REMOVAL OF CHROMIUM (III) FROM TANNERY WASTEWATERS WITH ACIDOPHILIC FUNGI

by

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ABSTRACT

Conventional methods for chromium removal from industrial effluents may be limited by technological or economical constraints, especially when they are applied to dilute metal solutions. Thus, biotechnological processes, which are efficient at low metal concentrations and require the use of fewer chemicals, may play an important role. The chromium recovery proposed here is based on the specific uptake of this metal by acidophilic fungi. Fifty acidophilic fungal isolates from the Río Tinto basin, an extreme acidic environment, were tested. Most of them were resistant to Cr(III) and Cr(VI) solutions at concentrations up to 10 mmol/L. The influence of different experimental conditions was evaluated (medium concentration, kinetics, requirement of induction etc.). Fungal isolate 143 was able to remove 63% of Cr(III) at 0.1 mmol/L, 74% at 1 mmol/L and 21% at 10 mmol/L. These are the best Cr(III)-fungal-uptake results at acidic pH described in the literature so far. It should be possible to use these acidophilic fungi, for example in tannery wastewater, as they can resist chromium concentrations and pH values found in these effluents (between 6.5-7.5 mmol/L Cr (III) and pH as low as 3-4).

OBJECTIVES

Chromium is a heavy metal with industrial applications in textile dyeing, dye and pigment production, mining operations, wood preservation, tanning processes, galvanometry, metal cleaning and surface treatment, among others.[1] Together with cadmium, its recovery is considered high priority due to the associated environmental risks in addition to the high depletion rate of chromium reserves.[2] In the tanning process, chromium (III) is used to give leather the desired characteristics of durability, resistance and flexibility. Consequently, wastewater from the tanning industry contains chromium. Wastewaters from the tanning and draining stages have the highest concentrations of chromium (III) and can be treated by a conventional precipitation process to recover and reuse the metal. However, in the later stages (retanning and subsequent steps), chromium cannot be recovered through this process and the wastewaters (pH = 3-4) are mixed with others and treated in physicochemical and biological water treatment plants where the chromium(III) is captured in the sludge. In this work, we describe a biotechnological methodology for recovering chromium(III) from tannery wastewaters. The basis of the process is the capacity of several acidophilic fungi that can grow in the presence of chromium and specifically uptake this heavy metal.

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INTRODUCTION TO CHROMIUM UPTAKE BIOTECHNOLOGY

Conventional methods for heavy metal removal from industrial effluents are chemical precipitation, ion exchange, electrochemical treatment, reverse osmosis and adsorption, among others[1]. However, the use of these treatment processes may be limited due to technological or economical constraints, especially when they are applied to dilute metal solutions. Subsequently, there is a need for the development of new competitive technologies.

There are several reasons for the increasing interest in the application of biological processes: they require fewer chemicals; they are eco-friendly, biodegradable, cost-effective alternative; and efficient at low contaminant concentrations. Such biological processes involve a combination of active and passive transport mechanisms, starting with the adsorption of metal ions to the surface of microbial biomass.

Metal accumulative bioprocesses are generally divided into two broad categories:

- Biosorption using living or non-living biomass. This may involve diffusion, adsorption, chelation, complexation or micro-precipitation mechanisms due to ionic interactions between microorganisms and the heavy metal, or due to the action of products excreted by cells.
- Bioaccumulation by living cells. This involves the retention of metals in the cytoplasm of microorganisms once they have been transported across the cell membrane by active transport proteins.

The soluble heavy metals can be either oxidized or reduced by the microorganisms and converted to less soluble species. Though microbial action on metal ion transformation is still poorly understood, two possibilities are considered: the oxidation/reduction of the heavy metal ion is carried out by extracellular enzymes (in which case metal ions do not enter the cell) or heavy metal ions are transported into the interior of the cell and converted to less soluble forms by intracellular enzymes.[3]

Biosorption that uses the ability of non-living biomass to accumulate heavy metals from wastewaters is considered a more competitive, effective and economically attractive treatment method than bioaccumulation, as it can be difficult to maintain viable biomass during the metal removal process. The application of biosorptive processes can make total treatment costs 28% cheaper than conventional ion exchange systems.[2]

However, a specific heavy metal may need to be removed from wastewater selectively in order to recover it and to reuse it. In these cases, bioaccumulation is the appropriate mechanism. Nonspecific biosorption (which relies on metal adsorption by charges on the cell surface) has been widely studied.[3-7] The type of microorganisms that are used tend to be bacteria, although some studies have been carried out with fungi and algae.[8-13] There are fewer reports on specific metal removal. Methods have been devised that involve certain metals and microorganisms in wastewater containing a single metal.[14] However, most of these methods involve tests on synthetic samples prepared in the laboratory.

