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BIOTREATMENT OF Cr(VI) - CONTAINING WASTEWATER MEDIATED BY INDIGENOUS BACTERIA

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Abstract

Indigenous *Pseudomonas veronii* 2E, *Klebsiella oxytoca* P2 and *Klebsiella ornithinolityca* 1P were tested as catalysts for the transformation of Cr(VI) to Cr(III) for Cr(VI)-containing wastewater biotreatments. The Cr(VI) biotransformation was assayed during bacterial growth and by using pre-grown quiescent cells. Proof of the unsuitability of a biotreatment based on Cr(VI)-reduction during bacterial growth was found: the reduction rate and the maximum Cr(VI) concentration able to be reduced were too low. On the other hand, high density suspensions of pre-grown quiescent cells presented the highest reduction rates, especially in the presence of an electron donor. The most efficient strain for Cr(VI) removal was *Pseudomonas veronii* 2E. Optimal pH and temperature for the biotransformation process resulted 7 and 32ºC respectively. The maximum initial reduction rate obtained in these conditions was 0.49 mg Cr(VI) h^{-1} g cell dry $weight^{-1}$ (9.4 µmol Cr(VI) h^{-1} g cell dry $weight^{-1}$). Proof of the feasibility and efficiency of this technology is provided by using in calcium alginate immobilized *P. veronii* 2E cells for the treatment of a real Cr(VI)-containing industrial wastewater.

Key words: bioremediation, Cr(VI)-biotransformation, industrial wastewater biotreatment, *Pseudomonas veronii*

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1. Introduction

In nature, chromium exists predominately in two oxidation states: Cr(III) and Cr(VI). While Cr(III) has a low toxicity, Cr(VI) is mutagenic, carcinogenic and extremely soluble in water with a consequent high mobility either in water or soils (Dogan et al., 2011; Focardi et al., 2011; Leita et al., 2011; Srinath et al., 2002; Vaiopoulou and Gikas, 2012). The use of Cr(VI) in a great variety of industries like metallurgy, leather tanning, electroplating and pigment production raised the necessity to develop economic and efficient methods to treat the generated effluents (Desai et al., 2008; Gürü et al., 2008; Poopal et al., 2009).

In many countries the law states that the concentration of Cr(VI) in an effluent must be nearly null in order to be discharged in water bodies and this tendency is becoming global (Dhal et al., 2013; Patra et al., 2010). Most commonly used methods to treat wastewaters that contain Cr(VI) consist in chemical and electrochemical reduction followed by Cr(III) precipitation (Barrera-Díaz et al., 2012; Sadeghi et al., 2017), while other described methodologies involve adsorption on ion exchange resins and reverse osmosis (Aguiar et al., 2017; Sharma and Adholeya, 2011; Thacker et al., 2006; Zhu et al., 2006).

All these treatments are expensive and in many cases ineffective, so it becomes very difficult for industries to comply with legal regulations (Colica et al., 2010). Many studies have reported that several bacterial and fungal strains are capable of reducing Cr(VI) to Cr(III) (Barrera-Díaz et al., 2012; Ghorbani and Younesi, 2016). This biotransformation can occur under aerobic or anaerobic conditions with low energy requirements and is also very effective at low concentrations of Cr(VI) (Elangovan and Phillip,

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2008). Regarding these advantages, developing wastewater treatment systems based on this biotechnology is a promissory alternative to improve Cr(VI) removal from industrial discharges. However, taking into account the design of the biotreatment and the model of the selected bioreactor, the operational conditions might be restricted to a population of adapted bacteria to ensure the stability of the catalytic activity. The use of autochthonous microorganisms that are easy to develop in non-expensive, simple and non-chemically defined culture media represents a way to obtain high density suspensions. The stability of cell viability and waste disposal avoid the introduction of exogenous species in the environment as well.

Previous studies consisted in the isolation of autochthonous chromium resistant bacteria and the evaluation of the chromium reduction ability of the isolated strains. The studies showed that *Pseudomonas veronii* 2E, *Klebsiella oxytoca* P2, *Klebsiella ornithinolityca* 1P were able to reduce Cr(VI) during growth, including in mixed cultures (Garavaglia et al., 2010). The aim of this work was to develop and implement a biotreatment process for Cr(VI) removal from electroplating wastes after determining which of the strains was suitable.

The biotransformation of Cr(VI) was tested either during bacterial growth or by using pre-grown quiescent cells, and parameters like biomass and electron donor concentration were fixed. For the most efficient strain in terms of Cr(VI) removal yields, optimal experimental conditions such as pH and temperature were selected and the biotreatment process was developed using immobilized cells by entrapment in calcium alginate.

2. Experimental

2.1. Microorganisms

Pseudomonas veronii 2E, *Klebsiella oxytoca* P2 and *Klebsiella ornithinolityca* 1P are autochthonous bacteria isolated from polluted environments belonging to soils or sediments associated to the Reconquista River basin (Buenos Aires Metropolitan Area). Biochemical characterization was performed by API multitest system (Bio Merieux) and bacteria were identified in previous works (Vullo et al., 2008) by 500 bp 16S r-RNA gene sequencing (MIDI Labs, USA) and with 1500 bp 16S r-RNA gene sequencing (MacroGen, Korea) with a 99.9% alignment with GenBank.

These bacteria are able to biosorb Cd(II), Zn(II) and Cu(II) and biotransform Cr(VI) from aqueous systems, as described in Garavaglia et al. (2010), Mendez et al. (2011) and Vullo et al. (2008). In all cases bacterial growth was achieved in PYG broth (5 g L^{-1} peptone, 2.5 g L^{-1} yeast extract and 1.1 g L^{-1} glucose).

2.2. Chromium (VI) biotransformation assays

2.2.1. Chromium (VI) biotransformation during bacterial growth

For Cr(VI) biotransformation during growth, 10 mL of a PYG broth-overnight culture in a 125 mL-Erlenmeyer flask (32ºC, 120 rpm) with 0.05 mM Cr(VI) were inoculated in 100 mL of fresh medium supplemented with 0.2 mM of Cr(VI) and incubated in equal conditions in a 500 mL-Erlenmeyer flask. Every 2 hours at 32ºC and 120 rpm, samples were taken and the concentration of Cr(VI) in supernatant and bacterial growth by Optical Density ($OD_{600\ nm}$) were monitored. Sterile PYG broth supplemented with 0.2 mM Cr(VI) was used as abiotic control.

