Journal of Hazardous Materials 176 (2010) 657–665

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Cr(Vi) reduction capacity of activated sludge as affected by nitrogen and carbon sources, microbial acclimation and cell multiplication

A.M. Ferro Orozco [a,*], E.M. Contreras [a,b], N.E. Zaritzky [a,b]

[a] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) CCT La Plata CONICET – Fac. de Cs. Exactas, UNLP. 47 y 116 (B1900AJJ) La Plata, Argentina
[b] Fac. de Ingeniería, UNLP. 47 y 1 (B1900AJJ) – La Plata, Argentina

ARTICLE INFO

Article history:
Received 16 July 2009
Received in revised form 9 November 2009
Accepted 15 November 2009
Available online 8 December 2009

Keywords:
Hexavalent chromium
Activated sludge
Biodetoxification
Biomass acclimation
Electron donors

ABSTRACT

The objectives of the present work were: (i) to analyze the capacity of activated sludge to reduce hexavalent chromium using different carbon sources as electron donors in batch reactors, (ii) to determine the relationship between biomass growth and the amount of Cr(VI) reduced considering the effect of the nitrogen to carbon source ratio, and (iii) to determine the effect of the Cr(VI) acclimation stage on the performance of the biological chromium reduction assessing the stability of the Cr(VI) reduction capacity of the activated sludge.

The highest specific Cr(VI) removal rate (q_{Cr}) was attained with cheese whey or lactose as electron donors decreasing in the following order: cheese whey ≈ lactose > glucose > citrate > acetate. Batch assays with different nitrogen to carbon source ratio demonstrated that biological Cr(VI) reduction is associated to the cell multiplication phase; as a result, maximum Cr(VI) removal rates occur when there is no substrate limitation. The biomass can be acclimated to the presence of Cr(VI) and generate new cells that maintain the ability to reduce chromate. Therefore, the activated sludge process could be applied to a continuous Cr(VI) removal process.

1. Introduction

Chromium is a transition metal often used in several industrial processes such as petroleum refining, metal finishing industries, leather tanning, iron and steel industries, inorganic chemical production, textile manufacturing and pulp production [1]. The hexavalent form of this metal, Cr(VI), has high water solubility, it is the most toxic among chromium species, and it is a known carcinogen. Besides, trivalent chromium, Cr(III), is less soluble in water, and it is an essential dietary element [2]. Therefore, reducing Cr(VI) to Cr(III) is beneficial in eliminating the toxicity of Cr(VI) of wastewaters.

For many years, conventional Cr(VI) removal was mainly achieved by chemical reduction [3,4], ion exchange or adsorption [5,6]. Recently, researchers have focused attention on biodetoxification of hexavalent chromium. In contrast to the conventional methods, biodetoxification is cost-effective [7]. A great number of bacterial genera were described as capable of reducing Cr(VI) to Cr(III) including *Escherichia* [8], *Pseudomonas* [9], *Bacillus* [10], *Shewanella* [11], *Serratia* [12], *Rhodobacter* [13] and *Arthrobacter* [14]. Besides, the bio-reduction of Cr(VI) to Cr(III) by using mixed cultures was also reported [15–21].

The activated sludge technology has been widely applied to treat municipal and some industrial wastewaters since its operation is simple and convenient [18,19]. In contrast to the pure cultures, the activated sludge biomass is easy to acclimate to different environments, it does not need to be manipulated under rigorous conditions, and the wastewater does not need to be sterilized before treatment [18,19]. Another important characteristic of the activate sludge microorganisms is the ability to flocculate. The flocculated aggregates exhibit technologically acceptable sedimentations rates. Gravity sedimentation is the only economically feasible way of separating biomass from treated wastewater in full-scale treatment plants [22]. Concerning Cr(VI)-containing wastewaters, within the pH range that activated sludge systems are usually operated, the Cr(III) produced during the biological reduction of Cr(VI) could generate insoluble chromium hydroxides [2]. These hydroxides could precipitate in the secondary clarifier along with the biomass; therefore, chromium could be easily removed from wastewaters when solids are purged from the reactor. For these reasons, the removal of Cr(VI) using an activated sludge system can be a suitable technological alternative.

In order to optimize the design and operation of the biological Cr(VI) reduction process in activated sludge reactors, a thorough understanding of the characteristics of microbial transformation of Cr(VI) is needed. Many factors, such as the presence of aerobic or anaerobic conditions [23], availability of energy sources such as sulfur [24], initial hexavalent chromium concentration [9] among

* Corresponding author. Tel.: +54 221 4254853; fax: +54 221 4254853.
E-mail address: mferro@cidca.org.ar (A.M.F. Orozco).

0304-3894/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2009.11.082

Nomenclature

Cr	exavalent chromium concentration ($mgCr\,L^{-1}$)
Cr_0	initial hexavalent chromium concentration ($mgCr\,L^{-1}$)
$F_{e-/e-}$	percentage of the organic substrate electrons that reduced Cr(VI) to Cr(III) (%)
i_B	nitrogen content of the biomass ($mgN\,gTSS^{-1}$)
N	nitrogen source concentration ($mgN\,L^{-1}$)
N_0	initial nitrogen source concentration ($mgN\,L^{-1}$)
N_0:S_0	initial nitrogen to carbon source ratio ($mgN\,gCOD^{-1}$)
$(N_0{:}S_0)_{CS}$	critical stoichiometric nitrogen to carbon source ratio ($mgN\,gCOD^{-1}$)
q_{Cr}	specific Cr(VI) removal rate ($mgCr(VI)\,gTSS^{-1}\,h^{-1}$)
q_S	specific organic substrate consumption rate ($gCOD\,gTSS^{-1}\,h^{-1}$)
S	organic substrate concentration ($mgCOD\,L^{-1}$)
S_0	initial organic substrate concentration ($mgCOD\,L^{-1}$)
X	biomass concentration ($mgTSS\,L^{-1}$)
X_0	initial biomass concentration ($mgTSS\,L^{-1}$)
$Y_{Cr/S}$	Cr(VI) removed per unit of organic substrate consumed ($mgCr(VI)\,gCOD^{-1}$)
$Y_{Cr/X}$	Cr(VI) removed per unit of biomass produced ($mgCr(VI)\,gTSS^{-1}$)
$Y_{X/S}$	biomass growth yield ($gTSS\,gCOD^{-1}$)
ΔCr	hexavalent chromium consumption ($mgCr\,L^{-1}$)
ΔS	organic substrate consumption ($mgCOD\,L^{-1}$)
ΔX	biomass production ($mgTSS\,L^{-1}$)
μ	specific growth rate (h^{-1})

others, affect the microbial Cr(VI) reduction. Taking into account the nutritional requirements of the microorganisms, in a previous work it was demonstrated that the presence of a suitable carbon source is necessary to enhance the Cr(VI) reduction capacity of activated sludge [25]. In addition, other researchers have reported that the Cr(VI) reducing activity of the microbial cells may vary in the presence of different carbon sources [26–28]. Therefore, choosing an appropriate electron donor is an important factor to be considered when a biological Cr(VI) removal process is used. With regard to Cr(VI)-containing wastewaters with low organic matter content, such as electroplating, pigmentation, and wood preservation [2], the carbon source has to be supplied externally; in those cases, the addition of cheese whey (a residue from dairy industries) could be a suitable alternative due to its low cost. Besides, presence of high organic matter along with Cr(VI) can also occur when wastewaters from more than one industry are mixed [21,29,30]; in this case, the availability of electron donors depends on the origin of the wastewater. Although the importance of the carbon source in biological Cr(VI) removal process has been assessed using pure cultures, the knowledge about the requirements of other nutrients related to cell multiplication, such as the availability of a nitrogen source, is scarce.

