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ScienceDirect
Journal of Hazardous Materials 156 (2008) 214–222
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Reduction of hexavalent chromium by *Sphaerotilus natans* a filamentous micro-organism present in activated sludges

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Received 25 June 2007; received in revised form 7 September 2007; accepted 6 December 2007
Available online 15 December 2007

Abstract

Wastewaters produced by various industries may contain undesirable amounts of hexavalent chromium (Cr(VI)), as chromate and dichromate, a hazardous metal affecting flora and animals of aquatic ecosystems as well as human health. One removal strategy comprises the microbial reduction of Cr(VI) to Cr(III), a less soluble chemical species that is less toxic than Cr(VI). In this work, the ability to reduce Cr(VI) of *Sphaerotilus natans*, a filamentous bacterium usually found in activated sludge systems, was evaluated. In aerobic conditions, *S. natans* was able to efficiently reduce Cr(VI) to Cr(III) from dichromate solutions ranging between 4.5 and 80 mg Cr(VI) l^{-1} in the presence of a carbonaceous source. A simultaneous evaluation of the microbial respiratory activity inhibition was also carried out to analyze the toxic effect of Cr(VI). Cr(VI) reduction by *S. natans* was mathematically modeled; chromium(VI) reduction rate depended on both Cr(VI) concentration and active biomass concentration. Although it is known that *S. natans* removes heavy metal cations such as Cr(III) by biosorption, the ability of this micro-organism to reduce Cr(VI), which behaves as an oxyanion in aqueous solutions, is a novel finding. The distinctive capacity to reduce Cr(VI) to Cr(III) than remain soluble or precipitated becomes *S. natans* a potential micro-organism to decontaminate wastewaters.

Keywords: Biological reduction; Cr(VI); Respirometry; *Sphaerotilus natans*; Filamentous micro-organisms

1. Introduction

Hexavalent chromium (Cr(VI)), as chromate and dichromate, is widely present in wastewaters from several industries such as pigment and dye production, leather tanning, electroplating and the steel industry [1]. Industrial discharges containing Cr(VI) and improper disposal procedures have resulted in large-scale environmental pollution. Cr(VI) is harmful for flora and animals of natural aquatic ecosystems, being highly toxic to all forms of life [2]. Owing to its high toxicity, this metal constitutes a serious risk for health whereas the chronic exposure to it, even at low concentrations, may produce mutagenesis and carcinogenesis [3]. The World Health Organization and the Environmental Protection Agency of the USA have set a maximum limit of Cr(VI) for domestic uses of water of 50 $\mu g\, l^{-1}$ [4].

The chromium concentration in wastewaters depends on the industry: in leather tanning, the reports indicate values ranging between 6.6 and 67.9 mg l^{-1} for total chromium and from 2.9 to 28.4 mg l^{-1} for Cr(VI) [5], while in plating industries, the effluents were reported to have 29.5 mg l^{-1} of total chromium and 28.2 mg l^{-1} of hexavalent chromium [6].

The conventional methods of Cr(VI) removal in wastewaters comprise chemical reduction followed by chemical precipitation, ion exchange and adsorption onto activated carbon. However, these methods are excessively energy-consuming and utilize large amounts of reagents [7]. Cr(VI) behaves as an oxyanion (CrO_4^{2-}, $Cr_2O_7^{2-}$) in water solution, so it cannot be precipitated nor bounded to negatively charged functional groups present in the biomass surface as the carboxylates. Microbial reduction of Cr(VI) to trivalent chromium (Cr(III)) can be applied to remediate contaminated waters where physicochemical methods are costly and produce secondary wastes [8]. Biological reduction of hexavalent chromium is economical,

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doi:10.1016/j.jhazmat.2007.12.014

Nomenclature

a	coefficient (mg $Cr(VI)_0$ l^{-1})
b	chromium(VI) reduction coefficient ((mg $Cr(VI)_0)^n$ (g $VSS_0)^{-n}$ h^{-1})
Cr(VI)	concentration of hexavalent chromium at time t (mg l^{-1})
$Cr(VI)_0$	concentration of hexavalent chromium at time = 0 (mg l^{-1})
COD	chemical oxygen demand (mg O_2 l^{-1})
D_0	ratio between initial Cr(VI) concentration and that of biomass (mg $Cr(VI)_0$ (g $VSS_0)^{-1}$)
FR_{OUR}	bacterial respiratory activity fraction based on respirometric technique (dimensionless)
k	coefficient ((g VSS)$^{-n}$ l^n h^{-1})
k'	apparent chromium(VI) reduction coefficient (h^{-1})
n	coefficient (dimensionless)
OUR	oxygen uptake rate (respirometry) (mg O_2 (l h)$^{-1}$)
$OUR_{(control)}$	oxygen uptake rate for control tests (mg O_2 (l h)$^{-1}$)
$OUR_{(chromium)}$	oxygen uptake rate for samples treated with chromium (mg O_2 (l h)$^{-1}$)
t	time (h)
VSS	volatile suspended solids
VSS_0	initial volatile suspended solids
X_0	initial biomass concentration (g VSS l^{-1})
$X_{(active)}$	biomass concentration with biological activity (g VSS l^{-1})

safe and sustainable [9]. For these reasons, characterization of the microbial reduction kinetics of Cr(VI) becomes essential. Cr(III) is more stable, less soluble and less toxic than Cr(VI) [10] and can be removed from the system as chromium hydroxide that precipitates at neutral pH [11].