2.2.2. Chromium (VI) biotransformation by pre-grown quiescent cells

In order to obtain the quiescent cell-suspensions, each strain was grown up to stationary phase in 400 mL of PYG broth (in 2 L-Erlenmeyer flask) for 20-24 hours at 32ºC with constant agitation at 120 rpm. Cells were harvested by centrifugation at 6300 *g* for 15 minutes. The obtained pellets were kept at -20ºC until the biotransformation assay took place, when the quiescent cells were thawed, washed and resuspended in 100 mM K_2HPO_4/KH_2PO_4 pH 7 buffer to a final density of 36 g cell dry weight L^{-1}.

A volume of 25 mL of the cell suspensions was spiked with 1 mM Cr(VI) and 20 mM glucose, 80 mM glucose or 1 mM NADH as alternative electron donors and incubated at 32ºC, 120 rpm. Every 2 hours, samples were taken and the concentration of Cr(VI) in the supernatant was determined. Cell-free 1 mM Cr(VI) solutions in the same buffer with the corresponding concentration of electron donor were used as control.

2.3. Effect of temperature and pH on Cr(VI) biotransformation capacity of P. veronii 2E

As a way to evaluate the influence of temperature on Cr(VI) biotransformation, cell pellets -obtained as described in section 2.2.2- were washed and suspended in 25 mL of 100 mM K_2HPO_4/KH_2PO_4 pH 7 buffer solution. This suspension (36 g cell dry weight L^{-1}) was spiked with 1 mM Cr(VI) and 20 mM glucose and incubated at five temperatures: 20ºC, 25ºC, 32ºC, 40ºC and 50ºC. Every 2 hours, samples were taken and the concentration of Cr(VI) in the supernatants was determined.

Quiescent cell pellets were suspended in 25 mL of different buffered solutions: 100 mM K_2HPO_4/KH_2PO_4 pHs 8 and 7, 50 mM Tris (tris[hydroxymethylaminomethane], pKa 8.1)-HCl pH 9 and 10 mM MES (2-[N-morpholino]ethanesulfonic acid, pKa 6.1) pHs 6 and 5.5, to evaluate pH effect on biological reduction of Cr(VI).

The suspensions were spiked with 1 mM Cr(VI) and 20 mM glucose, being the Cr(VI) in supernatants quantified every 2 hours up to 8 hours at 32ºC.

2.4. Chromium (VI) biotransformation by immobilized cells of P. veronii 2E by entrapment in calcium alginate beads

2.4.1. Temperature optimization

Frozen cell pellets of *P. veronii* 2E, obtained as in section 2.2.2, were thawed, washed and resuspended in 25 mL distilled water (72 g cell dry weight L^{-1}). This suspension was mixed with an equal volume of sodium alginate 1.5% (w/v). The mixture was then dripped into a 0.2 M $CaCl_2$ solution. The formed beads were stored in this solution at 4ºC for 1 hour, then washed with distilled water and suspended in 25 mL of an aqueous solution of 1 mM Cr(VI) and 20 mM glucose, adjusted to pH 7. The concentration of Cr(VI) was monitored every two hours at 20ºC, 25ºC, 32ºC, 40ºC and 50ºC during the first 8 hours. Cell free alginate beads incubated with 1 mM Cr(VI) and 20 mM glucose were used as control in each experiment.

2.4.2. Cr(VI) removal in batch systems

A biotreatment simulation was performed in sequential batches spiking an apparent volume of 50 mL of calcium alginate beads -prepared as described above- packed in 60 mL syringes (minireactors) with 25 mL of 1 mM Cr(VI)-20 mM glucose (pH 7) in each batch. The assay was carried out at 32ºC. Cr(VI) removal was evaluated every 2 hours after each 25 mL-chromium solution renewal.

A scale up process was carried out using a 1 L column equipped with an external thermal bath to keep the temperature at 32°C packed with an apparent volume of 1 L of the calcium alginate beads with entrapped cells. The column was filled with 300 mL of a 1 mM Cr(VI)-20 mM glucose solution or with a diluted chromic acid solution from a second rinse bath of an electroplating process (Cr(VI) final concentration: 1 mM). The chromic acid was neutralized with 6 M NaOH and supplemented with 20 mM glucose previously. Every two hours the Cr(VI) concentration in the supernatant was measured. When no Cr(VI) was detected, the column was refilled.

For this experiment the biomass was obtained harvesting the cells by centrifugation at 6300 *g* 10 batches of 1 L cultures and resuspending them in 500 mL of distilled water after washing them. The final cellular density of the suspension was 43 g cell dry weight L^{-1}.

2.4.3. Biotreatment of an industrial wastewater

Two industrial wastewaters from electroplating processes, wastewaters 1 and 2, with 0.26 and 0.69 mg L^{-1} Cr(VI) (0.005 and 0.013 mM) respectively were supplemented with 1.1 mM (200 mg L^{-1}) glucose as electron donor and pumped through a 1 L column packed with immobilized *P. veronii* 2E cells, as described above. The flow rate was adjusted in order to obtain a residence time of the wastewater inside the column of 30 minutes. Every two hours at 32ºC, the Cr(VI) concentration in the influent and effluent of the column was determined. At first, the two wastewaters were treated in separate, but after observation of the results, it came out that an optimal treatment was achieved when the wastewaters were treated sequentially.

So, the wastewater 2 was treated for 8-10 hours, after which the treatment continued with wastewater 1 for 12-14 hours. The total time of treatment was 96 hours. The work volume of the column was 210 mL. The chemical characterization of the two wastewaters tested in these biotreatments is resumed in Table 1.

2.5. Chromium (VI) analytical determination

The concentration of Cr(VI) was spectrophotometrically determined using an improved 1,5-diphenylcarbazide (DPC) method (Ahmad et al., 2010; Clesceri et al., 1998). Briefly, 1 mL of sample, 9 mL of 0.2 M H_2SO_4 and 200 µL of 0.25% (w/v) DPC prepared in acetone were mixed in a 10 mL volumetric flask. The mixture was then left for 10 minutes for color development. The absorbance of the solution was measured at 540nm. Distilled water was used as blank. All determinations were done by duplicate and experiments were repeated at least once.

