Hexavalent chromium removal using aerobic activated sludge batch systems added with powdered activated carbon

A Micaela Ferro Orozco¹, Edgardo M Contreras*, Nora C Bertola¹,², Noemi E Zaritzky¹,²

¹Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) – CONICET – Fac. de Cs. Exactas - UNLP.
(1900) La Plata, Argentina
²Fac. de Ingeniería – UNLP

Abstract

The addition of powdered activated carbon (PAC) has been proposed as a suitable technique to protect activated sludge against toxic wastewaters. However, the literature data describing the combined effect of PAC addition on Cr(VI) removal using activated sludge are scarce. The objectives of this study were to investigate the effect of the initial Cr(VI) concentration, PAC and an electron donor addition on Cr(VI) removal using aerobic activated sludge batch reactors.

The following Cr(VI) removal systems were tested: activated sludge alone; activated sludge with an external electron donor (5 g/ℓ of lactose); activated sludge with PAC addition (4 g/ℓ); activated sludge with both PAC and lactose; and PAC alone. The results reported here showed that activated sludges are capable of removing Cr(VI) via its reduction to Cr(III) only if a suitable electron donor (such as lactose) is available. For initial Cr(VI) concentration lower than 10 mg/ℓ, biomass alone can remove 100% of the Cr(VI). However, for higher initial Cr(VI) concentrations, removal efficiencies (R) of the system with PAC were higher than Rₚₜ corresponding to the system without PAC. In addition, as the initial Cr(VI) concentration increased, the rate of Cr(VI) removal and Rₚₜ values decreased reflecting loss of metabolic activity of the activated sludge due to the toxicity of Cr(VI); however, this inhibition was less in systems with PAC. Whereas the removal of Cr(VI) using powdered activated carbon (PAC) alone is negligible, the addition of PAC can improve the biological reduction of Cr(VI) due to the stimulating or protective effect against the Cr(VI) toxicity. This protective effect was also observed in respiratory activity of the biomass.

Keywords: activated sludge, powdered activated carbon, hexavalent chromium, trivalent chromium

Introduction

Heavy metal residues in contaminated habitats may accumulate in micro-organisms, aquatic flora and fauna, which in turn, may enter the human food chain and result in health problems. Among these metals, chromium and its compounds are placed on the priority list of toxic chemicals of many countries including the USA, the UK and Canada (Heddgectott, 1994). Chromium is usually found in the environment in oxidation states (III) and (VI). Each of the above-mentioned oxidation states has different biological and chemical properties. Typical hexavalent chromium compounds are highly soluble chromates (CrO₄²⁻, HCrO₄⁻) and dichromates (Cr₂O₇²⁻); these compounds are toxic and carcinogenic for a variety of organisms due to their strong oxidising capability (USEPA, 1998a). Trivalent chromium can form both anionic (e.g. Cr(OH)₄⁻, CrCl₄⁻) or cationic (e.g. Cr(H₂O)₆³⁺, Cr(OH)⁷⁺, Cr(OH)⁶⁻, Cr(OH)⁵⁻) compounds. These compounds are considered to be non-labile, inert species in the environment and essential for mammals in trace amounts (USEPA, 1999b). Due to its common presence in effluent discharge from steelworks, chromium electroplating, leather tanning and chemical manufacturing industries, chromium is often detected in sewage plants that treat a combination of industrial and municipal wastewater.

Conventional methods for removing Cr(VI) include chemical reduction to Cr(III) followed by a precipitation step under alkaline conditions (Beukes et al., 2000; Bojic et al., 2004) or adsorption using chitosan (Schmuhl et al., 2001) or activated carbon (Demirbas et al., 2004). All these methods have their own disadvantages; alkali precipitation produces large quantities of chemical sludge, whereas ion exchange and adsorption are generally costly and less specific for Cr(VI) removal in the presence of other ions. The search for new technologies has focused attention on the biotransformation of metals by using micro-organisms. A great number of bacterial genera were described as capable of reducing Cr(VI) to Cr(III) including Escherichia (Shen and Wang, 1994; Wang and Shen, 1997), Pseudomonas (Bopp and Ehrlich, 1988; Ishibashi et al., 1990), Vibrio (Clark, 1994; Rege et al., 1997), Bacillus (Wang and Xiao, 1995), Shewanella (Shen and Wang, 1994; Wang and Shen, 1997), Rhodobacter (Nepple et al., 2002), Arthrobacter (Asatiani et al., 2004). The main disadvantage of using pure cultures to remove Cr(VI) compounds is related to the use of sterile conditions to prevent the external microbial contamination. For this reason the removal of Cr(VI) using activated sludge is a promising technique.

