and the metal toxicity inactivation by precipitation with secreted metabolites (Novais, 1992).

An industrial biosorption process requires large quantities of biomass. Biomass can be cultivated specifically to be used as biosorbent, or alternatively waste biomass from industrial operations can be used (Tsezos, 1990). Last option can be economically more attractive, and the biomass uptake capacity can be improved by changes suffered during the process (Avery and Tobin, 1992).

An important step to the development of biosorption processes is the selection of optimal physicochemical conditions such as medium pH, temperature and biomass concentration, and to elucidate the effect of co-ions. Medium pH, affecting ion speciation in solution as well as the chemistry of biomass active sites, is extremely important (Tsezos, 1990).

The study of process kinetics and equilibrium is another fundamental step, giving important information about the uptake mechanism. Heavy metal uptake by biomass typically shows a rapid initial phase, associated to metabolism independent mechanisms (biosorption), followed by a slower one, associated to metabolism dependent processes (bioaccumulation) (Wehrheim and Wettern, 1994).

Equilibrium uptake may be described by adsorption isotherms. Langmuir and Freundlich models often fit reasonably well experimental data (Tsezos, 1990). These models, although being rather simplistic when applied to complex biological systems, give valuable information concerning biomass uptake capacities, describing adsorption equilibrium conditions of different types of biomass, and showing differences between different species and morphological types (Gadd, 1990).

Process viability also depends on its economical competitiveness with existing technologies. To be considered a good biosorbent, biomass should exhibit a rapid and efficient uptake and desorption, have a low production cost and be reusable, and be efficiently, rapid and economically separated from solution (Volesky, 1987).

The purpose of this work was to study Cr(III) and Pb(II) uptake by the flocculating yeast *S. cerevisiae* obtained as a residue from beer production. The use of this biomass is particularly interesting, as it is residual from beer production being available in large quantities with low costs. Furthermore, being flocculating, separation costs from the residual water are reduced and allows the use of high cellular density systems (Soares, 1995). The influence of pH, biomass concentration, the presence of a second ion (lead/chromium) and incubation temperature in the yeast uptake capacity was characterised.

2 Materials and methods

2.1 Microorganism

S. cerevisiae was obtained from UNICER, a Portuguese brewing industry, at the end of fermentation when it presents the highest flocculating capacity (Teixeira et al., 1991). Biomass suspension was prepared by washing 50 g of wet yeast in distilled water, followed by centrifugation (3 minutes, 3000 rpm). This procedure was repeated three times. Washed biomass was finally suspended in 100 ml of distilled water. This final biomass suspension was added to metal solutions in quantities depending on biomass concentration, referred as biomass dry weight/l, required in each essay.

2.2 Metal solutions

Metal solutions were prepared dissolving $CrCl_3 \cdot 6H_2O$ or $PbNO_3$ in distilled water.

2.3 Effect of medium pH

All essays were carried out with 100 ml of metal solutions (55 ppm) prepared in 250 ml flasks. The pH of chromium solutions was adjusted to 2.0, 3.4, 4.0, 4.5 and 5.0. A control solution was also prepared with pH 2.7. Lead solutions were adjusted to pH 3.0, 4.0 and 5.0. Control solution had a pH of 4.7. Control situation corresponds to experiments where pH was not adjusted. Medium pH corrections were made with HNO_3 or NaOH 0.1 M. Previously prepared yeast suspension (10 ml) was added to metal solutions, and flasks were incubated at 30 °C with orbital shaking (150 rpm). Samples from chromium and lead solutions were taken after 1 hour of incubation.

2.4 Effect of biomass concentration

250 ml of chromium and lead solutions were prepared in 500 ml flasks with different initial metal concentrations: 52, 55 and 60 ppm. 9.5, 25 and 50 ml of yeast suspension were respectively added to these solutions in order to obtain biomass concentrations around 1.5, 4 and 8 g dry weight/l. The pH of chromium solutions was adjusted to 5.0. The solutions were incubated at 30 °C with orbital shaking (150 rpm). During 26 hours, 10 ml samples were taken at different time intervals.

