

Chromium Biosorption by Waste Biomass of *Streptomyces Rimosus* Generated from the Antibiotic Industry

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Abstract: Biomass waste, mainly *Streptomyces rimosus* is generated from large scale antibiotic industry. In this study activated *S. rimosus* biomass was evaluated as biosorbent for the removal chromium from tannery waste water. As Cr^{3+} were bound to the biomass. The solution pH decreased, indicating that proton in the biomass was exchanged with chromium ion. The streptomyces rimosus bound Cr^{3+} at up to 65 mg/g at pH 4.8, where chromium does not precipitate. Reusability of biomass was examined by the desorption studies, in which H_2SO_4 eluted 95 % chromium. The rate of elimination of the chromium in the effluent of the tannery is about 83% after the sixteenth cycle.

Key words: Biosorption, *Streptomyces rimosus*, Uptake, Chromium

INTRODUCTION

Biosorption is an emerging and attractive technology which involves sorption of dissolved substances by biomaterial. It is a potential technique for the removal of heavy metals from solution^[24]. Chrome tanning, as done in modern industry, is done by the use of trivalent chromium salts. The use of a significant amount of chrome during tanning is also characteristic, under conditions which lead to little depletion. This system still produces sludge that has an excessive chrome concentration. The chrome concentration in the effluents is higher the regulation ceiling of 1 mg/l set by the authorities.

Cr (III) is environmental contaminant of major importance because of their toxic effect to man, animal and plant life^[4].

Conventional physical and chemical methods for the removal of Cr (III) from wastewaters, including ion-exchange resins, reverse osmosis, reduction and precipitation and coagulation, are highly expensive and also ineffective at lower concentration of metal ions.

Moreover, these methods also generate large quantity of toxic sludge^[1,17]. Biosorption process has gained importance due to its advantages over conventional separation technique^[18,19]. It employs the use of wide variety of biomass such seaweed^[26], microalgae^[9], fungi^[15], bacteria^[13], and various other plant materials^[6].

Non –living biomasses are preferred for their ease treatment process and avoidance of metal toxicity issues^[7,25]. Suitable biosorbents are often biopolymers

possessing an abundance of negatively charged ligands. These ligands readily form complexes with metal ions through electrostatic interactions^[12,21]. This complexation process is affected by a range of factory including pH, temperature and biosorbent loading^[14]. *Streptomyces rimosus*, mycelial bacteria can accumulate chromium. These properties are attributed to the high content of complexing functional groups in their cellular wall (e.g. amino, amide, hydroxyl, carboxyl, sulfhydryl, phosphate radicals)^[3,23,10]. In this study *S. rimosus* biomass from antibiotic industry was evaluated as a biosorbent material for the treatment of chromium bearing waste water. The dynamics of chromium biosorption were studied under pH varying and pH static conditions. Isotherm experiments were carried out at various pH and initial chromium concentration. The objectives of this work were to characterize the potential of industrial waste biomass to remove chromium from real leather (tannery) effluent and to investigate subsequent desorption processes to facilitate metal recovery.

MATERIALS AND METHOD

Effluents: Industrial tanning effluent was supplied by a tanning company (TAMEG Rouiba- Algeria) and was stored at 4°C without pre-treatment for a maximum of three months before use. No pH or colour change or precipitation occurred during storage. This effluent was also analysed, the gross characteristics of this effluent are presented in table 1.

Table 1: Effluent characteristic

Parameter	Value
pH	4.8
B.O.D ₅ (mg/l)	879
C.O.D (mg/l)	4056
Chromium(III) (g/l)	2.4
Total suspended solids (mg/l)	185

Biomass Preparation: The biomass used is the *Streptomyces rimosus* coming from the Saidal-complex manufacturing unit of antibiotics Medea-Algeria.

This antibiotic by product is a mud of mycelium resulting from the extraction of the fermented gelatine meat-broth for the manufacture of the oxytetracycline. This dead biomass underwent a pre-treatment which consists of repeated washing with distilled water until elimination of impurities, drying in a drying oven during 48 h, then crushing and sifting to obtain an easily storable powder of granulometric ranging from 50 to 160 μm. Then a chemical treatment is underwent which consists in introducing the dried biomass into a solution of hydroxide sodium of 0.1 N concentration. The biomass is maintained in suspension by agitation during 30 min at ambient temperature; filtered and dried biomass was stored in a desiccator and used as biosorbent in the sorption experiments.

