Journal of Hazardous Materials 175 (2010) 104–110

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Chromium (VI) biotransformation by β- and γ-Proteobacteria from natural polluted environments: A combined biological and chemical treatment for industrial wastes

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ARTICLE INFO

Article history:
Received 27 July 2009
Received in revised form
24 September 2009
Accepted 25 September 2009
Available online 2 October 2009

Keywords:
Cr(VI) biotransformation
Industrial waste biotreatment
Pseudomonas veronii
Bioremediation

ABSTRACT

The high solubility of Cr(VI) in aqueous systems, together with carcinogenic and mutagenic effects on living organisms, make industrial effluents receive specific treatments for Cr(VI) elimination. Biotreatments, based on biotransformation of Cr(VI) to Cr(III) which is immobilized as $Cr(OH)_3$, are the most effective methods for the removal of Cr(VI) concentrations below 2 mM. The aim of our study is the application of pure or mixed bacterial cultures for Cr(VI) biotransformation followed by chemical flocculation of $Cr(OH)_3$ as a combined treatment for industrial wastes. *Pseudomonas veronii* 2E, *Delftia acidovorans* AR, *Klebsiella oxytoca* P2 and *Klebsiella ornithinolytica* 1P, isolated from polluted environments showed a decrease from 38.83 to 74.32%, in 0.05 mM of initial Cr(VI). As revealed DGGE experiments, *P. veronii* 2E and *K. ornithinolytica* 1P could develop together in cocultures and in these conditions a 72.88% of Cr(VI) present was removed. Although the pH of the cultures was 8, no $Cr(OH)_3$ sediment was detected. The results of total chromium quantification support this observation. The precipitation of Cr(III) was induced using different commercial flocculants. Best yields were obtained using Na_2CO_3 0.1 M, which allowed the flocculation of almost 100% of Cr(III) present. This combined treatment would be an economical and ecological way to remove Cr(VI).

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1. Introduction

Hexavalent chromium frequently appears in many industrial discharges, especially from tanneries and electroplating activities in the form of chromate and dichromate [1]. Its high solubility in aqueous systems, its permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids [2,3] together with carcinogenic and mutagenic effects on living organisms [4], makes industrial effluents receive specific treatments for Cr(VI) elimination. For that purpose, many chemical processes such as chemical reduction followed by precipitation, ion exchange and adsorption on activated charcoal are applied [5], but with Cr(VI) concentrations below 2 mM, these methodologies result ineffective. Besides, these techniques are generally costly and can themselves produce other waste problems. In Argentina, maximal permissible levels of Cr(VI) in industrial wastewaters to be discharged into either sewage or surface water courses should be less than 0.2 mg/L (0.0038 mM) (Resolución 336/03 de la Provincia de Buenos Aires and Resolución 79.179/90 de Recursos Hídricos de la Nación Argentina). The monthly average Cr(VI) maximum value informed in literature is 0.077 mg/L for industrial wastewater discharges [6]. European Union recommends total chromium limits of 0.05 and 0.1 mg/L for potable and industrial wastewater respectively [http://europa.eu/]. That is why other alternative technologies are necessary to remove Cr(VI) to fulfil the national legislation requirements.

Microbial treatments, based on biotransformation of Cr(VI) to Cr(III) which is easily immobilized as $Cr(OH)_3$, are still efficient with low Cr(VI) amounts. These methods offer an economical as well ecofriendly option for chromate detoxification and bioremediation [7]. A number of chromium resistant microorganisms had been reported to detoxify hexavalent chromium [8], where the method of detoxification could be periplasmic biosorption, intracellular bioaccumulation and biotransformation directly through enzymatic reaction or indirectly with metabolites [5].