Most of the laboratory scale biological systems used for the treatment of Cr(VI) containing wastewater are operated in batch mode, due to the eventual loss of active biomass as a result of metal toxicity [9]. Several authors [8,23,27] reported that the rate and extent of Cr(VI) reduction in batch cultures depend on the initial biomass concentration regardless of the subsequent growth. These authors postulated that the new cells generated in the presence of hexavalent chromium lose the chromate reduction capacity due to the mutagenic effects of chromium. The new mutant cells could generate a decrease in the Cr(VI) transport inside the cells as a resistance mechanism [8,23,27]. As a result, the Cr(VI) reduction capacity of the mutant cells would decrease. In this context, the capability of the biomass to reduce Cr(VI) is not stable. This implies that it would not be possible to continuously remove Cr(VI) on a long-term basis without intermittently reseeding the biological system. However, there are recent reports concerning stable biological Cr(VI) reduction in continuous systems which could contradict this theory [21,31,32].

The main objectives of the present work were: (i) to analyze the capacity of activated sludge to reduce hexavalent chromium using different carbon sources as electron donors in batch reactors, (ii) to determine the relationship between biomass growth and the amount of Cr(VI) reduced considering the effect of the nitrogen to carbon source ratio, and (iii) to determine the effect of the Cr(VI) acclimation stage on the performance of the biological chromium reduction assessing the stability of the Cr(VI) reduction capacity of the activated sludge.

2. Materials and methods

2.1. Biological and chemical materials

All reagents used in the present work were commercial products of reagent grade from Anedra (San Fernando, Argentina).

Activated sludges used in all the experiments were harvested from an aerobic laboratory-scale (4.5 L) activated sludge reactor with partial biomass recycle. The reactor was fed with a synthetic wastewater with the following composition: dehydrated cheese whey 1.5 g, $(NH_4)_2SO_4$ 0.94 g, and $NaHCO_3$ 1.03 g dissolved in 1 L of tap water. Soluble chemical oxygen demand (COD_S) of the synthetic wastewater was $1500\,mg\,L^{-1}$. The hydraulic retention time was 2 d; the sludge age was maintained at 45 d by daily wasting of the mixed liquor directly from the reactor. During the experiments the temperature of the reactor was $20 \pm 2\,^{\circ}C$. Under steady-state conditions dissolved oxygen concentration (DO) was above $4\,mg\,L^{-1}$, pH was 7.5 ± 0.4, COD_S of the effluent ranged between 30 and $80\,mg\,L^{-1}$, and total suspended solid (TSS) concentration ranged from 3000 to $4000\,mgTSS\,L^{-1}$.

2.2. Cr(VI) removal experiments by activated sludges

2.2.1. Effect of the type of the carbon source

In order to study the effect of the type of the carbon source on Cr(VI) removal by activated sludge, batch assays were performed in 250 mL aerated vessels. Five organic substrates, cheese whey, lactose, glucose, acetate, and citrate were tested as electron donors for Cr(VI) reduction. As it was mentioned previously, wastewater from different sources can be mixed prior to the treatment; therefore, it would not be unusual to find cheese whey, lactose, or glucose (dairy industry wastewaters) [33,34], acetate (municipal wastewaters) [35] or citrate (food processing wastewaters) [36] along with hexavalent chromium in the mixed wastewater.

All Cr(VI) removal assays were performed at constant temperature ($20 \pm 2\,^{\circ}C$) and pH 7.0 ± 0.1; this pH was selected because it is the optimum value for the metabolic activity of most of the microorganisms that are present in a typical activated sludge [22]. pH was measured continuously using a polymer body pH probe (Broadley-James Corp.) connected to an on/off pH controller (Masstek, Argentina). Sulfuric acid or sodium hydroxide (1N) was used to maintain pH of the cultures close to the desired set-point. Small air pumps were employed to aerate and to agitate the vessels. Air was pumped near the bottom of each vessel via a flexible hose. Aeration was sufficient to maintain the oxygen concentration above $4\,mgO_2\,L^{-1}$. Inoculum was obtained from the activated sludge reactor previously described (Section 2.1); biomass was washed three times with phosphate buffer (KH_2PO_4 $2\,g\,L^{-1}$, K_2HPO_4 $0.5\,g\,L^{-1}$, pH

7) before performing the assays. In all the experiments the initial biomass concentration was 3000 ± 200 mgTSS L^{-1}. The medium composition was the following: organic substrate 5 gCOD L^{-1}, ammonium sulfate 212 mgN L^{-1} (nitrogen source), and micronutrient solutions M1 and M2 (1 mL L^{-1}). The composition of M1 was (expressed as g/100 mL): $FeSO_4 \cdot 7H_2O$ 1.5, $ZnSO_4 \cdot 7H_2O$ 0.5, $MnSO_4 \cdot H_2O$ 0.3, $CuSO_4 \cdot 5H_2O$ 0.075, $CoCl_2 \cdot 6H_2O$ 0.015, and citric acid 0.6. M2 solution contained the following (g/100 mL): $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.05, BO_3H_3 0.01, KI 0.01.

A Cr(VI) stock solution (10 gCr(VI) L^{-1}) was prepared using analytical grade $K_2Cr_2O_7$; in all the experiments an appropriate volume of this solution was added to obtain 25 mgCr(VI) L^{-1}. At predetermined time intervals samples were taken to determine biomass (X), organic substrate (S), total ammonia nitrogen (TAN), and Cr(VI) concentrations. The Cr(VI) removal rates were obtained from the slope of Cr(VI) as a function of time; then, from the ratio between the Cr(VI) removal rate and biomass concentration, the specific Cr(VI) removal rate (q_{Cr}) was obtained. In addition, the amount of Cr(VI) removed per unit of organic substrate consumed ($Y_{Cr/S}$) was calculated from the slope of Cr(VI) as a function of S. Organic substrate and total ammonia nitrogen were measured to verify the presence of both substrates during the determination of Cr(VI) removal rates. For each tested substrate, abiotic control experiments (without biomass) were also performed.

2.2.2. Effect of the nitrogen to carbon source ratio on Cr(VI) removal by activated sludge

Nitrogen is one of the main constituents of the biomass and several studies showed that the nitrogen content for most activated sludges is a constant [33]. For this reason, by modifying the initial nitrogen source concentration in a batch assay, the amount of biomass produced can be controlled; this strategy was used in the present work.

Cr(VI) removal batch assays similar to those described in the previous section were performed. Tested nitrogen source (ammonium sulfate) concentrations ranged from 0 to 250 mgN L^{-1}. Lactose (5 g L^{-1}) was present in all the tests as carbon source. In all cases the initial biomass concentration was 700 ± 50 mgTSS L^{-1}. The objective of these experiments was to study the relationship between biomass growth and the amount of Cr(VI) removed, therefore, a low initial biomass concentration allowed us to measure the increase of biomass without significant errors. At predetermined time intervals samples were taken to measure biomass (X), organic substrate (S), total ammonia nitrogen (TAN), and Cr(VI) concentrations. For each batch assay, the following coefficients that characterize activated sludge growth and Cr(VI) removal kinetics were calculated: specific growth rate (μ, h^{-1}), biomass growth yield ($Y_{X/S}$, gTSS gCOD^{-1}), Cr(VI) removed per unit of biomass produced ($Y_{Cr/X}$, mgCr(VI) gTSS^{-1}), specific organic substrate consumption rate (q_S, gCOD gTSS^{-1} h^{-1}), specific Cr(VI) removal rate (q_{Cr}, mgCr(VI) gTSS^{-1} h^{-1}), and Cr(VI) removed per unit of organic substrate consumed ($Y_{Cr/S}$, mgCr(VI) gCOD^{-1}). In all cases, only data corresponding to the exponential growth phase (e.g. without limitation on nitrogen or carbon sources) were considered for calculations. The observed specific growth rate (μ) was calculated from the slope of the linear part of the plot of ln(X) as a function of time. The biomass growth yield was obtained from the slope of X as a function of S, and the amount of Cr(VI) removed per unit of biomass produced was calculated from the slope of Cr(VI) as a function of X. Then, coefficients q_S, q_{Cr}, and $Y_{Cr/S}$ were calculated as follows:

$$q_S = \frac{\mu}{Y_{X/S}} \quad (1)$$

$$q_{Cr} = \mu Y_{Cr/X} \quad (2)$$

$$Y_{Cr/S} = Y_{X/S} Y_{Cr/X} \infty \quad (3)$$

2.2.3. Experimental estimation of stoichiometric coefficients

Experimental stoichiometric coefficients such as $Y_{X/S}$, $Y_{Cr/S}$ and $Y_{Cr/X}$, generically described by $Y_{M/N}$, were estimated as the slope of the linear part of the plot of M as a function N, as was mentioned in the previous section. For example, the amount of Cr(VI) removed per unit of organic substrate consumed ($Y_{Cr/S}$) was calculated from the slope of Cr(VI) as a function of S. This calculation is based on the following considerations. The instantaneous $Y_{Cr/S}$ value can be defined as the ratio between Cr(VI) and organic substrate consumption rates [33]:

$$Y_{Cr/S} = \frac{dCr/dt}{dS/dt} = \frac{dCr}{dS} \quad (4)$$

where Cr and S are the Cr(VI) and organic substrate concentrations respectively. If during a batch test, $Y_{Cr/S}$ is constant within a certain time interval, then Eq. (4) can be integrated yielding the following expression:

$$(Cr_0 - Cr) = Y_{Cr/S}(S_0 - S) \quad (5)$$

where Cr_0 and S_0 are the initial Cr(VI) and organic substrate concentrations respectively, and Cr and S the instantaneous values at time t. The expressions between parentheses in Eq. (5) correspond to Cr(VI) and organic substrate consumptions (ΔCr, ΔS) at time t, respectively. Eq. (5) can be reordered to obtain the following expression:

$$Cr = Cr_0 - Y_{Cr/S} S_0 + Y_{Cr/S} S \quad (6)$$

Thus, the linear part of the plot of Cr as a function of S corresponds to the amount of Cr(VI) removed per unit of organic substrate consumed ($Y_{Cr/S}$). A similar procedure was used for the determination of the other stoichiometric coefficients, $Y_{Cr/X}$ and $Y_{X/S}$.

2.2.4. Acclimation of the biomass and stability of the Cr(VI) reduction capacity

In order to evaluate the acclimation of the biomass to the presence of hexavalent chromium and the stability of the Cr(VI) reduction capacity of the microbial community, experiments using five consecutive Cr(VI) removal batch reactors were performed. In these experiments the inoculum of each batch reactor was obtained from the previous one. Before starting each assay, the biomass obtained from the previous batch was harvested and washed with phosphate buffer until all Cr(VI), lactose and ammonia remnant from the previous batch were eliminated. Then, the washed biomass was diluted with fresh culture medium to serve as inoculum for the next Cr(VI) removal batch test. Each consecutive batch reactor was fed with fresh medium at the beginning; therefore, initial conditions were the same for all the consecutive Cr(VI) removal assays, except the nature of the biomass. The initial concentrations for each batch experiment were the following: 25 mgCr(VI) L^{-1}, 7.5 g lactose L^{-1}, 212 mgN L^{-1} (ammonium sulfate), and 600 ± 50 mgTSS L^{-1}. At different times, samples were taken to determine biomass (X), organic substrate (S), total ammonia nitrogen (TAN), and Cr(VI) concentrations. For each batch assay coefficients μ, $Y_{X/S}$, $Y_{Cr/X}$, q_S, q_{Cr}, and $Y_{Cr/S}$ were calculated as was previously described.

2.3. Analytical techniques

Total suspended solids (TSS) were used to measure biomass concentration. Known sample volumes (8 mL in this work) were poured into pre-weighted centrifuge tubes, centrifuged and washed twice with distilled water, and placed at 105 °C for 24 h; TSS of each sample was calculated as the difference between final weight (dry

sample + tube) and initial weight (tube alone) divided by the sample volume. Organic substrate concentration was determined as chemical oxygen demand (COD). Soluble COD (CODs) was determined as follows: 3 mL of culture samples were centrifuged for 5 min at 13000 rpm (Eppendorf 5415C); then, the supernatant was filtered through 0.45 μm cellulosic membranes (Osmonics Inc.). Finally, soluble COD of the filtrate was determined using commercial reagents (Hach Company, Loveland, CO). Total ammonia nitrogen (TAN) concentration of the filtrate was measured by the Nessler colorimetric method using commercial reagents (Hach Company, Loveland, CO). Cr(VI) of the filtrate was determined colorimetrically using a spectrophotometer (Hach DR 2000) at 540 nm by reaction with 1,5-diphenylcarbazide in acid solution [37].

All the results reported in the present work are averages of at least two experiments.

3. Results and discussion

3.1. Effect of the type of electron donors on the removal of Cr(VI) by activated sludge

Hexavalent chromium removal batch assays were performed to study the effect of the type of the carbon source as electron donor on Cr(VI) removal by activated sludge. Abiotic control experiments demonstrated that the removal of Cr(VI) in the absence of biomass was negligible within the tested time interval. However, chromium reduction in the presence of activated sludge was observed for all the tested electron donors, although the rate and extent of chromate reduction varied significantly comparing the five carbon sources analyzed. Fig. 1 shows the effect of the organic substrate on the specific Cr(VI) removal rate ($q_{Cr,}$ mgCr(VI) $gTSS^{-1}$ h^{-1}) and on the amount of Cr(VI) removed per unit of organic substrate consumed ($Y_{Cr/S}$, mgCr(VI) $gCOD^{-1}$). Concerning q_{Cr}, Fig. 1 shows that the performance of the activated sludge to remove hexavalent chromium with the five tested electron donors decreased as follows: cheese whey ≈ lactose > glucose > citrate > acetate. In general, high q_{Cr} values were obtained when fermentable substrates, such as cheese whey, lactose, and glucose, were tested; on the contrary, relatively low specific Cr(VI) removal rates were obtained with non-fermentable substrates such as citrate and acetate. When Cr(VI) removal experiments were performed without substrate addition, the lowest specific Cr(VI) removal rates were obtained. These results indicate that the presence of suitable substrates that serve as electron donors enhanced Cr(VI) removal rates. Therefore, considering the need of adding a carbon source for the biological removal of hexavalent chromium, cheese whey could be an inexpensive choice, because it constitutes a residue from the dairy industries.

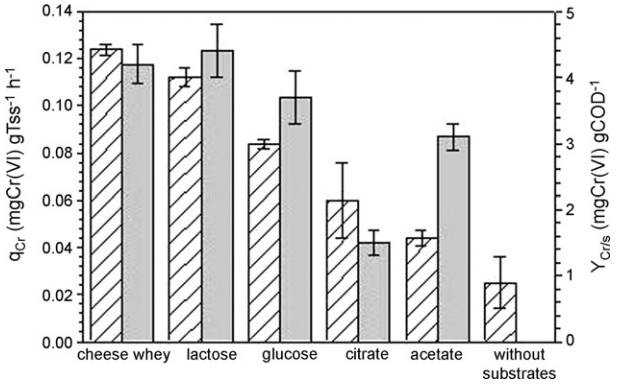

Fig. 1. Effect of the type of carbon source on the specific Cr(VI) removal rate (q_{Cr}) (dashed bars) and on the amount of Cr(VI) removed per unit of organic substrate consumed ($Y_{Cr/S}$) (solid bars).

Results shown in Fig. 1 demonstrate that the specific Cr(VI) removal rate (q_{Cr}) was strongly dependent on the type of organic substrate. Bae et al. [28] studied the ability of *Escherichia coli* ATCC 33456 to reduce Cr(VI) in batch cultures; those authors found that the order of preference of *E. coli* ATCC 33456 with respect to substrates was similar to the obtained in the present paper using activated sludges.

