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Bioremoval Performances of Hexavalent Chromium by Suspended and Immobilised Microbial Biomass onto Pozzolana: Studying the Self-purification Mechanism

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Abstract

Hexavalent chromium is one of the hazardous metals that can be found in industrial effluents such as wood treatment units, mining, leather tanning, pigment, steel and electroplating industry. The present work aimed to evaluate the self-purification capacity of the microbial flora taken from the domestic wastewater effluent of Chlef City, with the perspective of designing a microbial bed in the secondary sewage treatment to prevent an industrial Cr(VI) contamination. Under various operating conditions, the bioremoval performance of Cr(VI) was evaluated by using suspended biomass and immobilised biomass (fixed onto pozzolana grains).

The results showed that the immobilised biomass was more efficient and more resistant to Cr(VI) toxicity than the suspended biomass. Indeed, the Cr(VI) was totally eliminated within 96 h for an initial concentration of 50 mg l^{-1} by the immobilised biomass, while this rate was obtained after 120 h for the suspended biomass. The kinetic data fit well to the pseudo-first order kinetic model. The intraparticle diffusion kinetic model suggested that the diffusion process of Cr(VI) was greatly influenced by the initial concentration. The microbial flora present in domestic wastewater is a promising biosorbent that is able to treat effluent containing Cr(VI).

Keywords

Bioremoval, hexavalent chromium, pozzolana, biosorption

1 Introduction

Heavy metals are the main inorganic contaminants whose removal from polluted sites presents a major challenge for the environmental institutions.[1] Chromium and its derivatives are the most hazardous chemicals. The hexavalent form of chromium, which can be found in industrial discharges, is known for its toxic effects, *i.e.*, carcinogenic, mutagenic, and teratogenic. In addition, its high solubility leads to its high mobility in ecosystems.[1,2] That's why decontamination of wastewater from these chemical species is necessary to protect the ecosystems and human health.

Therefore, several biological, physical, and chemical methods have been developed for the treatment of metalliferous effluents, such as chemical oxidation/precipitation, biosorption, coagulation-flocculation, membrane separation, filtration, ion exchange, *etc.*[3–7] Although these methods have proven high effectiveness, they often present a high investment cost, especially when applied for the treatment of high volume and concentrated effluents, and also produce unmanageable by-products.[8]

Much research has focused on exploiting the "natural depollution" phenomenon, by using *in situ* living organisms for water purification through biosorption and/or biotransformation mechanisms.[8] Among these organisms are bacteria,[9] yeasts,[10] fungi,[11] algae,[12] *etc.* These biosorbents are more attractive economically due to their characteristics, abundance, and low cost.[13]

The comparison between biofilm systems and planktonic cell systems has been investigated through several studies. *Pan et al.*[14] reported that the immobilised biomass of a *Bacillus subtilis* strain has higher capacity to reduce hexavalent chromium than the biofilm, while the latter has a greater ability to fix the hexavalent chromium. Nevertheless, several studies have indicated that the biofilm presents a higher tolerance to hexavalent chromium. *Teitzel and Parsek*[15] reported that *Pseudomonas aeroginosa* biofilm is more resistant to hexavalent chromium than suspended cells.

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The support can play an important role in immobilised cell systems due to its surface specificity and its physicochemical characteristics. The most used natural supports in the biological reduction of hexavalent chromium are activated carbons,[16,17] zeolites,[18] and kaolin.[19] These materials are characterised by a microporous surface that favours the attachment of bacteria leading to the good adhesion of the biofilm. However, some studies have shown that pozzolana can be a very good support in biological filtration[20] without being exploited in the reduction processes of hexavalent chromium using glucose as an electron donor. According to *El Hameur et al.*,[21] pozzolana, with its properties such as microporous surface, positive charge, and hydrophobicity, is a suitable carrier, especially for anaerobic bacteria strain. These characteristics are encouraging in aero-anaerobic biofilm systems.

In order to prevent the incoming effluent of metallic pollution charged on chromic ions, this work is a preliminary design study of a microbial bed that can be installed in the wastewater treatment plant of Chlef City. The ability of natural autochthonous microbial flora, present in the domestic wastewater influent of the wastewater treatment plant of Chlef City, was investigated for the bio-reduction of hexavalent chromium. Under the influence of various operating parameters such as aero-anaerobic conditions, contact time, initial hexavalent chromium concentration, and kinetic studies, the microbial flora was studied in two states: suspended and immobilised onto pozzolana grains for the biosorption efficiency of hexavalent chromium.

2 Materials and methods

2.1 Support

Pozzolana has an alveolar texture and strong microporosity. This material was collected from the El Kelkoul (Bouhamidi) deposit, located 25 km from Béni-Saf, Tlemcen, West Algeria. The particle-size distribution analysis showed that the grain diameter used was between 3 and 4 mm. The physical and chemical composition properties are listed respectively in Tables 1 and 2.

Before using the pozzolana as support in the immobilization process, the material was pre-treated. This pre-treatment served to eliminate iron oxides, aluminium, silica residuals from the pozzolana surface that could react and interfere with microorganisms. The pozzolana samples were washed with tap water, dried in air, then crushed and sieved through a series of standardised sieves (ISO 3310-1). A fraction of 300–500 μm particle size was soaked in sodium hydroxide solution (0.1 N) during 24 h, and rinsed thoroughly with distilled water until pH neutralization.

2.2 Preparation of chromium(VI) solutions

In this study, analytical grade potassium chromate (K_2CrO_4) was purchased from Merck, and used to prepare a stock-concentrated solution of Cr(VI) at 1000 mg l^{-1}. In order to obtain a desired concentration of Cr(VI), further low-concentration solutions were prepared by simple dilutions from the stock solution.

