Biotechnological Sequestering of Chromium(III) from Post-Tanning Effluents: First Results

by

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Abstract

Heavy metals in waste waters and sludge may cause significant environmental problems, and it is known that conventional recovery technologies cannot always achieve satisfactory treatment. For example, they are inappropriate to completely recover the chromium in the case of waste waters from the tanning process. Chromium can be recovered (by precipitation) from effluents that contain high concentrations of this metal. However, effluents from the later stages in present day tanning process often have a low concentration of chromium that cannot be recovered and is found in the sludge of the wastewater treatment plant (WWTP). The aim of our research is to recover and reuse the chromium (III) from post-tanning effluents by means of a biotechnological sequestering method using acidophilic fungi. In this study, we tested acidophilic fungi capable to grow in the presence of chromium in waste waters from various stages of a real post-tanning process. When the post-tanning process was carried out on a pilot plant scale in which conventional rechroming and neutralisation stages were undertaken and the use of additional chemicals was avoided, chromium (III) sequestration values of above 95% were obtained. As these results are so promising, further studies will focus on searching for more resistant fungal strains and determining which of the chemicals used in the post-tanning process can be avoided or replaced by alternatives.

Resumen

Metales pesados en efluentes y lodos podrían causar significantes problemas al medio ambiente, y es conocido que la recuperación por medio de tecnologías convencionales no puede siempre ser un tratamiento satisfactorio. Por ejemplo, son inapropiados para la recuperación completa de cromo en ciertos casos de los desechos hídricos en el proceso de curtición. Cromo puede ser recuperado (por precipitación) de efluentes conteniendo altos niveles de concentración de este metal. Sin embargo, efluentes de las etapas posteriores provenientes del proceso de curtición de hoy en día, que muy a menudo contienen concentraciones tan bajas, que no es recuperable y se encuentra entonces el cromo en el lodo de la planta de tratamiento de desechos líquidos (WWTP). El objetivo de nuestras investigaciones es recuperar y reutilizar el cromo (III) en los efluentes del recurtido por medio de un método secuestrante biotecnológico empleando hongos acidófílos. En este estudio, probamos hongos acidofílos capaces de reproducirse en presencia de desperdicios líquidos de las varias etapas reales del recurtido. Cuando se efectuó a escala de planta piloto el proceso en las operaciones de recromado y neutralizado convencionales y el empleo de agentes químicos adicionales fue obviado, se obtuvieron resultados de secuestro del cromo (III) superiores al 95%. Ya que los resultados prometen tanto, estudios posteriores se enfocarían en la búsqueda de cepas micóticas más resistentes y determinando cuales de los productos químicos usados en los procesos posteriores al curtido pueden ser evitados o sustituidos por alternativos.

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GOAL OF THE PROJECT

Part of the chromium(III) used for tanning is recovered from waste waters by means of a precipitation-redisolution process. Although this is a well known process, it is not possible to be applied to all waste waters generated in the tanning process. This is due to the low concentration of chromium(III) and to the presence of other substances, as dyes or fats, in the effluents of the post-tanning steps (retanning, dyeing, etc.). If this chromium(III) is not recovered, it will be finally found in the sludge from waste water treatment plants (WWTP), preventing the use of this sludge in agriculture and, thus, having to be disposed in landfills. The main goal of this project is the development of alternative biotechnology based methodologies, for the post-tanning effluents treatment which contain low concentrations of Cr(III). The selected methodology is based on the chromium(III) sequestering properties by means of acidophilic fungi.

INTRODUCTION

The tanning process involves several stages: liming, tanning, post-tanning and finishing. The first three of these are wet stages that require large volumes of water. The waste waters that are generated contain a high level of organic matter, among other pollutants.

From the tanning phase onwards, waste waters contain a high level of Cr(III): a heavy metal that leads to a series of additional problems. In recent decades, the tanning industry has worked to resolve or reduce this problem, for example by using other processes instead of chromium tanning[1,2] or by trying to recover as much chromium as possible from the waste waters.[3-8] To date, the results have not totally solved the problem.