While q_{Cr} is a kinetic coefficient that evaluates the potential of the tested biomass to remove Cr(VI), $Y_{Cr/S}$ is a measure of the efficiency of a given substrate to provide electrons to reduce Cr(VI). Considering standard deviation, Fig. 1 shows that cheese whey, lactose, and glucose had similar performances to reduce Cr(VI); in other words, the fraction of available substrate electrons used to reduce Cr(VI) was similar for these substrates. The same results were observed when $Y_{Cr/S}$ values corresponding to glucose and acetate were compared. On the other hand, Fig. 1 also shows that the $Y_{Cr/S}$ value corresponding to citrate were slightly lower that those obtained with the four previously mentioned substrates. Obtained $Y_{Cr/S}$ values in the present work (Fig. 1) fall within the range reported by other authors. Bae et al. [28] reported $Y_{Cr/S}$ values ranging between 1.5 and 2.9 mgCr(VI) $gCOD^{-1}$ for *E. coli* ATCC 33456 grown in glycerol. Caravelli and Zaritzky [31] found an $Y_{Cr/S}$ value of about 1.0 mgCr(VI) $gCOD^{-1}$ corresponding to the removal of Cr(VI) by *Sphaerotilus natans* in continuous culture with citrate as carbon and energy source. From the results reported by Villegas et al. [38], $Y_{Cr/S}$ values corresponding to yeasts isolated from Cr(VI) contaminated sites ranged between 2 and 7 mgCr(VI) $gCOD^{-1}$.

3.1.1. Calculation of the fraction of the total transferable electrons of the substrate that were used to reduce Cr(VI)

Based on the values of $Y_{Cr/S}$, expressed as gCr $gCOD^{-1}$, the fraction of electrons from the substrate that reduced Cr(VI) to Cr(III) can be calculated using the electron equivalence concept and an electron balance [39]. If 32 gCOD substrate (1 mol of O_2) are consumed, then 4 mol of electrons must be transferred to biomass, molecular oxygen, and Cr(VI) for maintaining the electron balance. Considering that 3 mol of electrons are necessary to reduce 1 mol of Cr(VI) to Cr(III) (52 gCr), the percentage of electrons of the organic substrate that reduced Cr(VI) to Cr(III) (F_{e^-/e^-}) is:

$$F_{\frac{e^-}{e^-}}(\%) = 100 Y_{Cr/S} \frac{32\ \text{gCOD}}{4\ \text{mol}\ e^-} \frac{3\ \text{mole}\ e^-}{52\ \text{gCr}} \quad (7)$$

By applying Eq. (7) to data of $Y_{Cr/S}$ shown in Fig. 1 it was possible to obtain the percentage of electrons that were transferred to the hexavalent chromium. In all cases, the electron transfer efficiency from the tested substrates to Cr(VI) was very low. Calculations showed that in the best case obtained in the present work (lactose as carbon source), less than 0.2% of the total transferable electrons were used to reduce Cr(VI) to Cr(III); the other 99.8% was employed on biomass synthesis, and on energy production by reducing molecular oxygen. Therefore, in order to improve the biological reduction of Cr(VI), more research is needed concerning the factors affecting the electron transfer efficiency.

3.2. Relationship between biomass growth and Cr(VI) removal: effect of the nitrogen to carbon source ratio

Fig. 2 shows some examples of batch Cr(VI) removal assays performed in the presence of different initial total ammonia nitrogen (TAN) concentrations; in these cases tested TAN concentrations were 0 (without addition of a nitrogen source), 70, and 210 mgN L^{-1}. In the experiment with TAN = 0 mgN L^{-1} (triangles in Fig. 2), no significant changes of hexavalent chromium (Fig. 2a), TAN (Fig. 2b), soluble COD (Fig. 2c) or biomass (Fig. 2d) concen-

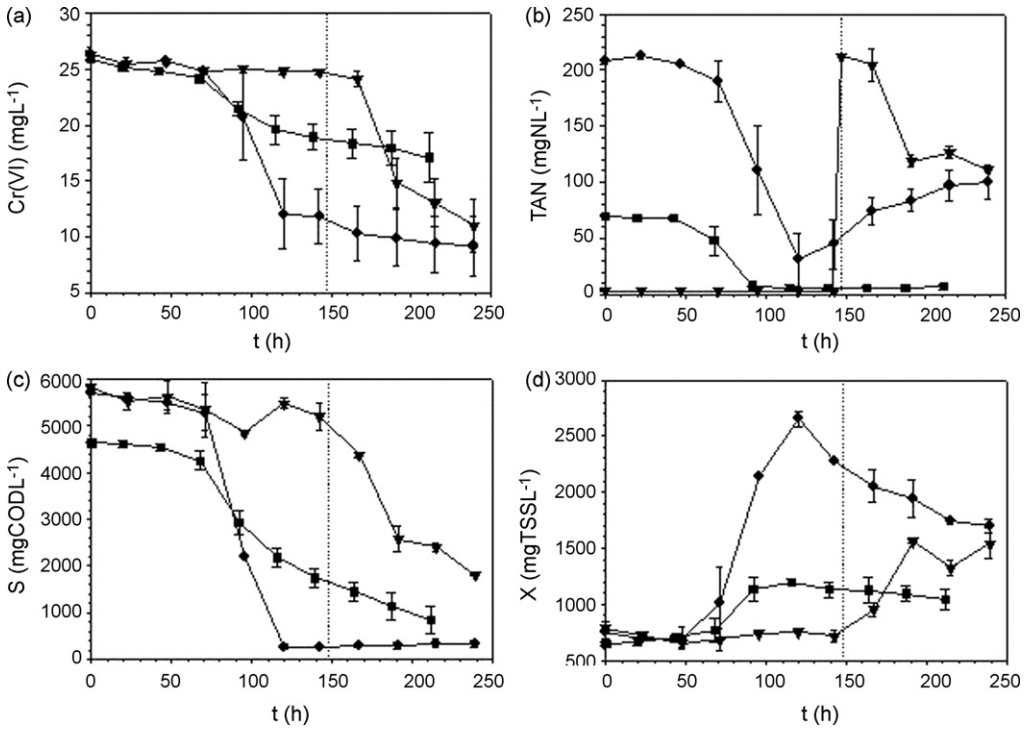

Fig. 2. Examples of batch Cr(VI) removal assays in the presence of different initial total ammonia nitrogen (TAN) concentrations: (▼) control experiment with no addition of nitrogen source, (■) 70 mgN L^{-1}, and (●) 210 mgN L^{-1}. Dotted lines indicate the time at which the control experiment was spiked with ammonium sulfate (220 mgN L^{-1}). Lactose (5 gCOD L^{-1}) was used as carbon source. Bars indicate the standard deviation.

trations were observed during the first 147 h. After this period, ammonium sulfate (220 mgN L^{-1}) was added to the culture (dotted lines in Fig. 2). Then, about 24 h after spiking with the nitrogen source, Cr(VI) reduction started, TAN and soluble COD concentrations decreased and biomass concentration increased as a function of time. These results indicate that the non-acclimated activated sludge used in this experiment could tolerate 25 mgCr(VI) L^{-1} during 170 h without a significant loss of metabolic activity or Cr(VI) reduction capacity. In addition, these results suggest that the Cr(VI) reduction could be associated with the cell multiplication stage.