Table 1. Chemical characterization of the electroplating effluents 1 and 2

Property	***Effluent***	
	1	***2***
Conductivity	897 µS cm^{-1}	1121 µS cm^{-1}
Hardness	398 mg L^{-1}	450 mg L^{-1}
Alkalinity	421 mg L^{-1}	384 mg L^{-1}
Sulfate	54 mg L^{-1}	46 mg L^{-1}
Chloride	21.6 mg L^{-1}	81.5 mg L^{-1}
Calcium	93.0 mg L^{-1}	115 mg L^{-1}
Nitrate	27.9 mg L^{-1}	78.5 mg L^{-1}
Magnesium	36.9 mg L^{-1}	44.2 mg L^{-1}
Sodium	48.3 mg L^{-1}	47.3 mg L^{-1}
Silica	77.2 mg L^{-1}	77.4 mg L^{-1}
pH	8	7.7
Cr(VI)	0.26 mg L^{-1} (0.005 mM)	0.69 mg L^{-1} (0.013 mM)

3. Results and discussion

3.1. Chromium (VI) biotransformation during bacterial growth and by pre-grown quiescent cells

After monitoring Cr(VI) concentration at every 2h-samples during aerobic growth, similar reduction rates were observed with the three strains (Fig. 1). Cr(VI) could not be totally removed by growing cells (71% for *P. veronii* 2E, 87% for *K. oxytoca* P2 and 88% for *K. ornithinolityca* 1P) during the tested 24 hours of culture development. The low reduction rate and metal biotransformation were directly associated with the obtained biomass yield (< 1 g cell dry weight L^{-1}) since the reduction of Cr(VI) is linearly dependant on it (Zhu et al., 2006). Higher concentrations of Cr(VI) could not be assayed because bacterial growth was strongly inhibited above 0.2 mM. Thus, the use of a biotreatment based on Cr(VI) biotransformation during bacterial growth is only suggested when a low -or subtoxic- Cr(VI) concentration is present.

In preliminary experiments, Cr(VI) reduction was carried out with harvested *K. oxytoca* P2 cells in both exponential and stationary phases with 1.1 g cell dry weight L^{-1} each. 20 mM glucose as electron donor and 0.2 mM Cr(VI) were added to the bacterial suspensions at buffered pH 7. After 10 hours at 32°C, only a 45% of Cr(VI) was removed in presence of stationary phase-harvested cells, while a 35% Cr(VI) reduction was detected with exponential phase-harvested cells in the same conditions. These exploratory and unpublished results led to work directly with stationary phase-harvested cells, easier to be collected than exponential phase cells.

When Cr(VI) reduction was assayed using suspensions of pre-grown stationary-phase cells (36 g cell dry weight L^{-1}), results obtained with 20 mM glucose were similar to those obtained with 1 mM NADH. Thus, *P. veronii* 2E was the most efficient strain with a 100% of Cr(VI) biotransformation even without the addition of an external electron donor in 4-6 hours (Fig. 2).

Fig. 2 also shows that the three strains had a similar behavior when 80 mM of glucose was added for they were able to reduce Cr(VI) at a high reduction rate. A 100% Cr(VI) removal for *P. veronii* 2E and *K. oxytoca* P2, while a 84% for *K. ornithinolityca* 1P were achieved in 8-10 hours. When using quiescent cells, diverse compounds are needed to be added to the reaction mixture as electron donors in order to stimulate Cr(VI) biotransformation (Barrera-Díaz et al., 2012).

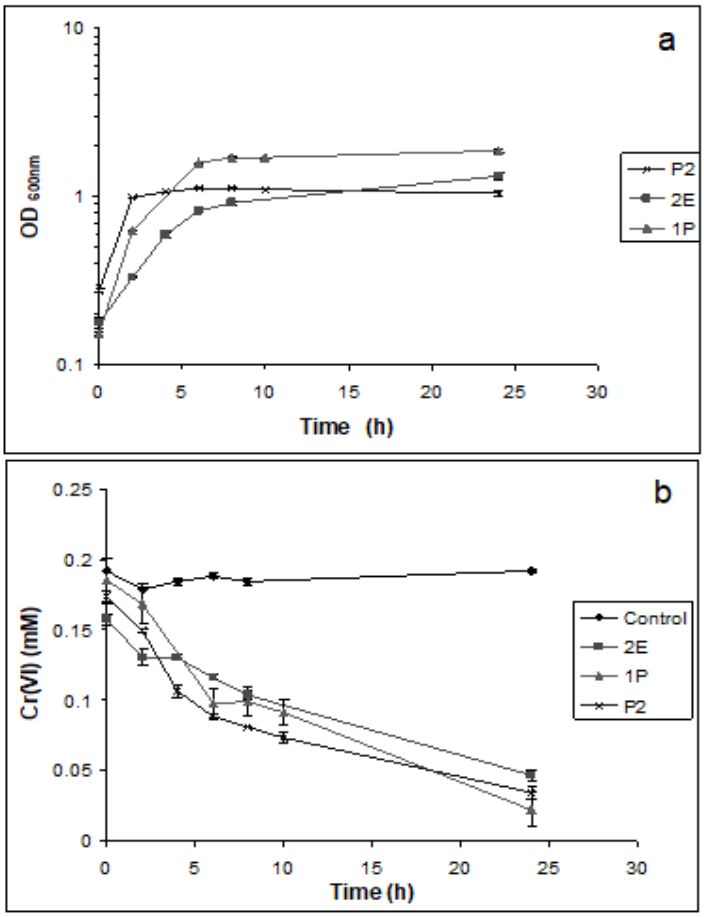

Fig. 1. (a) Bacterial development (as OD_{600nm}) during cultures, (b) Cr(VI) quantification in culture supernatants during cell growth. 2E: *Pseudomonas veronii* 2E (■), P2: *Klebsiella oxytoca* P2 (X) and 1P: *Klebsiella ornithinolityca* 1P (▲)