The objective of the present study is the application of either axenic or mixed cultures for Cr(VI) biotransformation followed by chemical flocculation of $Cr(OH)_3$ as a combined treatment for industrial wastes, with Cr(VI) concentrations below 2 mM. The microorganisms to be tested were isolated from polluted soil, surface water and sediments of industrial areas of Buenos Aires Metropolitan Area, Argentina. In order to accomplish the general goal, the microorganism's tolerance to chromium (VI) will be

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doi:10.1016/j.jhazmat.2009.09.134

determined. To assess the potential of these chromium resistant bacteria for this ion bioreduction, the best conditions (concentration of metal, culture media composition, inoculation, temperature and pH) for the batch cultures will be tested. Pure and mixed cultures will be used in order to establish the most efficient combination to decrease the concentration of chromium in the effluent to permitted values.

2. Methods

2.1. Selection of bacterial strains and molecular characterization

Microorganisms were isolated from samples collected from polluted environments in the Buenos Aires Metropolitan Area, as described in previous studies [9]. Bacteria were identified by 500 bp 16S r-RNA gene sequencing (MIDI Labs, USA) and 1500 bp 16S bp r-RNA gene sequencing (MacroGen, Korea) as: *Klebsiella ornithinolytica* 1P, *Pseudomonas veronii* 2E, *Klebsiella oxytoca* P2, *Delftia acidovorans* AR and *Ralstonia taiwanensis* M2. The microorganisms were maintained in semisolid medium inside test tubes.

2.2. Determination of bacterial resistance to Cr(VI)

The minimal inhibitory concentration (MIC) of chromium at which no growth occurred was determined by both the visual method (no turbidity was observed as a signal of bacterial growth inhibition) and the "spectrophotometrical" method (turbidity measured as the absorbance at 600 nm).

2.2.1. Visual method

Two or three colonies of bacterial strains were suspended in 5 mL of physiological solution to get turbidity equivalent to the test tube 1 (standard) in the Mc Farland scale [10]. MIC was determined in duplicate by inoculating 0.1 mL of the suspension in 3 mL of PYG broth (casein peptone 2.5 g/L, yeast extract 1.25 g/L, glucose 0.5 g/L) with 0.1, 0.25, 0.50, 0.75, 1, 2 and 5 mM Cr(VI). Cultures were incubated at 32 °C for at least 1 week, checking bacterial development by comparing with the control cultures Cr(VI)-free.

2.2.2. Spectrophotometrical method

This method was used only for *K. ornithinolytica* 1P, because all the other strains form aggregates that interfere with the absorbance measurements at low bacterial densities. MIC was determined in duplicate in PYG broth with the same concentrations of Cr(VI) mentioned before and the bacterial development by absorbance at 600 nm using a Turner spectrophotometer (SP 830 model) every 24 h. MIC was estimated as the first dilution which completely inhibits bacterial growth in PYG medium.

2.3. Cr(VI) removal in batch cultures

The isolated bacteria were screened for the chromium removal potentiality conditions. Potassium chromate (K_2CrO_4) was used as a source of hexavalent chromium. The initial inoculates were prepared in 125 mL conical flasks containing 10 mL of PYG broth, Cr(VI) 0.05 mM and each separated strain. Cultures were incubated at 32 °C and 200 rpm for 3 days.

2.3.1. Pure cultures

Metal retention was tested in batch cultures by inoculating 100 mL of PYG medium containing the same concentration of Cr(VI) with 10 mL of the previous culture and incubating at 32 °C and 200 rpm. Samples were taken at different time intervals depending on the strain used: (a) for *K. oxytoca* P2, five samples were extracted every 2 h and one last sample after 24 h, (b) for *D. acidovorans* AR, six samples were analyzed every 2 h and (c) for all the other strains (*K. ornithinolytica* 1P, *P. veronii* 2E and *R. taiwanensis* M2), three samples were taken every 2 h, being a fourth taken after 24 h. The volume of all the aliquots was 12 mL.

2.3.2. Mixed cultures

The same methodology was used for mixed cultures, adding 10 mL of each pure culture. Four samples were taken every 2 h and a fifth sample was analyzed after 24 h. The mixed cultures used were:

(1) *K. ornithinolytica* 1P, *P. veronii* 2E.
(2) *K. ornithinolytica* 1P, *K. oxytoca* P2
(3) *K. oxytoca* P2, *P. veronii* 2E.
(4) *K. ornithinolytica* 1P, *P. veronii* 2E, *K. oxytoca* P2.