2.5 Effect of co-ions

In 500 ml flasks, 250 ml of metal solutions were prepared according to Table 1 without pH adjustment.

Each solution was inoculated with 10 ml of yeast suspension and incubated at 30 °C for about 24 hours, 10 ml samples taken at different time intervals. Another set of experiments was done with chromium and mixture solutions pH corrected to 5.0. The pH of lead solutions wasn't adjusted as its initial value was already around 5.0.

2.6 Kinetics experiments

Metal solutions (250 ml, 55 ppm) were prepared in 500 ml flasks. The pH of chromium solutions was adjusted to 5.0. Solutions were inoculated with 25 ml of yeast suspension and incubated at 10, 25, 30 and 35 °C for 24 hours. During this period 10 ml samples were removed at different time intervals.

2.7 Equilibrium experiments

Yeast suspension (10 ml) was added to 100 ml of metal solutions with concentrations from 5 to 200 ppm. Chromium solutions pH was adjusted to 5.0. After 1 hour of incubation at 10, 25, 30 and 35 °C, 10 ml samples were collected.

2.8 Biomass dry weight

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

2.9 Analytical methods

Chromium and lead concentration in the samples was determined by AAE after biomass removal by filtration. Samples were preserved by acidification to pH 2 with concentrated HNO_3 and kept at 4 °C.

3 Results and discussion

3.1 Effect of medium pH

Figure 1 shows that yeast uptake capacity increases with pH, being higher at pH 5.0 for both metal ions. Higher pH

Table 1. Metal solutions used to study co-ions effect

Mixture solutions	Control solutions
25 ppm Cr/25 ppm Pb	25 ppm Cr/0 ppm Pb 0 ppm Cr/25 ppm Pb
100 ppm Cr/100 ppm Pb	100 ppm Cr/0 ppm Pb 0 ppm Cr/100 ppm Pb

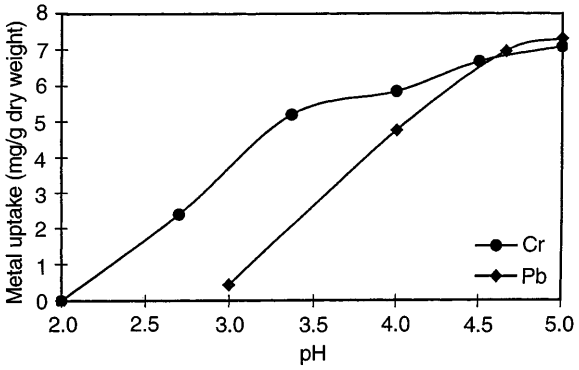

Fig. 1. Effect of initial pH on metal ions uptake

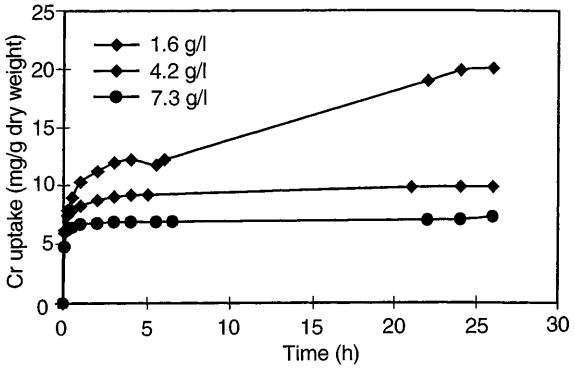

Fig. 2. Effect of biomass concentration in chromium uptake

caused metals precipitation. This behaviour can be explained by the effect of pH on the cell wall functional groups and on the ionic species present in solution, particularly in the case of chromium (Ramos et al., 1995). The cell wall has an amphoteric behaviour according to medium pH. Above the isoelectric point, the net charge of the yeast cell wall is negative due to the ionisation of functional groups as $-COOH$, $-OH$ and $-NH_2$, explaining the greater metal uptake capacity at higher medium pH.