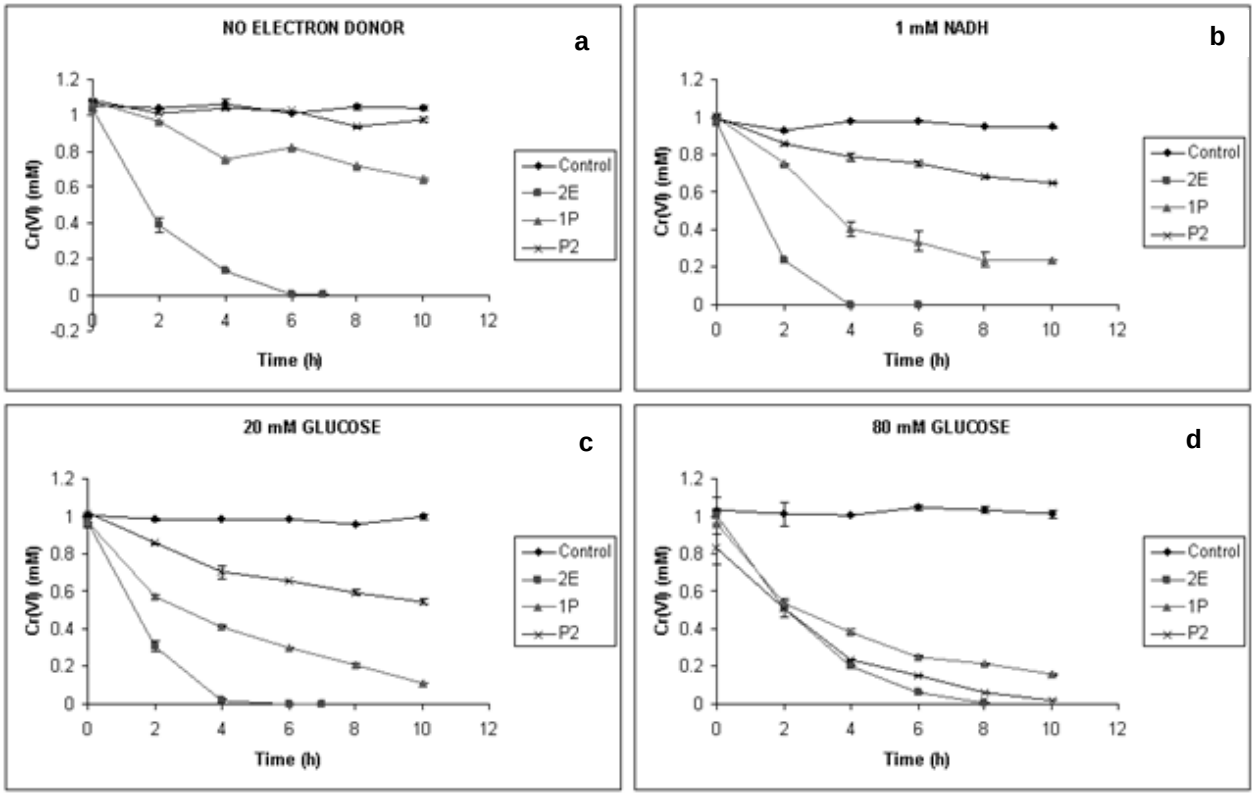

Fig. 2. Cr(VI) removal by quiescent cells in presence of different electron donors: (a) no electron donor present, (b) 1 mM NADH, (c) 20 and (d) 80 mM glucose. 2E: *Pseudomonas veronii* 2E (■), P2: *Klebsiella oxytoca* P2 (X) and 1P: *Klebsiella ornithinolityca* 1P (▲)

This enhancement of Cr(VI) reduction by the presence of electron donors was reported by many authors, where 71% improvement in anaerobic reduction rates was registered in presence of lactate (Zhu et al., 2006), or an increase from 5 to 20 times with acetate in aerobic or anaerobic conditions respectively (Xu et al., 2011a).

The capacity of Cr(VI) reduction without an electron donor addition may be attributed to the presence of extracellular oxidizable compounds which could not be eliminated by washing procedures since they probably were bound to cell envelopes. *Geobacter sulfurreducens* is a well known case of external electron donors because of the presence of cell bound extracellular cytochromes that accumulate electrons during growth phases and discharge them once an electron acceptor like Cr(VI) is present (Schrott et al., 2011). In the same way, *P. veronii* 2E could have compounds bounded to the cell matrix that could act as electron donors explaining the high reduction rate obtained without the addition of an electron donor. Preliminary results showed that *P. veronii* 2E is a good producer of exopolymers (ES), either soluble or bounded to cells. These ES could behave as endogenous electron donors. Being the best candidate, *P. veronii* 2E was then selected for the development of an aerobic biotreatment process for 1 mM Cr(VI) removal in presence of 20 mM glucose as an economic alternative for electron donor compound.

3.2. Effect of pH and temperature on Cr(VI) biotransformation capacity of P. veronii 2E

Biotransformation experiments at regulated pH were carried out as described in section 2.2. at 32ºC, 20 mM glucose -as electron donor extracted from the results previously obtained - and pH 5.5, 6, 7, 8 and 9 (Fig. 3a). Total Cr(VI) reduction (100%) was obtained at pH 7 and 8 in less than 6 hours (Fig. 3a).

The reduction kinetics at different temperatures is shown in Fig. 3b. Although the optimal temperature for Cr(VI) reduction resulted 32ºC, acceptable reduction yields were also obtained at 25ºC and 40ºC with total Cr(VI) removal in 8 hours. At 50ºC, an 85% reduction was achieved in 8 hours. Additional control experiments performed with quiescent cells pre-heated at 100ºC revealed no reduction activity.

The reduction rates obtained at the different pH and temperature values are depicted in Fig. 3c and Fig. 3d respectively. Maximal initial reduction rate of 0.49 mg Cr(VI) h^{-1} g cell dry $weight^{-1}$ (9.4 μmol Cr(VI) h^{-1} g cell dry $weight^{-1}$) corresponded to pH 7 and 32ºC, being the optimal experimental conditions to work with quiescent cells in future biotransformation processes. Experiments carried out at different pHs and temperatures showed that pH affected Cr(VI) reduction kinetics immediately after the beginning of the process, while the temperature seemed to affect the reduction since the fourth hour of the process.