Assays with addition of a nitrogen source from the beginning of the experiment exhibited a lag phase of 70 hours approximately; after that, removal of hexavalent chromium (Fig. 2a), consumptions of the nitrogen source (Fig. 2b) and of soluble COD (Fig. 2c) and biomass growth (Fig. 2d) started. Maximum Cr(VI) removal rates were observed during the exponential growth phase, where no carbon or nitrogen limitation occurs. In the case that the initial TAN concentration was 210 mgN L^{-1} (circles in Fig. 2), lactose was depleted at t = 120 h but TAN concentration was around 25 mgN L^{-1}; this case corresponded to a carbon limited batch assay. At this time, Cr(VI) removal rate decreased drastically and TAN concentration increased due to the biomass decay. When the initial TAN concentration was 70 mgN L^{-1} (squares in Fig. 2), nitrogen source was depleted at t = 95 h. At that time, the biomass growth stopped, but the carbon source concentration was around 3000 mgCOD L^{-1}. Hence, in this case, the biomass growth was limited by the nitrogen source. Because lactose was also the energy source, biomass concentration remained almost constant; however, Cr(VI) removal rate decreased markedly due to the depletion of the nitrogen source. These results confirmed that it is necessary the presence of both carbon and nitrogen sources to obtain high Cr(VI) removal rates. However, it must be pointed out that when one of both substrates is depleted, Cr(VI) removal continues but at a lower rate. Therefore, from the obtained results, it can be concluded that the observed biological Cr(VI) removal was the consequence of two processes: (1) a fast Cr(VI) removal process, associated to the biomass growth, and (2) a slow removal process that is independent of the presence of the carbon and nitrogen sources. Although the second process may be important under certain conditions, such as in continuous cultures in which substrate concentrations are normally low, this process is out of the scope of the present work.

For each batch assay performed in the presence of different initial total ammonia nitrogen (TAN) concentrations, biomass production (ΔX) and Cr(VI) consumption (ΔCr) at the end of the exponential growth phase were calculated. Fig. 3 shows that ΔX and ΔCr increased as a function of the ratio between initial nitrogen and carbon source concentrations (N_0:S_0), demonstrating the relationship between biomass growth and Cr(VI) removal process. In these cases, biomass growth was limited by the nitrogen source. When the tested N_0:S_0 ratios were higher than a certain critical N_0:S_0 ratio ranging between 28 and 38 mgN $gCOD^{-1}$, no further

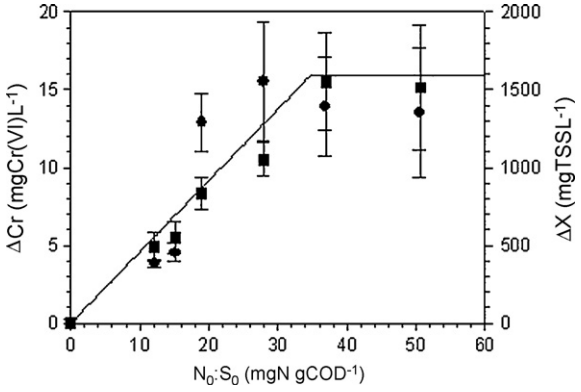

Fig. 3. Biomass production (ΔX, ■) and Cr(VI) consumption (ΔCr, ●) as a function of the initial nitrogen to carbon source ratio (N_0:S_0). Continuous line was calculated by means of Eq. (10a, 10b). Bars indicate the standard deviation.

increases of ΔX and ΔCr were obtained; in these cases, microbial growth and Cr(VI) removal were limited by the carbon source.

The obtained results demonstrate that biological Cr(VI) removal is associated to the cell multiplication phase, as a result, maximum Cr(VI) removal rates occur when there is no limitation in carbon or nitrogen sources. Therefore, for all the initial TAN tested concentrations, coefficients that characterize activated sludge growth and Cr(VI) removal kinetics during the exponential growth phase were calculated. Table 1 shows that the coefficients were almost independent of the initial TAN concentration, for this reason, overall mean values of the specific growth rate (μ), biomass growth yield ($Y_{X/S}$), and specific organic substrate consumption rate (q_S) were calculated. Coefficients shown in Table 1 are within the range reported by other authors. Stasinakis et al. [40] studied the growth kinetics of activated sludge using acetate as a carbon source; for non-acclimated biomass growing in the presence of 25 mgCr(VI) L^{-1}, those authors reported μ values ranging from 0.007 to 0.029 h^{-1}, and $Y_{X/S}$ values between 0.20 and 0.25 gTSS gCOD^{-1}, respectively. Yetis et al. [41] reported a biomass growth yield of 0.53 gTSS gCOD^{-1}, which was slightly higher than the obtained in the present work. However, it must be pointed out that those authors used a complex synthetic wastewater with protease-peptone as a carbon source; on the contrary, in the present work a basal medium with lactose was used. Table 1 shows that coefficients q_{Cr}, and $Y_{Cr/S}$ describing the Cr(VI) removal process were in accordance with those depicted in Fig. 1 corresponding to lactose as the carbon source.

3.3. Maximum Cr(VI) removal as a function of the initial nitrogen (N_o) and carbon source (S_o) concentrations

Stoichiometric coefficients $Y_{X/S}$ and $Y_{Cr/X}$ are useful to calculate the maximum Cr(VI) removal as a function of the initial nitrogen (N_0), and carbon (S_0) source concentrations used in a batch assay. Results obtained in the present work demonstrated that during the exponential growth phase Cr(VI) removal and biomass production are proportional, being $Y_{Cr/X}$ the proportionality coefficient. Moreover, when N or S was completely consumed, both biomass growth and Cr(VI) removal stopped. Thus, the Cr(VI) consumed at the end of the exponential growth phase (ΔCr) can be calculated as follows:

$$\Delta Cr = Y_{Cr/X}\Delta X \quad (8)$$

where ΔX is the biomass production at the end of the exponential growth phase. It must be remarked that ΔCr in Eq. (8) represents the amount of Cr(VI) that potentially could be removed if the Cr(VI) removal process is not limited by its own concentration. In the cases when $Cr_0 < \Delta Cr$, a Cr_F concentration close to zero could be achieved.

The biomass production at the end of the exponential growth phase (ΔX) depends on the initial growth limiting substrate concentration, which can be the nitrogen source (N_0) or the carbon source (S_0). The critical stoichiometric N_0:S_0 ratio, $(N_0{:}S_0)_{CS}$, can be defined as the initial N_0:S_0 ratio at which both N and S are depleted at the same time; in this case both N and S are limiting substrates simultaneously. If $N_0{:}S_0 > (N_0{:}S_0)_{CS}$ then N is in excess with respect to the stoichiometric requirements and S is the growth limiting substrate, therefore, ΔX is proportional to S_0. On the contrary, when $N_0{:}S_0 < (N_0{:}S_0)_{CS}$, the nitrogen source is the limiting substrate for biomass growth and ΔX results proportional to N_0. Taking into account the critical stoichiometric N_0:S_0 ratio, ΔX can be calculated as a function of the initial S and N concentrations as follows:

$$\Delta X = Y_{X/S}S_0 \quad if \quad N_0 : S_0 > (N_0 : S_0)_{CS} \quad (9a)$$

$$\Delta X = N_0/i_B \quad if \quad N_0 : S_0 < (N_0 : S_0)_{CS} \quad (9b)$$

where i_B (mgN gTSS^{-1}) corresponds to the nitrogen content of the biomass. Then, combining Eqs. (8) and (9a, 9b) the following is obtained:

$$\Delta Cr = Y_{Cr/X}Y_{X/S}S_0 \quad if \quad N_0 : S_0 > (N_0 : S_0)_{CS} \quad (10a)$$

$$\Delta Cr = (Y_{Cr/X}N_0)/i_B \quad if \quad N_0 : S_0 < (N_0 : S_0)_{CS} \quad (10b)$$

According to several authors [33,39], if assimilation (incorporation of the nitrogen source into biomass) is the only nitrogen consumption process (e.g. when nitrification can be neglected), the critical stoichiometric N_0:S_0 ratio can be calculated as the product between the nitrogen content of the biomass (i_B), and the biomass growth yield ($Y_{X/S}$). Considering the most accepted value for i_B (i_B = 120 mgN gTSS^{-1}), and the average experimental $Y_{X/S}$ value shown in Table 1 ($Y_{x/s}$ = 0.29 gTSS gCOD^{-1}), the critical stoichiometric N_0:S_0 ratio results 35 mgN gCOD^{-1}, which is within the range of the experimental critical N_0:S_0 ratio obtained in the present work. In addition, Fig. 3 shows that ΔCr calculated using Eq. (10a, 10b) is close to the experimental values within the range of tested initial N_0:S_0 ratios. Therefore, Eqs. (10a, 10b) could be useful for process design and operation of the Cr(VI) containing wastewater treatment using batch reactors.