Much less references exist on the bioaccumulation of metals within microbial living cells.[15-18] The dominant pollutant degraders are bacteria and fungi. Their small size means that there is a high surface-to-volume ratio, which is ideal for rapid pollutant uptake. Bacteria are generally smaller and more active than fungi and are most frequently used at near-neutral pH.[19,20] Fungi are more relevant at acidic pH and for degrading complex substances[18, 21].

Published studies with bacteria show satisfactory results for chromium bioaccumulation at circumneutral pH.[16, 19, 20, 22, 23] However, less satisfactory results have been reported at acidic pH.[24, 25] As can be seen in Table I, most of the studies use bacteria and only some experiments with fungi and yeasts have been recently described. The use of fungi for bioaccumulating chromium is a very recent field of research and promising results have been obtained[18] with *Aspergillus versicolor* to treat textile wastewaters containing dyes, Cr(VI), Cu(II) and Ni(II).

It must be noticed that most of the studies found in the literature referred to Cr(VI) and only few experiments with Cr(III) bioaccumulation by yeasts have been described.[26, 27] These experiments reached a 35-95% of Cr(III) uptake using a huge amount of microbial biomass (400mg yeast dry biomass in 10mL). It is already known[28] that Cr(III) ions can enter cells very slowly only via simple diffusion or phagocytosis since biological membranes are practically impermeable to Cr(III), while Cr(VI) crosses the cell membrane at a much higher rate.

Thus, summarizing, yeasts and fungi are interesting microorganisms to test chromium uptake when wastewaters have an acidic pH, which is the case of surface treatment and tanning processes, (with wastewaters pH ranging from 1.5 to 4). Moreover, the chromium in tanning wastewater is Cr(III) whose bioaccumulation is scarcely studied in the literature. In this paper, some preliminary results using acidophilic fungi to recover Cr(III) from acidic water solutions are presented. Acidophilic fungi, which were isolated from an extreme acidic environment, Río Tinto (Iberian Pyrite Belt, Southwestern Spain), can withstand the presence of heavy metals which are soluble in the acidic conditions in which they grow and specifically bioaccumulate some of the heavy metals existing in the environment depending on the type of fungi selected.[29-31]

TABLE I
Chromium(VI) bioaccumulation described in the literature.

Type of microorganism	Growth conditions	C_0 (mmol Cr(VI)/L)	% Cr bioacc	q_e (mg Cr/g biomass)	Reference
Bacteria	15 days, pH=7-9 30ºC	1.3 - 3.5	38-85%	21-116	[16]
Bacteria	24h, pH=2,5 28ºC	1 - 2.9	No data	32-38	[25]
Bacteria	5 days, pH=7-9 20ºC	0.5 – 0.6	95-98%	10,9-28,4	[19]
Bacteria	5 days, pH=7-8 20ºC	0.6 – 3.5	No data	10,9-109,45	[19]
Bacteria	6 days, pH=8, 2%NaCl 20ºC	2 - 10	98,2-98,8%	21,7-56,7	[20]
Mixed cultures isolated from industrial wastewaters	5-7 days, pH=7-9, 0-6% NaCl, 20ºC	0.5 - 6	20-90%	25-60	[23]
Bacteria	pH=5,5, 30ºC	1 – 3.8	No data	13,3	[22]
Fungi *A.versicolor*	7 days, pH=6, 30ºC	1 – 2.9	41-99%	No data	[18]
Fungi	4 days, pH=5-5.5, 30ºC	0.5 - 1	10-90%	5-18	[21]
Yeasts *Saccharomyces cerevisiae*	4 h, pH=5-6, 25ºC 400 mg yeast biomass in 10mL	0.2-2mmol/L	15-62% 35-95% Cr(III)	No data	[26] [27]

METHODS

A series of experiments were designed to determine the feasibility of using active acidophilic fungi in the recovery of chromium from wastewater. The chromium resistance and Cr(III) uptake capacities of different fungi were evaluated and the influence of some experimental conditions determined. No experiments were made to determine if the chromium was bioaccumulated by the fungal cells, biosorpted or both, thus, the verb uptake has been used throughout the manuscript. The uptake of metals is described[28] as a biphasic process with a first biosorption step (which takes few minutes and depends on the pH, the temperature and the ratio between initial metal and biomass concentrations, among other experimental parameters) and a bioaccumulation step, which is a slower process and depends on the temperature and the presence of metabolism inhibitors. In living cells it is very difficult to separate both processes.