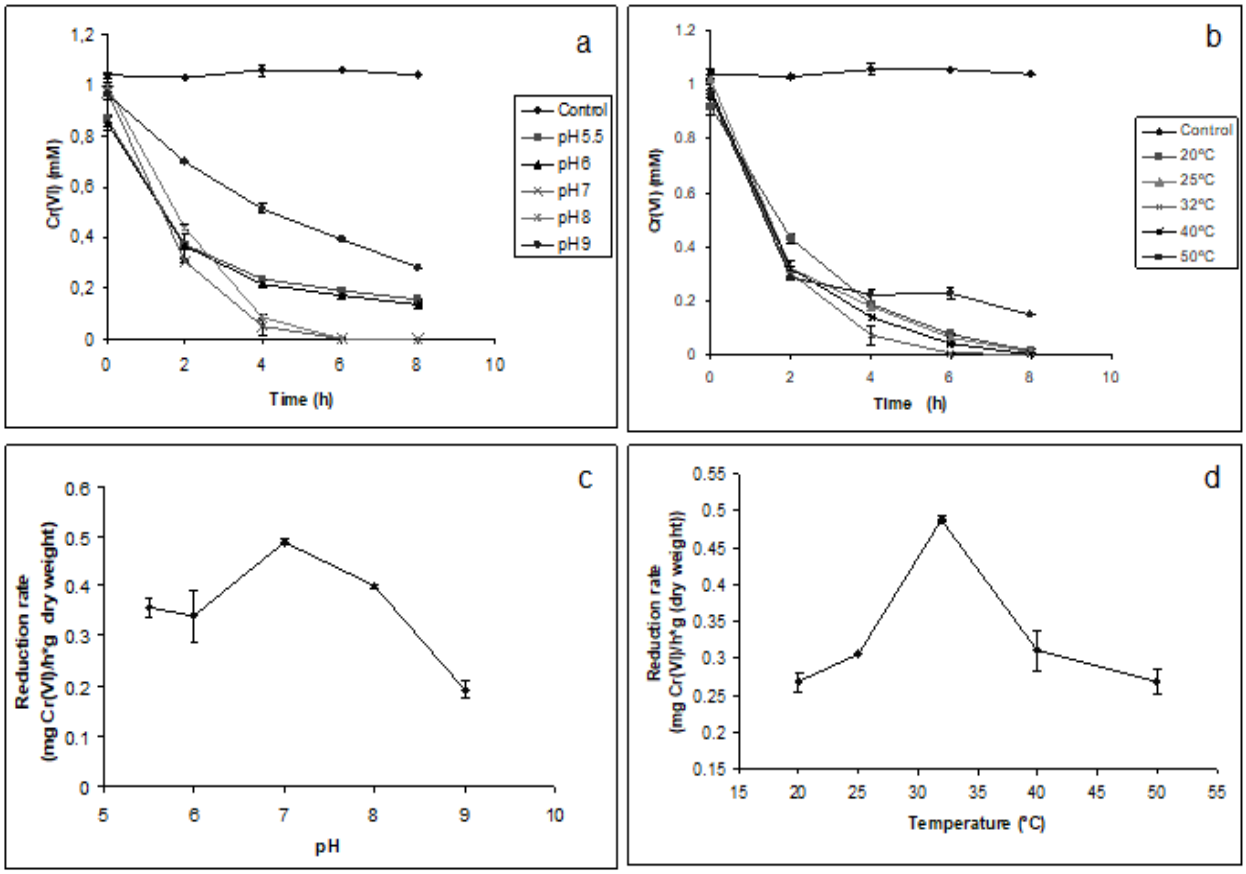

Fig. 3. (a) Cr(VI) reduction kinetics of *Pseudomonas veronii* 2E quiescent cells at different pH (♦ abiotic control, ■ pH=5.5, ▲ pH=6, X pH=7, ∗ pH=8 and • pH=9) and (b) temperatures (♦ abiotic control, ■ 20ºC, ▲ 25ºC, X 32ºC, ∗ 40ºC and • 50ºC). (c) Cr(VI) reduction rates as function of pH and (d) temperature

Experiments carried out at different pHs and temperatures showed that pH affected Cr(VI) reduction kinetics immediately after the beginning of the process, while the temperature seemed to affect the reduction since the fourth hour of the process. This inactivation behavior clearly implied that the process of Cr(VI) biotransformation was of enzymatic nature. Several works have reported the existence of a soluble chromate reductase for different *Pseudomonas* (Park et al., 2000; Suzuki et al., 1992) and a membrane-associated chromate reductase of *Bacillus megaterium* (Cheung et al., 2006).

3.3. Chromium (VI) biotransformation by immobilized cells of P. veronii 2E by entrapment in calcium alginate beads.

3.3.1. Temperature optimization

For temperature optimization experiments using immobilized cells, the pH was adjusted to 7 but not regulated in a buffer solution in order to explore the bioreduction yields under the simplest experimental conditions possible to reduce future costs in the process. An average of a 40% of Cr(VI) retention into the Ca-alginate beads was evidenced by the decrease in concentration of supernatants obtained when the cell free Ca-alginate beads were used as control experiments at different temperatures (Fig. 4). The highest Cr(VI)-removal initial rate was obtained at 40ºC (1.1 µmol Cr(VI) h^{-1} g cell dry $weight^{-1}$) accompanied by a maximal Cr(VI) diffusion rate inside the alginate matrix, without affecting the reducing activity as occurred at 50ºC (Fig. 4).

In summary, 100% Cr(VI) removal was most efficiently achieved with *P. veronii* 2E immobilized cells in 4 hours at 40ºC, while at 32ºC 8 hours were required.

Working with entrapped cells in calcium alginate decreased the reduction time in a half at 40ºC. This effect was not observed at 32ºC, 20ºC or 25ºC, registering a delay in the total Cr(VI) removal of more than 8 hours.

Immobilization experiments evidenced that chromium could be retained in the cell-free alginate beads. However, this retention might not be translated as an abiotic reduction: most of the Cr(VI) could be recovered from the beads after consecutive washing procedures, meaning that it only represented a concentrating step, consequence of a Cr(VI) diffusion phenomena. Cr(VI) reduction rate with free quiescent cells at 40°C resulted significatively lower (6.0 µmol Cr(VI) h^{-1} g cell dry $weight^{-1}$) than the one registered with immobilized cells, being possibly related to the Cr(VI) pre-concentration in the beads.

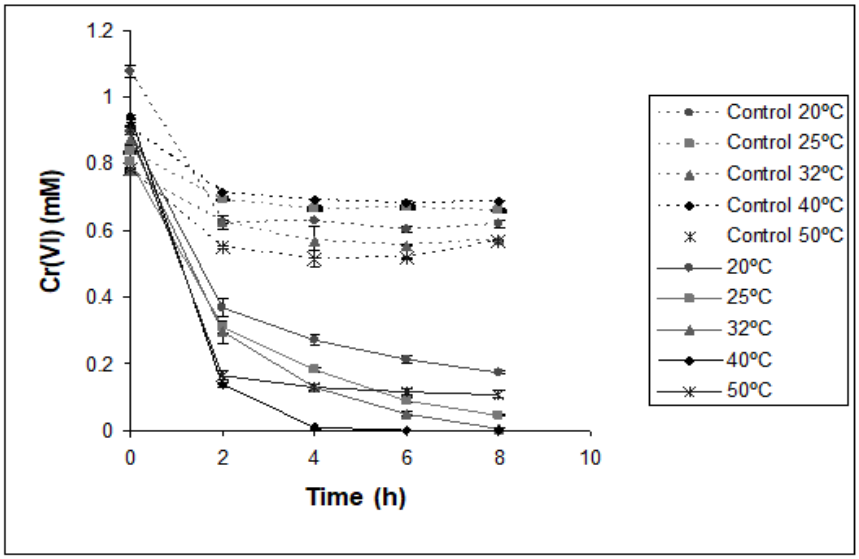

Fig. 4. Biotransformation of Cr(VI) by immobilized cells of *Pseudomonas veronii* 2E at different temperatures (• 20ºC, ■ 25ºC, ▲ 32ºC, ♦ 40ºC and ∗50ºC; dotted lines correspond to cell free control experiments)

In contrast, at 32ºC, the removal rates of immobilized cells were lower (8.0 µmol Cr(VI) h^{-1} g cell dry $weight^{-1}$) than the values obtained with free quiescent cells (9.4 µmol Cr(VI) h^{-1} g cell dry $weight^{-1}$) at the same temperature. This could be explained by the introduction of a limiting factor in the process: the diffusion rate of the substrates across the alginate matrix.