3.4. Analysis of the biomass acclimation and stability of the Cr(VI) reduction capacity

In the previous section of the present study the relationship between biomass growth and Cr(VI) removal was demonstrated. However, as was mentioned, several authors [8,23,27] reported that the rate and extent of Cr(VI) reduction in batch cultures depends on the initial biomass concentration, regardless of subsequent growth. In this context, in the literature it was assumed that the new cells generated in the presence of Cr(VI) have lost the chromate reduction activity. In order to verify this hypothesis, Cr(VI) removal experiments using five consecutive batch reactors were performed (n = 5); in these experiments, the biomass generated in the previous reactor ($n-1$) was used as inoculum for the next one (n). Then, fresh culture medium was added to obtain the same initial conditions for all the consecutive assays. These experiments allowed studying the acclimation of the biomass and the stability of the chromate reduction capacity of the microbial community.

Fig. 4 shows that a decrease of hexavalent chromium concentration, biomass growth, organic substrate and ammonium consumption was observed in the five consecutive batch assays. In addition, all these processes started at about the same time demonstrating the strong relationship between Cr(VI) reduction and biomass growth. Results shown in Fig. 4 demonstrated that the new cells obtained at the end of each Cr(VI) removal batch assay had the same potential to remove Cr(VI) than the initial biomass that was harvested from the activated sludge reactor.

Based on the data shown in Fig. 4, coefficients that characterize activated sludge growth (μ, q_S, $Y_{X/S}$) and Cr(VI) removal (q_{Cr}, $Y_{Cr/X}$, $Y_{Cr/S}$) were calculated. Fig. 5 shows that the coefficients corresponding to the first batch were in agreement with those depicted in Fig. 1 and in Table 1. Then, a marked increase of the kinetic coefficients μ, q_S, and q_{Cr} corresponding to batch #2 was observed with respect to batch #1. In the subsequent reactors (#2 to #5), these coefficients remained constant. This effect was attributed to the acclimation of the biomass to the new environment; the inoculum of the first batch was obtained from the activated sludge reactor that was feed with a model wastewater containing cheese whey as a carbon source and without hexavalent chromium. However, lactose was used as the carbon source in the consecutive Cr(VI) removal batch assays; thus, differences between the first batch and the subsequent ones can be attributed to an acclimation effect.

Table 1
Kinetic and stoichiometric coefficients that characterize activated sludge growth and biological Cr(VI) removal as a function of the initial nitrogen source concentration (N_0).

						Mean value ± IC95%
N_0 (mgN L^{-1})	70	110	130	210	250	
S_0 (mgCOD L^{-1})	4700	5500	4700	5700	4900	
N_0:S_0 (mgN $gCOD^{-1}$)	14.9	20.0	27.7	36.8	51.0	
μ (h^{-1})	0.016	0.014	0.012	0.019	0.014	0.015 ± 0.003
$Y_{X/S}$ (gTSS $gCOD^{-1}$)	0.280	0.200	0.268	0.369	0.308	0.29 ± 0.08
$Y_{Cr/X}$ (mgCr $gTSS^{-1}$)	7	15	16	6	10	10.8 ± 5.6
q_S (gCOD $gTSS^{-1}$ h^{-1})	0.057	0.070	0.045	0.051	0.045	0.054 ± 0.013
q_{Cr} (mgCr $gTSS^{-1}$ h^{-1})	0.112	0.210	0.192	0.114	0.140	0.15 ± 0.06
$Y_{Cr/S}$ (mgCr $gCOD^{-1}$)	2.0	3.0	4.3	2.2	3.1	2.9 ± 1.1

In all cases $Cr(VI)_0$ = 25 mgCr L^{-1}, X_0 = 700 ± 50 mgTSS L^{-1}. All coefficients were calculated during the exponential growth phase.

Literature data show that the specific Cr(VI) removal rate (q_{Cr}) is a function of the bacterial genera tested, and on the acclimation degree of the studied biomass to Cr(VI). In general, pure cultures have higher q_{Cr} values than mixed cultures (e.g. activated sludges); likewise, acclimated cultures exhibit higher q_{Cr} values than non-acclimated ones [8,28,42]. Because in the present study a non-acclimated mixed culture (activated sludge) was used to remove Cr(VI), q_{Cr} value corresponding to the first batch (q_{Cr} = 0.10 ± 0.06 mgCr $gTSS^{-1}$ h^{-1}) was lower than the obtained in the subsequent reactors (q_{Cr} = 0.43 ± 0.05 mgCr $gTSS^{-1}$ h^{-1}). The latter q_{Cr} value, corresponding to acclimated activated sludge, is close to those reported by other authors. For example, q_{Cr} corresponding to *E. coli* ATCC 33456 with glucose as carbon source was 0.62 mgCr $gTSS^{-1}$ h^{-1} [28]. Villegas et al. [38] studied the removal of Cr(VI) by yeasts isolated from Cr(VI) contaminated sites; based on the results reported by those authors, q_{Cr} values ranged from 0.6 to 1.0 mgCr(VI) $gTSS^{-1}$ h^{-1}.

With regard to the stoichiometric coefficients, $Y_{X/S}$ and $Y_{Cr/X}$, an acclimation effect, similar to the previously described can be observed in Fig. 5. In this case, $Y_{X/S}$ increased and $Y_{Cr/X}$ decreased from batch #1 to #2; after this effect, coefficients remained constant in all the consecutive batch experiments. On the contrary, coefficient $Y_{Cr/S}$ was constant for all the Cr(VI) removal assays. These results indicate that biomass was completely acclimated after the first batch.

It must be remarked that the procedure employed in the present work to perform the five consecutive Cr(VI) removal batch assays introduces an important difference between these results and other reported data. For example, in Cr(VI) removal experiments using fed-batch reactors [43] or sequential batch reactors (SBR) where a partial replacement of the culture medium is performed [32], results may reflect the accumulation of potentially toxic by-products. In addition, if only the carbon source but not the entire culture medium is respiked [8] then an imbalance of nutrients may occur. In these cases, loss of Cr(VI) reduction capacity may be due to the depletion of other substrates such as the nitrogen source, for example. In the present work, all these problems that hindered the interpretation of results were solved because consecutive Cr(VI) removal assays were performed using fresh medium at the beginning of each experiment. Therefore, results obtained in the present work reflected only the previous history of the biomass, but not the influence of accumulation of potentially toxic by-products or a nutrient imbalance.