Waste waters from the tanning and sammying stages contain the highest concentrations of chromium(III) and can be treated by a precipitation/dissolution process to recover chromium for reuse. However, in the stages that follow (retanning and successive stages) chromium cannot be recovered by this process. Consequently, these waste waters are added to other waste waters from the process and are treated in physico-chemical and biological treatment plants. This results in sludge that contains much higher levels of chromium(III) than permitted for agricultural uses. For example, in the case of the tanning industry of Igualada (Spain), although the industry was recovering the chromium by precipitation from the tanning and sammying stages, still large amounts of chromium were found in the WWTP sludge coming from the post-tanning steps. About 20 t of sludge were generated every day with a chromium content of about 10 g Cr(III) per kg of sludge.[9] Therefore, it is important to develop a process that enables chromium(III) to be recovered from effluents before they are treated in a waste water treatment plant.

A fungi treatment to recover Cr(III) from tanning effluents can be found in the literature.[10] It describes a biosorption process to recover the chromium remaining in the water after the WWTP.

In this project, we studied the possibility of recovering Cr(III) from waste waters generated in the post-tanning process (figure 1). Our aim was to use acidophilic fungi isolated from Río Tinto (Iberian Pyritic Belt in south-western Spain) to recover the Cr(III) from the effluent before the WWTP.

From the Tinto River in Spain, we isolated fungi that can survive in extreme conditions, acidic pH and the presence of heavy metals.[11,12] Some acidophilic fungal isolates are resistant to the presence of chromium and are capable of sequestering this metal.[13] The specific sequestration method is not known at present. However, we do know that it involves a mechanism of specific active metal transport into the cell.

Previous tests undertaken by the authors indicated that some fungal isolates from Río Tinto were capable of resisting and sequestering chromium at concentrations of 1 mM[9]. These fungi are therefore appropriate for sequestering chromium from post-tanning waste waters, which, as shown below, may contain chromium at concentrations of 6.5-7.5 mM.

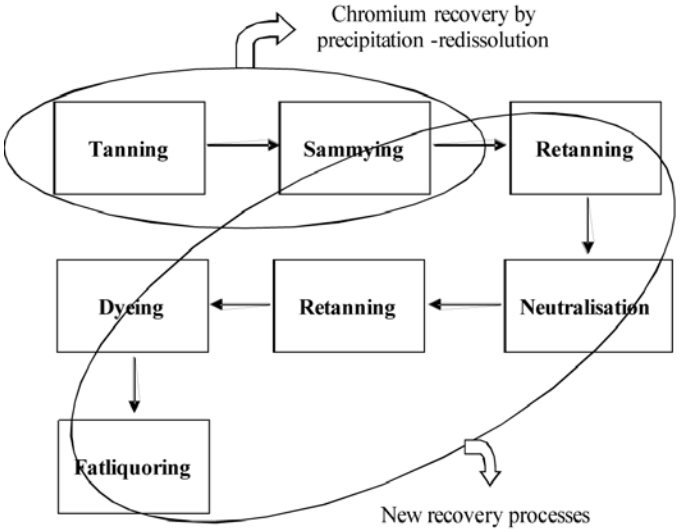

Figure 1. Stages in the tanning process which generate waste waters containing Cr(III).

EXPERIMENTAL

Waste water experiments

To study the possibility of using acidophilic fungi in the specific sequestration of chromium(III) from post-tanning waste waters, we first analysed waste waters from various stages of the post-tanning process to determine the chromium

concentration. The post-tanning process varies significantly depending on the required characteristics of the leather. Therefore, once we had undertaken specific analyses of real samples from various post-tanning processes, we compiled a table (Table I) to illustrate the range of the values of various parameters that can be found in these processes, depending on the required end product.