At pH 2 chromium is mainly in the form of Cr^{3+} (Ramos et al., 1995). Since there was no chromium uptake at this pH, it is reasonable to admit that *S. cerevisiae* doesn't adsorb Cr^{3+}. At pH 4 chromium is in the form of Cr^{3+}(40%) and $Cr(OH)^{2+}$ (60%), and at pH 5 the predominant species are $Cr(OH)^{2+}$ (70%) and $Cr(OH)_4^{5+}$ (20%) (Ramos et al., 1995).

Considering chromium speciation with pH, the uptake at pH 4 mainly results from $Cr(OH)^{2+}$ biosorption, admitting that Cr^{3+} isn't adsorbed. At pH 5 the uptake increment may be related to the additional biosorption of $Cr(OH)_4^{5+}$.

The influence of medium pH in lead uptake is mainly due to the changes in the yeast cellular wall, since there is no ionic speciation of this metal in aqueous solutions.

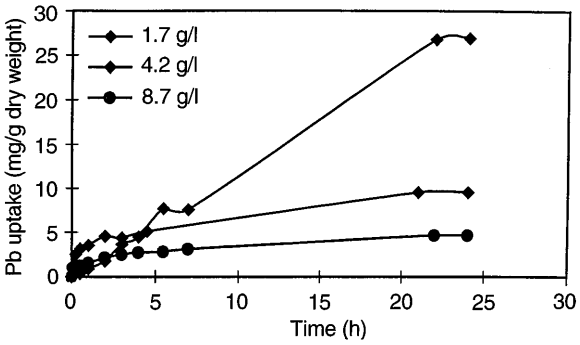

Fig. 3. Effect of biomass concentration in lead uptake

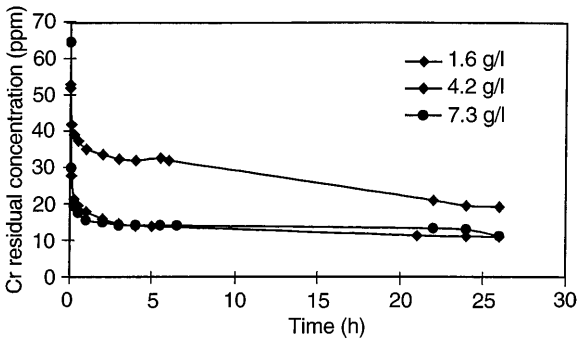

Fig. 4. Evolution of the residual chromium concentration for different biomass concentrations

3.2
Biomass concentration

Data from Figs. 2 and 3 shows that chromium and lead specific uptake decrease with increasing biomass concentration.

The effect of biomass concentration in chromium uptake kinetic is represented in Fig. 4. It is possible to see that the essay carried out with 1.6 g dry weight/l presents a slower uptake rate and lower uptake efficiency (metal removed/initial metal concentration) after 26 hours (near 20% lower), than the essays with higher biomass concentration of 4.2 and 7.3 g dry weight/l.

During the first hours of incubation lead uptake kinetic (Fig. 5) was slower using 1.7 g dry weight/l compared with the essays using 4.5 and 8.7 g dry weight/l. However, after 21 hours lead was removed with 100% efficiency in all essays.