Although this problem could be circumvented by rising the temperature, a compromise should be taken between the optimal temperature of function of the catalyst and the temperature that allows a rapid diffusion through the immobilization matrix, always considering the lower energy consumption as possible. This diffusion resistance of the matrix and its influence in the reduction rate were studied by Xu et al. (2011b). They clearly identified four stages of Cr(VI) biotransformation, being the diffusion the limiting one that controlled the reduction rate.

3.3.2. Cr(VI) removal in batch systems

Sequential batch procedures for Cr(VI) removal were assayed with the immobilized bacteria. Results shown in Fig. 5a revealed a 100% Cr(VI) removal after the three first batches of 8 hours each. During the fourth batch, a decrease of 79% in Cr(VI) was detected after 24 hours, indicating a slowdown in the biotransformation rate.

The scale up of the sequenced batches was performed with a simulated wastewater or a diluted chromic acid solution from a second rinse bath of an electroplating process. The treatment applied to the synthetic wastewater achieved a 100% reduction in less than 6 hours during the first three batches. In the fourth batch, total Cr(VI) reduction was reached in 10 hours.

During the last batch Cr(VI) reduction was of 84.3% in 8 hours. Fig. 5b resumes the results obtained in the designed reactor with the diluted chromic acid solution. A 100% removal of Cr(VI) was achieved in less than 4 hours during the first four batches. In the fourth batch, the same percentage of Cr(VI) removal was obtained after 5 hours. In the fifth batch the biotransformation percentage was reduced to 96.2% in 8 hours.

In sequential batch systems a slowdown in the biotransformation rate was observed as the treatment proceeded. This decrease in the reduction yield could be attributed to a partial loss of the catalytic activity, possibly associated to the Cr(III) accumulation in the beads. So, the accumulation of Cr(III) and the potential replacement of Ca(II) with Cr(III) in the gel structure is a fact to be considered (Araujo and Teixeira, 1997). During the sequential batches carried out in this case, no changes were observed in beads´ integrity, making the process useful for at least 3 times.

In the scale up experiment the number of times that the beads could be reused and the Cr(VI) biotransformation rate were higher than in the experiments carried out at a minor scale. This improvement in Cr(VI) removal could be attributed to the fact that in the scale up experiment the relationship between the apparent volume of the alginate beads and the volume of the simulated wastewater was 3.3:1 while at a minor scale the relationship used was 2:1. A major number of beads were in contact with the wastewater in the scale up experiment, explaining the improvement in the Cr(VI) biotransformation rate.

3.3.3. Biotreatment of an industrial wastewater

The experiments carried out with the wastewater 2 alone showed that after 18 hours Cr(VI) (36 work volumes) started to accumulate in the column reaching a concentration of 0.09 mg L^{-1} (0.0017 mM). In contrast, no Cr(VI) was detected after 100 hours of treatment (200 work volumes) of the wastewater 1 when it was treated alone.

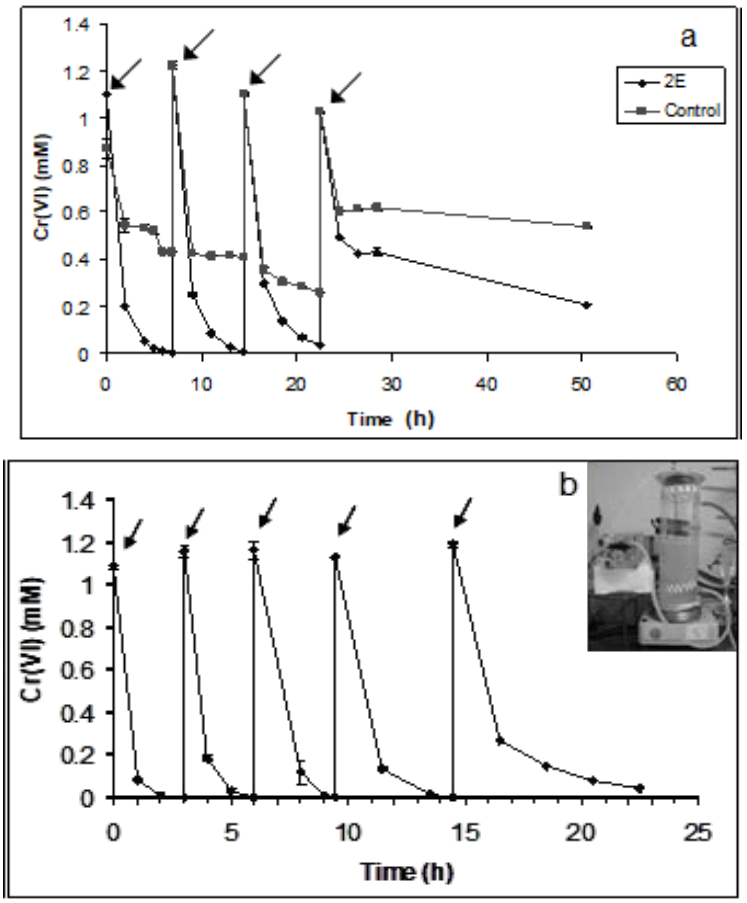

Fig. 5. (a) Cr(VI) removal by *Pseudomonas veronii* 2E immobilized in calcium alginate beads in 25 mL-batch minireactors. Arrows indicate Cr(VI) solution renewal after each 8 hours of treatment. ♦ immobilized cells in calcium alginate; ■ cell-free calcium alginate as abiotic control. (b) Scale up of Cr(VI) removal by *Pseudomonas veronii* 2E immobilized in calcium alginate beads in 300 mL-batch bioreactor. Arrows indicate the renewal -after each treatment- of the diluted chromic acid solution belonging to a second rinse bath of an electroplating process

To avoid the chromium saturation in the column when treating wastewater 2, both wastewaters were treated in a sequential mode. After 8-10 hours of treatment of the wastewater 2 the influent of the column was changed by the wastewater 1 which was treated for 12-14 hours. Fig. 6 shows the Cr(VI) concentration in the influent and effluent of the column during this procedure. The concentration of Cr(VI) in the effluent never surpassed the 0.1 mg L^{-1} (0.0019 mM). A total of 42 L or 200 column volumes were treated of which 23.6 L (112.4 column volumes) correspond to wastewater 1 and 18.4 L (87.6 column volumes) correspond to wastewater 2.