2.3 Preparation of the biomass

The inoculum used was provided from natural microflora (biomass) present in the domestic wastewater outfall of a sewage treatment plant of Chlef City. The biomass was inoculated into a synthetic solution at volume ratio of 5 % (v/v), at constant temperature of 20 ± 2 C with an initially adjusted pH to 6.8, and a stirring speed of 100 rpm. The composition of the synthetic solution was:[22] glucose 10 g; casein peptone 0.2 g; NH_4NO_3 0.571 g; KH_2PO_4 0.351 g were added to one litre of natural water to obtain a C/N ratio equal to 10,[23] while the C/P ratio was equal to 50.[24,25] The solution thus prepared was sterilised before its inoculation with vacuum microfiltration by passage over a microporous cellulose acetate membrane with a porosity of 0.22 °μm.

2.4 Preparation of immobilised biomass

The inoculum contained mainly chemo-organotrophs (bacteria and fungi). The implantation of these microorganisms, as well as the supporting colonization was achieved by inoculating a mixture of 15 ml of inoculated biomass in 285 ml of the synthetic solution. In three identical 300-ml beakers of the inoculated solution, 10 g of pozzolana was introduced. The experimental devices were kept in a static mode for 24 h to allow the adhesion of the biomass onto the pozzolana surface. After this phase, growing experiments were carried out in an oscillating stirring table at 150 rpm and constant temperature of 22 ± 2 °C in order to develop the biofilm.

Table 1 – Physical characterisation of pozzolana[3]

Characteristic	S_{BET} / $m^2\,g^{-1}$	V_{pores} / $cm^3\,g^{-1}$	D_{pores} / Å	Density / –	$D_{particles}$ / cm
Pozzolana	4	0.011	109.58	1.02	3–5

Table 2 – Chemical composition (% by weight) of pozzolana[3]

Composition	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	SO_3	PAF
Percentage	46.10	17.40	10.60	10.40	3.80	3.50	1.50	0.40	4.41

It should be noted that the biomass was initially adapted to Cr(VI), using a volume ratio of 10 % (v/v) of the prepared suspensions.

2.5 Preparation of suspended biomass

The same procedure described previously for the preparation of immobilised biomass was used to generate the suspended biomass, with a slight modification in biomass growth, which was obtained without the pozzolana support.

2.6 Study of chromium adsorption onto pozzolana grains

The adsorption experiments were carried out in an oscillating stirring table at 150 rpm (Ikabortechnik, KS 501) using 300 ml flasks at room temperature (22 ± 2 °C). A pozzolana/synthetic water ratio of 30 % was used (30 g of pozzolana with particle size between 3 and 5 mm was introduced into 100 ml of Cr(VI) solution at 10 mg l^{-1}). The pH of the solution was kept at 7.

The residual concentration of Cr(VI) was determined after centrifugation by analysing the supernatant using a spectrophotometer (OPTIZEN 2120) at 540 nm after complexation with diphenylcarbazide reagent. The efficiency of the hexavalent chromium removal is defined by the following equation:

$$E(\%) = \frac{c_0 - c_t}{c_0} \cdot 100 \quad (1)$$

where c_0 and c_t are the initial and instantaneous chromium concentrations (mg l^{-1}), respectively.

The adsorbed quantity of Cr(VI) at time t (q_t), is expressed by the following equation:

$$q_t\left(\text{mg g}^{-1}\right) = (c_0 - c_e)\frac{V}{m} \quad (2)$$

where c_e is the equilibrium Cr(VI) concentration (mg l^{-1}), V is the volume of solution (l), and m is the amount of adsorbent used (g).

2.7 Biosorption of hexavalent chromium

The biosorption study consisted of evaluating the removal capacity and comparing the performances of the adapted suspended biomass (suspended microbial flora) and immobilised biomass (microbial flora fixed onto pozzolana).

In a 200 ml batch reactor, 20 ml of Cr(VI) solution at 10 mg l^{-1} was mixed with 180 ml of inoculated biomass (volume ratio of 10 %). The mixture was then agitated on a rotary shaker at a constant speed of 150 rpm. All experiments were conducted at ambient temperature (22 ± 2 °C) and initially adjusted pH to 7 ± 2.

The effect of different parameters was evaluated, as follows:

- Biotic medium: in order to assess the microorganisms capacity to remove hexavalent chromium, urban wastewater solution was mixed with a volume ratio of 10 % with Cr(VI) solution of 10 mg l^{-1} containing 5 g of glucose. Periodic sampling (every 1 h) was carried out under aseptic operating conditions for total flora enumeration (Colony-Forming Unit – CFU). The number of colonies was monitored over 8 h.
- Abiotic medium: in the batch reactor, Cr(VI) solution of 10 mg l^{-1} was mixed with glucose solution at concentration of 2 g l^{-1} without inoculated biomass.
- Glucose content: in batch reactor, the bioreduction of Cr(VI) was evaluated at different glucose contents: 0, 1, 3, 5, 7, and 10 g l^{-1}, with an initial Cr(VI) concentration of 10 mg l^{-1}.
- Biomass state: a comparative study was conducted in the batch reactor (volume ratio of 10 %) between suspended biomass and biomass immobilised onto pozzolana for the removal of hexavalent chromium (10 mg l^{-1}), at the optimum glucose content.
- The effect of contact time on the biodegradation of Cr(VI) by immobilised and suspended biomass was studied for up to 7 days, keeping all the operating conditions as in the previous experiments.
- The influence of Cr(VI) concentration on the bio-removal process was studied at different initial Cr(VI) concentrations ranging from 50 to 100 mg l^{-1}, at aforementioned optimised parameters. This effect was also evaluated on the microbial biomass by enumerating the total flora at each concentration of Cr(VI).