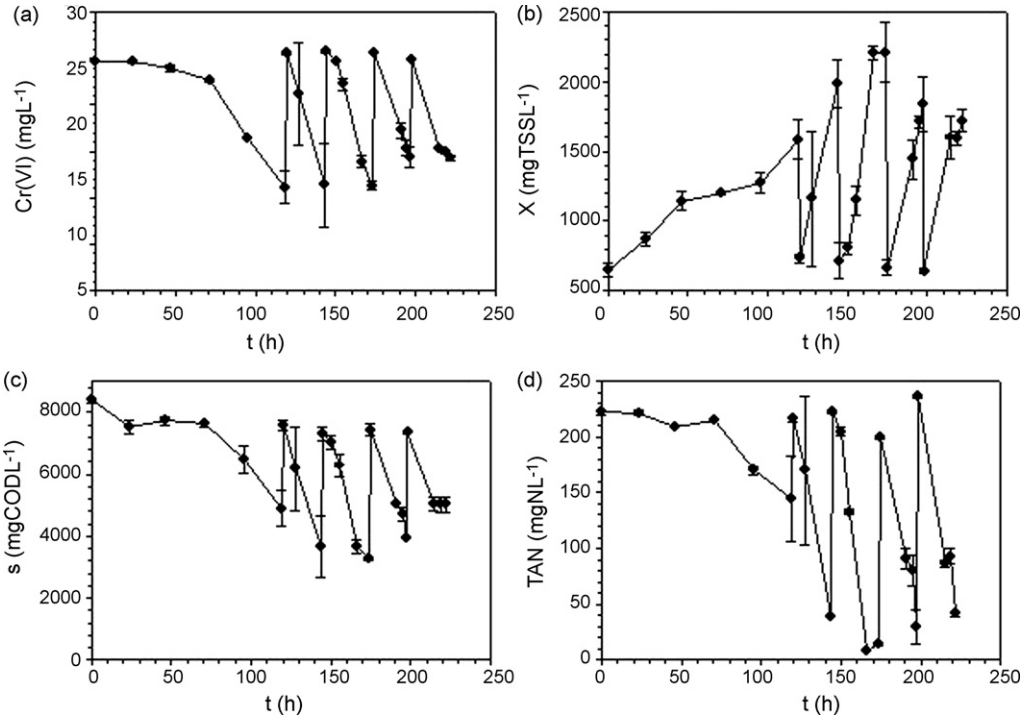

Fig. 4. Cr(VI) removal in consecutive batch assays. (a) Cr(VI) concentration, (b) biomass concentration, (c) organic substrate concentration and (d) total ammonia nitrogen concentration as a function of time. Bars indicate the standard deviation.

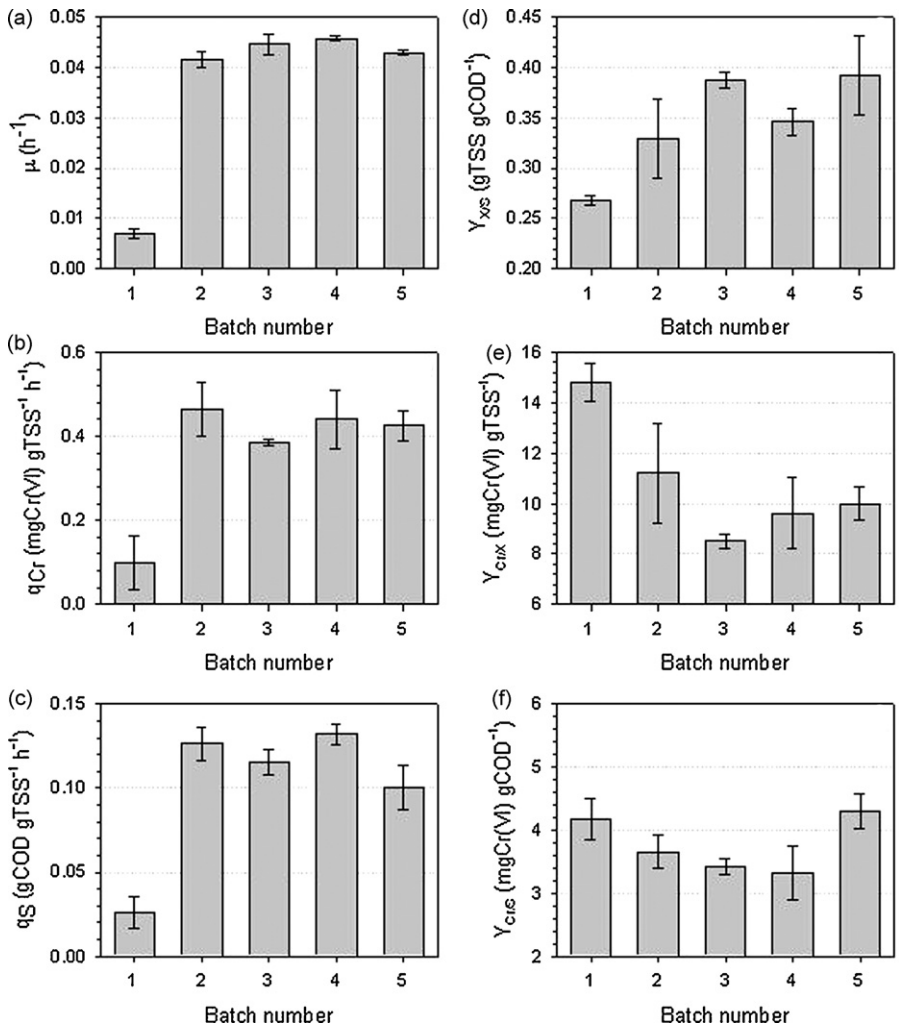

Fig. 5. Coefficients characterizing activated sludge growth (μ, q_S, $Y_{X/S}$) and Cr(VI) removal kinetics (q_{Cr}, $Y_{Cr/X}$, $Y_{Cr/S}$) as a function of the sequential batch number. Bars indicate the standard deviation.

4. Conclusions

Considering the addition of different carbon sources as electron donors for Cr(VI) reduction by activated sludge, results show that the rate and extent of chromate reduction varied significantly with the five tested carbon sources. High values of the specific Cr(VI) removal rate (q_{Cr}) were obtained when cheese whey, lactose, and glucose, were tested. On the contrary, relatively low q_{Cr} values were obtained with citrate and acetate. Therefore, cheese whey may be utilized as a technological alternative due to its low cost because it constitutes a residue from the dairy industries. Besides, calculations showed that less than 0.2% of the total transferable electrons of each organic substrate were used to reduce Cr(VI), being the other 99.8% employed on biomass synthesis and on energy production by molecular oxygen.

In the present work it was demonstrated that biological Cr(VI) reduction is associated to the cell multiplication phase, as a result, maximum chromate removal rate occurs when there is no limitation in carbon or nitrogen sources. The stoichiometric coefficients, $Y_{X/S}$ and $Y_{Cr/X}$, could be used to calculate the maximum hexavalent chromium removal as a function of the initial nitrogen and carbon source concentrations.

Considering the consecutive Cr(VI) removal batch assays, results showed that the microbial community can acclimate to the new environment in the presence of chromate. In addition, the new cells generated in the presence of hexavalent chromium maintain the ability to reduce Cr(VI). These results indicate that the activated sludge process could be applied to achieve Cr(VI) removal in continuous system.

Acknowledgments

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

References

[1] J.W. Patterson, Industrial Wastewater Treatment Technology, Butterworth Publisher, Stoneham, MA, 1985.
[2] S.A. Katz, H. Salem, The Biological and Environmental Chemistry of Chromium, VCH Publishers, Inc, New York, USA, 1994.
[3] J.P. Beukes, J.J. Pienaar, G. Lachmann, The reduction of hexavalent chromium by sulphite in wastewater, Water SA 26 (2000) 393–396.
[4] A. Bojic, M. Purenovic, D. Bojic, Removal of chromium (VI) from water by micro-alloyed aluminium composite (MAIC) under flow conditions, Water SA 30 (2004) 353–360.
[5] E. Demirbas, M. Kobya, E. Senturk, T. Ozkan, Adsorption kinetics for the removal of chromium (VI) from aqueous solutions on the activated carbons prepared from agricultural wastes, Water SA 30 (2004) 533–540.