The methodology involved three types of experiments: an initial screening of 50 fungal isolates to test their resistance to Cr(III) and Cr(VI), the selection of some isolates to test Cr(III) uptake and finally some tests to determine the influence of different parameters in Cr(III) uptake. In the initial screening, we evaluated the resistance of 50 fungal isolates to increasing concentrations of Cr(III) and Cr(VI). The source of Cr(VI) was potassium dichromate salt ($K_2Cr_2O_7$) and the source of Cr(III) was the chromium trichloride solid salt ($CrCl_3{\cdot}6H_2O$). Some of the previous isolates were selected to simultaneously evaluate the resistance and the Cr(III) uptaking capacity. In this case the source of Cr(III) was the chrome liquor matrix used for tanning.

Subsequently, a series of experiments with only two isolates were carried out, in which the influence of different parameters in the efficiency of Cr(III) uptake was determined. The parameters studied were: the amount of energy available in the culture medium, the chromium concentration, the pH and the kinetics of chromium uptake. Only Cr(III) removal has been studied because no Cr(VI) is found in the tanning wastewaters.

EXPERIMENTAL SECTION

As mentioned before, two sources of Cr(III) were used: $CrCl_3 \cdot 6H_2O$ solid salt (in the screening of the 50 fungal isolates), and Cr(III) solutions prepared from chrome tanning liquor (in all other tests). Chrome tanning liquor was a solution of Cr(III) containing 2000-2500 mmol/L of chromium. It was obtained by glucose reduction of a Cr(VI) solution, which is a by-product of the vitamin K production process. Chrome liquor is the tanning agent that is usually employed in tanning industries. Tests were carried out with different dilutions of chrome liquor (see Table II).

TABLE II
Physicochemical properties of the chrome liquor dilutions used in this work.

Cr(III) concentration (mmol/L)	pH	Conductivity (µS/cm)
0.1	4.52	599
1.0	3.69	857
10	3.08	1457
50	2.88	5056
Cr liquor: 2200	2.27	32500

All components of fungal growth media were supplied by Cultimed Panreac

Conservation of the Fungal Isolates

Fungi were isolated from Rio Tinto, in which the concentration of chromium can reach values of around 381 ppm (7.33 mmol/L).To preserve fungal isolates in a viable way, they were frozen in 2 mL cryogenic vial (NUNC cryotubes[1]). For this purpose, 1 mL of 40% glycerol was added to a block of fungi from a Petri dish and the samples were kept at -20ºC.[29]

The solid growth medium was potato dextrose agar. This medium was prepared by dissolving 42 g of potato dextrose agar in 1L of water and sterilized in an autoclave at 0.5 atm and 100ºC for 30 minutes.

Procedure to Test the Resistance and Uptake Capacity of Fungal Isolates

The procedure for growing the fungal isolates from their frozen state consists on growing them firstly on solid medium (potato dextrose agar) and then on the liquid medium (YEPD) in which they will be tested. This procedure was as follows (see Figure 1):

The selected fungi were grown on a solid medium. A small piece of agar of the solid medium in which the isolate was grown was put into 100 mL of liquid medium (YEPD) in 250 mL Erlenmeyer flasks. They were then incubated for different periods of time at 30ºC whilst shaking at 140 rpm in a small incubator shaker (Aerotron Infors AG) with the incubation chamber sealed.

The culture liquid medium that supported fungal growth was YEPD (12 g/L glucose, 12 g/L peptone, 6 g/L of yeast extract and pH 5.5, adjusted with sulphuric acid 3 M). Different dilutions of YEPD growth medium were prepared (100%, 50% or 10%). All experiments were done in duplicate.