All chemicals used in this study were of analytical grade. The chromium stock solution was prepared using Cr₂(SO₄)₃. In order to adjust the pH, 0.1 M NaOH and HCl solution were used. In all experiments the vessels were rigorously mixed using a stirrer equipped in the system.

Physical Properties: The zeta potential of biomass particles was measured using a Zeta Potential Analyser (model 1200 Micromeritics) (T=20°C; sample density 1 g cm⁻³; Test duration 150s; Conductivity cell constant: 0.803 cm⁻¹; Intensity I=7mA; pH=5.8 for the NaOH-treated biomass).

The effective surface area of the biosorbent was approximated as the external surface of biomass particles. Assuming that the biomass particles are spherical, their external surface per unit volume of test solution is:

$$S = \frac{6m}{d_p \rho_{app} V_{sol}} \quad (1)$$

Where m is the sorbent mass suspended in the test solution, d_p is particle size diameter, ρ_{app} is the apparent density of the sorbent, and V_{sol} is the solution volume.

The specific surface area of the biomass S_p was calculated from the following expression:

$$S_p = \frac{6}{d_p \rho_{app}} \quad (2)$$

Equilibrium Sorption Experiment: Serial dilutions of the tanning effluent were prepared using deionised distilled water to give solutions ranging in concentration from full strength to 1 in 20 dilutions To evaluate the sorption capacity of the biomass, chromium biosorption isotherm were obtained at different pH. The isotherm experiments were carried out with 0.3 g biomass in 100 ml with the initial chromium concentration ranging from 0 to 2400 mg/l. Suspension were agitated at room temperature (20°C ± 2°C) and the pH was controlled using 0.1 M NaOH. Samples were taken periodically to analyse the chromium concentration.

Measurement of Chromium Uptake: Before analysis of chromium concentration, all samples were filtered through 0.45 μm pore size. The dissolved chromium concentration of samples was analysed using an atomic absorption spectrometer (Perkin Elmer 2380). The metal uptake (q) was calculated from the mass balance as follows:

$$q = \frac{V(C_i - C_f)}{M} \quad (3)$$

Where C_i and C_f refer to the initial and residual metal concentration (mg.l⁻¹), respectively. V is the volume of solution (l) and M is the mass of the biosorbent *Streptomyces rimosus* (g).

Desorption Experiments: To evaluate the desorption efficiency the Cr³⁺ loaded biomass was dried at 60°C for 24 h after equilibrium sorption at pH 4.8. The dried biomass was contacted with 1 M H₂SO₄ or 1 M HCl for 4 h to allow chromium to be released from the biomass.

There after, the desorbed chromium was analysed and desorption efficiency was calculated as follows:

$$\text{Desorption efficiency (\%)} = \frac{\text{released chromium (mg)}}{\text{Initially sorbed chromium (mg)}} * 100 \quad (4)$$

RESULTS AND DISCUSSION

The physical properties of the biosorbent are shown in Table 2.

Table 2: The physical characteristics of the biomass.

	NaOH-treated biomass
Particle size d_p (μm)	50-160
Humidity (%)	4.4
ρ_{app} (g cm^{-3})	0.41
S (m^{-1}) ^a	418
S_p ($\text{m}^2 \text{g}^{-1}$) ^a	0.14
Zeta potential (Volt)	-0.072

^aAn average value of d_p was used for calculation.

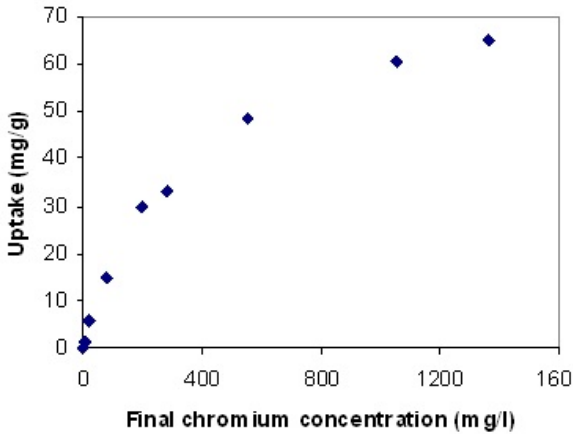


Fig. 1: Isotherm of chromium biosorption by *Streptomyces rimosus* during 5 h (Biomass dosage: 3g/l ; pHil = 4.8 ; T = 20°C)