[6] R. Schmuhl, H.M. Krieg, K. Keizer, Adsorption of Cu(II) and Cr(VI) ions by chitosan: kinetics and equilibrium studies, Water SA 27 (2001) 1–8.
[7] Y. Li, G.K.-C. Low, J.A. Scott, R. Amal, Microbial reduction of hexavalent chromium by landfill leachate, J. Hazard. Mater. 142 (2007) 153–159.
[8] Y.T. Wang, H. Shen, Modeling Cr(VI) reduction by pure bacterial cultures, Water Res. 31 (1997) 727–732.
[9] Y. Wang, C. Xiao, Factors affecting hexavalent chromium reduction in pure cultures of bacteria, Water Res. 29 (1995) 2467–2474.
[10] L. Philip, L. Iyengar, C. Venkobachar, Cr(VI) reduction by *Bacillus coagulans* isolated from contaminated soils, J. Environ. Eng. 124 (1998) 1165–1170.
[11] C.R. Myers, B.P. Carstens, W.E. Antholine, M.J. Myers, Chromium (VI) reductase activity is associated with the cytoplasmic membrane of anaerobically grown *Shewanella putrefasciens* MR-1, J. Appl. Microbiol. 88 (2000) 96–106.
[12] M.A. Mondaca, V. Campos, R. Moraga, C.A. Zaror, Chromate reduction in *Serratia marcescens* isolated from tannery effluent and potential application for bioremediation of chromate pollution, Scientific World J. 2 (2002) 972–977.
[13] B.B. Nepple, J. Kessi, R. Bachofen, Chromate reduction by *Rhodobacter sphaeroides*, J. Ind. Microb. Biotechnol. 25 (2002) 198–203.
[14] N.V. Asatiani, M.K. Abuladze, T.M. Kartvelishvili, N.G. Bakradze, N.A. Sapojnikova, N.Ya. Tsibakhashvili, L.V. Tabatadze, L.V. Lejava, L.L. Asanishvili, H.-Y. Holman, Effect of chromium (VI) action on *Arthrobacter oxydans*, Curr. Microbiol. 49 (2004) 321–326.
[15] E.N. Chirwa, Y.T. Wang, Simultaneous chromium (VI) reduction and phenol degradation in an anaerobic consortium of bacteria, Water Res. 34 (2000) 2376–2384.
[16] A.S. Stasinakis, N.S. Thomaidis, D. Mamais, T.D. Lekkas, Investigation of Cr(VI) reduction in continuous-flow activated sludge system, Chemosphere 57 (2004) 1069–1077.
[17] E. Dermou, A. Velissariou, D. Xenos, D.V. Vayenas, Biological chromium (VI) reduction using a trickling filter, J. Hazard. Mater. B126 (2005) 78–85.
[18] Y. Chen, G. Gu, Preliminary studies on continuous chromium (VI) biological removal from wastewater by anaerobic-aerobic activated sludge process, Bioresour. Technol. 96 (2005) 1713–1721.
[19] Y. Chen, G. Gu, Short-term batch studies on biological removal of chromium from synthetic wastewater using activated sludge biomass, Bioresour. Technol. 96 (2005) 1722–1729.
[20] P.E. Molokwane, K.C. Meli, E.M. Nkhalambayausi-Chirwa, Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading: Brits culture (South Africa), Water Res. 42 (2008) 4538–4548.
[21] R. Elangovan, L. Philip, Performance evaluation of various bioreactors for the removal of Cr(VI) and organic matter from industrial effluent, Biochem. Eng. J. 44 (2009) 174–186.
[22] J. Wanner, Activated Sludge Bulking And Foaming Control, Technomic Publishing Company, Inc, USA, 1994.
[23] Y.T. Wang, H. Shen, Bacterial reduction of hexavalent chromium, J. Ind. Microbiol. 14 (1995) 159–163.
[24] M. Viera, G. Curuchet, E. Donati, A combined bacterial process for the reduction and immobilization of chromium, Int. Biodeterior. Biodegrad. 52 (2003) 31–34.
[25] A.M. Ferro Orozco, E.M. Contreras, N.C. Bertola, N.E. Zaritzky, Hexavalent chromium removal using aerobic activated sludge batch systems added with powdered activated carbon, Water SA 33 (2007) 239–244.
[26] S. Llovera, R. Bonet, M. Simon-Pujol, F. Congregado, Chromate reduction by resting cells of *Agrobacterium radiobacter* EPS-916, Appl. Environ. Microbiol. 59 (1993) 3516–3518.
[27] H. Shen, Y.T. Wang, Biological reduction of chromium by *E. coli*, J. Environ. Eng. 120 (1994) 560–572.
[28] W.C. Bae, T.G. Kang, I.K. Kang, Y.J. Won, B.C. Jeong, Reduction of hexavalent chromium by *Escherichia coli* ATCC 33456 in batch and continuous cultures, J. Microbiol. Microbiol. Soc. Korea 38 (2000) 36–39.
[29] E.U. Cokgor, O. Karahan, D. Orhon, The effect of mixing pharmaceutical and tannery wastewaters on the biodegradation characteristics of the effluents, J. Hazard. Mater. 156 (2008) 292–299.
[30] E.U. Cokgor, G. Insel, E. Aydin, D. Orhon, Respirometric evaluation of a mixture of organic chemicals with different biodegradation kinetics, J. Hazard. Mater. 161 (2009) 35–41.
[31] A.H. Caravelli, N.E. Zaritzky, About the performance of *Sphaerotilus natans* to reduce hexavalent chromium in batch and continuous reactors, J. Hazard. Mater. 168 (2009) 1346–1358.
[32] A. Coreño-Alonso, F.J. Acevedo-Aguilar, G.E. Reyna-López, A. Tomasi, F.J. Fernández, K. Wrobel, K. Wrobel, J.F. Gutiérrez-Corona, Cr(VI) reduction by *Aspergillus tubingensis* strain: role of carboxylic acids and implications for natural attenuation and biotreatment of Cr(VI) contamination, Chemosphere 76 (2009) 43–47.
[33] B. Atkinson, F. Mavituna, Biochemical Engineering and Biotechnology Hanbook, Stockton Press, New York, 1991.
[34] A.E. Ghaly, M.S.A. Tango, M.A. Adams, Enhanced lactic acid production from cheese whey with nutrient supplement addition. Agricultural Engineering International: the CIGR J. Scientific Res. Develop. Manuscript FP 02 009. May, 2003.
[35] A.S.M. Chua, H. Takabatake, H. Satoh, T. Mino, Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent, Water Res. 37 (2003) 3602–3611.
[36] E. Contreras, L. Giannuzzi, N. Zaritzky, Growth kinetic of the filamentous microorganism *Sphaerotilus natans* in a model system of a food industry wastewater, Water Res. 34 (2000) 4455–4463.
[37] APHA, Standard methods for the examination of water and wastewater, 17 ed., American Public Health Association, Washington DC, 1992.
[38] L.B. Villegas, P.M. Fernández, M.J. Amoroso, L.I.C. De Figueroa, Chromate removal by yeast isolated from sediments of a tanning and a mine site in Argentina, Biometals 21 (2008) 591–600.
[39] D. Orhon, N. Artan, Modeling of Activated Sludge Systems, Technomic Publishing Company, Inc, USA, 1994.
[40] A.S. Stasinakis, D. Mamais, N.S. Thomaidis, T.D. Lekkas, Effect of chromium (VI) on bacterial kinetics of heterotrophic biomass of activated sludge, Water Res. 36 (2002) 3342–3350.
[41] U. Yetis, G.N. Demirer, C.F. Gokcay, Effect of chromium (VI) on the biomass yield of activated sludge, Enzyme Microb. Technol. 25 (1999) 48–54.
[42] E.A. Schmieman, D.R. Yonge, M.A. Rege, J.N. Petersen, C.E. Turick, D.L. Johnstone, W.A. Apel, Comparative kinetics of bacterial reduction of chromium, J. Environ. Eng. 124 (1998) 449–455.
[43] K. Yamamoto, J. Kato, T. Yano, H. Ohtake, Kinetics and modeling of hexavalent chromium reduction in Enterobacter cloacae, Biotech. Bioeng. 41 (1993) 129–133.