3 Results and discussions

3.1 Study of pozzolana adsorption capacity

Pozzolana is the support material for the microbial flora, so it should not react with chromium. The characterisation of pozzolana revealed a very low specific surface, a macroporous structure with the presence of iron oxide. It was therefore imperative to measure the Cr(VI) adsorption capacity of pozzolana. The obtained results are shown in Fig. 1. The adsorbed quantity of chromium was clearly insignificant

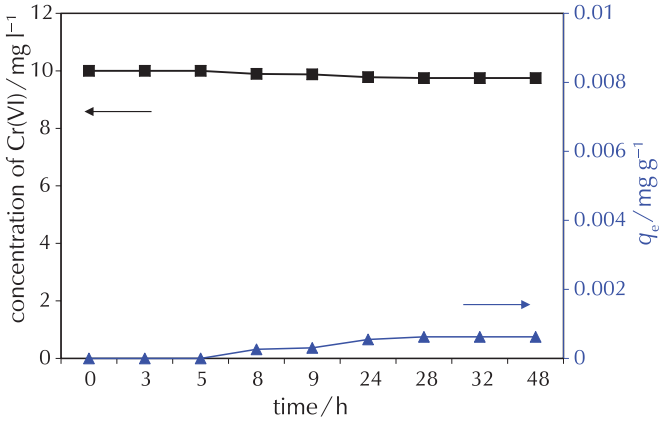

Fig. 1 – Cr(VI) adsorption onto sterilised pozzolana grains

over time, and the initial concentration remained almost invariable. Indeed, the concentration slightly dropped below 9.75 mg l^{-1}, thus confirming the low adsorption capacity of pozzolana, in the order of 0.0006 mg Cr(VI)/g of pozzolana.

These tests allowed us to conclude that the treated pozzolana weakly adsorbed Cr(VI), which made it a suitable support in the packed bed bioreactors.

3.2 Study of bio-reduction of chromium(VI) by domestic microflora

Fig. 2 reveals that the change in the environmental operating conditions led to a latency or adaptation phase of microbial flora. Determining the transition time was a crucial step for the flora to resume metabolic activity, which is revealed by their degradation capacity and multiplication. The graph shows that, during the first 5 h of contact, the microbial flora underwent a regression, which had resulted in a population of $4.3 \cdot 10^6$ CFU/ml. Beyond this period, the same population grew to $9 \cdot 10^6$ CFU/ml after 7 h, and $69 \cdot 10^6$ CFU/ml after 8 h. These findings indicated that the biomass acclimatisation period (8 h), obtained in our study, was shorter than those obtained by *Iddou and Ouali*.[26]

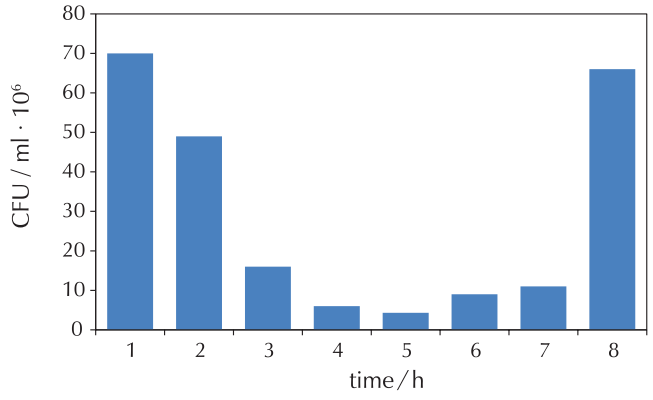

Fig. 2 – Evolution of the adaptation period of microbial flora to the chromic solution

3.3 Factors affecting bio-elimination

3.3.1 Effect of the abiotic medium

This study consisted of verifying whether the synthetic medium reacts with Cr(VI) ions. The results presented in Fig. 3 shows that the reduction of Cr(VI) by the substrate is very low. After 5 h, a 6 % reduction in Cr(VI) was registered, and reached 10 % after 9 h of contact.

3.3.3 Effect of glucose content

Fig. 4 shows the bio-reduction of Cr(VI) using different glucose contents with an initial Cr(VI) concentration of 10 mg l^{-1}. After 192 h (8 days) of contact, a slight decrease in the initial concentration of Cr(VI) around 3 mg l^{-1}

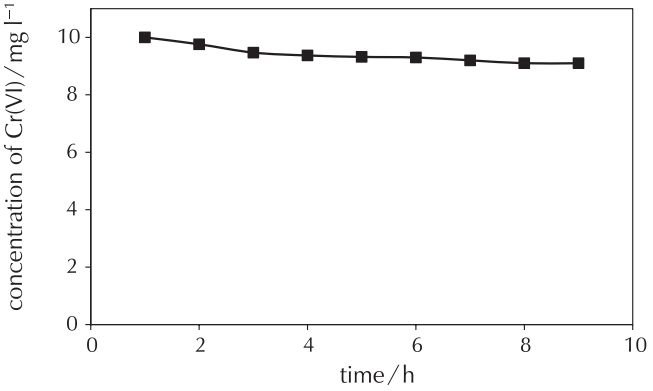

Fig. 3 – Effect of the abiotic medium on the kinetics of removal of Cr(VI)

at 3 g l^{-1} of glucose content was noted, and the removal amount recorded was only 30 %. With an increase in glucose content up to 5 g, a noticeable decrease in the concentration of Cr(VI) was observed, in which the Cr(VI) was totally removed after 7 days of contact. This enhancement of hexavalent chromium was certainly ensured by the microbial activity. The further increase in glucose content up to 10 g l^{-1} led to no improvement of the removal rate; contrariwise, the elimination of Cr(VI) had decreased compared to the increasing tendency of glucose content.