D. acidovorans AR and *R. taiwanensis* M2 were not used for mixed culture experiments because of their development of an antimicrobial activity against other bacterial strains.

In both types of cultures, bacterial growth was tested measuring absorbance at 600 nm ($A_{600\,nm}$) and each sample's pH was stated. All samples were centrifuged (3000 × *g*, 15 min) and supernatants analyzed for Cr(VI) and total Cr contents to estimate total removal. Cell free medium supplemented with Cr(VI) was tested simultaneously as control and all experiments were performed in duplicates.

2.4. Molecular analysis for the mixed culture composition studies

DNA analysis was carried out either in pure or mixed cultures in order to characterize the microbial composition of the samples. The selected time intervals were the same ones than in the Cr(VI) decay experiments. The DNA extraction procedure was the CTAB (N-cetyl-N,N,N-trimethylammoniumbromide) method modified for soil samples [11]. First of all, in order to estimate DNA quantities obtained by this technique, DNA was extracted from *K. oxytoca* P2 and *P. veronii* 2E cultures and checked in an agarose gel 0.8% electrophoresis (Horizon 58 model), revealed with ethidium bromide and ultraviolet light (320 nm, GelDoc 200 Biorad). The DNA concentration was defined using nanodrop (ND1000-Nanodrop technology).

Due to the presence of big amounts of RNA in the extracts, it was necessary to eliminate it using the corresponding enzyme. The presence or absence of each strain at the different time intervals in batch pure or mixed cultures was determined by the PCR-DGGE technique (polymerase chain reaction-denaturating gradient gel electrophoresis) [12]. For 16S rRNA gene amplification by PCR, F341-GC (5′ CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCC-CGCCCG*CCTACGGGAGGCAGCAG* 3′) and R518 (5′ CCGTCAATTCCTT-TRAGTTT 3′) primers were used and electrophoresis was carried out in a 40% (acrylamide/bisacriylamide) polyacrilamide gel with 30% to 60% of denaturant (urea/formamide) at 60 °C and 120 V for 4.5 h with TAE buffer (20 mM Tris–acetate, pH 7.4; 10 mM sodium acetate and 0.5 mM Na_2–EDTA). To visualize DNA bands, SYBR Gold (Molecular Probes, Eugene, OR) and ultraviolet light transillumination (320 nm, GelDoc 200 Biorad) were implemented.

2.5. Cr(III) chemical flocculation

Preliminary tests for Cr(III) flocculation were performed using 5 mL aliquots of a 0.0192 M solution of $CrCl_3 \cdot 6H_2O$ (pH 4, 18 MΩ water, Millipore) and different volumes of 0.1 M Na_2CO_3 solution (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL). The samples obtained were centrifuged (3000 × *g*, 10 min), the pH of the all the supernatants was determined before separating them. The mass of each precipitate was weighed with an electronic balance (±0.0001 g) and compared

Table 1
Cr(VI) minimal inhibitory concentration (MIC) in PYG Broth of five of the isolates, estimated by the visual method.

Strain	MIC (mM)
K. ornithinolytica 1P	0.50
P. veronii 2E	0.50
K. oxytoca P2	0.75
D. acidovorans AR	0.50
R. taiwanensis M2	0.75

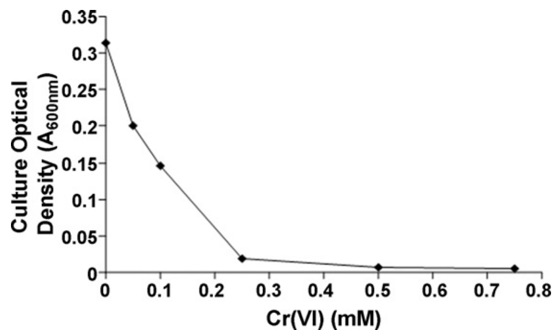


Fig. 1. Cr(VI) MIC estimation by turbidimetry for *Klebsiella ornithinolytica* 1P.

to the initial amount of the cation. The same procedure was followed using commercial flocculants and coagulants instead (0.5 mL COAG-C-6 and 0.5 mL Flox-A-6) adjusting the final pH to 9 using 0.1 M NaOH or 0.2 M H_2SO_4. Attempts to estimate the Cr(III) concentration that remained in the supernatants after coagulation were performed by oxidation to Cr(VI) and spectrophotometrical determination of this cation as described in the analytical procedures.