These results suggest that chromium and lead uptake kinetics and efficiency wasn't significantly improved using biomass concentration higher than 4.5 g dry weight/l. This

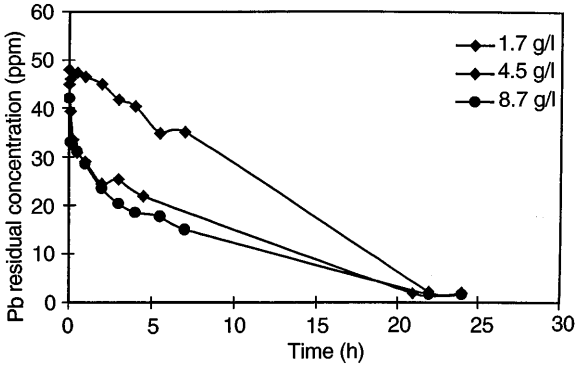

Fig. 5. Evolution of the residual lead concentration for different biomass concentrations

may be explained by the augmentation of electrostatic interactions at high biomass concentrations that inhibit metals biosorption, and by the existence of mass transfer limitations inside yeast flocs eventually representing a controlling step.

3.3
Co-ions effect

Figures 6 and 7 represent the metal uptake in mixtures and pure solutions without pH adjustment, each metal with initial concentration of approximately 25 (Fig. 6) and 100 ppm (Fig. 7). In general metals uptake was slower in mixture solutions, suggesting a competition between the two ions for binding sites in yeast cellular walls. *S. cerevisiae* seems to have more affinity to lead, showing a higher uptake capacity and kinetics to this metal. Chromium uptake only begins when nearly all lead in solution has been already removed. In mixture solution with 100 ppm of each metal, chromium wasn't removed at all.

Data from experiments carried out with initial pH correction of chromium and mixture solutions are presented in Figs. 8 and 9. It is interesting to notice that pH adjustment at 5 seems to decrease competition effect between the two metal ions in study. In mixtures solutions lead presented a faster uptake than in pure ones. This difference in the sorption behaviour may be due to changes in yeast binding sites or to different chromium species present in solution, that might not compete with lead ions.

3.4
Kinetics experiments

Typical biosorption processes exhibit a rapid initial uptake, followed by a slower one. From Fig. 10 it is possible to conclude that chromium uptake follows the standard biosorption kinetics. At 25, 30 and 35 °C, about 50% of the total uptake occurred in the first 30 minutes of incubation. At 10 °C this value only reached 41%.

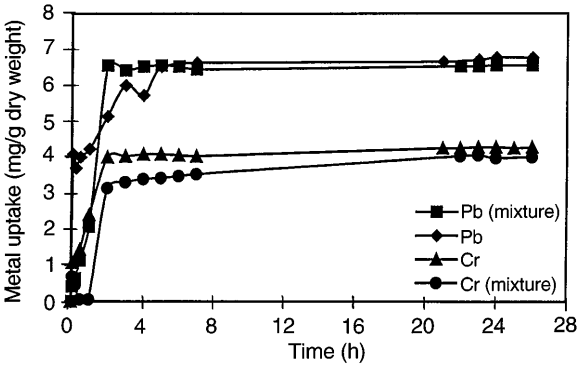

Fig. 6. Metals uptake in control and mixture solutions (Ci = 25 ppm)

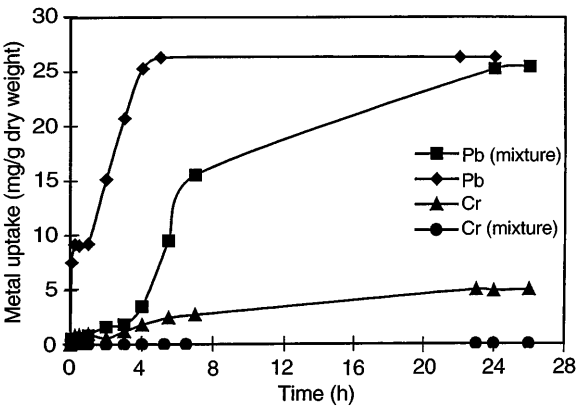

Fig. 7. Metals uptake in control and mixture solutions (Ci = 100 ppm)