The presence of glucose in the medium is the primary source of carbon for the microbial flora, to be used in their growth and metabolic activities. In front of the oxidative stress caused by Cr(VI), the microbial activity passes through an adaptation phase (around 48 h), this resistance leads to the elimination of chromium.[23] In our study, 5 g l^{-1} was the optimal dose for the microbial activity, in this case Cr(VI) served a useful biological purpose to living organisms. The microbial flora resort to taking the oxygen present in the hydrogen chromate ions ($HCrO_4^-$), which is the dominant form in study conditions according to *Bahador et al.*,[27] to oxidize the organic matter taken from the glucose, thereby reducing hexavalent chromium to trivalent chromium.

The small amount of hexavalent chromium removed from the aqueous solution obtained in the case of 10 g l^{-1} of

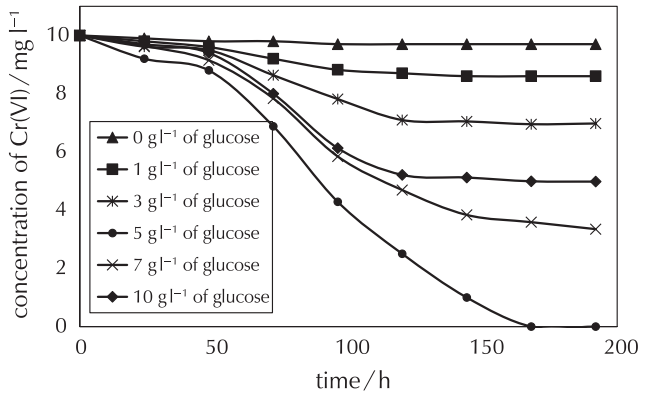

Fig. 4 – Effect of glucose content (in g l^{-1}) on the residual chromium concentration

glucose content was reflected by the non-use of the oxygen carried by the hydrogen chromate ions. As reported by *Iddou and Ouali*,[23] in the abundance of carbon, microbial flora becomes slothful and reduces the metabolic activity induced by their heterotrophic behaviour.

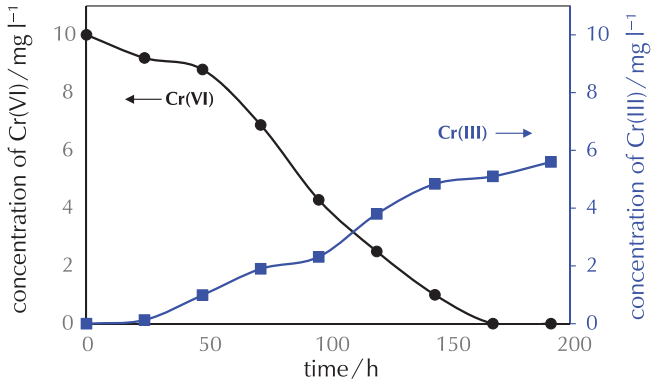

Fig. 5 – Amount of Cr(VI) reduced to Cr(III) at 5 g l^{-1} of glucose content

3.3.3 Effect of biomass state

A comparative study between suspended biomass and biomass immobilised onto pozzolana for the elimination of hexavalent chromium is shown in Fig. 6. It can be seen that the removal rate is clearly faster in the case of immobilised biomass than the suspended biomass during the first 40 h. The rate increased with time to reach 100 % after 48 h for immobilized biomass, while this rate was obtained after 120 h in the case of suspended biomass.

These results are in good agreement with the findings of *Quintelas and Tavares*[28] and *Micaela et al.*[29] regarding the primacy efficiency of the biofilm supported on granular activated carbon for the removal of Cr(VI) and cadmium(II) from aqueous solution.

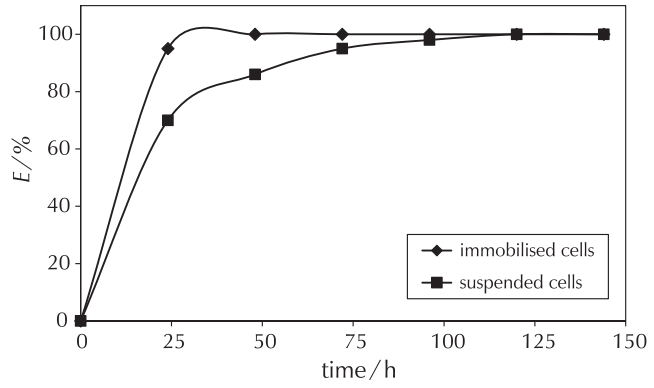

Fig. 6 – Effect of biomass state on the removal of chromium(IV)

The supremacy of immobilised biomass over suspended biomass seems to be due to the density of cells in contact with Cr(VI) ions. According to *Wang and Shen*[30] and *Turick et al.*,[31] high cell density is always required for obtaining a better reduction rate of hexavalent chromium. *Losi et al.*[32] explained this supremacy by the reduction mechanisms of chromium involved in the case of suspended cells. This reduction is due to the presence of soluble protein reductases NADH (Nicotinamide adenine dinucleotide) which serve as electron donor in redox reactions. Whereas, in immobilised cells, Cr(VI) is reduced, playing the role as final acceptor of electrons in the respiratory chain.

In addition, other authors have shown that greater elimination is achieved with fixed cells than with suspended cells, because the adhesion of cells to the solid support offers more protection from the toxic effect of chromium.[33–35]

3.3.4 Effect of contact time

This parameter is important for determining the required time to reach equilibrium state. Analysis of the chromium removal rates shown in Fig. 7, below 120 h, confirmed our earlier findings regarding the supremacy of the immobilised biomass. Cr(VI) was completely eliminated after 96 h of contact for immobilised biomass, while this rate was achieved after 144 h for the suspended biomass.