Many bacterial genera have been reported to reduce Cr(VI) to Cr(III) including *Bacillus* [12], *Pseudomonas* [13], *Escherichia* [14], *Arthrobacter* [15], *Ochrobactrum* [16], *Brevibacterium* [17], *Shewanella* [18].

The toxic effect of Cr(VI) on pure and mixed bacterial cultures has been studied by several methods such as microbial viability by the total plate count technique [10], optical density as an indirect measurement of biomass [15], cellular morphology [19] and oxygen uptake rate [20]. This last method is an index of the metabolic activity of aerobic micro-organisms, and has been extensively used to study the toxicity of several chemical agents [21].

Sphaerotilus natans is a filamentous bacterium usually found in the ecological communities of heavily polluted freshwater and in activated sludge systems mainly with filamentous bulking problems [22–24]. There are reports in literature on *S. natans* dealing biosorption of heavy metal cations (Ag(I), Pb(II), Cd(II), Zn(II), Cu(II), Cr(III)) by living and lyophilized biomass [25–28]; this micro-organism has a protein–lipopolysaccharidic sheath that is probably responsible for the metals removal [29]. However, information about the capacity of this micro-organism to reduce an oxyanion such as hexavalent chromium was not found in literature.

The objectives of the present work were: (a) to determine the capacity of *S. natans* to decrease Cr(VI) concentration in dichromate solutions using aerated batch reactors; (b) to evaluate the reduction capacity of this micro-organism to convert Cr(VI) to Cr(III); (c) to analyze the toxic effect of chromium species on the microbial respiratory activity; (d) to develop a mathematical model that describes the decay of Cr(VI) concentration as a function of contact time and active biomass concentration.

2. Materials and methods

2.1. S. natans culture

S. natans ATCC #29329 was grown in a chemostat with the following culture medium: monohydrate citric acid, 3480 mg l^{-1}; $(NH_4)_2SO_4$, 1000 mg l^{-1}; $MgSO_4 \cdot 7H_2O$, 400 mg l^{-1}; $CaCl_2 \cdot 2H_2O$, 50 mg l^{-1}; KH_2PO_4, 250 mg l^{-1}; $Na_2HPO_4 \cdot 12H_2O$, 1000 mg l^{-1}; vitamin B12, 100 μg l^{-1}; $FeSO_4.7H_2O$, 15 mg l^{-1}; $ZnSO_4.7H_2O$, 5 mg l^{-1}; $MnSO_4 \cdot H_2O$, 3 mg l^{-1}; $CuSO_4 \cdot 5H_2O$, 0.75 mg l^{-1}; $CoCl_2 \cdot 6H_2O$, 0.15 mg l^{-1}; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.5 mg l^{-1}; BO_3H_3, 0.1 mg l^{-1}; KI, 0.1 mg l^{-1}. The medium was sterilized at 121 °C for 60 min except vitamin B12, that was sterilized by membrane filtration (0.45 μm Millipore HA) and then added to the sterile medium.

The pH of the culture medium was adjusted to 7.0 with NaOH before autoclaving. A constant pH 7.0 was maintained throughout the course of the chemostat operation by automatic addition of 1M H_2SO_4.

A chemostat containing the culture medium (1 l) was initially inoculated with *S. natans* (agar slant inocula) and operated aseptically. Micro-organisms were grown at 30 °C with an air flow rate of 2 l min^{-1}, rotor speed of 600 rpm, and dissolved oxygen concentration above 2 mg O_2 l^{-1}. Biomass concentration was measured by chemical oxygen demand (COD) and transformed into volatile suspended solids (VSS) using a calibration curve previously determined [30].

The chemostat was operated as a batch for several hours until the biomass reached a concentration of 0.5 g VSS l^{-1}, then the chemostat was operated under continuous regime. Dilution rates ranged between 0.11 and 0.13 h^{-1} corresponding to cellular and hydraulic residence times of 9.09 and 7.69 h respectively. The system was considered to run under steady-state conditions after operating for a period of at least five residence times.

2.2. Cr(VI) biosorption and reduction experiments

The *S. natans* pure culture growing in the chemostat was harvested by centrifugation and resuspended in distilled water (pH 7.0). Samples of 200 ml from this microbial suspension (pre-grown cells) were transferred to 250 ml aerobic stirred batch reactors to be treated with Cr(VI) concentrations ranging

between 4.5 and 80 mg l^{-1} and different contact times (3–285 h). A stock $K_2Cr_2O_7$ solution of 4 g Cr(VI) l^{-1} was used to obtain the desired concentrations.

A first set of experiments were conducted to evaluate the Cr(VI) biosorption capacity by *S. natans* active micro-organisms to decrease Cr(VI) concentration in dichromate solutions. These experiments were performed at 20 °C, with different pH values ranging between 3.0 and 7.0, without an oxidizable substrate.

In another set of experiments, the reduction capacity of Cr(VI) promoted by the biological activity of *S. natans* was tested, using glucose as oxidizable substrate at an initial pH of 7.0. A volume of 5 ml glucose solution (8.0%, w/v, pH 7.0) was added to the stirred batch reactor to obtain an initial substrate concentration of 2100 mg l^{-1} expressed as soluble COD (determined after centrifugation of samples). Glucose solution concentration was controlled during the test in order to assure oxidizable substrate concentration higher than 1000 mg l^{-1} (as soluble COD). The biomass concentration used in all the experiments ranged between 690 and 2090 mg VSS l^{-1}. In all the experiments, at regular intervals samples were centrifuged at 14,000 rpm for 5 min to determine chromium species concentrations.