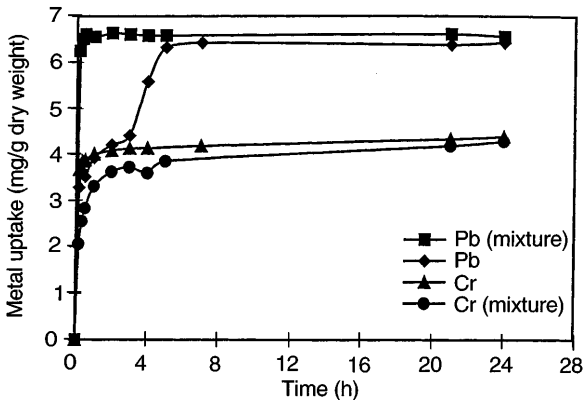

Fig. 8. Metals uptake in control and mixture solutions (Ci = 25 ppm)

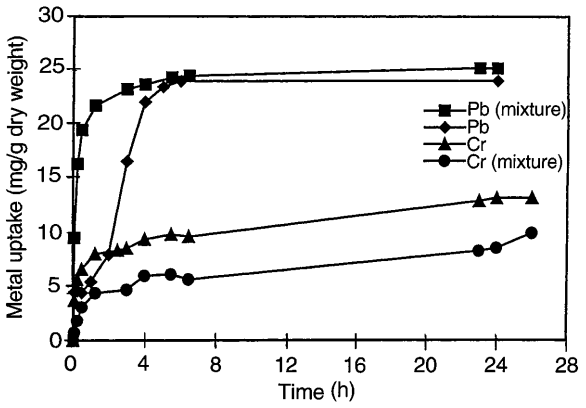

Fig. 9. Metals uptake in control and mixture solutions (Ci = 100 ppm)

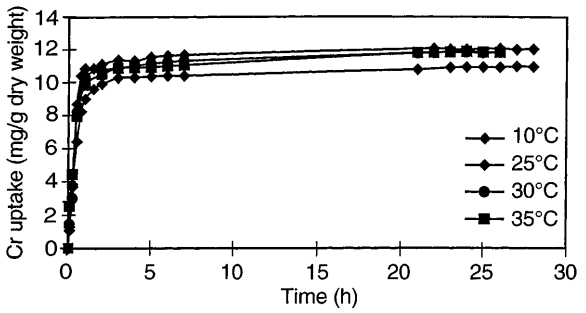

Fig. 10. Chromium uptake at different incubation temperatures (Ci ≈ 55 ppm)

Temperature seems to have little influence in chromium uptake, only a slight reduction at 10 °C may be observed. At these conditions, total uptake was 10.94 mg Cr/g dry weight. This phenomenon is probably due to the lower mobility of chromium ions and the reduction in the active uptake at lower temperatures. At 25, 30 and 35 °C the uptake values reached after 24 hours were very similar: 12.03, 11.82 and 11.83 mg Cr/g dry weight, respectively.

Lead uptake was very fast in the first 5 minutes of incubation, corresponding to 53.8% of total uptake at 10 °C, 34% at 25 °C, 37.2% at 30 °C and 26.5% at 35 °C (Fig. 11). In the following 55 minutes the uptake was nearly insignificant, but during the second and third hours of incubation lead uptake was completed. In the essay carried out at 10 °C the uptake increased progressively, missing the second rapid step.

During the second step with rapid uptake kinetics, medium pH increased reaching values higher than 7. This phenomenon may be a consequence of damages in the yeast cell walls caused by metal ions. The damages may cause changes in cell permeability, allowing positive ions (as K^+) to exit the cell. Positive ions can be replaced by H^+ ions, increasing medium pH. At high pH lead can precipitate explaining its fast removal from solution. The fact that at 10 °C total lead uptake was only 56% may be explained by the absence of cell damages, possibly due to a lower bioaccumulation caused by low ion mobility and metabolic inhibition.

In the first 5 minutes lead uptake was higher than chromium uptake during the same period, suggesting that *S. cerevisiae* has higher affinity to lead. The affinity difference may be related to ion electronegativity, lead being more electronegative than chromium.