2.6. Analytical procedures

Hexavalent chromium was determined spectrophotometrically using the s-diphenylcarbazide method [13,14] with a detection limit of 5 μg/L. The blank was prepared using water (18 MΩ, Millipore) and absorbance was measured at 540 nm ($A_{540\,nm}$) using a Perkin Elmer Spectrophotometer (Lambda 20). A calibration curve was constructed using standard Cr(VI) solutions of concentrations: 0.00195, 0.00500, 0.01000, 0.01500 and 0.01900 mM.

Total chromium was quantified by air-acetylene flame atomic absorption spectroscopy in a Varian AA-50B, wavelength 357.9 nm, after digestion of samples with H_2SO_4(c) and H_2O_2 in a microwave digester.

2.7. Quality control and quality assurance

The standard reference material of Cr (p.a.) was used for the calibration and quality assurance for each analytical batch. All solutions were prepared using p.a. reagents and deionised water (18 MΩ, Millipore). The methodologies used in this work were selected in order to fulfil the general standards for quality controls required for microbiological analyses [14,15]. The values informed for the analytical determinations agree with the precision of the instruments used, on the other hand the uncertainty values for the microbiological determinations are lower than 10%.

3. Results and discussion

3.1. Determination of bacterial resistance to Cr(VI)

3.1.1. Visual analysis

The bacterial growth of all the strains was inhibited only at relatively high concentrations of Cr(VI) (Table 1). These experimental results prove that all the selected microorganisms are tolerant and/or resistant to the presence of this cation, specially in comparison with data obtained by other authors [16–19] in similar culture media.

The inhibition values that were obtained are around the same range of concentrations for all the microorganisms. The higher values were for *K. oxytoca* P2 and *R. taiwanensis* M2. The five strains were selected then for Cr(VI) biotransformation experiments in batch cultures.

3.1.2. Spectrophotometrical method

MIC was determined by this method only for *K. ornithinolytica* 1P, with 0, 0.05, 0.1, 0.25, 0.50 and 0.75 mM Cr(VI). Results are shown in Fig. 1.

At 0.25 mM the absorbance values are low but at 0.50 mM values are almost 0. The estimation of MIC by this method confirmed the one obtained by the less accurate visual method.

3.2. Cr(VI) removal in batch cultures

In all control experiments, cell free PYG supplemented with Cr(VI) showed non-significative changes in Cr(VI) concentrations during the 32 °C, 200 rpm and 3 days incubation. This is a confirmation of the inexistence of any possible abiotic Cr(VI) reduction, due to the presence of organic matter in the culture medium.

3.2.1. Pure cultures

Batch culture Cr(VI) removal was evaluated for each isolate, to make an adequate selection based on the maximum metal microbial elimination in growth conditions. Many bacterial strains detoxify Cr(VI) by reducing it to Cr(III) both aerobically and