2.3. Cr(VI) and total chromium concentrations

Cr(VI) concentrations in the supernatant were determined by a spectrophotometric method at 540 nm, using diphenylcarbazide [31].

Considering that the micro-organism could reduce Cr(VI) to Cr(III), the concentrations of soluble Cr(III) and precipitated Cr(III) (chromium hydroxide) were determined at each time from the following mass balance: $[Cr]_{total} = [Cr(VI)]_{soluble} + [Cr(III)]_{soluble} + [Cr(III)]_{precipitated}$. The total chromium concentration in the supernatant ($[Cr]_{soluble\ total} = [Cr(VI)]_{soluble} + [Cr(III)]_{soluble}$) was measured using potassium permanganate and ammonium persulfate (to oxidize Cr(III) to Cr(VI)) followed by the reaction with diphenylcarbazide previously described. The Cr(III) concentration present in solution (soluble Cr(III)) was calculated by the mass balance as the difference between total soluble chromium concentration and that of hexavalent chromium. The total Cr(III) concentration (soluble and precipitated fractions) was calculated as the difference between the initial Cr(VI) concentration and the residual Cr(VI) concentration determined at each time. The percentage of soluble Cr(III) with respect to the total Cr(III) concentration in the different experiments was also reported.

The performance of Cr(VI) reduction by *S. natans* was calculated as follows:

$$\text{Cr(VI) reduction}\,\% = 100\frac{\text{Cr(VI)}_0 - \text{Cr(VI)}}{\text{Cr(VI)}_0} \quad (1)$$

where Cr(VI) is the concentration at time t, and $Cr(VI)_0$ the initial value.

2.4. Respirometric assessment of Cr(VI)-induced microbial inactivation

Respirometric tests were performed to evaluate the toxic effect of hexavalent chromium on the microbial respiratory activity. Control tests were performed with micro-organisms not submitted to Cr(VI). The equipment included a vessel containing a polarographic oxygen probe (YSI Incorp., Ohio, USA), an aerator and a magnetic stirrer; the signal from the oxygen probe was connected to a computer. The oxygen uptake rate (OUR) in the control tests ($OUR_{(control)}$) and those of the samples exposed to Cr(VI) ($OUR_{(chromium)}$) were expressed as mg O_2 $(l\,h)^{-1}$.

OUR values of control microbial samples were measured by placing 15 ml of the *S. natans* biomass suspension (pre-grown cells suspended in distilled water, pH 7.0) in the respirometer and adding a pulse (0.35 ml) of a glucose stock solution (8%, w/v) as the oxidizable substrate. After one minute of contact time, the mixture was aerated for 1 min. Once aeration was stopped, a linear decrease of the dissolved oxygen concentration was recorded as a function of time, the slope representing the value of $OUR_{(control)}$.

$OUR_{(chromium)}$ values of *S. natans*, for different Cr(VI) concentrations and contact times (3–285 h) in the aerobic stirred batch reactor, were determined using aliquots of 15 ml *S. natans* microbial suspensions placed in the respirometer. The values of $OUR_{(chromium)}$ were measured after glucose addition and aeration. The effect of chromium on the biomass respiratory activity was expressed as follows:

$$FR_{OUR} = \frac{OUR_{(chromium)}}{OUR_{(control)}} \quad (2)$$

where FR_{OUR} is the fraction of bacterial respiratory activity exposed to different initial Cr(VI) concentrations and contact times with respect to the control samples.

2.5. Statistical analysis

Analysis of variance was done using Systat software. Nonlinear regressions were conducted in the Sigma Plot 2.0 software (Jandel Scientific, Chicago, IL, USA).

3. Results and discussion

3.1. Cr(VI) reduction performance

Experiments carried out without adding glucose showed that in the absence of an oxidizable substrate living *S. natans* biomass did not produce a decrease of Cr(VI) concentration from dichromate solutions in any of the tested cases (pH 7.0 and 3.0). Considering that Cr(VI) behaves as an oxyanion (CrO_4^{2-}, $Cr_2O_7^{2-}$) in solution, it would be expected that these anions interact strongly with positively charged ligands [32]. However in the experiments where the pH value was lowered from 7.0 to 3.0 (in order to change the overall charge of the microbial surface from negative to positive), the Cr(VI) biosorption process was not observed.