3.5 Equilibrium experiments

Biosorption equilibrium was characterised by Langmuir and Freundlich models. Langmuir model is expressed by:

$$q = (q_m b C_{eq})/(1 + bC_{eq}) \ , \qquad (1)$$

where q is the metal uptake capacity (mg of metal/g dry weight) and C_{eq} is the concentration of the metal ion in solution when equilibrium is reached (ppm). Parameter q_m represents the uptake capacity when the surface is completely covered with metal ions, being an indication of the biosorbent maximum uptake capacity. Constant b is related to adsorption energy (Özer et al., 1994), reflecting quantitatively the affinity between the biosorbent and the metal ion (Holan and Volesky, 1994). These parameters are useful to compare the uptake performance (Holan and Volesky, 1994).

Freundlich model is expressed by:

$$\ln q = \ln K + (1/n)\ln C_{eq} \ , \qquad (2)$$

K is a biosorption equilibrium constant, representative of the uptake capacity, and n a constant indicative of biosorption intensity. Freundlich model doesn't predict biosorbent saturation. Figure 12 shows chromium isotherms after 1 hour of incubation. Uptake increases with chromium concentration until biomass reaches saturation.

Corresponding Langmuir parameters to chromium sorption are presented in Table 2. Correlation coefficient (r) confirms the applicability of Langmuir model to these isotherms.

Comparing q_m values at the studied temperatures, the uptake capacity increases in the following order: 10 °C < 30 °C < 35 °C < 25 °C, differing from kinetics results. Temperature effect was not evident in the uptake kinetics essays, where only at 10 °C chromium uptake was significantly lower. Once biomass was obtained from a brewing industry, making impossible to control uniformity of its physiologic characteristics, experimental results may be influenced by differences in the biomass used, thus justifying the apparent incoherence of the data.

According to b values, biomass affinity to chromium follows the order: 10 °C > 30 °C > 35 °C > 25 °C. Against the expected order, this suggests that essays with the lower uptake capacity have higher affinity and vice-versa. Due to the error associated with b calculation, b values obtained

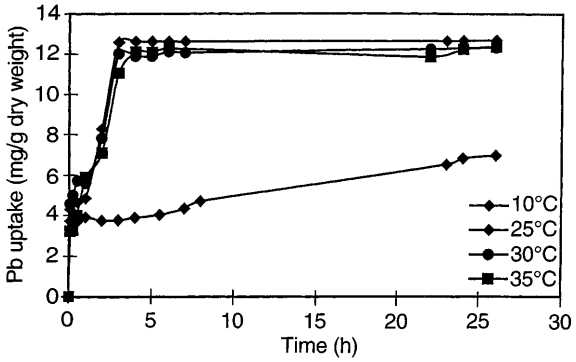

Fig. 11. Lead uptake at different incubation temperatures (Ci ≈ 52 ppm)

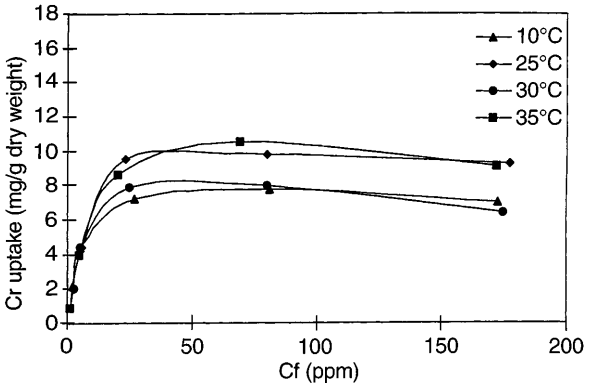

Fig. 12. Chromium isotherms

Table 2. Langmuir parameters for chromium uptake

Temp. (°C)	Langmuir		
	q_m	b	r
10	8.35	0.1727	0.9939
25	13.26	0.0704	0.9988
30	9.16	0.1195	0.9666
35	12.94	0.0806	0.9990

for *S. cerevisiae* should only be used as an indication of the magnitude order of chromium affinity to biomass.