Subsequently, we undertook tests that involved seeding and growing acidophilic fungi in real waste water from the post-tanning process. Several types of experiments were carried out: experiments with combined waste waters from all of the post-tanning stages; experiments with waste waters from each individual stage (to discover which baths contained substances that inhibited fungal growth); experiments without induction; and experiments with induction (to adapt fungal isolates to increasing concentrations of chromium). The fungi selected for this study were isolate 143 (in previous studies[9], this isolate sequestered 75% of the chromium(III) present in 1 mM solutions prepared in the laboratory) and isolates 50, 37 and 128.

Procedure to test the fungal isolates

The procedure for maintaining and growing the fungal isolates was as follows:

The selected fungi, which were grown on a solid medium, were introduced into a liquid medium in Erlenmeyer flasks (a small piece of the agar of the solid medium in which the isolate was grown was placed in a liquid culture medium) and incubated at 30ºC whilst shaking at 140 rpm for one week. The culture medium that supported the fungi was YEPD (yeast extract peptone dextrose, with 12 g/L glucose, 12 g/L peptone, 6 g/L of yeast extract and pH 5.5 adjusted with sulphuric acid 3 M).

The method used to determine the resistance and sequestration capacity of the fungi is described as follows:

The growth medium for the fungi was a 90:10 mixture of chromium(III) containing effluent:YEPD. The final concentration of the metal in all cases was determined.

Resistance:

To determine the profile of resistance, fungi were placed in a nutrient-rich medium and exposed to increasing concentrations of the metal. We observed the concentration at which the fungi stopped growing. 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). The final volume was adjusted to 100 mL (this solution contained an approximate proportion of 90:10 of the Cr(III):YEPD solution, as mentioned above). The experiment was replicated for each kind of fungal isolate. Solutions were incubated at 30ºC and 140 rpm for one week (see Figure 2). If fungi developed, they were considered resistant to this concentration of the metal. To measure the growth, the weight of dry biomass in 100 mL was calculated. Fungi were weighed after filtration and dried in a drying chamber at 100ºC.

Figure 2. Isolates 128 and 143.

Sequestration capacity:

To assess the fungal capacity to sequester Cr(III), we analysed the metal present in the solution before the introduction of the fungi and the concentration of metal that remained in the solution after growth. The difference in these two chromium concentrations provided us with a measure of the fungi's sequestration capacity (in % of Cr(III) sequestered).

We then introduced the selected fungal isolates into solutions of chromium(III):YEPD, as described above. For each concentration studied, we prepared a control solution, i.e. a medium containing chrome liquor:YEPD in a ratio of 90:10 with no fungi. This sample served as a reference to follow the chromium solution behavior. After a week of growth, the contents of the Erlenmeyer flasks were filtered using a vacuum pump and the chromium content was analysed in the biomass and in the filtrate. This analysis of chromium was carried out by inductively coupled plasma (ICP) spectrophotometry. The sequestering efficiency was calculated using the following equation:

$$\%\ Cr\ sequestered = 100 - \frac{mg/L\ Cr\ in\ final\ solution\ (after\ fungi\ sequestering)}{mg/L\ Cr\ control\ solution\ (no\ fungi\ treatment)} * 100$$

Induction tests:

Induction was undertaken to adapt the fungal isolates to increasing concentrations of the metal. Induction consists on growing the fungal isolates at very low concentrations of metal at first and gradually increasing this concentration until the desired level.

We prepared a solution of a low concentration of Cr(III) (0.1 mM or 1mM), introduced one of the selected fungi (inoculum of 1/100), and adjusted the final volume to 100 mL. The solution was then incubated for one week (at 30ºC and 140 rpm), after which an aliquot was extracted (1/100) and transferred to an Erlenmeyer flask with a higher concentration of the metal. The solution was incubated again under the same temperature and agitation conditions. This operation was repeated until it was observed that the fungi no longer developed. Samples were taken to determine the growth and sequestration capacity at each concentration. The following induction protocol was usually undertaken: 1mM - 10mM - 50mM

RESULTS AND DISCUSSION

Analysis of the waste waters to be treated

We analysed waste waters from the post-tanning stage in a company that tans cowhide for shoes and other leather goods. Post-tanning involves rechroming (or chromium retanning), neutralisation, retanning, dyeing and fatliquoring.