Biosorption Isotherms: Biosorption isotherms are important for the description of how biosorbate will interact with a biosorbent and are critical in optimizing the use of biosorbent. Equilibrium adsorption isotherms with no pH controls shown in figure 1. Adsorption isotherms represent the equilibrium distribution of metal ions between the aqueous and solid phases, when the concentration increases. The curve is concave to the x-axis which is characteristic of microbial metal binding although saturation uptake levels were not attained at the equilibrium solution concentrations involved^[22,5].

Moreover, uptake data have frequently been successfully fitted to the Langmuir adsorption model. The Langmuir model suggests monolayer sorption or a homogeneous surface without interaction between sorbed molecules. In addition, the model assumes uniform energies of sorption onto the surface and no transmigration of the sorbate.

When the data were transformed to the reciprocal Langmuir format^[11], a clearly non-linear plot resulted as seen in figure 2. This non-conformity to idealized Langmuir behaviour may be interpreted as indicating complex absorption processes involving multilayer, interactive or multiple site type binding or some combination of these phenomena.

Scatchard analysis used here to estimate the number of site type and their relative affinity for

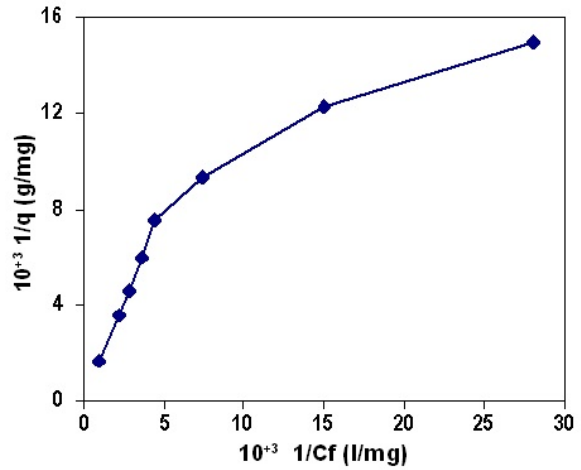


Fig. 2: Langmuir isotherm plot the biosorption of Cr (III) onto *Streptomyces rimosus* Biomass (biomass dosage: 3g/l, pH. 4.8, Temperature 20°C)

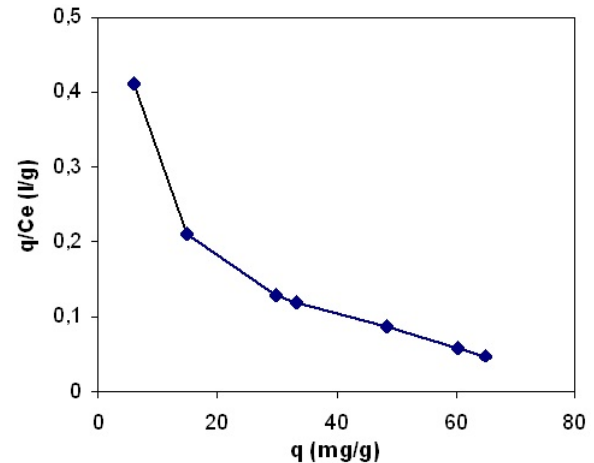


Fig. 3: Scatchard plot for chromium Adsorption by *Streptomyces Rimosus*.

metal ion. The data were further transformed to the Scatchard format^[16] as shown in figure 3 which also resulted in a clearly non-linear plot. The presence of more than one inflection point on a plot based on Scatchard analysis usually indicates the presence of more than one type of binding site in the biomass and/or multiple forms of chromium in solution. Moreover, the authors^[2,20] show that chromium may exist in the form of Cr(III), $\text{Cr}(\text{OH})^{+2}$, CrSO_4^+ , $\text{Cr}_2((\text{OH})_2(\text{SO}_4))^{2+}$, CrOHSO_4 , $\text{Cr}_2(\text{OH})_2(\text{SO}_4)_2$.