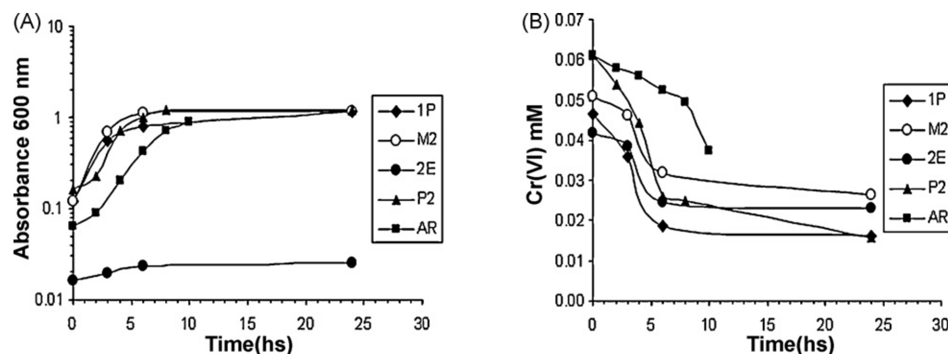


Fig. 2. Time course of Cr(VI) removal in pure batch cultures of *Pseudomonas veronii* 2E (2E), *Klebsiella ornithinolytica* 1P (1P), *Klebsiella oxytoca* P2 (P2), *Delftia acidovorans* AR (AR) and *Ralstonia taiwanensis* M2 (M2). (A) Bacterial growth evolution. (B) Cr(VI) concentration as function of incubation time.

anaerobically [20]. Cr(VI) affects the growth of microorganisms and interferes with nucleic acid synthesis, thus in order to grow in a medium containing this metal, bacteria have to detoxify it, action achieved by several mechanisms such as reduction, accumulation inside the bacterium or adsorption of Cr(VI) on its surface [21]. The reduction to Cr(III) may be related to the involvement of chromate reductase activity [22]. In all cases, culture pH was registered, being the initial culture pH between 6 and 7 and the final culture pH between 7 and 8 at the stationary phase of growth. This last value is theoretically adequate for Cr(III) removal as the insoluble $Cr(OH)_3$ (Ksp(25 °C) = 7×10^{-31} [http://bc.barnard.columbia.edu/~schapman/bc2001/handouts/Ksp.pdf]).

The duplication time for each isolate could be calculated from the exponential phase of growth, extracted from Fig. 2A, and it could be noticed as well that the cultures reached the stationary stage after 24 h at a Cr(VI) concentration of 0.05 mM.

The two isolates with the maximum growth were *K. oxytoca* P2 and *R. taiwanensis* M2, meanwhile *P. veronii* 2E presents the lowest growth of them all. This could be a consequence of the formation of numerous aggregates, which is an inconvenient to get an accurate absorbance value. With the values of the corresponding slopes at exponential phase of growth, the specific cell growth rate (μ) and duplication time (t_d) were calculated for each strain.

The fastest growth was detected in the *D. acidovorans* AR culture (1.0 h duplication time) and the lowest in *P. veronii* 2E (10 h duplication time) possibly being due to the particular way of aggregate growing. In all the cases all the cultures were started with the same number of initial cells. The exponential state was reached before the 6 h when growth began to stop until reaching a maximum value of absorbance near 1.00, corresponding to the stationary phase.

Along with the microbial growth a decrease of the concentration of Cr(VI) was detected as shown in Fig. 2B. These experimental facts suggest that the microorganisms could be using this metal as an electron acceptor in their metabolism.

The initial concentration of Cr(VI) was similar for all the pure cultures (0.05–0.06 mM) reaching final values between 0.015 and 0.035 mM. All the bacterial isolates showed relatively high disappearance percentages of this metal, being *K. oxytoca* P2 the most efficient one with a 74.32% Cr(VI) disappearance. In spite of the aggregates formed, *P. veronii* 2E showed an important decrease in the concentration of Cr(VI) (44.47%).

All the strains have a high capacity of Cr(VI) biotransformation using initial concentrations simulating the ones present in effluents. The final values are still slightly high compared to the accepted values so studies to determine the best conditions and combined elimination techniques are required.

3.2.2. Mixed cultures

The same data treatment was used for mixed cultures and the results obtained are shown in Fig. 3.

The microbial growth was similar for all the mixed cultures resulting *K. ornithinolytica* 1P + *K. oxytoca* P2 combination the fastest one (1.3 h duplication time). The exponential phase of growth was attained after 3 h while the stationary one is reached after 6 h. The pH variation detected went from an initial value of 6 to a final one of 8 in all the cultures. Again the microbial growth took place at the same time that the decrease in Cr(VI) concentration did (Fig. 3B).