In general, biological treatment is negatively affected by the heavy metals due to their toxicity for micro-organisms. However, the literature data with respect to the toxic effect of Cr(VI) on the activated sludge process (e.g. substrate removal, respiration activity, or bacterial growth) are controversial. For example, Stasinakis et al. (2002) reported that Cr(VI) concentrations higher than 10 mg/ℓ inhibited the growth of non-acclimatised activated sludge. Chua (1998) found that the effect of 0.05 mg/ℓ of Cr(VI) on the performance of sequencing batch reactors was a function of the sludge age. Yetis et al. (1999) reported

Available on website http://www.wrc.org.za
ISSN 0378-4738 = Water SA Vol. 33 No. 2 April 2007
ISSN 1816-7950 = Water SA (on-line)
the stimulation of an acclimatised sludge in the presence of 25 mg Cr(VI)/ℓ. Vankova et al. (1999) found that the Cr(VI) concentration that inhibited 50% of the respiration rate ranged between 40 and 90 mg/ℓ. However, Madoni et al. (1999) reported that 100 mg Cr(VI)/ℓ reduced the activated sludge respiration rate by only 21.5%.

The addition of powdered activated carbon (PAC) into the aeration basin has been proposed as a suitable technique to protect the activated sludge against toxic wastewaters (Orshansky and Narkis, 1997; Costa and Márquez, 1998). However, data reported in the literature describing the combined effect of PAC addition on Cr(VI) removal using activated sludge are scarce.

The objectives of this study were to investigate the effect of the initial Cr(VI) concentration, PAC and an electron donor addition on Cr(VI) removal using batch reactors of aerobic activated sludge. Five different Cr(VI) removal systems were analysed:

1. Activated sludge alone
2. Activated sludge with an external electron donor
3. Activated sludge with PAC addition
4. Activated sludge with both PAC and an electron donor
5. PAC alone.

Materials and methods

Activated sludge

The activated sludge used in this experimental set-up was cultured in a laboratory-scale (4.5 ℓ) Eckenfelder aerobic reactor type. The plant was fed with a synthetic wastewater containing dehydrated cheese whey (1 500 mg), (NH₄)₂SO₄ (94 mg) and NaHCO₃ (1 030 mg) dissolved in 1 ℓ of tap water. The C: N: P ratio of the used cheese whey was 100:7.0:6. (Bertola et al., 2001).

Hydraulic retention time was 2 d; sludge age was maintained at 45d by the daily wasting of mixed liquor directly from the reactor. During the experiments the temperature of the reactor was 19 ± 2°C; under steady state conditions dissolved oxygen (DO) concentration was above 5 mg/ℓ; pH was 7.5 ± 0.4; soluble chemical oxygen demand (COD) in the effluent ranged between 30 and 80 mg/ℓ; and the biomass concentration ranged from 3 500 to 4 000 mgCOD/ℓ.

Chromium (VI) removal potential of the used activated sludge

In order to determine if the micro-organisms present in the activated sludge were capable of reducing hexavalent chromium, a Cr(VI) removal assay using an activated sludge batch reactor with lactose (5 g/ℓ) as substrate was performed; in this experiment the biomass concentration was 1 800 mgCOD/ℓ and the initial Cr(VI) concentration was 26 mg Cr/ℓ. Samples were taken at predetermined time intervals and they were centrifuged at 13 000 r/min for 5 min (Eppendorf centrifuge 5415C, Hamburg, Germany) to eliminate the biomass and/or PAC, upon which the total chromium and Cr(VI) concentrations were determined in the supernatant. Soluble Cr(III) concentration was calculated as the difference between total Cr and soluble Cr(VI) concentrations. All measurements were performed in duplicate. The Cr(VI) removal efficiency (Rₜₐₚ) of each tested system was calculated using the following expression:

\[ Rₜₐₚ = 100 \left( \frac{C₀ - C}{C₀} \right) \]

where:

Co and C were the initial Cr(VI) and the Cr(VI) concentration at time t respectively.

Respirometry

Oxygen uptake rate (OUR) is a widely used index of metabolic activity of aerobic micro-organisms (Vanrolleghem et al., 1994) and has been used to study toxicity of several chemical agents (Ros, 1993; Vanrolleghem et al., 1994). This technique was used to evaluate the effect of Cr(VI) and PAC on the microbial respiration. The tested Cr(VI) concentrations were 0, 10, 25, 50, 75 and 100 mg/ℓ; in the systems with PAC the concentration was 4 g PAC/ℓ.