Effect of Ph on Chromium (Iii) Biosorption: Earlier studies on heavy metal biosorption have shown that pH was the single most important parameter affecting the biosorption process.

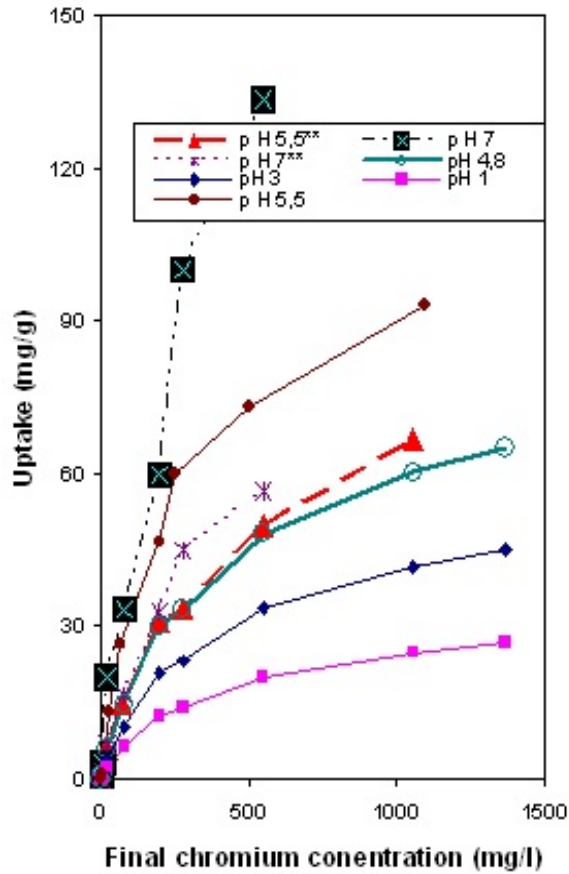


Fig. 4: Effect of pH and influence of precipitation on uptake (** indicate where precipitation effects have been subtracted)

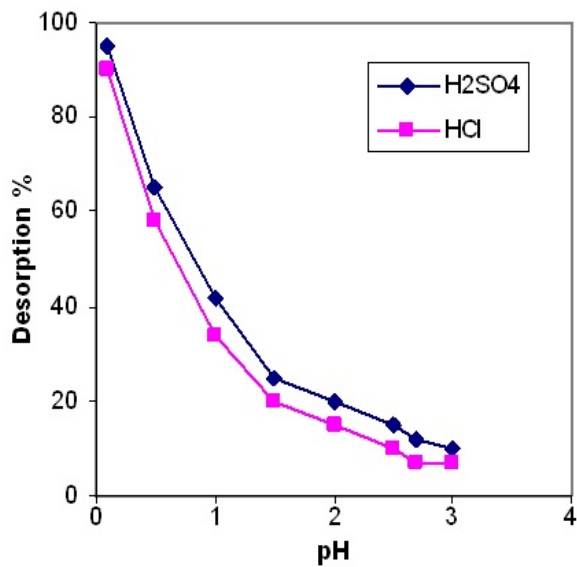


Fig. 5: Effect of desorption agents (H2SO4, HCl) pH on desorption efficiency.

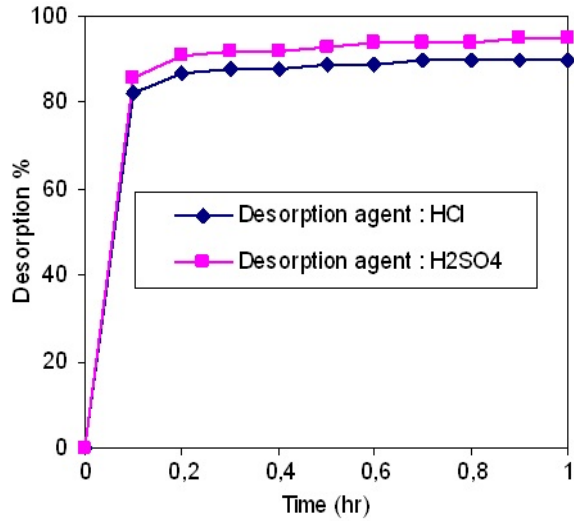


Fig. 6: Time-course profiles for the desorption of Chromium from chromium-loaded biomass

An isotherm is an equilibrium relationship of sorbate distribution between aqueous solution and sorbent phase at the pH- static condition. It is useful for evaluate the capacity of (bio) sorbents.