The retanning processes used to obtain leather for shoes and leather goods can be broadly divided into three classes, depending on the types of chemical products that are used:

a. Chromium-synthetic-vegetable retanning

b. Chromium-synthetic retanning

c. Chromium-aldehyde-synthetic retanning

We analysed waste waters from different retanning processes (for the three types of retanning mentioned above) and we drew up one table (Table I) that shows the most common stages in a post-tanning process and the most likely range of values for the parameters. The highest concentrations of chromium were found in the chromium retanning stage, which was also the stage in which the most variation was observed.

The results show that the acidophilic fungi under study should be resistant to pH values of 3-4 and conductivity values of 2-12 mS/cm. The concentration of Cr varies considerably

TABLE I

Interval of values for effluents from different stages in the post-tanning process

	pH	Cond. mS/cm	Red. Pot. mV	COD mg/L O2	Cr (ppm)	Cr (mM)
Wash 1	3.1 – 3.5	10.2 – 11.7	303 – 309	2688 – 7085	230 – 282	4.4 – 5.4
Cr. retanning	2.8 – 3.3	7.1 – 11.7	307 – 343	3936 – 7123	933 – 1931	18 – 37
Neutralisation	4.2 – 5.0	7.9 – 17.1	232 – 280	3805 – 10886	562 – 996	11 – 19
Synthetic retanning	4.1 – 4.3	8.2 – 15.6	206 – 285	13284 – 16800	30 – 258	0.6 – 5.0
Wash 2	4.2 – 4.3	7.0 – 7.9	243 – 282	2624 – 10168	52 – 138	1.0 – 2.6
Dye 1	3.5 – 4.1	4.4 – 10.3	215 – 292	4723 – 9053	27 – 194	0.5 – 3.7
Wash 3	3.7 – 3.9	2.8	233 – 273	1312 – 5712	21 – 32	0.4 – 0.6
Dye 2	3.4 – 4.0	2.3 – 11.2	170 – 267	2016 – 6384	19 – 59	0.4 – 1.1
Wash 4	3.6 – 4.0	1.5 – 5.2	197 – 281	525 – 3528	27 – 48	0.5 – 0.9
Fatliquoring	4.0 – 4.4	3.0 – 5.4	192 – 253	6888 – 44352	23 – 72	0.4 – 1.4
Fixed fatliquoring	3.3 – 3.5	3.2 – 5.6	218 – 278	1574 – 39648	16 – 60	0.4 – 1.1
Final top	3.2 – 3.6	2.6 – 6.2	227 – 289	5986 – 6569	50 – 53	1

between the various baths and according to the type of process considered. The highest chromium concentration was found in the rechroming bath (from 18 to 37 mM of Cr(III)). When the waste water from all of these stages was mixed, the concentration of Cr(III) was approximately 340-390 ppm (6.5-7.5 mM). The fungi under study should be able to sequester chromium at these concentrations.

Tests with acidophilic fungi in real post-tanning waste waters

Below, we describe the results of four experiments undertaken with real waste waters from the post tanning process. These experiments show that there are real possibilities of using this technology, which is still in the research phase.

Combined post-tanning waste waters: company process.

A combined sample was taken from a residual bath of a tanning company. The concentration of chromium in the sample was determined by ICP spectrophotometry and found to be 390 ppm (7.5 mM).

A preliminary study was undertaken to verify whether acidophilic fungi could grow in waste waters taken from a real process that contained chromium and other types of pollutants.

We tested fungal isolates 50, 37 and 143. All three fungi grew when they were introduced into solutions containing a chromium concentration of 0.1 mM (prepared from real residual baths diluted until about 0.1 mM of Cr). However, more efficient growth was observed in the controls (no chromium).