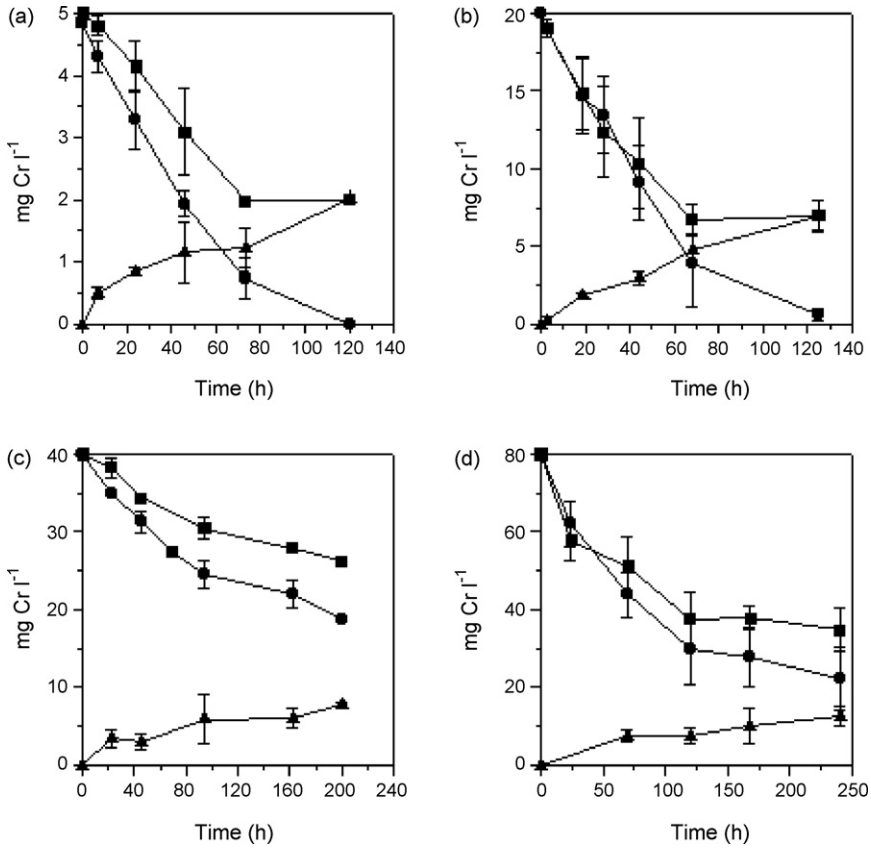

Fig. 1. Effect of initial Cr(VI) and biomass concentrations on Cr(VI) reduction by *Sphaerolitus natans*: (a) 5 mg Cr(VI) l^{-1} (X_0 = 1.008 g VSS l^{-1}), (b) 20 mg Cr(VI) l^{-1} (X_0 = 1.033 g VSS l^{-1}), (c) 40 mg Cr(VI) l^{-1} (X_0 = 0.965 g VSS l^{-1}) and (d) 80 mg Cr(VI) l^{-1} (X_0 = 2.091 g VSS l^{-1}). (●) Soluble Cr(VI); (■) total soluble Cr; (▲) soluble Cr(III). The standard deviations are indicated by error bars.

However *S. natans* was able to reduce Cr(VI) to Cr(III) in presence of glucose, thus an energy source was essential for the biological reduction process.

Concentrations of soluble hexavalent chromium, total soluble chromium, and soluble trivalent chromium were plotted as a function of contact time with *S. natans* (Fig. 1a–d) for different initial Cr(VI) concentrations ranging between 4.5 and 80 mg l^{-1}. As can be observed, the time required for the total reduction of Cr(VI) increased with increasing initial Cr(VI) concentration.

A general analysis of the results observing Fig. 1a and b shows that for initial Cr(VI) concentrations of 5 and 20 mg l^{-1} respectively and a similar biomass concentration of about 1 g VSS l^{-1}, Cr(VI) reduction of 100% and 97% was achieved after 120 h, while the amount of soluble Cr(III) produced in each case was of 40% and 36% of the reduced chromium. For an initial Cr(VI) concentration of 40 mg l^{-1} (Fig. 1c) and for the same contact time (120 h) with a comparable biomass concentration, 43% of Cr(VI) was reduced leaving in solution 36% of the Cr(III) produced. It can be observed that in these experiments using similar biomass concentrations, the percentage of Cr(VI) reduction decreased with increasing initial chromium concentration. Besides more than 50% of the Cr(III) produced is not soluble and remained in the system attached to *S. natans* cells and/or as precipitated $Cr(OH)_3$, without adding chemical agents, therefore it can be easily removed.

It must be emphasized that in all the Cr(VI) reduction experiments, *S. natans* was obtained from a chemostat fed with a culture medium without chromium; thus a previous contact with Cr(VI) was not required to induce the biological reduction process.

The effect of D_0 (ratio between initial Cr(VI) concentration to that of biomass) on Cr(VI) reduction can be also qualitatively analyzed. As an example, results from experiments carried out with an initial Cr(VI) concentration of 80 mg l^{-1} and a biomass concentration of 2 g VSS l^{-1} (Fig. 1d) can be compared with results of Fig. 1c corresponding to an initial Cr(VI) concentration of 40 mg l^{-1} and a biomass concentration of 1 g VSS l^{-1}; in both cases the D_0 value was approximately the same (40 mg $Cr(VI)_0$ (g $VSS_0)^{-1}$). Fig. 1d shows that 62% of Cr(VI) was reduced in 160 h and a similar relative amount of Cr(VI) was reduced during the same contact time in Fig. 1c, demonstrating that D_0 affected Cr(VI) reduction.

During Cr(VI) reduction experiments (Fig. 1a–d), biomass concentration did not change significantly. Chromium(VI) reduction by *S. natans* was observed to be independent of cell growth but dependent on the presence of metabolically active cells.