Resistance

To determine the resistance profile, fungi were placed in a nutrient-rich medium (YEPD) and exposed to increasing concentrations of metal (induction tests). The higher metal concentrations at which fungal growth was observed were determined. A solution of known concentration of Cr(III) was prepared and one of the selected fungi was introduced (in an inoculum of 1/100, i.e. 1 mL of fungi in 100 mL of medium). Solutions were incubated for one week at 30ºC and 140 rpm. If fungi developed, they were considered resistant to this metal concentration. To measure fungal growth yield, the weight of dry biomass was obtained, after filtration with qualitative filter paper (Filter-lab), and dried at 100ºC.

Uptake capacity

To assess Cr(III) uptake capacity, we analyzed the metal concentration of the solution before the introduction of the fungi and after fungal growth. The difference between these chromium concentrations provides the amount of Cr(III) removed from solution. Selected fungal isolates were exposed to different concentrations of chromium. Control solutions for each concentration were prepared to evaluate the evolution of the metal in the absence of fungi (thus, discarding chromium precipitation if any). After a week of growth, the contents of the Erlenmeyer flasks were filtered and the chromium content was analyzed in the biomass and in the filtrate. The chromium analysis was carried out by inductively coupled plasma (ICP) spectrophotometry (Perkin Elmer, Optima 2100 DV). The uptake efficiency was calculated using the following equation:

$$\%\ Cr\ uptake = 100 - \frac{mg\ Cr/L\ in\ final\ solution\ (after\ fungal\ growth)}{mg\ Cr/L\ in\ control\ solution\ (no\ fungal\ treatment)}\ x\ 100$$

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[a] Sterile polypropylene cryogenic vials intended for cryogenic transportation and storage of biological material.

Results and Discussion

Resistance to Cr(III) and Cr(VI) Profile of 50 Acidic Fungal Isolates

A total of 50 fungal isolates were tested to evaluate their resistance to increasing concentrations of chromium (Table III). Although only Cr(III) is used in the tanning process, the resistance was tested both for Cr(III) and Cr(VI) in order to use the Cr(VI) information for other industrial processes. Some of the tested fungi have been already characterized at the genus level using phenotypic and genotypic properties. Others have not yet been classified. The growth medium was 100% YEPD and the concentration of chromium was achieved with the addition of the corresponding quantities of salts. The fungal resistance was tested by the procedure described in the Methods section using incubations of 7 days for each chromium concentration. The induction sequence was: 0.1 mmol/L 1 mmol/L 10 mmol/L 50 mmol/L 100 mmol/L 200 mmol/L.

The resistance results (Figure 2) show that most of the fungal isolates resisted concentrations between 0.1 and 10 mmol/L of chromium. Only few isolates were able to grow at 50 mmol/L (isolates 93, 100, 102, 106, 108, 117, 122, 128, 129, 133, 143