The treatment conducted in the 1 L column alternating between the two wastewaters showed promissory results for a scaling up process. This procedure allowed to wash the column with the more diluted wastewater (wastewater 1) avoiding the accumulation of Cr(VI) which was produced after treatment of wastewater 2. The saturation of the column when treating only wastewater 2 could be attributed to the fact that the rate of entry of Cr(VI) into the column was greater than the rate of Cr(VI) reduction.

The Cr(VI) concentration in the treated effluent alternating both wastes were below the limit set by the Argentinean laws for industrial discharges which is 0.2 mg L^{-1} (0.0038 mM) (ANL 26221, 2007). Even more, the total chromium concentration was below the 0.1 mg L^{-1} (0.0019 mM) that establishes the Code of Federal Regulations of the United States as the maximum contaminant level in surface water (CFR, 2014). Other studies have reported the use of immobilized bacteria for Cr(VI)-containing biotreatment (Ahmad et al., 2010; Elangovan and Philip, 2008) but in those cases the immobilization was achieved by biofilm formation over different matrices and not by entrapment in polymeric substances as in this study. The treatment developed in this study was very simple and removed chromium in a single step. It seems that Cr(III) remained inside the beads since it was not detected in the effluent even after washing with a pH 2 acidic solution. This remarkable fact differs from the results obtained by Ahmad et al. (2010) and Elangovan and Philip (2008) where Cr(III) was still present in solution and it had to be removed by additional processes.

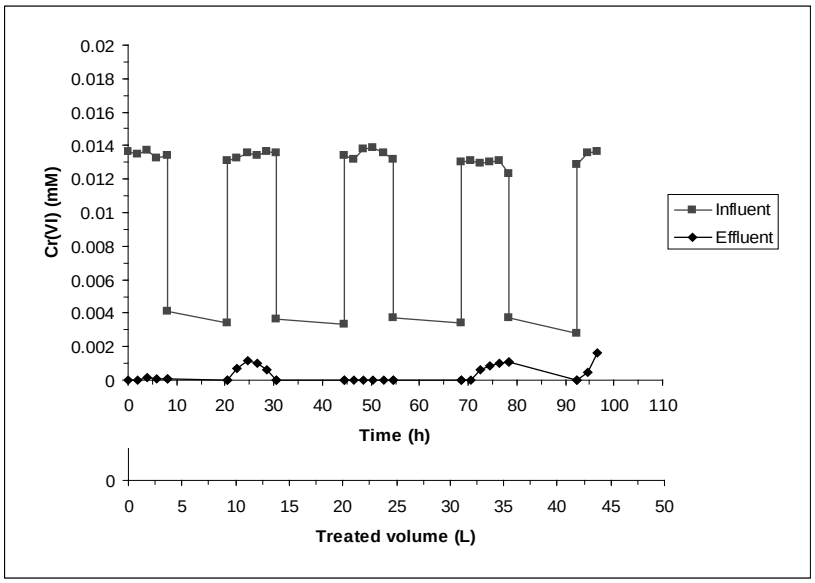

Fig. 6. Cr(VI) removal in a sequential biotreatment of the industrial effluents 2 and 1 (0.69 mg L^{-1} (0.013 mM) and 0.26 mg L^{-1} (0.005 mM) Cr(VI) respectively). Cr(VI) concentration was monitored both in the influent (■) and effluent (♦) of the immobilized-cell bioreactor

4. Conclusions

Cr(VI) biotransformation was optimal with a suspension of *P. veronii* 2E quiescent cells immobilized in calcium alginate at 40ºC, uncontrolled pH and 20 mM glucose. While the highest reduction rate was obtained using free cells at 32ºC and pH 7, the reduction rate of immobilized cells was lower at the same temperature, indicating that the substrate diffusion represented a limiting factor.

Scale up experiments showed the feasibility of application of this technology in wastewater biotreatment. Further research will focus on an industrial scale bioreactor design, the search for a more economic medium for biomass production and Cr(III) recovery.

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References

Aguiar F.P., Espinola J.G.P., Fonseca M.G., Arakaki L.N.H., Teotonio E.E.S., de Oliveira S.F., (2017), Silica gel modified with amino imine groups as an adsorbent for metallic cations, *Environmental Engineering and Management Journal*, **16**, 213-224.

Ahmad W.A., Zakaria Z.A., Khasim A.R., Alias M.A., Ismail S.M.H.S., (2010), Pilot-scale removal of chromium from industrial wastewater using the ChromeBac system, *Bioresource Technology*, **101**, 4371-4378.

Clesceri L.S., Greenberg A.E., Eaton A.D., (1998), *Chromium*, In: *Standard Methods for the Examination of Water and Wastewater*, Clesceri L.S., Greenberg A.E., Eaton A.D. (Eds.), 20th Edition, American Public Health Association, American Water Works Association and Water Environment Federation, United Book Press, Inc., Baltimore, Maryland, USA, 3- 65-67.

Araujo M.M., Teixeira J.A., (1997), Trivalent chromium sorption on alginate beads, *International Biodeterioration & Biodegradation*, **40**, 63-74.

ANL 26221, (2007), Argentinean National Law No. 26221, Article 122: Drinking Water and Wastewaters, Annex B, *Official Bulletin* 31107, 1, Buenos Aires, On line at: http://infoleg.mecon.gov.ar/infolegInternet/anexos/125000-129999/125875/norma.htm

Barrera-Díaz C.E., Lugo-Lugo V., Bilyeu B., (2012), A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction, *Journal of Hazardous Materials*, **223-224**, 1-12.

Cheung K.H., Lai H.Y., Gu J.D., (2006), Membrane associated hexavalent chromium reductase of *Bacillus megaterium* TKW3 with induced expression, *Journal of Microbiology and Biotechnology*, **16**, 855-862.