Even less growth was observed when the fungi were introduced into solutions containing a higher concentration of chromium (1 mM). Under these conditions, the fungus that grew best was isolate 143.

Finally, at 7.5 mM of chromium, isolates 50 and 37 did not grow. Isolate 143 grew slightly. As it is difficult to take representative samples from companies because processes change daily, we decided to undertake an experiment with waste water from a tanning process carried out in a pilot plant, following a specific formula provided by the company.

Combined post-tanning waste waters: pilot plant process, company formulation.

In a pilot plant, we carried out a tanning process using the same formulation and chemical products as those used by the company. The post-tanning waste waters (obtained by combining the waste waters from each of the stages) were found to have a pH of 4 and a concentration of Cr(III) of 340 ppm (6.54 mM).

We tested isolates 143 and 128. They grew well in 0.1 mM solutions of chromium, although not as well as in the control (no chromium). The fungi stopped growing in 1 mM solutions of chromium.

Therefore, we concluded that real post-tanning waste waters contain a chemical product that inhibits the growth of acidophilic fungi, as fungal growth resisted a maximum concentration of 1mM. Further studies should examine the resistance of these fungi in baths from different post-tanning stages to see which ones contain fungicides.

TABLE II

Analysis of the various post-tanning baths studied

Analysis of the baths	mg/L Cr	mM Cr	pH
First wash bath 300% of the bath	202.5	3.89	3.28
Rechroming bath 100% of the bath	1175	22.60	3.52
Neutralisation bath 150% of the bath	350	6.73	5.03
Synthetic retanning bath 150% of the bath	95	1.83	4.26
Wash from the first dye 200% of the bath	15.35	0.30	4.78
Bath from the first fixed dye (penetration dye) 150%	18.72	0.36	3.94
Second dye bath 100% of the bath	34.96	0.67	3.79
Wash bath before fatliquoring 200% of the bath	0.24	0.00	3.92
Fatliquoring bath 150% of the bath	34.16	0.66	3.73
Water repellent bath 150% of the bath	31.84	0.61	3.39

Post-tanning waste waters stage by stage: company process

We decided to undertake tests of the growth of isolate 143 in each of the baths from the post-tanning stages, as the results obtained with combined waste waters (both from the company and from the pilot plant) were not good. We collected samples from each of the baths in the company and analysed the chromium contents (by atomic absorption, AA) and the pH of each one of them (see Table II).

An initial growth test was undertaken with fungi 143 in waste waters from the different baths, at a chromium concentration of approximately 1 mM (see Table III). Baths that had chromium concentrations over 1 mM were diluted and the rest were left as they had been taken from the factory. Due to the variation in output concentrations, the values are approximate. Isolate 143 only grew in three of the ten baths tested (see Table III): the rechroming bath, the neutralisation bath and the first dye bath (with 57.5%, 46% and 37% of Cr sequestered, respectively).

Results were negative when baths contained substances that inhibit fungal growth. However, this was difficult to determine a priori, as many commercial products that have unknown formulations are used in post-tanning stages. Some retanning agents, dyes or fatliquors are inhibitors. When the formulations were studied in more detail, sulphonic acids with fungicidal properties were discovered in the retanning agents. An induction study was carried out on the baths in which positive growth had occurred, to discover the concentration at which growth stopped and the % of chromium that the fungi could sequester (see Table IV).

In the case of rechroming baths, chromium sequestering was maintained at around 25% (at a concentration of 1 mM), even when the incubation period was increased from 1 to 2 weeks. In the first experiment, 57% sequestration occurred in the first week (Table III). In the neutralisation bath, the maximum sequestration was 69.5% (Table IV), whilst in the first experiment (Table III) it was 46%. The behaviour of the dye baths varied. Sequestration occurred in some cases (37%, Table III), but not in others (Table IV). The variation between Table III and Table IV in the percentage of chromium sequestered is due to the fact that the 1 mM chromium concentration is approximate. The real concentration varied between the two tables.