This fact can be explained as follows: chromate ions will be adsorbed onto the surface of the microbial wall cell; in the absence of oxygen, Cr(VI) can be reduced enzymatically *via* a protein associated with the membrane.[36] This protein is located on the outer surface of the inner membrane, meaning that the reduction of chromium would occur in the periplasm of the microbial. Due to low solubility in water at pH above 5, the trivalent form of chromium cannot cross the membrane which protects the cell.

In some cases, chromate is absorbed by the microbial wall cell, and the reduction of Cr(VI) could occur inside the cytoplasm, when there is low chromate reductase activity in the periplasmic. As explained by *Bopp and Ehrlich*,[37] in the presence of glucose and under anaerobic conditions, the enzyme which catalyses the reaction would be repressed. Since in this work, glucose was used as a source of carbon, based on *Shen and Wang*[38] studies, the reduction of chromium would be associated with the respiratory chain

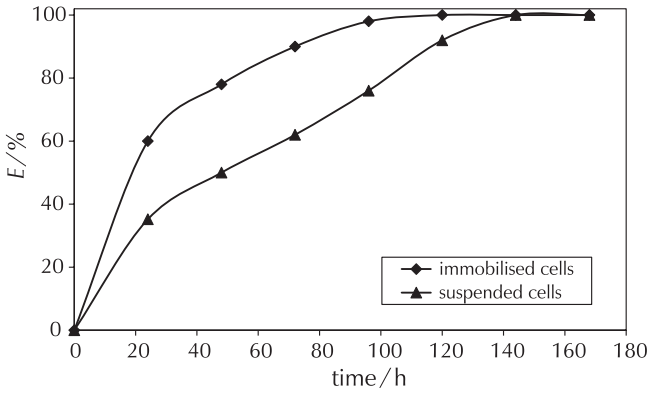

Fig. 7 – Effect of contact time on the removal of Cr(VI)

located in the internal membrane. Two cytochromes, b and d, of the respiratory chain would transfer the electrons necessary for the reduction of Cr(VI). It seems that in this strain, the chromate reductase activity is due to NADH soluble enzyme. However, where the proteins have not been identified, it is unclear whether the chromate reductase activity in aerobic and anaerobic activity involves the same enzymes.[34]

3.3.5 Effect of the initial chromium(VI) concentration

The effect of Cr(VI) initial concentration on the removal efficiency of the suspended and immobilised biomass is clearly shown in Fig. 8 (a and b). With increasing Cr(VI) concentration, the removal rate decreased significantly. Indeed, after 120 h of contact time, the Cr(VI) was completely eliminated from the aqueous solution at initial concentration of 50 mg l^{-1} for both suspended and immobilised biomass. At initial concentration of 60 mg l^{-1}, the maximum uptake was more than 90 % in the case of immobilised biomass, while less than 70 % was registered in the case of suspended biomass. For the higher concentrations (70, 80, 90, and 100 mg l^{-1}), less than 60 % was registered in the case of suspended biomass, while this rate exceeded 75 % in the case of immobilised biomass. This can be explained by the decrease in the number of electron donors of the biological matrix necessary for reducing and/or eliminating the Cr(VI) from aqueous solution. At higher Cr(VI) concentrations it became more toxic, which caused a high mortality rate, thereby reducing the number of microbial flora in the solution. The low uptake capacity obtained for the suspended biomass was due to the suspended biomass being more vulnerable to the toxicity of Cr(VI) than the was the more protected immobilised biomass. This confirmed our previous results regarding the protected activity of fixed cells.

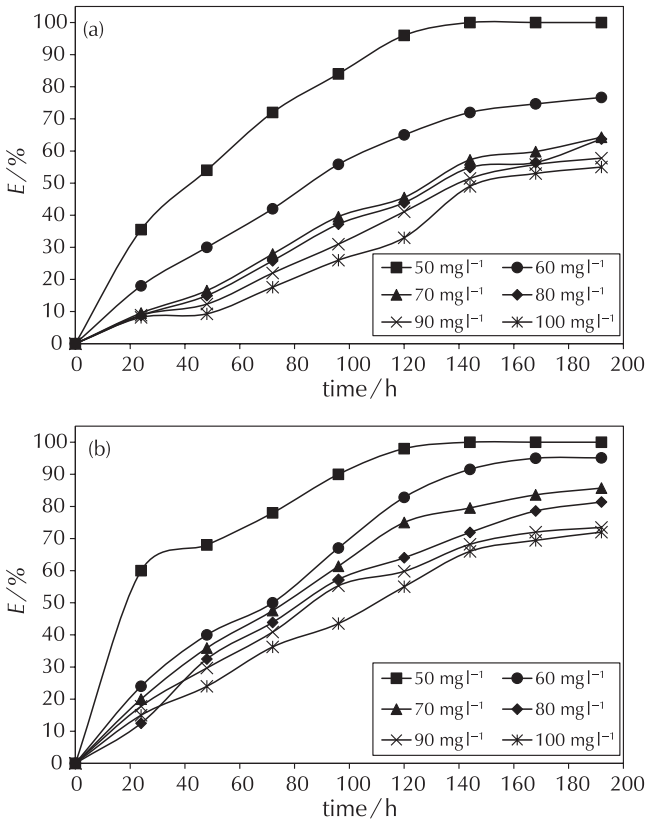

Fig. 8 – Effect of initial chromium concentration on the removal efficiency of (a) suspended biomass, and (b) immobilised biomass

The same findings were reported by *Quintelas and Tavares*[28] and *Micaela et al.*,[29] using an initial Cr(VI) concentration of 100 mg l^{-1} with biomass immobilised onto powdered and granulated activated carbon.