The initial concentration in all cases was similar except in the first case (1P + 2E) but this could be because of operational errors caused by the presence of aggregates. The final concentration was between 0.015 and 0.020 mM showing an improved Cr(VI) biotransformation performance but as in the case of pure cultures the values are still a little high compared to accepted ones. The best combination resulted *K. ornithinolytica* 1P and *P. veronii* 2E with a 72.88% Cr(VI) removal.

3.3. Molecular analysis for the mixed culture composition studies

The 16S rDNA amplification was obtained in the section determined by F341-GC and R518 primers using PCR. Fig. 4 shows the results obtained for the DGGE developed for pure cultures and mixed cultures at 0 (t_0), 3 h (t_1), 6 h (t_2) and 24 h (t_3) of incubation time. Using DGGE, it was determined that only *P. veronii* 2E + *K. ornithinolytica* 1P could develop in a coculture (Fig. 4A). Both strains were present at any state of the batch culture while in all other combinations only one strain was able to grow, predominating over the other strains.

The mixed culture *P. veronii* 2E–*K. ornithinolytica* 1P demonstrated the capacity of developing together in mixed cultures. Since an improved Cr(VI) removal was achieved thanks to biotransformation by this coculture, this combination will be used in further studies in growing or non-growing conditions by chromate reductase activity analysis.

3.4. Total Cr quantification

Total chromium was quantified in all fractions of batch cultures. Total Cr concentration was determined in supernatants and cells

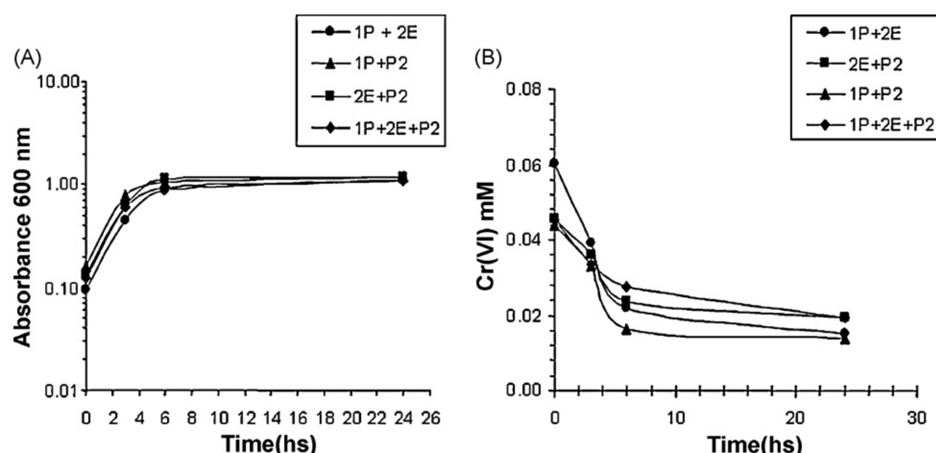


Fig. 3. Time course of Cr(VI) removal in mixed batch cultures of *Klebsiella ornithinolytica* 1P and *Pseudomonas veronii* 2E (1P + 2E); *K. ornithinolytica* 1P and *Klebsiella oxytoca* P2 (1P + P2); *K. oxytoca* P2 and *P. veronii* 2E (P2 + 2E); *K. ornithinolytica* 1P, *P. veronii* 2E and *K. oxytoca* P2 (1P + 2E + P2). (A) Bacterial growth evolution. (B) Cr(VI) concentration as function of incubation time.