Combined post-tanning waters: pilot plant experiment with own formula

A final post-tanning pilot plant experiment was carried out, in which conventional rechroming and neutralisation stages were undertaken (see Table V), and the use of additional chemicals was avoided. The neutralisation bath was analysed by atomic absorption spectrometry, and a chromium concentration of 11 mM was found.

The results of chromium sequestration obtained with fungi 128 in the neutralisation bath (11 mM of Cr(III)) were very good, as values of 95-99% were obtained (see Table VI).

These results show that if the formulation used in the post-tanning process is considered careful and the use of fungicides is avoided, specific biosequestration could be an efficient methodology for reducing the amount of chromium in waste waters from a tanning process. Further studies

TABLE III

Growth of isolate 143 in 1 mM (approximate concentration) in the various post-tanning baths

Analysis of the growth of fungi 143	Growth	g biomass/ 100 mL	% Cr sequestered
First wash bath 300% of the bath	Negative		
Rechroming bath 100% of the bath	Positive	0,1126	57,5
Neutralisation bath 150% of the bath	Positive	0,0867	46,3
Synthetic retanning bath 150% of the bath	Negative		
Wash bath from the first dye 200% of the bath	Positive	0,1482	37,3
Bath from the first fixed dye (penetration dye) 150%	Negative		
Second dye bath 100% of the bath	Negative		
Wash bath before fatliquoring 200% of the bath	Negative		
Fatliquoring bath 150% of the bath	Negative		
Water repellent bath 150% of the bath	Negative		

TABLE IV

Growth and sequestering of chromium in some of the real post-tanning baths (fungi 143)

Type of bath	g biomass/ 100 mL	% Cr sequestration
Rechroming (1mM)	0.0518	24.7
Neutralisation (1 mM)	0.0048	69.5
Dyeing (0.3 mM)	0.0321	-
Rechroming (10 mM)	0.0076	1.4
Neutralisation (6.73 mM)	0.0098	21.3

TABLE V

Formula used for the rechroming and neutralisation process

Operation	Formula	Operation	Formula
Rechroming	10% of water at 50ºC 4% of chrome liquor (42% basicity and 11% Cr_2O_3) 2% of organo-chromium(12% of Cr_2O_3) Tumble for 2 hours, adjust pH to 4 and empty	Neutralisation	Wash with 200% of water containing 0.7 g/L formic acid (pH=4) Tumble for 15 minutes, empty and collect sample

TABLE VI

Results of the induction tests with fungi 128 in a neutralisation bath after rechroming

Induction 0.1 mM - 1mM - 5mM	g biomass/100 mL	%Cr sequestered	mgCr / g biomass
Results in 5 mM of Cr(III)	0.125	99.4	178.6
Induction 0.1 mM - 1mM - 5mM - 11mM	g biomass/100 mL	%Cr sequestered	mgCr / g biomass
Results in 11 mM of Cr(III)	0.186	95.5	185.9

should be undertaken to isolate and identify more resistant fungi that can sequester chromium from real industrial waste waters and to determine which chemical products are inhibitors that should be avoided (or replaced by others) in post-tanning stages.

CONCLUSIONS

Acidophilic fungi could be a solution to recover the chromium(III) from post-tanning waste waters. The results obtained, with pilot plant waste waters from the neutralisation step containing 11 mM of chromium, show that the fungal isolate 128 can recover more than 95% of chromium. This result clearly shows that it is possible to apply the proposed technology to sequestering the chromium from post-tanning waste waters.

However, when applying acidophilic fungi to the integrated post-tanning waste waters coming from real industries, the fungi did not grow as expected. This is due to the presence of inhibitory substances (like sulphonic acids for example).

Therefore, further research is needed either to select more resistant fungi or to substitute these inhibiting substances in the post-tanning process.

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