CFR, (2014), National Primary Drinking Water Regulations: Maximum Contaminant Levels and Maximum Residual Disinfectant Levels, *Code of Federal Regulations*, Title 40, Chapter 1, Subchapter D, Part 141, Subpart G, Washington, DC, On line at: http://www.ecfr.gov.

Colica G., Mecarozzi P.C., De Philippis R., (2010), Treatment of Cr(VI)-containing wastewaters with expolysaccharide-producing cyanobacteria in pilot flow through and batch systems, *Applied Microbiology and Biotechnology*, **87**, 1953-1961.

Desai C., Jain K., Madamwar D., (2008), Hexavalent chromate reductase activity in cytosolic fractions of *Pseudomonas sp.* G1DM21 isolated from Cr(VI) contaminated industrial landfill, *Process Biochemistry*, **43**, 713-721.

Dhal B., Thatoi H.N., Das N.N., Pandey B.D., (2013), Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: A review, *Journal of Hazardous Materials*, **250–251**, 272-291.

Dogan N.M., Kantar C., Gulcan S., Dodge C.J., Yilmaz B.C., Mazmanci M.A., (2011), Chromium (VI) Bioremoval by *Pseudomonas* bacteria: Role of Microbial Exudates for Natural Attenuation and Biotreatment of Cr(VI) Contamination, *Environmental Science and Technology*, **45**, 2278-2285.

Elangovan R., Philip L., (2008), Performance evaluation of various bioreactors for the removal of Cr(VI) and organic matter from industrial effluent, *Biochemical Engineering Journal*, **44**, 174-186.

Focardi S., Pepi M., Landi G., Gasperini S., Ruta M., Di Biasio P., Focardi S.E., (2011), Hexavalent chromium reduction by whole cells and cell free extract of the moderate halophilic bacterial strain *Halomonas sp*. TA-04, *International Biodeterioration & Biodegradation*, **66**, 63-70.

Garavaglia L., Cerdeira S.B., Vullo D.L., (2010), Chromium (VI) biotransformation by β- and γ-*Proteobacteria* from natural polluted environments: A combined biological and chemical treatment for industrial wastes, *Journal of Hazardous Materials*, **175**, 104-110.

Ghorbani F., Younesi H., (2016), Bioremediation of Chromium(VI) from aqueous solution by *Aspergillus niger:* optimization of process parameters, *Environmental Engineering and Management Journal*, **15**, 2551-2558.

Gürü M., Venedik D., Murathan A., (2008), Removal of trivalent chromium from water using low-cost natural diatomite, *Journal of Hazardous Materials*, **160**, 318-323.

Leita L., Margon A., Sinicco T., Mondini C., (2011), Glucose promotes reduction of hexavalent chromium in soil, *Geoderma*, **164**, 122-127.

Mendez N., Ramirez S.A.M., Ceretti H.M., Zalts A., Candal R., Vullo D.L., (2011), *Pseudomonas veronii* 2E surface interactions with Zn(II) and Cd(II), *Global Journal of Environmental Science and Technology*, **1**, 1-9.

Park C.H., Keyhan M., Wielinga B., Fenford S., Matin A., (2000), Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase, *Applied and Environmental Microbiology*, **66**, 1788-1795.

Patra R.M., Malik S., Beer M., Megharaj M., Naidu R. (2010), Molecular characterization of chromium (VI) reducing potential in Gram positive bacteria isolated from contaminated sites, *Soil Biology and Biochemistry*, **42**, 1857-1863.

Poopal A.C., Laxman S.R., (2009), Studies on biological reduction of chromate by *Streptomyces griseus*, *Journal of Hazardous Materials*, **169**, 539-545.

Sadeghi S., Moghaddam M.R.A., Arami M., (2017), Techno-economical evaluation of hexavalent chromium removal by electrocoagulation process with the aid of polyaluminum chloride as coagulant: optimization through response surface methodology, *Environmental Engineering and Management Journal*, **16**, 93-104.

Schrott G.D., Bonanni P.S., Robuschi L., Esteve-Nuñez A., Busalmen J.P., (2011), Electrochemical insight into the mechanism of electron transporting biofilms of *Geobacter sulfurreducens*, *Electrochimica Acta*, **56**, 10791-10795.

Sharma S., Adholeya A., (2011), Detoxification and accumulation of chromium from tannery effluent and spent chrome effluent by *Paecilomyces lilacinus* fungi, *International Biodeterioration & Biodegradation*, **65**, 309-317.

Srinath T., Verma T., Ramteke P.W., Garg S.K., (2002), Cr(VI) biosorption and bioaccumulation by chromate resistant bacteria, *Chemosphere*, **48**, 427-435.

Suzuki T., Miyata N., Horitsu H., Kawai K., Takamizawa K., Tai Y., Okazaki M., (1992), NAD(P)H-dependent chromium (VI) reductase of *Pseudomonas ambigua* G-1: a Cr(VI) intermediate is formed during the reduction of Cr(VI) to Cr(III), *Journal of Bacteriology*, **174**, 5340-5345.

Thacker U., Parikh R., Shouche Y., Madamwar D., (2006), Hexavalent chromium reduction by *Providencia sp*., *Process Biochemistry*, **41**, 1332-1337.

Vaiopoulou E., Gikas P., (2012), Effects of chromium on activated sludge and on the performance of wastewater treatment plants: A review, *Water Research*, **46**, 549-570.

Vullo D.L., Ceretti H.M., Daniel M.A., Ramírez S.A., Zalts A., (2008), Cadmium, zinc and copper biosorption mediated by *Pseudomonas veronii* 2E, *Bioresource Technology*, **99**, 5574-5581.

Xu L., Luo M., Li W., Wei X., Xie K., Liu L., Jiang C., Liu H., (2011a), Reduction of hexavalent chromium by *Pannonibacter phragmitetus* LSSE-09 stimulated with external electron donors under alkaline conditions, *Journal of Hazardous Materials*, **185**, 1169-1176.

Xu L., Luo M., Yang L., Wei X., Lin X., Liu H., (2011b), Encapsulation of *Pannonibacter phragmitetus* LSSE-09 in alginate-carboxymethyl cellulose capsules for reduction of hexavalent chromium under alkaline conditions, *Journal of Industrial Microbiology and Biotechnology*, **38**, 1709-1718.

Zhu W., Chai L., Ma Z., Wang Y., Xiao H., Zhao K., (2006), Anaerobic reduction of hexavalent chromium by bacterial cells of *Achromobacter sp.* Strain Ch1, *Microbiological Research*, **163**, 616-623.