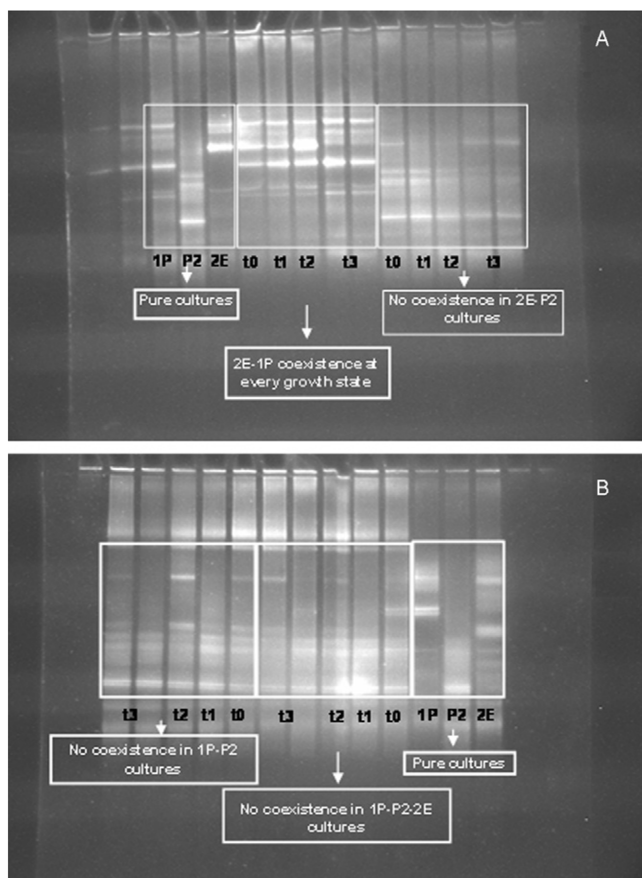


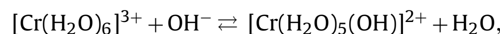
Fig. 4. Mixed culture composition analysis by Denaturating Gradient Gel Electrophoresis (DGGE). A. Starting left: Pure cultures (*K. ornithinolytica* 1P (1P), *K. oxytoca* P2 (P2) and *P. veronii* 2E (2E)), *K. ornithinolytica* 1P and *P. veronii* 2E coculture (1P + 2E) and *K. oxytoca* P2 and *P. veronii* 2E coculture (P2 + 2E). B. Starting left: *K. ornithinolytica* 1P and *K. oxytoca* P2 coculture (1P + P2), *K. ornithinolytica* 1P, *P. veronii* 2E and *K. oxytoca* P2 coculture (1P + 2E + P2) and pure cultures.

to establish the mass balance. Surprisingly, while Cr(VI) concentration decreased as shown in Fig. 2, total Cr remained at culture supernatants, only traces were found in cell fractions (Fig. 5).

The Cr(III) concentrations in the supernatants (shown in Fig. 5) are all very near the initial value of 0.05 mM, showing a quantitative reduction together with the fact of this ion remaining in solution in spite of the pH reached at the end of the experiments (between 7 and 8) that seems to be adequate for the precipitation of the hydroxide. The possible reactions between the extremely stable hexaaquochromium (III) complex and hydroxide ions and the corresponding values for the logarithm of its equilibrium constants are shown below.

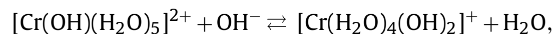
Cr(III) with NaOH 0.1 M:

(a) Precipitation steps:



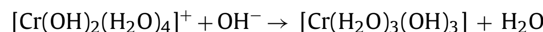
$$[Cr(H_2O)_6]^{3+} + OH^- \rightleftarrows [Cr(H_2O)_5(OH)]^{2+} + H_2O,$$

$log\,K(20\,°C) = 9.41$ [23, 24]



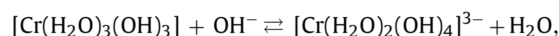
$$[Cr(OH)(H_2O)_5]^{2+} + OH^- \rightleftarrows [Cr(H_2O)_4(OH)_2]^{+} + H_2O,$$

$log\,K(20\,°C) = 17.30$ [23, 24]



$$[Cr(OH)_2(H_2O)_4]^{+} + OH^- \rightarrow [Cr(H_2O)_3(OH)_3] + H_2O$$

(b) Precipitate redissolution:



$$[Cr(H_2O)_3(OH)_3] + OH^- \rightleftarrows [Cr(H_2O)_2(OH)_4]^{3-} + H_2O,$$

$log\,K(20\,°C) = 29.90$ [24, 25]