The performance of *S. natans* to reduce Cr(VI) to Cr(III) can be compared to that of other micro-organisms; however, in order to compare the results obtained with *S. natans* with

Table 1
Effect of initial Cr(VI) concentration on the Cr(VI) reduction rates and specific Cr(VI) reduction rates by *Sphaerotilus natans* at different contact times

Initial Cr(VI) concentration ($Cr(VI)_0$, mg l^{-1})	Cr(VI) reduction rate (mg Cr(VI) $(l\,h)^{-1}$)			Specific Cr(VI) reduction rate (mg Cr(VI) (g VSS $h)^{-1}$)		
	24 h	72 h	120 h	24 h	72 h	120 h
5	0.064	0.056	0.042	0.063	0.055	0.041
20	0.239	0.236	0.161	0.231	0.228	0.155
40	0.234	0.177	0.162	0.242	0.183	0.167
80	0.743	0.501	0.403	0.355	0.239	0.192

those reported in literature, it must be considered that in our experiments, *S. natans* was suspended in distilled water with glucose as the only electron donor to reduce Cr(VI), while in the majority of the published works on chromium reduction, nutrient media to support microbial growth were generally utilized.

Our results show that *S. natans* was able to reduce an initial chromium concentration of 80 to 20 mg l^{-1} (75%) in 240 h. In comparison *Bacillus* sp., isolated from chromate contaminated soil, reduced Cr(VI) from 80 to 40 mg l^{-1} after 42 h in a nutrient medium, however Cr(VI) reduction ceased at higher contact times [12].

Ochrobactrum sp., isolated from chromium-contaminated soil samples and inoculated in Luria broth containing hexavalent chromium, was able to reduce a Cr(VI) concentration of 150 to 10.2 mg l^{-1} in 127 h [16]. However, in this case the optical density of the *Ochrobactrum* sp. cultures increased more than 40 times during the incubation time, while in our work the biomass concentration of *S. natans* remained approximately constant. In most of the research works, for instance those conducted by Thacker and Madamwar [16], nutrient-rich media with yeast extract and tryptone were used [33,34]. In such conditions, the real toxicity of Cr(VI) may have been masked or decreased due to complexation of Cr(VI) with organic components so the microbial metabolism was probably less affected. Keyhan et al. [19] reported that several pure cultures of bacterial strains (*Pseudomonas putida*, *Escherichia coli*, *Shewanella putrefaciens*) in Luria broth produced a more rapid Cr(VI) reduction than those suspended in minimal salt media supplemented with glucose.

3.2. Initial Cr(VI) reduction rates

In order to compare our results with those of the literature, the initial slopes of the curves were calculated to obtain the initial Cr(VI) reduction rates. From the information of Fig. 1a–d, the highest rate of Cr(VI) reduction (0.743 mg Cr(VI) $(l\,h)^{-1}$) and the highest specific rate of reduction that was determined taking into account the biomass concentration (0.355 mg Cr(VI) (g VSS $h)^{-1}$) were observed over the first 24 h of contact time with an initial Cr(VI) concentration of 80 mg Cr(VI) l^{-1} (Table 1). The initial slopes of the curves (initial reduction rates) were similar to those reported by Wang and Xiao [35] for *Pseudomonas fluorescens* LB 300 (10^{10} cells ml^{-1}) in the same period (24 h) and for initial Cr(VI) concentrations of 70 and 90 mg l^{-1}. Values slightly lower were reported by Wang and Xiao [35] for *Bacillus* sp. (10^{10} cells ml^{-1}) with initial reduction rates of 0.48 and 0.44 mg Cr(VI) $(l\,h)^{-1}$ for initial Cr(VI) concentrations ranging between 50 and 70 mg l^{-1} respectively.

Table 1 shows the effect of initial Cr(VI) concentration on the Cr(VI) reduction rates (mg Cr(VI) $(l\,h)^{-1}$) and on the specific Cr(VI) reduction rates (mg Cr(VI)(g VSS $h)^{-1}$) calculated from the slopes of the curves Cr(VI) vs. time. The rate of Cr(VI) reduction by *S. natans* increased with increasing initial Cr(VI) concentrations (Table 1).

3.3. Respirometric tests

Control tests conducted in the absence of hexavalent chromium showed that the respiratory activity of *S. natans* gradually increased over time until reaching about twice of the initial value after 40–48 h. This increase may have been caused by the adaptation of the micro-organism to the carbon and energy source utilized in the batch tests.

In all the experiments carried out with Cr(VI) (4.5–80 mg l^{-1}), a marked inhibition of the respiratory activity of *S. natans* was found between 24 and 48 h of contact time with the solution, the respiratory activity reaching only 15–25% of the initial value. However, such activity was progressively recovered to attain 50–85% of the initial value at the end of the experiments. These changes observed in the respiratory activity may be ascribed to adaptation of the micro-organisms and/or to lower Cr(VI) concentration in solution, owing to the reduction of Cr(VI) to Cr(III) produced by this bioprocess.

Our results are in agreement with those of Vaňková et al. [20]; they reported that initially the respiratory activity of activated sludge decreased as a function of contact time with chromium (2–800 mg Cr(VI) l^{-1}) and was recovered later.

The fraction of *S. natans* respiratory activity (FR_{OUR}) as a function of initial Cr(VI) concentration and contact times was determined using Eq. (2) for all the tested conditions (Fig. 2). The values of FR_{OUR} decreased drastically during the first hours of contact with hexavalent chromium, and gradually increased during 24–48 h reaching values of FR_{OUR} between 0.5 and 0.65 depending on the initial Cr(VI) concentration.