Regarding the toxicity effect of Cr(VI) on the microbial flora, microbiological analyses were performed to determine the number of colonies remaining in the suspended cell bioreactor at the end of each experience. As shown in Fig. 9, the microbiological analysis revealed that the cell density decreased as the concentration of chromium increased. In fact, the microbial flora decreased from $70 \cdot 10^6$ to $3.2 \cdot 10^5$ CFU/ml when the concentration of Cr(VI) increased from 1 to 100 mg l^{-1}.

Based on the obtained results, the microbial flora loaded in biofilms was more resistant and less exposed to environmental changes, such as nutrient limitations, variation of pH, and antimicrobial substances, than were those on the surface or in suspension. Also, these differences were due to the structure and cellular organisation in the biofilm, which moderated the effects of environmental changes.[33,35]

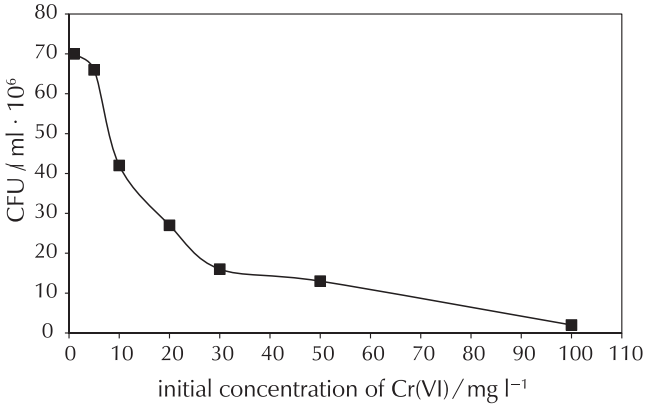

Fig. 9 – Effect of initial concentration of Cr(VI) on the microbial biomass

3.3.6 Bioreduction kinetics of Cr(VI)

The autochthonous microbial flora living in the effluent of Chlef City showed a high bioreduction ability of Cr(VI). The bioreduction kinetics process of Cr(VI) at different initial concentrations of Cr(VI) was studied by the exponential decay (Eq. 3).[39]

$$c / c_0 = a \cdot e^{-k t} \quad (3)$$

Linearized form of Eq. (3) is:

$$\ln(c / c_0) = \ln(a) - k\,t \tag{4}$$

where *a* and *k* are constant and rate constant, respectively.

From the curves presented in Figs. 10a and 10b, it was observed that the reduction process of Cr(VI) over time fitted well with the exponential decay, showing a coefficient correlation close to unity for all the initial concentrations.

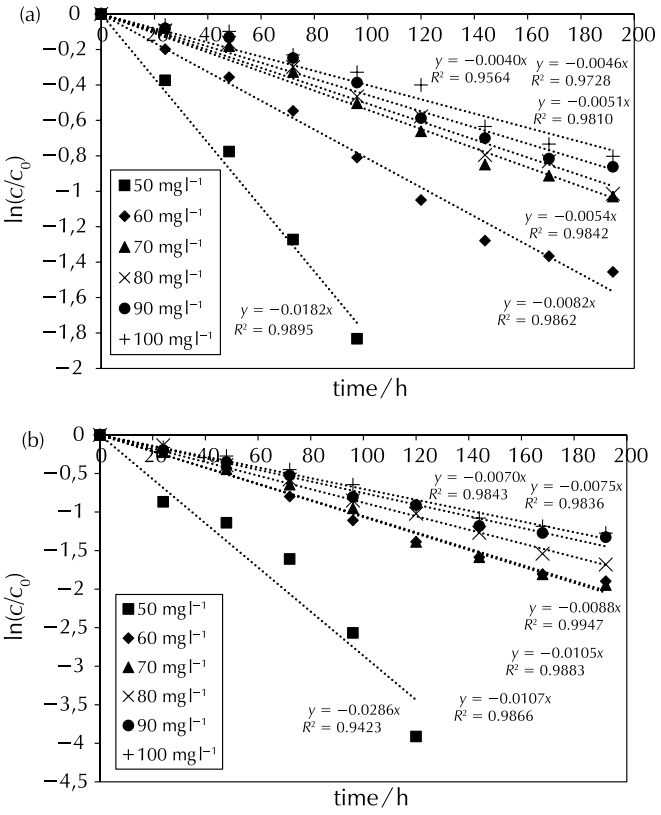

Fig. 10 – Bioreduction kinetics of Cr(VI) at different initial concentrations of Cr(VI) by (a) suspended biomass, and (b) immobilised biomass

Fig. 11 shows that the bioreduction rate of Cr(VI) by autochthonous microbial flora living in the effluent of Chlef City had decreased with increased initial concentration, thus confirming the toxicity effect of Cr(VI) on the microbial flora.

3.3.7 Biosorption kinetics of chromium(VI)

A kinetics study of Cr(VI) biosorption was carried out to understand the removal process and evaluate the reaction order in both systems, suspended and immobilized biomass.