The latter assay was performed using the supernatant of the centrifuged outlet stream of the treatment plant; the final soluble COD ranged between 30 and 80 mg/ℓ. Lactose was chosen as the electron donor because this sugar was the main component of the cheese whey used to feed the wastewater treatment plant. For the Cr(VI) removal systems using activated sludge (Systems 1 to 4) the biomass concentration was 2 000 ± 300 mgCOD/ℓ. In the experiments with PAC addition (Systems 3 to 5), 4 g/ℓ of a wood based PAC (Clarimex SA, Type 061) was used; Table 1 shows the characteristics of the activated carbon used in the experiments. Cr(VI) stock solutions were freshly prepared using analytical grade K₂Cr₂O₇; tested Cr(VI) concentrations were 10, 25, 50 and 100 mg/ℓ. All batch experiments lasted about 200h; samples were taken at predetermined time intervals and they were centrifuged at 13 000 r/min for 5 min (Eppendorf centrifuge 5415C, Hamburg, Germany) to eliminate the biomass and/or PAC, upon which the total chromium and Cr(VI) concentrations were determined in the supernatant. Soluble Cr(III) concentration was calculated as the difference between total Cr and soluble Cr(VI) concentrations. All measurements were performed in duplicate. The Cr(VI) removal efficiency (Rₜₐₚ) of each tested system was calculated using the following expression:

\[ Rₜₐₚ = 100 \left( \frac{C₀ - C}{C₀} \right) \]

TABLE 1

| Characteristics of the PAC used |
|----------------------------------|
| Properties                        | Value               |
|-----------------------------------|---------------------|
| Surface area, BET (N/77°K)       | 889 m²/g            |
| Methylene blue adsorption         | 260 mg/g            |
| Iodine adsorption                 | 800 mg/g            |
| Bulk density                      | 0.29 g/cm³          |
| Moisture                          | 12 %                |
| pH (1% suspension)                | 6.0 - 8.0           |
| Screen analysis,                  |                     |
| Passes mesh #325                  | 60 – 80 %wt         |

The Cr(VI) removal efficiency (Rₜₐₚ) of each tested system was calculated using the following expression:

\[ Rₜₐₚ = 100 \left( \frac{C₀ - C}{C₀} \right) \]

where:

Co and C were the initial Cr(VI) and the Cr(VI) concentration at time t respectively.
to a computer. OUR was measured by placing 20 ml of the activated sludge suspension in the respirometer. A volume of 1 ml cheese whey solution was added as the oxidisable substrate; after 10 min the system was aerated during 1 min. Once aeration was stopped, a linear decrease of dissolved oxygen concentration with time was found, OUR being the slope of the line.

The effect of Cr(VI) on activated sludge respiratory activity was evaluated using the following expression:

\[ Fr = \frac{OUR_{Cr(VI)}}{OUR_{control}} \]  

where:

- \( Fr \) is the bacterial respiratory activity fraction based on determinations of the OUR on untreated samples (OUR\(_{control}\)) and after the contact with hexavalent chromium (OUR\(_{Cr(VI)}\)) in the presence or absence of PAC.

All assays were performed in duplicate.

**Analytical methods**

Cr(VI) in the supernatant was determined by the diphenylcarbazide method (Standard Methods, 1989). In order to measure the total chromium concentration, supernatants were treated with ammonium persulphate and potassium permanganate to oxidise Cr(III) to Cr(VI); Cr(III) concentration was estimated as the difference between oxidised (total Cr) and non-oxidised (Cr(VI)) samples. Precipitated and/or adsorbed (non-soluble) Cr was calculated as the difference between the initial total Cr and the total Cr concentration at time \( t \). The COD was determined using Hach reagents (Hach Company, Loveland, CO). The biomass concentration was determined as COD using a procedure previously developed (Contreras et al., 2002).

**Results and discussion**

**Chromium (VI) removal potential of the activated sludge used in the experiments**

This experiment was performed to demonstrate that the microorganisms present in the activated sludge were capable of removing Cr(VI). Figure 1 shows that Cr(VI) concentration decreased from 26 to 17 mg/ℓ within the first 67 h. At this time, the biomass was harvested by centrifugation (indicated by an arrow) and afterwards no subsequent change in Cr(VI) concentration was observed. As neither detectable adsorption of chromium species, reduction of Cr(VI) nor the oxidation of Cr(III) on the vessel walls were produced, this experiment demonstrated that the removal of Cr(VI) was associated with the presence of the biomass.