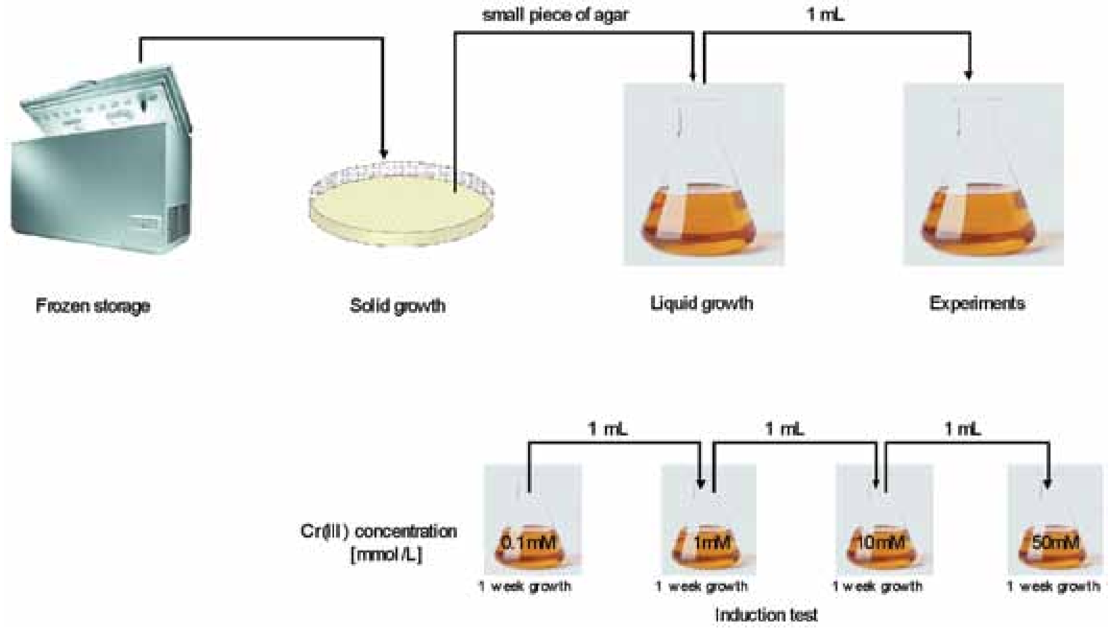

Figure 1. Experimental procedure for fungi storage and growth.

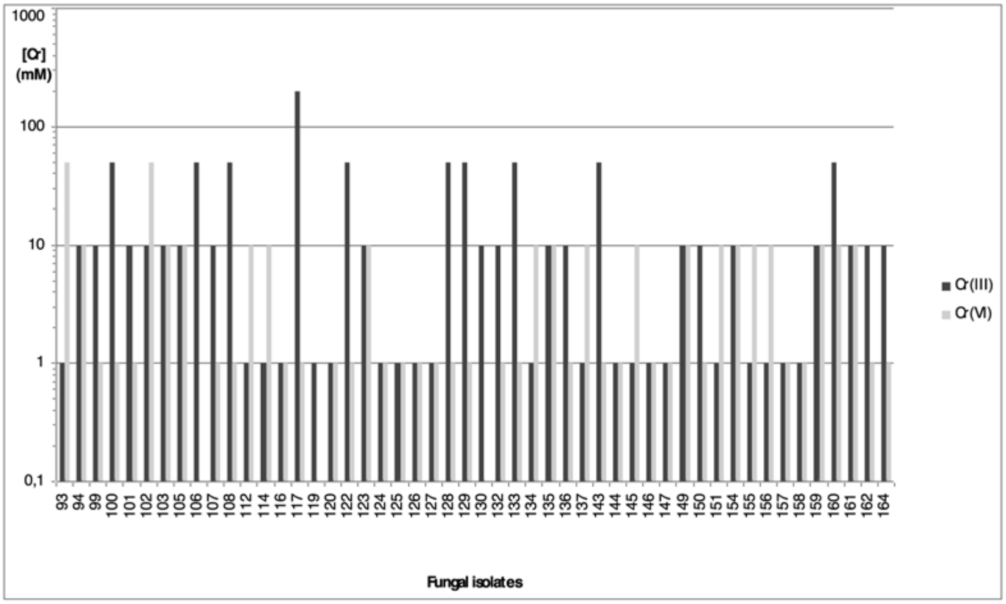

Figure 2. Resistance of different fungal isolates to Cr(III) and Cr(VI).

TABLE III
Fungal isolates tested
(nd: not determined).

Fungi	Characterization	Fungi	Characterization
93	*Penicillium* sp.	**130**	
94	*Trichoderma* sp.	**132**	*Penicillium* sp.
99	*Geniculisporium* sp.	**133**	*Penicillium* sp.
100	*nd*	**134**	*Penicillium* sp.
101	*Nodulisporium* sp.	**135**	*Nodulisporium* sp.
102	*Trichoderma* sp.	**136**	*Penicillium* sp.
103	*Trichoderma* sp.	**137**	*nd*
105	*Penicillium* sp.	**143**	*Penicillium janthinellum*
106	*nd*	**144**	*nd*
107	*Penicillium* sp.	**145**	*nd*
108	*Penicillium* sp.	**146**	*nd*
112	*Penicillium* sp.	**147**	*nd*
114	*nd*	**149**	*nd*
116		**150**	*Penicillium grancanariae*
117	*Aspergillus* sp.	**151**	*Nodulisporium sp.*
119	*nd*	**154**	*Trichoderma* sp.
120	*Penicillium* sp.	**155**	*Penicillium* sp.
122	*Penicillium* sp.	**156**	*Trichoderma* sp.
123	*Penicillium* sp.	**157**	*Penicillium* sp.
124	*nd*	**158**	*Trichoderma* sp.
125	*nd*	**159**	*Phialomyces* sp.
126	*Trichoderma* sp.	**160**	*nd*
127	*Trichoderma* sp.	**161**	*Trichoderma* sp.
128	*Penicillium* sp.	**162**	*nd*
129	*nd*	**164**	*Paecilomyces* sp.