Data from lead biosorption equilibrium is represented in Fig. 13, and in Table 3. Lead isotherms have a very different shape compared to chromium ones. At 25, 30 and 35 °C uptake increases with initial lead concentration, never reaching biomass saturation. This phenomenon is another indication to the existence of mechanisms other than biosorption in lead removal.

Since lead uptake does not follow Langmuir model, the Freundlich isotherms fitting was tested. Freundlich isotherms, with the exception of the 10 °C essay, are a good description of lead removal phenomenon by yeast biomass (Table 3).

K values indicate the following order of the uptake capacity: 10 °C <25 °C <35 °C <30 °C, while *n* values indicate an increase of the biomass affinity to lead with increasing temperature.

3.6 Comparison to other systems

Compared to other biological systems used to remove trivalent chromium and lead from aqueous solution, metals uptake by residual brewer's yeast used in these essays presents similar efficiencies, with the advantage of the reduced cost of the biomass used.

Our data indicate that chromium uptake efficiency varies from 74%, in 10 ppm solutions, to 62% in 100 ppm solutions. Removal efficiencies of the same order of magnitude have been reported using other microorganisms: 30 to 35% with *Aspergillus carbonaris*, 75.5% with *A. giacomell*, 46% with *S. noursei* (Al-Asheh, and Duvnjack, 1995) and 35 to 75% with waste sludges (Naidoo, and Kasan, 1996).

Lead removal efficiencies referred in literature indicate 100% efficiency with *Ecklonia radiata*, 50% with *P. badius*, 21% with *P. radiata* and 35% with *S. cerevisiae* (Matheickal, and Yu, 1996). Our values show that the use of residual brewer's *S. cerevisiae* for lead removal is an attractive alternative, since uptake efficiencies varied from 87% in 10 ppm solutions to 100% in 100 ppm solutions.

4 Conclusions

When using residual biomass from a brewing industry for heavy metal removal, chromium and lead uptake increased with medium pH, being higher at pH 5.0. The pH effect in both metals uptake results from changes in the cell wall functional groups. The presence of different ion species in solution depending on medium pH, phenomenon occurring only with chromium ions, also affects the metal uptake. Optimal biomass concentration either for chromium or lead is 4.5 g dry weight/l. When simultaneous removal of chromium and lead was tested at pH values below 5, biomass presented a higher affinity to lead ions. This competition effect was reduced at pH 5.

Chromium biosorption kinetics presented a rapid initial uptake, characteristic of a metabolism independent mechanism, followed by a slower uptake, associated to metabolism dependent mechanisms. Incubation temperature in the range 25–35 °C didn't affect significantly chromium uptake. Only at 10 °C the uptake showed lower removal efficiency.

Lead uptake didn't follow the typical biosorption kinetics, suggesting the involvement of different uptake mechanisms and possibly lead precipitation.

Chromium biosorption equilibrium was best described by Langmuir model, while lead biosorption equilibrium was best described by Freundlich model, in the range of studied metal concentrations. Biomass was saturated for initial chromium concentration around 50 ppm, while for lead, no biomass saturation was observed using metal solutions with initial concentration up to 200 ppm, with the exception of the essay at 10 °C.

The residual biomass from brewing industry tested in this work to remove Cr(III) and Pb(II) from solution has similar results compared to other biological systems developed with the same purpose. Considering this fact, moreover the low cost and availability of this biomass, it can be considered as an attractive alternative to traditional technologies used to treat residual waters contaminated with heavy metals.