The effect of initial Cr(VI) concentration on FR_{OUR} of *S. natans* during the first 24 h contact time was evaluated from the initial slopes of Fig. 2. FR_{OUR} is a dimensionless value that starts from 1, decreasing with time; thus negative slopes (S) were obtained. Fig. 3 shows S values as a function of initial Cr(VI)

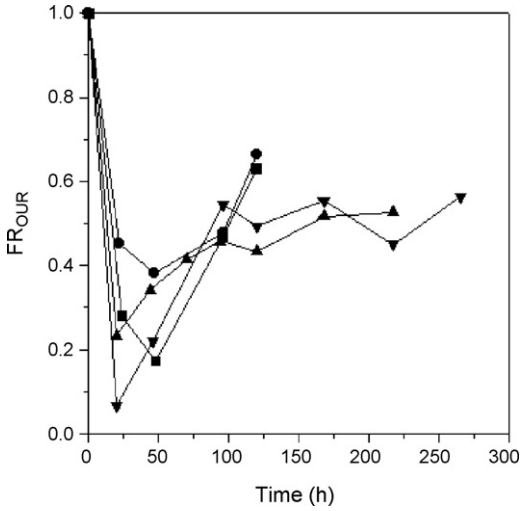

Fig. 2. *S. natans* bacterial respiratory activity fraction (FR_{OUR}) as a function of contact time for different initial Cr(VI) concentrations (mg l^{-1}): (●) 5, (■) 20, (▲) 40 and (▼) 80 (initial biomass concentration $X_0 = 936 \pm 81$ mg VSS l^{-1}).

concentration. As can be observed, *S. natans* respiratory activity is strongly dependent on initial Cr(VI) concentration.

3.4. Mathematical modelling of Cr(VI) reduction using S. natans

3.4.1. Simplified model to assess the effect of Cr(VI) concentration on reduction rate

Cr(VI) reduction rate by *S. natans* in a medium containing glucose as energy source was modeled in a first stage by a simple first-order equation with respect to Cr(VI) as follows:

$$\frac{d\text{Cr(VI)}}{dt} = -k'\text{Cr(VI)} \quad (3)$$

where k' is the apparent chromium(VI) reduction coefficient (h^{-1}).

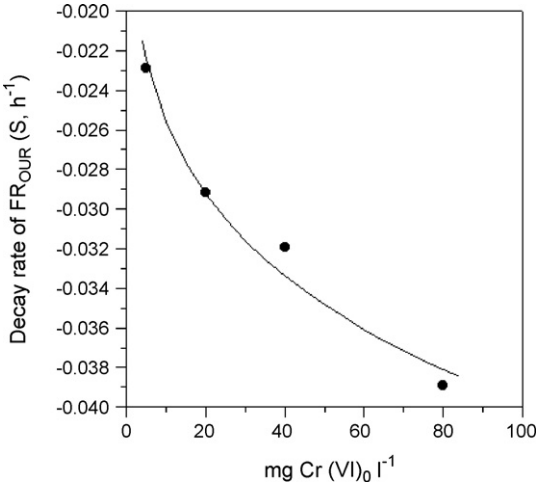

Fig. 3. Decay rate (S, h^{-1}) of *S. natans* respiratory activity fraction (FR_{OUR}) during the first 24 h contact time with different initial Cr(VI) concentrations (original data of FR_{OUR} vs. time are shown in Fig. 2).

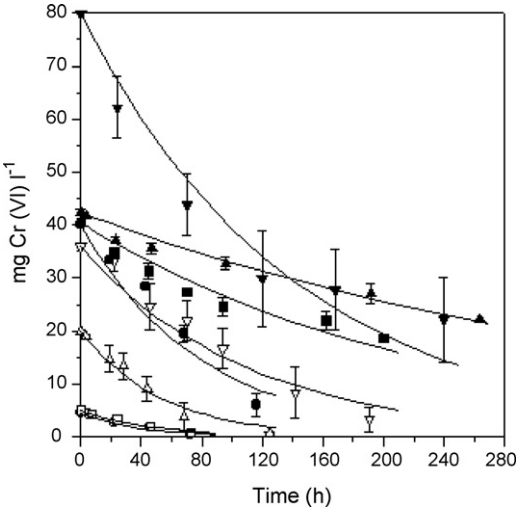

Fig. 4. Experimental data and modeling of Cr(VI) reduction by *S. natans* for different initial Cr(VI) and biomass concentrations (X_0, g VSS l^{-1}): (◯) 4.5 mg Cr(VI) l^{-1} ($X_0 = 1.596$); (□) 5 mg Cr(VI) l^{-1} ($X_0 = 1.008$); (△) 20 mg Cr(VI) l^{-1} ($X_0 = 1.033$); (▽) 36 mg Cr(VI) l^{-1} ($X_0 = 0.877$); (●) 40 mg Cr(VI) l^{-1} ($X_0 = 1.559$); (■) 40 mg Cr(VI) l^{-1} ($X_0 = 0.965$); (▲) 42.5 mg Cr(VI) l^{-1} ($X_0 = 0.691$); (▼) 80 mg Cr(VI) l^{-1} ($X_0 = 2.091$). (—) predictions by Eq. (4). The standard deviations are indicated by error bars.