and 160) and one isolate at 200 mmol/L of Cr(III) (isolate 117). The tested isolates were usually more resistant to Cr(III) than Cr(VI), as expected due to the greater toxicity of Cr(VI). However, there were some exceptions (isolates 93 and 102). Many isolates resisted similar concentrations of Cr(VI) and Cr(III) (i.e. isolates 94, 103, 105, 116, 120, 123, 124, 125, 126, 127, 135, 144 and 146, among others). Four of the tested isolates were selected to evaluate whether chromium resistance was an inherent phenotypic characteristic of the fungi or induction by exposure to increasing concentrations of the metal was required. Isolates 93, 117, 122 and 154 were inoculated to the maximum concentration of chromium they had resisted in the previous experiment, but none of them were able to grow directly at this concentration. It can be concluded that the induction process favors the chromium resistance of the tested fungal isolates.

Characterization of Tannery Wastewater

Wastewater with Cr(III) from different stages of the post-tanning process has been characterized to determine the range of pH and chromium concentrations that fungi must resist. The analyses have revealed pH values between 3 and 4 and conductivity of 2-12 mS / cm. The concentration of Cr(III) varies considerably, from 0.45 to 37 mmol/L, depending on the stage and type of process considered. When the wastewater from all these post-tanning stages is mixed, the concentration of Cr(III) falls and is approximately 6.5-7.5 mmol/L.[31]

Systematic Screening of Resistance and Specific Uptake of Cr (III)

Six fungi were selected to test them using the chrome liquor solution, used in tanning. These six isolates were selected to evaluate their ability to uptake Cr(III). Although it is well-known that the resistance and the uptake capacity are independent processes,[28] the criteria to select the six isolates was their resistance to concentrations of Cr(III) higher than 1mmol/L and their different characteristics. The selected isolates were: 102, 106, 117, 128, 143 and 144. Where, isolate 117 (*Aspergillus* sp.) was the most resistant Cr(III) fungi; isolate 102 (*Trichoderma sp.*) was more resistant to Cr(VI) than to Cr(III); isolates 128 (*Penicillium sp.*), 143 (*P. janthinellum)* and 144 were resistant to both Cr(III) and Cr(VI) and isolate 106 was only resistant to Cr(III). A series of experiments were performed to determine the efficiency of the selected acidophilic fungi to uptake chromium at increasing concentrations of Cr(III) liquor solutions. Fungi were grown in 100% YEPD. These tests were performed to determine which fungi were the most suitable for Cr(III) uptake in leather tanning processes to further optimize their operation conditions.

Fungi 128 and 143 uptook important quantities of chromium and were able to grow up to 50 mmol/L, although their chromium uptake efficiecy dropped when the concentration of

TABLE IV
Screening results (100% YEPD and Cr(III) liquor solutions).

Induction Tests	Cr(III) concentration							
	0.1mmol/L		1 mmol/L		10 mmol/L		50 mmol/L	
Fungal isolates	g biom/ 100mL	% Cr uptaken.	g biom/ 100mL	% Cr uptaken.	g biom / 100mL	% Cr uptaken.	g biom / 100mL	% Cr uptaken.
102	-	-	0.58	0	0.54	5.4	0.08	7.1
106	-	-	0.36	12.1	0.68	7.2	0.76	5.4
117	-	-	1.01	6.4	0.85	7.4	0.02	6.7
144	-	-	1.24	11.0	1.03	4.4	0.01	8.7
128	**0.78**	**30.0**	**0.85**	**37.3**	**0.63**	**1.5**	**0.64**	**0.0**
143	**0.86**	**35.6**	**0.64**	**32.8**	**0.73**	**2.3**	**0.80**	**0.8**

chromium was higher than 1 mmol/L. As shown in Table IV, isolates grown in 100% YEPD produced between 0.5-1 g of biomass per 100 mL of culture, some of them even at the highest chromium concentration tested, 50 mmol/L. Three of the isolates, 102, 117 and 144, did not grow at 50 mmol/L Cr(III) concentrations. The difference found for isolate 117 with the experiment reported in Fig 2 could be due to the chrome liquor matrix that was used here which has a different composition than the $CrCl_3·6H_2O$ salt used before. Isolates 128 and 143 were selected for further optimization because they were already characterized and they have an efficient biomass production.