**Chromium (VI) removal performance of the different systems**

Figure 2 shows an example of Cr(VI) removal experiments using the different tested systems; in this case the initial Cr(VI) concentration was 100 mg/ℓ. Cr(VI) removal rates of Systems 1 (activated sludge alone), 3 (activated sludge with PAC), and 5 (PAC alone) were slower than removal rates in System 2 (activated sludge with lactose). In addition, the maximum Cr(VI) removal rate was obtained in System 4 (activated sludge with lactose and PAC). At 190 h the Cr(VI) concentration in Systems 1, 3, and 5 ranged between 90 and 95 mg/ℓ. However, in Systems 2 and 4, where lactose was added, the Cr(VI) concentrations after 190 h were 67 and 14 mg/ℓ respectively. The results using different Cr(VI) initial concentrations showed a similar tendency.

**Effect of the electron donor addition (lactose)**

Figure 3 shows the Cr(VI) removal efficiencies (R\(_ s \)) corresponding to the tested systems as a function of time for different Cr(VI) initial concentrations. For systems without lactose (Fig. 3a; c) R\(_ s \) values were lower than in the systems with lactose (Fig. 3b; d). One of the main mechanisms proposed in the literature for Cr(VI) removal using biomass (pure and mixed cultures) under aerobic conditions is the reduction of Cr(VI) to Cr(III) (Imai and Gloyna, 1990; Wang and Shen, 1994; 1995; Wang and Xiao, 1995). In our experiments the systems with lactose (Fig. 3b; d) also had higher removal efficiencies than the systems without this sugar (Fig. 3a; c).

**Effect of PAC addition**

Figure 2 shows that the rate of Cr(VI) removal using PAC alone (System 5) was very low; the removal efficiency was lower than 7% for all the tested initial Cr(VI) concentrations. However,
Cr(VI) removal efficiencies in the presence of biomass with PAC (Fig. 3c, d) were higher than those of the systems without PAC (Fig. 3a, b) for initial Cr(VI) concentrations higher than 25 mg/l.

Comparing the systems without lactose, it was observed that the Cr(VI) removal performance of System 3 (activated sludge with PAC) was higher than the 

In all cases the contact time was 190 h and the initial Cr(VI) concentration was 10 mg/l. The Cr(VI) values of both Systems 4 (with PAC) and 2 (without PAC) were 100%. However, for Cr(VI) initial concentrations higher than 10 mg/l, it was observed that the capacity to reduce Cr(VI) in the activated sludge (Fig. 3b) decreased as the initial Cr(VI) concentration increased, therefore the rate of Cr(VI) removal and R_E values decreased reflecting loss of metabolic activity of the activated sludge due to the toxicity of Cr(VI). In System 2 (biomass with lactose), the R_E final values (at 190 h) were 77, 65 and 34 % for the initial Cr(VI) concentrations of 25, 50 and 100 mg/l respectively (Fig. 3b). The loss of metabolic activity was observed to a lesser extent when PAC was present; the R_E final values for the system containing lactose and PAC (Fig. 3d) were 98, 90 and 86 % when the initial Cr(VI) concentrations were 25, 50 and 100 mg/l respectively. These results suggest a protective and/or stimulating effect of the activated carbon on the micro-organisms.

Distribution of the different chromium species in the tested systems

Total chromium and Cr(VI) concentrations were determined in the supernatant of each system. Soluble Cr(III) concentration was 10 mg/l the 

In the systems containing lactose, when the initial Cr(VI) concentration was 10 mg/l the 

As the activated sludge treatment plant was fed with cheese whey, these nitrogen-containing compounds could be present in
the outlet stream. Therefore, the higher soluble Cr(III) concentrations found in the tested systems suggested the formation of coordination complexes that increased the Cr(III) solubility.

Although the highest Cr(VI) removal was obtained using System 4 (activated sludge with lactose and PAC), the highest soluble Cr(III) concentration was found in System 2 (activated sludge with lactose). This result suggested that in System 4 the potential ligands of Cr(III) could be adsorbed onto the PAC decreasing the Cr(III) solubility. Previous experiments performed in our laboratory showed that the specific adsorption of cheese whey onto the used activated carbon was 83 mg COD/g PAC; thus, the addition of 4 g PAC/l could potentially adsorb about 332 mg COD/l. This value was much higher than the soluble COD in the outlet stream (30 to 80 mg COD/l); therefore all the potential ligands of Cr(III) could be adsorbed. If this was the case, soluble Cr(III) concentrations in systems with PAC (System 4) must be lower than in systems without PAC (System 2), in accordance with the experimental observations (Fig. 5).