Table 3. Freundlich parameters for lead uptake

Temp. (°C)	Freundlich		
	K	*n*	*r*
10	0.799	3.536	0.8630
25	1.072	3.052	0.9479
30	1.755	2.688	0.9991
35	1.183	2.674	0.9892

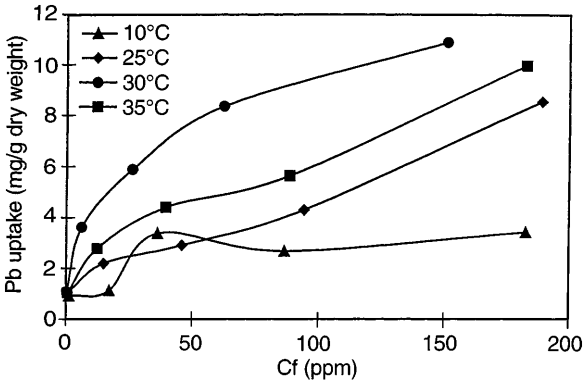

Fig. 13. Lead isotherms

References

1. **Al-Asheh, S.; Duvnjak, Z.:** Adsorption of Copper and Chromium by *Aspergillus carbonaris*. Biotechnology Progress, 11 (1995) 638–642
2. **Avery, S.V.; Tobin, J.M.:** Mechanisms of Strontium Uptake by Laboratory and Brewing Strains of *Saccharomyces cerevisiae*. Applied and Environmental Microbiology, 58 (1992) 3883–3889
3. **Gadd, G.M.:** Fungi and Yeasts for Metal Accumulation. In Microbial Mineral Recovery, Environment Biotechnology. H. L. Lehrlich and C. L. Brierley editors, MacGraw Hill Publishing Company; (1990) 249–275
4. **Holan, Z.R.; Volesky, B.:** Biosorption of Lead by Biomass of Marine Algae, Biotechnology and Bioengineering, 43 (1994) 1001–1009

5. **Matheickal, J.T.; Yu, Q.:** Biosorption of Lead from Aqueous Solutions by Marine Algae *Ecklonia radiata*. Water Science and Technology, 34 (1996) 1–7
6. **Novais, J.M.:** Biological Removal of Metals from Industrial Effluents. Profiles of Biotechnology, Villa, T. G. and Abald, J., Eds., University of Santiago, (1992) 367–375
7. **Özer, D.; Aksu, Z.; Kutsal, T.; Caglar, A.:** Adsorption Isotherms of Lead (II) and Chromium (VI) on *Cladophora crispata*. Environmental Technology, 15 (1994) 439–448
8. **Ramos, R.L.; Rubio, L.F.; Coronado, R.M., G. and Barron, J.M.:** Adsorption of Trivalent Chromium from Aqueous Solutions onto Activated Carbon. J. Chem. Tech. Biotechnol. 12 (1995) 64–67
9. **Rus, E.; Soter, S.; Lakhala, F.:** A Spiral Bioreactor for Removal and Recovery of Metals from Aqueous Wastes. Bioprocess Engineering, 13 (1995) 13–17
10. **Soares, E.J.V.:** Caracterização dos Mecanismos Fisiológicos de Floculação em Leveduras. PhD Thesis, Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto, 1995
11. **Teixeira, J.M.; Teixeira, J.A.; Mota, M.; Guerra, M.M.B.; Cruz, J.M.M.; Almeida, A.M.S.:** The Influence of Cell Wall Composition of a Brewer's Flocculent Lager Yeast on Sedimentation During Successive Industrial Fermentations. European Brewery Convention- Proceedings of the 23rd congress-Lisbon 1991 (1991) 241–248
12. **Tsezos, M.:** Engineering Aspects of Metal Binding by Biomass. In Microbial Mineral Recovery, Environment Biotechnology. H. L. Lehrlich and C. L. Brierley editors, MacGraw Hill Publishing Company; (1990) 325–340
13. **Volesky, B.:** Biosorbents for Metal Recovery. Tibtech, 5 (1987)
14. **Wehrheim, B.; Wettern, M.:** Biosorption of Cadmium, Copper and Lead by Isolated Mother Cell Walls and Whole *Cells of Chlorella fusca*. Appl. Microbiol. Biotechnol. 41 (1994) 725–728