Cr (III) Uptake Tests with Fungal Isolates 128 and 143

Experiments with Different Concentration of Yepd Growth Media

Induction tests (from 0.1 to 50 mmol Cr(III) / L) were conducted with different concentrations of YEPD (100%, 50% and 10%) to assess the influence of energy and carbon source on the growth and uptake efficiencies for both fungal isolates.

The best result was obtained for isolate 143 at 1 mmol/L Cr(III) using 10% YEPD as it removed 73.6% of Cr(III) from solution. Both isolates (143 and 128) gave reasonable uptake values using the lowest concentration of YEPD at 10 mmol/L Cr(III) concentration. However, the highest uptake values were obtained at 0.1 and 1 mmol/L of Cr(III).

As shown in Table 5, the greater the amount of YEPD (culture medium) the higher the growth efficiency (0.7 to 0.8 g biomass/100 mL). However, it appears that chromium removal was higher at the lowest concentration of the medium components. The best Cr(III) uptake results were obtained with 10% YEPD. These results agreed with the ones described in the literature for chromium removal with yeast,[27] where metal uptake was enhanced by increasing glucose concentration from 10 mmol/L to 60 mmol/L while decreasing in the case of chromium. Although it is clear that the medium composition has an important effect in the removal of Cr(III) from the solution, further research has to be performed to clarify this issue.

The best Cr(III) uptake results in terms of amount of Cr(III) taken up per gram of fungal biomass (dry weight) were obtained with 10% YEPD at 50 mmol/L Cr(III) solution (see Table V).

ph Variation Over Time

During the growth period the pH of the solution was monitored at the beginning (initial pH) and the end of each period (final pH). It must be pointed out that, to obtain the initial solution chrome liquor is mixed with the culture media. Thus, the initial pH of each experiment was higher than the pH shown in Table III for pure chrome liquor dilutions.

The pH results showed that:

- At concentrations of Cr(III) below 10 mmol/L, the initial pH was about 4-5. and after one week growth the pH increased up to 7.
- When the concentration of Cr(III) was about 10 mmol/L or higher the pH of the media varied from 4-4.5 initially to 6.5 at the end of the week.

So, it is clear that fungal growth tend to neutralize the pH of the medium. This suggests that not all the Cr(III) removed is bioaccumulated; part of it may be adsorbed onto the surface of the fungi or precipitated since Cr(III) precipitates at pH 7-8.

Uptake Kinetics

Experiments were conducted allowing fungi to grow for an extended period of time: two weeks instead of one. Analyses conducted at the end of two weeks showed that the percentage of Cr(III) uptake was similar than at one week.

Some experiments were performed to analyze the evolution of the chromium uptake for shorter periods of time. The results show (Table VI) that both isolates managed to uptake similar amounts of chromium in 4 days than in one week. Hence, the fungal growth period could be reduced, if necessary, by nearly a half, which is important for future industrial applications.

Comparison of Results with those Described in the Literature

The literature review described previously show that, although biotechnology is an interesting option when dealing with diluted metal solutions, few references exist regarding bioaccumulation using living microorganisms. The most common microorganisms used are bacteria, fungi and yeasts, all of them having different metabolic characteristics. In the case of chromium bioaccumulation most experiments refer to bacteria and very few to fungi and yeasts. It has also been shown that the majority of experiments deal with Cr(VI) bioaccumulation and not Cr(III).

Nevertheless, Cr(III) removal by high concentration of yeasts biomass (400mg in 10mL) was described, at pH 5-6 for 0.2-2mmol/L solutions, achieving 35-95% Cr(III) uptake in 4h, from solutions prepared dissolving chromium trichloride salt in pure water.[27] In our study (with low concentration of fungal biomass, 7.2 mg in 10 mL) we have obtained Cr(III) removal values of 73.6% at 1 mmol/L using industrial chrome liquor solutions (pH 4-5 and containing also organic matter) instead of pure chromium trichloride salt solutions.

The fungal isolates studied in this work have a resistance to Cr(III) up to 100 mmol/L and Cr(VI) up to 50 mmol/L, much higher than the concentrations described for Cr(VI) in the literature (0.5-3 mmol/L). We also found that these fungi can resist and uptake chromium using 10% of the usual rich growth media, which is an interesting result for scaling it to industrial applications. In view of these preliminary results

TABLE V

Results of Cr(III) uptake efficiencies with different percentages of YEPD.