Protective effect of PAC against Cr(VI) toxicity in the activated sludge

Considering that Cr(VI) removal in System 5 (using PAC alone) was almost negligible (Fig. 2) and that the presence of PAC in Systems 3 and 4 improved the Cr(VI) removal efficiencies (Fig. 3c, d), this improvement cannot be attributed to the presence of the activated carbon itself but to a metabolic stimulation of the biomass or a protective effect of PAC against the Cr(VI) toxicity.

Different authors reported that different metabolic activities of the activated sludge were stimulated by the addition of PAC in the aeration tanks (Taniguchi et al., 1993; Orshansky and Narkis, 1997; Okada et al., 2000; Morinaga et al., 2003). The stimulation of bacterial activity when cells are adhered on activated carbon may be due to different mechanisms:

(a) The concentration of substrates is high at the surface of the activated carbon (Kalinske, 1972)

(b) Substrates adsorbed on activated carbon can be supplied directly to the adhered bacteria by desorption (Li and DiGiano, 1983)

(c) Higher oxygen concentration on activated carbon surface (Kalinske, 1972; Ying and Weber, 1979)

(d) Longer contact times between the cells and adsorbed substrates

(e) The adsorption of inhibitory or toxic substances on the activated carbon surface (Sublette et al., 1982).

Experiments performed in our laboratory showed that the used PAC adsorbed about 17 mg of lactose per gram of activated carbon; therefore, mechanisms (a), (b), and (d) cannot be discarded as possible explanations of the observed stimulating or protective effect of PAC against Cr(VI) toxicity. Our results showed that the Cr(VI) removal using PAC alone (System 5) was negligible in comparison to Systems 2 (activated sludge-lactose) and 4 (activated sludge-lactose-PAC), thus, the hypothesis (e) about the adsorption of Cr(VI) on the PAC surface could be discarded.

The protective effect of PAC on the metabolic activity of the micro-organisms in the presence of Cr(VI) was analysed by measuring the activated sludge respiration rate. The measurements were performed under different initial Cr(VI) concentrations in the absence and in the presence of PAC. Results were expressed as a fraction of the respiration rate (Fr) as defined in Eq. (4). Figure 6 shows that Fr values in the presence of PAC were higher than in its absence for all the tested Cr(VI) concentrations. Therefore, the addition of PAC protected the biomass decreasing the loss of metabolic activity of the activated sludge due to the Cr(VI) toxicity. Sher et al. (2000) studied the effect of zinc on activated sludge with and without PAC addition. These authors found that although zinc was not adsorbed on the activated carbon, the micro-organisms in this system resisted the zinc toxicity better than in the system containing activated sludge without PAC.

Conclusions

Activated sludges are capable of removing Cr(VI) via its reduction to Cr(III) only if a suitable electron donor (such as lactose) is available. For initial Cr(VI) concentration lower than 10 mg/l, biomass alone can remove 100 % of the Cr(VI). However, for higher initial Cr(VI) concentrations, removal efficiencies of system with PAC were higher than Rc corresponding to system without PAC. In addition, as the initial Cr(VI) concentration increased, the rate of Cr(VI) removal and Rc values decreased reflecting loss of metabolic activity of the activated sludge due to the toxicity of Cr(VI); however, this inhibition was less in systems with PAC. Whereas the removal of Cr(VI) using PAC alone is negligible, the addition of PAC can improve the biological reduction of Cr(VI) due to the stimulating or protective effect against the Cr(VI) toxicity. This protective effect was also observed in respiratory activity of the biomass.

Thus, it was necessary for the biomass, a suitable electron donor and PAC to be present simultaneously in order to achieve high Cr(VI) removal efficiencies when the Cr(VI) initial concentration was higher than 10 mg/l.

Acknowledgements

The financial support given by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (Proyecto 09-11211) and Universidad Nacional de La Plata, is gratefully acknowledged.

References

ASATIANI NV, ABULADZE MK, KARTVELISHVILI TM, BAKRADZE NG, SAPOJNIKOVA NA, TSIBAKHASHVILI NY, TABATADZE LV, LEJAVA LV, ASANISHVILI LL and HOLMAN H (2004) Effect of chromium (VI) action on Arthrobacter oxydans. Current Microbiol. 49 321-326.

BERTOLA NC, BEVILACQUA AE and ZARITZKY NE (2001) Mod-