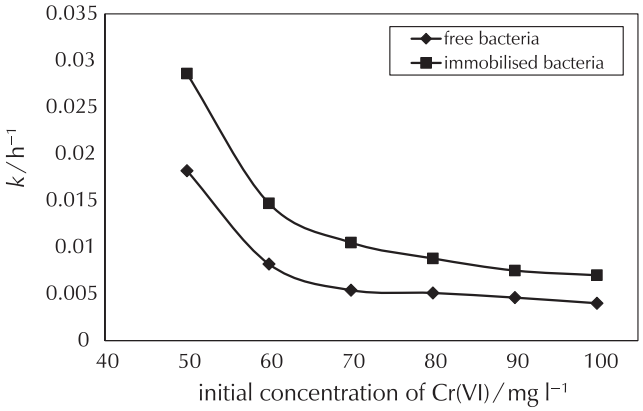

Fig. 11 – Bioreduction rate constant (*k*) of Cr(VI) at different initial concentrations

Pseudo-first order kinetic model

Lagergren's law (Eq. (5)) was tested to treat the kinetic experimental data:[40]

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.3}t \tag{5}$$

where, q_e and q_t are the adsorbed quantity of chromium at equilibrium time at time *t* (h), respectively (mg g^{-1}), and k_1 is the pseudo-first order kinetics constant (h^{-1}).

Pseudo-second order kinetic model

The kinetic model of pseudo-second order, proposed by *Ho and McKay*,[41] Eq. (6) was also tested to evaluate the biosorption process of Cr(VI).

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{6}$$

where k_2 is pseudo-second order kinetic constant (l mg^{-1} h^{-1})

Intraparticle diffusion model

The intraparticle diffusion model proposed by Weber and Morris was employed to identify the diffusion mechanism:[42]

$$q_e = k_p t^{0.5} \tag{7}$$

where k_p is an intraparticle diffusion kinetic model constant (g mg^{-1} h$^{-0.5}$).

For each model, the calculated values of parameters are given in Table 3.

The results regrouped in Table 3 indicate that the biosorption kinetics of Cr(VI) by suspended and immobilised bio-

mass fitted well with the pseudo-first order kinetic model. This good agreement was translated by the calculated values of the correlation coefficient (R^2) obtained by the pseudo-first order kinetic model, which were closer to unity than those obtained by the pseudo-second order kinetic model in both suspended and immobilised biomass cases.

Several studies have shown that biosorption kinetics of chromium by microorganisms is mostly described by the pseudo-first order model. Indeed, *Iddou and Ouali* confirmed such results for the biosorption of hexavalent chromium by activated sludge.[23]

The pseudo-first and pseudo-second order kinetic models cannot determine whether the film or pore diffusion is the controlling step of the biosorption rate. As reported by *Weber and Morris*, when intraparticle diffusion is dominant in the biosorption process, then a plot of $t^{0.5}$ *versus* the biosorption amount would be linear.[43]

Fig. 12 represents the plot of q_t *versus* $t^{0.5}$ for the initial Cr(VI) concentrations of 50, 60, 70, 80, 90, and 100 mg l^{-1} for both cases (suspended and immobilised biomass). The biosorption kinetics data fit well with the intraparticle diffusion model (see Table 3).

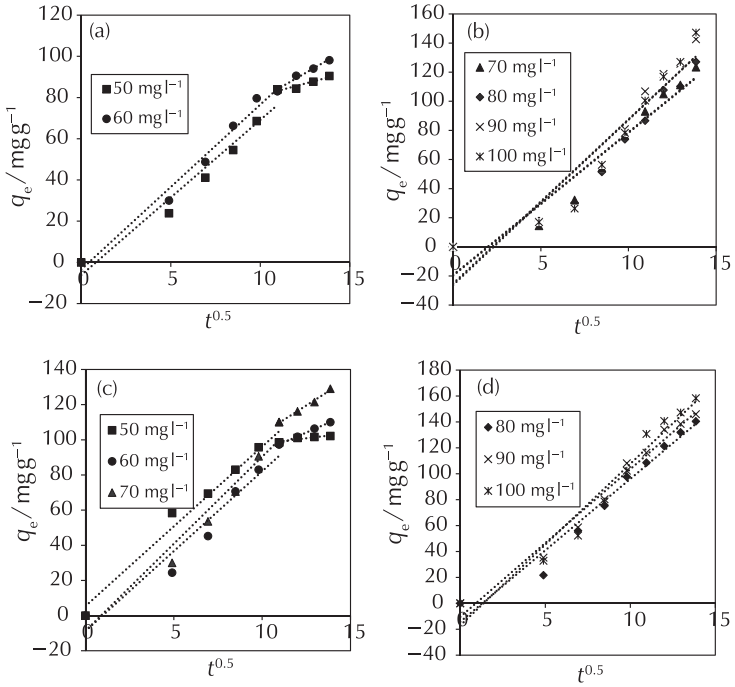

Fig. 12 – Intraparticle diffusion plots for Cr(VI) biosorption by suspended and immobilised biomass, (a) suspended biomass low concentration, (b) suspended biomass high concentration, (c) immobilised biomass low concentration, (d) immobilised biomass low concentration

As reported by several other authors,[44,45] when the microorganisms are exposed to a contaminated environment, they immediately undergo mutation to develop resistance and an efficient protective system against the lethal effects of Cr(VI).