Fungi		128			143		
		10% YEPD	50% YEPD	100% YEPD	**10% YEPD**	50% YEPD	100% YEPD
0.1 mmol/L Cr (III)	g biomass / 100mL	0.135	0.452	0.780	0.082	0.043	0.860
	mg Cr / g biomass	2.10	0.58	0.20	4.00	2.04	0.22
	% Cr uptaken	**54.6**	50.0	30.0	**63.0**	16.9	35.6
1 mmol/L Cr (III)	g biomass / 100mL	0.075	0.038	0.850	0.072	0.030	0.640
	mg Cr / g biomass	22.67	25.18	2.28	36.54	27.04	2.67
	% Cr uptaken	**32.7**	18.4	37.3	**73.6**	15.6	32.8
10 mmol/L Cr (III)	g biomass / 100mL	0.156	0.255	0.630	0.113	0.107	0.730
	mg Cr / g biomass	88.67	36.50	1.24	96.64	77.85	11.80
	% Cr uptaken	26.6	17.9	1.50	21.0	16.02	2.27
50 mmol/L Cr (III)	g biomass / 100mL	0.069	0.442	0.640	0.079	0.247	0.801
	mg Cr / g biomass	**128.12**	0.00	0.00	**82.28**	0.00	0.01
	% Cr uptaken	3.4	0	0	2.5	0	0.8

TABLE VI
Kinetics of Cr(III) uptake for fungal isolates 128 and 143.

Cr(III) 0.1 mmol/L		**% uptake**					
Fungi		Day 1	Day 2	Day 3	Day 4	Day 7	Day 14
128	10% YEPD	27.0	28.0	35.0	47.5	50.1	50.0
143	10% YEPD	32.6	39.7	44.5	55.5	63.0	64.0

Cr(III) 1 mmol/L		**% uptake**					
Fungi		Day 1	Day 2	Day 3	Day 4	Day 7	Day 14
128	10% YEPD	11.7	11.7	19.1	24.9	26.2	26.5
143	10% YEPD	19.9	39.8	55.0	65.7	73.6	74.0

acidophilic fungi seem to be appropriate for specific Cr(III) uptake from post-tanning wastewaters although more experiments to scale and optimize the process must be undertaken. Poljsak reported[28] that age and fungal initial concentration greatly affects uptake capacity and Congeevaram that the optimal growth temperature for some fungi is 35ºC.[17] These parameters should be studied in future trials.

Conclusions

The results of screening 50 acidophilic fungal isolates showed that most of them are resistant to concentrations of chromium up to 10 mmol/L, which is the chromium concentration detected in the environment from which they were isolated. Resistance to Cr(III) is in general higher than resistance to Cr(VI), although some isolates can resist similar concentrations of Cr(III) and Cr(VI). An induction process helps to adapt acidophilic fungi to increasing concentrations of chromium, promoting higher levels of resistance and uptake efficiencies at higher metal concentrations. The tested fungal isolates can develop in an acidic medium in the presence of chromium and can uptake and remove chromium from the medium. The best results were achieved at Cr(III) concentrations of 0.1 and 1 mmol/L with 10% of YEPD. The highest value of uptake (74%) was obtained with the isolate 143 at a Cr(III) concentration of 1 mmol/L (using a tanning chromium liquor matrix). One week of growth at a given concentration is sufficient to induce the fungal resistance and uptake capacity for Cr(III). Nevertheless, the incubation time can be reduced to 4 days, if necessary, with almost similar removal efficiencies. The uptake results are dependent on the experimental conditions. Further studies on the influence of additional variables (age and initial concentration of the fungus; influence of the matrix; etc.) should be carried out.

The final conclusion from these studies is that the application of acidophilic fungi to recover Cr(III) from tannery wastewater is potentially feasible as they resist concentrations of up to 10 mmol/L of Cr(III) at a pH of about 4. The concentration of chromium in post-tanning wastewater[31] is 6.5 to 7.5 mmol/L and the pH is 3-4. So, more experiments with fungi in real tanning wastewaters must be performed to optimize the process because these wastewaters contain other substances (dyes, fats, and other chemicals) that can affect fungi metabolism. It would also be interesting to check their ability in biodegradation and recovery of Cr(III) from tanning wastes, as recently described in the literature for other microorganisms.[32]

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