By integrating Eq. (3), the following was obtained:

$$\ln\frac{\text{Cr(VI)}}{\text{Cr(VI)}_0} = -k't \quad (4)$$

where Cr(VI)_0 is the concentration of hexavalent chromium at $t = 0$ and Cr(VI) the concentration at any time (t).

Eq. (4) satisfactorily fitted the experimental data of Cr(VI) as a function of time for the tests comprising initial Cr(VI) concentrations ranging between 4.5 and 80 mg l^{-1} and initial biomass concentrations (X_0) between 0.690 and 2.090 g VSS l^{-1} (Fig. 4). Table 2 shows the fitted parameters obtained during the study of the kinetics of hexavalent chromium decay by the action of *S. natans*. Each experimental point was the average of duplicates, measured in two runs carried out under the same conditions of Cr(VI)_0 and X_0.

3.4.2. Mathematical model including the effect of the biological activity of the biomass

As can be observed in Table 2, k' is not a kinetic constant because it depends on the biomass concentration. Then, a more detailed equation was proposed to model experimental results of Cr(VI) reduction including the effect of the biomass. The following dependence was proposed in terms of the biomass with biological activity:

$$\frac{d\text{Cr(VI)}}{dt} = -k\text{Cr(VI)}[X_{(\text{active})}]^n \quad (5)$$

where k is the coefficient ((g VSS)$^{-n}$ l^n h^{-1}), $X_{(\text{active})}$ is the biomass concentration with biological activity (g VSS l^{-1}) and n is an empirical coefficient.

Comparing Eq. (3) with Eq. (5) results:

$$k' = k[X_{(\text{active})}]^n \quad (6)$$

Table 2
S. natans apparent chromium (VI) reduction coefficient (k', h^{-1}) obtained by fitting Eq. (4) to the experimental data

$Cr(VI)_0$ (mg l^{-1})	X_0 (g VSS l^{-1})	D_0 (mg $Cr(VI)_0$ (g $VSS_0)^{-1}$)	k' (h^{-1})
4.5	1.596	2.81	0.02521 (0.00329)[a]
5	1.008	4.96	0.02124 (0.00157)
20	1.033	19.35	0.01894 (0.00158)
36	0.877	41.05	0.00926 (0.00094)
40	1.559	25.65	0.01276 (0.00200)
40	0.965	41.93	0.00445 (0.00034)
42.5	0.691	61.33	0.00254 (0.00017)
80	2.091	38.25	0.00711 (0.00068)

X_0 = initial biomass concentration; D_0 = ratio between initial Cr(VI) concentration to that of biomass. k' = apparent chromium(VI) reduction coefficient.
[a] Standard deviation between parenthesis.

As was previously indicated, an increase of initial Cr(VI) concentration produces a decrease in the respiratory activity of *S. natans* and in its metabolic activity. Thus, the biomass concentration with biological activity ($X_{(active)}$) depends on the initial Cr(VI) concentration.

The following phenomenological expression was proposed to represent the effect of initial Cr(VI) concentration on the biomass concentration with biological activity:

$$X_{(active)} = X_0 f(Cr(VI)_0) \tag{7}$$

where X_0 is the initial biomass concentration and f is the function of the initial Cr(VI) concentration ($Cr(VI)_0$). Then, the active biomass is proportional to the initial biomass concentration affected by a function that depends on $Cr(VI)_0$ concentration because high concentrations of Cr(VI) has a toxic effect on the biomass.

A simple relationship was considered as a first approach for the function f:

$$f(Cr(VI)_0) = \frac{a}{Cr(VI)_0} \tag{8}$$

where a is the coefficient (mg $Cr(VI)_0$ l^{-1}).

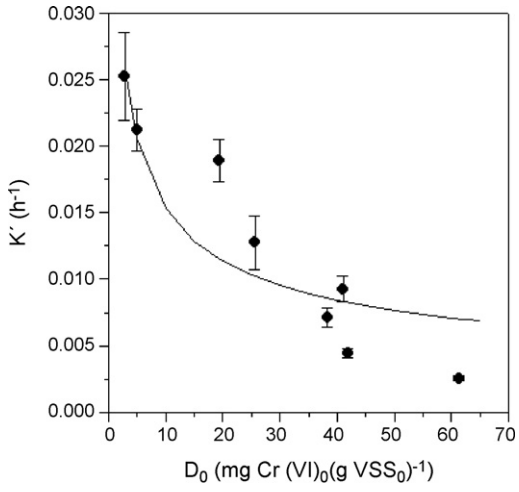

Fig. 5. Effect of D_0 (ratio between initial Cr(VI) concentration to that of biomass, mg $Cr(VI)_0$ (g $VSS_0)^{-1}$) on k' (apparent chromium(VI) reduction coefficient, h^{-1}). (●) experimental data. (—) Eq. (12). The standard deviations are indicated by error bars.

then

$$X_{(active)} = \frac{X_0 a}{Cr(VI)_0} \tag{9}$$

Eq. (9) indicates that high initial Cr(VI) concentrations decreases the biological activity of the biomass.