Due to the structural similarity between chromate ion (CrO_4^{2-}) and tetrahedral sulphate ion (SO_4^{2-}), Cr(VI) could be transported into microbial cells *via* SO_4^{2-} transport pathway present in cell membranes. Once the Cr(VI) enters the cytoplasm cell, it induces ChrR activity, which drives its reduction into Cr(III). In aerobic conditions, intracellular enzyme-mediated Cr(VI) reduction mainly uses NADH, NADPH, acetate, and glucose as an electron donor.[46] While in anaerobic conditions, Cr(VI) itself serves as a terminal electron acceptor in the respiratory chain, in which the electron transport pathway occurs by cytochrome b (Cytb) or cytochrome c (Cytc) along the respiratory chains.[47] Usually, the reduced chromium is released to the outside of

Table 3 – Calculations of kinetic constant parameters of Cr(VI) biosorption by suspended and immobilised biomass

Biomass type	c_0 / mg l^{-1}	$q_{e,\,exp}$	Pseudo-first order			Pseudo-second order			Intraparticle diffusion	
			k_{1p}	q_{e_cal}	R^2	k_{2p}	$q_{e,\,cal}$	R^2	k_p	R^2
Suspended	50	90.48	0.0232	133.39	0.96	$1.44 \cdot 10^{-4}$	116.28	0.85	7.19	0.97
	60	98.13	0.0218	132.86	0.95	$1.94 \cdot 10^{-4}$	116.28	0.91	7.50	0.98
	70	123.43	0.0207	227.63	0.81	$1.59 \cdot 10^{-5}$	263.16	0.25	9.81	0.93
	80	127.17	0.0200	236.99	0.76	$2.04 \cdot 10^{-5}$	238.10	0.32	9.81	0.93
	90	142.63	0.0208	278.24	0.77	$8.58 \cdot 10^{-6}$	357.14	0.15	11.28	0.91
	100	147.20	0.0201	290.70	0.72	$7.10 \cdot 10^{-6}$	384.62	0.12	11.34	0.90
Immobilised	50	102.13	0.0279	101.19	0.95	$5.68 \cdot 10^{-4}$	109.89	0.99	7.31	0.92
	60	110.02	0.0233	172.43	0.95	$9.21 \cdot 10^{-5}$	149.25	0.79	8.82	0.97
	70	129.03	0.0221	209.68	0.89	$8.02 \cdot 10^{-5}$	169.49	0.79	10.09	0.98
	80	140.44	0.0218	243.86	0.84	$4.16 \cdot 10^{-5}$	212.77	0.59	11.03	0.97
	90	145.92	0.0228	245.75	0.88	$7.12 \cdot 10^{-5}$	192.31	0.80	11.40	0.98
	100	158.24	0.0224	285.03	0.84	$4.33 \cdot 10^{-5}$	227.27	0.66	12.47	0.96

the cytoplasm by the plasmid, and partially adsorbed by the functional group.[48] A small amount of reduced chromium could be bioaccumulated inside the cell *via* thiol-Cr complex formation and vacuolar compartmentation.[49]

4 Conclusion

In this study, the microbial flora taken directly from the main outfall entrance of the WWTP (wastewater treatment plant) was gradually adapted to the chromic medium from low concentrations to higher concentrations. The autochthonous microbial flora living in the effluent of Chlef City showed a high bio-elimination ability of Cr(VI).

The obtained results showed that the removal efficiency had been greatly affected by the initial concentration of Cr(VI), glucose content, and the rate efficiency depending on the biomass state. In addition, when the initial concentration of Cr(VI) increased, the elimination rate decreased. This regression in the metabolic activity of biomass is due to the toxicity of Cr(VI). The biomass immobilised onto the pozzolana surface grain was found to be more efficient than the suspended biomass. For higher concentrations of Cr(VI), the reduction efficiency for the immobilised biomass was higher compared to the suspended biomass – the registered rates were 100 and 70 %, respectively. This fact is due to the microbial flora inside the biofilm supported onto the pozzolana being more protected against the Cr(VI) toxicity. The bioreduction kinetics process at different initial concentrations of Cr(VI) fitted exponential decay. The biosorption kinetics of Cr(VI) were described well by the pseudo-first kinetic model. The Weber and Morris intraparticle diffusion kinetic model, showed that the initial concentration of Cr(VI) influenced the diffusivity of Cr ions into the microbial cells. At low concentrations, the intraparticle diffusion was not the rate-limiting step, while at high concentrations the pore diffusion was the dominant step of Cr(VI) biosorption process.

It can be concluded that the process using biomass immobilized onto pozzolana could be an excellent alternative to the use of activated carbon or zeolites for the treatment of chromic water.

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SAŽETAK

Učinkovitost biološkog uklanjanja šesterovalentnog kroma suspendiranom i imobiliziranom mikrobnom biomasom na pucolanu: proučavanje mehanizma samočišnjenja

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Šesterovalentni krom je jedan od opasnih tvari koja se može naći u otpadnim tokovima drvne industrije, rudarstva, štavljenja kože, industrije pigmenta, čelika i galvanizacije. Cilj ovog rada bio je procijeniti kapacitet samopročišćavanja mikrobne flore uzete iz komunalnih otpadnih voda grada Chlefa, s perspektivom projektiranja mikrobnog sloja primjenjivog za sekundarnu obradu industrijskih voda s ciljem sprječavanja kontaminacije kromom(VI). Učinak biološkog uklanjanja šesterovalentnog kroma procijenjen je pri različitim radnim uvjetima primjenom suspendirane te imobilizirane biomase (fiksirane na zrna pucolana).

Rezultati su pokazali da je imobilizirana biomasa učinkovitija i otpornija na toksičnost kroma(VI) od suspendirane biomase. Primjenom imobilizirane biomase, 50 mg l^{-1} kroma(VI) je potpuno uklonjeno tijekom 96 h dok je uz primjenu suspendirane biomase bilo potrebno 120 h. Kinetika odgovara modelima pseudo prvog reda. Kinetički model unutarčestične difuzije ukazao je na veliki utjecaj početne koncentracije kroma(VI) na proces njegove difuzije. Mikrobna flora prisutna u komunalnim otpadnim vodama obećavajući je sorbens koji se može primijeniti za pročišćavanje voda koje sadrže krom(VI).

Ključne riječi
Biološko uklanjanje, šesterovalentni krom, pucolan, biosorpcija

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