As can be seen in figure 4, the uptake of Cr^{3+} by streptomyces rimosus biomass was obtained by varying the initial concentration of chromium. When the pH value was raised from 1 to 4.8, the adsorption capacity was enhanced significantly from 27 to 64 mg/g biomass. Uptake was enhanced, probably because of proton competition to Cr^{3+} binding. Adsorption at pH 5.5 and 7.0 marked precipitation effects augmented the biosorption removal of chromium. From solution resulting in apparent sequestration levels of in excess of 93 and 133 mg/g respectively illustrated in figure 4. As can be seen in the figure, when the precipitation component is subtracted the net biosorption values are in good agreement with each other and those observed at pH 4.8^[22].

If the biosorption takes place under conditions that chromium may precipitate, a chemical sludge is generated which should be treated via appropriate solid waste management methods. From a practical point of view, therefore, chromium biosorption process is better operated at pH 4.8.

Chromium Desorption: To recover heavy metals on reuse the biosorbent, desorption efficiency should be considered. In this study, the chromium bearing biomass was contacted with hydrochloric acid or sulphuric acid (figure.5)

When a low pH was used the desorption efficiency was 95 %. At pH values of 0.5. and 1.0 only 66 and 51 % chromium was recovered respectively.

Table.3: Adsorption/ desorption cycles for Cr with biomass. The initial concentration of Cr at the first cycle was 2.4 g/litre; (Biomass concentration = 3g/l; pH_i (adsorption) = 4.8; pH_{desorption} = 0; T = 20°C)

Number of cycles	Adsorption					Desorption		
	Q _{ads} (mg/g) (a)	C _{ads} (mg/l) (b)	C _c (mg/l) (c)	C _R (mg/l) (d)	% Elim (e)	Q _{des} (mg/g) (f)	C _{des} (mg/l) (g)	C _{des-c} (mg/l) (h)
1	60.0	180.0	180.0	2220	7.5	57	171.0	171
2	57.0	171.0	351.0	2049	14.6	54.1	162.4	333
3	54.5	163.2	514.2	1885	21.4	51.4	154.3	487
4	51.4	154.3	668.5	1731	27.8	48.8	146.6	633
5	48.8	146.6	815.1	1584	33.9	46.4	139.2	772
6	46.4	139.2	954.3	1445	39.7	44.1	132.3	904
7	44.1	132.3	1086.6	1313	45.2	41.9	125.7	1029
8	40.9	125.7	1212.3	1187	50.5	39.8	119.4	1148
9	39.8	119.4	1331.7	1068	55.4	37.8	113.4	1261
10	37.8	113.4	1445.1	954	60.2	35.9	105.0	1366
11	35.9	107.7	1552.8	847	64.7	34.1	102.3	1468
12	34.1	102.3	1655.2	744	68.9	32.4	97.2	1565
13	32.4	97.2	1752.4	647	73.0	30	92.4	1657
14	30.8	92.4	1844.8	555	76.8	29.2	87.6	1744
15	29.2	87.7	1932.6	467	80.5	27.7	83.3	1827
16	27.7	83.3	2015.1	383	83.5	26.4	79.2	1906

- (a) Chromium uptake (mg/g biomass)
- (b) Chromium uptake (mg/ L effluent)
- (c) Cumulative uptake over 14 successive cycles (mg/ L effluent)
- (d) Residual chromium ion Concentration in effluent over 14 successive cycles (mg/L)
- (e) Cumulative percentage elimination (percentage adsorption) over 14 successive cycles
- (f) Chromium quantity desorption by utilising H₂SO₄ desorption agent (mg/ g biomass)
- (g) Chromium quantity desorption in H₂SO₄ desorption agent (mg / L)
- (h) Cumulative desorption in H₂SO₄ desorption agent over 14 successive cycles (mg/L)

Chromium desorption increased with decreasing desorption agents pH (including HCl and H₂SO₄) to a maximum value of Cr 95% at approximately zero pH. Variation of the hydrochloric acid or Sulphuric acid caused non-difference in desorption over the experimental range investigated as shown in figure 5.