Considering that $Cr(VI)_0/X_0$ corresponds to D_0 previously defined as the ratio between initial Cr(VI) concentration and that of biomass, Eq. (9) can be expressed as follows:

$$X_{(active)} = \frac{a}{D_0} \tag{10}$$

By replacing Eq. (10) in Eq. (5) results:

$$\frac{dCr(VI)}{dt} = -kCr(VI)\frac{a^n}{D_0^n} \tag{11}$$

By comparing Eq. (11) with Eq. (3) the following is obtained:

$$k' = \frac{ka^n}{D_0^n} = \frac{b}{D_0^n} \tag{12}$$

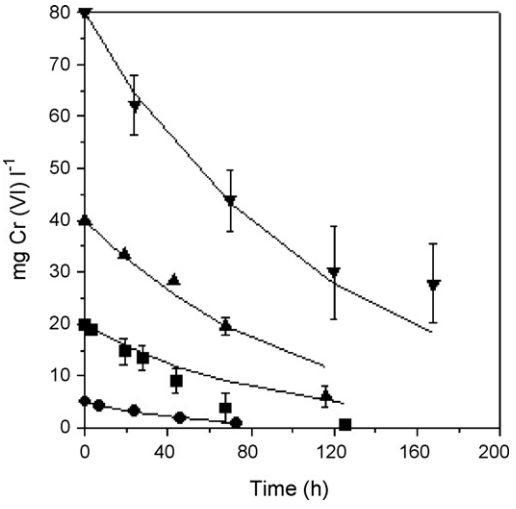

Fig. 6. Experimental data and modeling of Cr(VI) reduction by *S. natans* for different initial Cr(VI) concentration and ratio between initial Cr(VI) concentration and that of biomass (D_0, mg $Cr(VI)_0$ (g $VSS_0)^{-1}$): (●) 5 mg Cr(VI) l^{-1} (D_0 = 4.96); (■) 20 mg Cr(VI) l^{-1} (D_0 = 19.35); (▲) 40 mg Cr(VI) l^{-1} (D_0 = 25.65); (▼) 80 mg Cr(VI) l^{-1} (D_0 = 38.25). (—) predictions by Eq. (13). The standard deviations are indicated by error bars.

where b is the chromium(VI) reduction coefficient ((mg $Cr(VI)_0)^n$ (g $VSS_0)^{-n}$ h^{-1}). Values of k' shown in Table 2 were plotted as a function of D_0. The apparent chromium(VI) reduction coefficient k' decreased while increasing D_0. This evident relationship constitutes a novel finding on Cr(VI) biological reduction process.

Coefficients $b = 0.0412$ (mg $Cr(VI)_0)^n$ (g $VSS_0)^{-n}$ h^{-1} (S.D. = 0.00858 (mg $Cr(VI)_0)^n$ (g $VSS_0)^{-n}$ h^{-1}) and $n = 0.423$ (S.D. = 0.0939) of Eq. (12) were determined by non linear regression analysis using the software Sigma Plot 2.0 (Fig. 5, $p < 0.05$).

The following integrated first-order kinetic equation with respect to Cr(VI) was obtained by combining Eqs. (11) and (12):

$$\ln\frac{Cr(VI)}{Cr(VI)_0} = -\frac{b}{D_0^n}t \quad (13)$$

Eq. (13) allows to predict Cr(VI) concentration as a function of time and the ratio between initial Cr(VI) concentration and that of biomass; results are shown in Fig. 6. This equation allowed to model the biological reduction of Cr(VI) considering also the influence of biomass concentration and the effects of initial Cr(VI) concentration on *S. natans* biological activity.

4. Conclusions

The present work demonstrates that *S. natans*, a filamentous bacterium from activated sludge systems, was able to efficiently reduce hexavalent chromium to trivalent chromium from dichromate solutions with concentrations ranging between 4.5 and 80 mg Cr(VI) l^{-1} using a carbonaceous source under aerobic conditions. The concentration of soluble Cr(VI) and precipitated and soluble Cr(III) were determined along the experiments.

Although it is well known that *S. natans* removes heavy metal cations by biosorption, the ability of this micro-organism to reduce Cr(VI) by means of a biological process requiring a source of energy is a novel finding.

Previous contact of *S. natans* with Cr(VI) was not required to induce the biological reduction process. In comparison to other micro-organisms, nutrient-rich media was not necessary to achieve a good performance. Besides biomass concentration of *S. natans* remained approximately constant during all the Cr(VI) reduction experiments, diminishing the problems of biomass final disposition.

Chromium(VI) reduction rate by *S. natans* depended on both initial Cr(VI) concentration and biomass concentration. The ratio between the initial concentration of Cr(VI) to that of biomass constitutes an important parameter that determines the apparent chromium(VI) reduction coefficient for this micro-organism. Biomass concentration is a relevant parameter that must be considered in kinetic studies of Cr(VI) biological reduction. A kinetic equation that considers the effect of active biomass concentration and the effects of initial Cr(VI) concentration on biological activity was developed; experimental results were satisfactorily modeled by the proposed equations.

S. natans can be used as a promising micro-organism for Cr(VI) reduction from dichromate solutions considering its excellent reduction activity as well as the actual possibility of using wastes from activated sludge systems, where *S. natans* is a typical micro-organism, for detoxification of metal-contaminated industrial effluents.

The distinctive capacity to reduce Cr(VI) to Cr(III) becomes *S. natans* a potential micro-organism to decontaminate wastewaters from tanneries as well as it can be exploited in bioremediation strategies.

Acknowledgements

The authors gratefully acknowledge the financial support given by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de La Plata, and Agencia Nacional de Promoción Científica y Tecnológica, ARGENTINA.

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