The kinetics desorption of Cr from the chromium metal loaded biomass is demonstrated in Figure 6. It can clearly be seen that metals desorbed very rapidly, and the desorption reached equilibrium within 12 min. The desorption efficiency was around 95% (H₂SO₄ desorption agent), 90% (HCl desorption agent) for chromium recovered.

The effect of increasing acid concentrations was investigated (figure 7). At each elutant concentration the percentage desorbed chromium was essentially independent of chromium loading on the biomass as shown in figure. 7. Desorption stages with increasing

acid concentrations resulted in a recovery exceeding 80% of the isolated chromium although the use of 2 N acid caused visible alteration of the biomass structure

Adsorption /Desorption Cycles: To make the adsorption process more economical it is necessary to regenerate the adsorbent. Repeated adsorption/ Desorption (A/D) operations were performed to examine the reusability and metal recovery efficiency of the biomass. As shown in table. 3 and Fig. 8, with sixteen A/D cycles, the Cr adsorption capacity of the biomass decreased slightly from 60 mg/g died biomass (cycle = 1) to about 27.7 mg/g died biomass (cycle = 16), and for all cycles nearly 94% of adsorbed Cr was recovered with H₂SO₄ (1N) - driven desorption.

The concentration of chromium in the effluent of the tannery passes from 2, 4 g/l to 0, 38 g/l at the end of the sixteenth cycle, what corresponds to a rate of

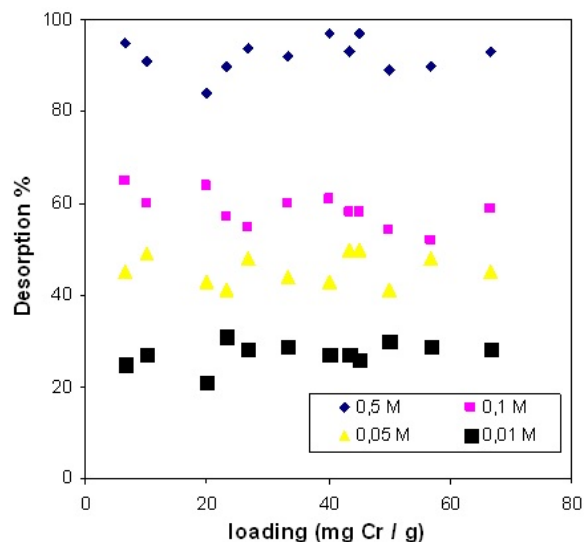


Fig. 7: Cumulative desorption efficiency with successive treatments of increasing eluant (H_2SO_4) concentration

elimination of chromium in the effluent of the tannery of 83.5%, as well as the cumulative concentration of the chromium desorbed in the sulphuric acid at 1 N is about 1.906 g/l. The concentration of the chromium in the sewage of the tannery passes from 2.4 g/l to 0.38 g/l at the end of sixteenth cycle what corresponds has a rate of elimination of the chromium in the effluent of the tannery of 83.5% ven if the sorption capacities decrease with increasing number of cycles, it is remarkable that *Streptomyces rimosus* already used, then reactivated, preserved its ability for sorption. This capacity for reactivation could constitute a technical and/or an economical argument for the utilisation of *Streptomyces rimosus* in waste water detoxification process.

Conclusion: The biosorbent which was investigated in this study (*streptomyces rimosus*) was capable of adsorbing chromium from tannery effluent; the optimal pH for adsorption of chromium by mycelial by-products of *streptomyces rimosus* was around 4.8. The desorption efficiency was around 95% (H_2SO_4 desorption agent), 90% (HCl desorption agent) for chromium recovered.

Repeated adsorption/desorption (A/D) operations made it possible to lower the concentration of the chromium in the sewage of the tannery from 2.4 g/l to 0.38 g/l at the end of sixteenth cycle what corresponds has a rate of elimination of the chromium in the effluent of the tannery of 83.5%.

This biological material, inexpensive, is transformed in dry and stabilised powder and mixed in

batch system with the contaminated water, can be an economical alternative method to depollute effluents of tannery.

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