Considering a Cr(III) concentration around 0.045 mM, the corresponding Ksp (solubility product constant) value for the chromium (III) hydroxide at 298 K ($Cr(OH)_3$, 7×10^{-31}) and the water ionization equilibrium, the exact pH for the beginning of precipitation must be 5.4. Due to the amphoteric character of the chromium (III) hydroxide, several coordination complexes between the metal cation and hydroxide ions are possible [23–25] in basic solutions. Only a limited number of literature studies were done to study the speciation and solubility of this hydroxide in several mixtures. Based on these results, different alternatives for the complete flocculation of the cation were studied.

3.5. Cr(III) chemical flocculation

In spite of the success obtained for the bacterial growth of all the strains in the presence of Cr(VI) and the high percentages of concentration decrease of this ion in the analyzed cultures, the Cr(III) produced by the reduction remains in solution. For the biotreatment to be complete, the total precipitation of Cr(III) has to be achieved. The final pH was theoretically adequate for the formation of the hydroxide but due to the extremely low concentration of the cation, this process is not detected in the isolated supernatants of the cultures. Different precipitation agents were studied using standard solutions of Cr(III), resulting the sodium carbonate solution the most effective one considering not only the amount

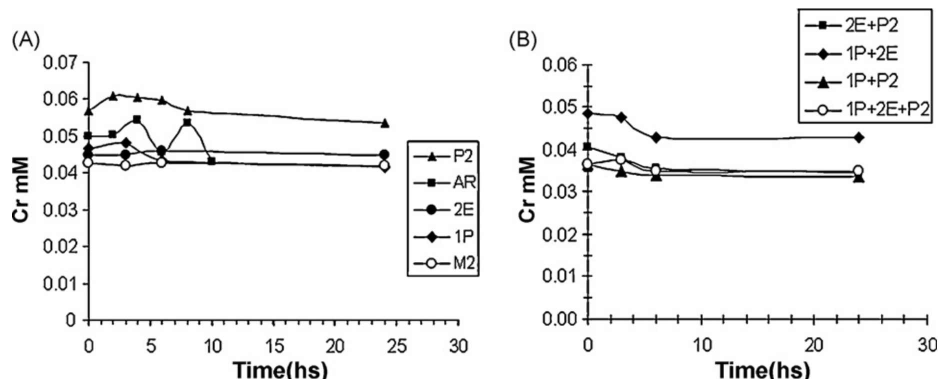


Fig. 5. Total chromium concentration quantification in culture supernatants as function of incubation time of: (A) pure cultures of *P. veronii* 2E (2E), *K. ornithinolytica* 1P (1P), *K. oxytoca* P2 (P2), *D. acidovorans* AR (AR) and *R. taiwanensis* M2 (M2) and (B) mixed cultures of *K. ornithinolytica* 1P and *P. veronii* 2E (1P + 2E); *K. ornithinolytica* 1P and *K. oxytoca* P2 (1P + P2); *K. oxytoca* P2 and *P. veronii* 2E (P2 + 2E); *K. ornithinolytica* 1P, *P. veronii* 2E and *K. oxytoca* P2 (1P + 2E + P2).

Table 2
Cr(III) precipitation with Na_2CO_3 0.1 M at different molar ratios.

Initial Cr(III) solution (mol)	Na_2CO_3 (0.1 M) (mL)	Molar ratio $Cr^{3+}:CO_3^{2-}$	$Cr(OH)_3$ (mol)	$Cr(OH)_3(H_2O)_3$ (mol)
9.58×10^{-5}	1.5	1.00:1.56	1.23×10^{-4}	8.09×10^{-5}
	2.0	1.00:2.08	1.33×10^{-4}	8.73×10^{-5}
	2.5	1.00:2.61	1.24×10^{-4}	8.15×10^{-5}
9.62×10^{-5}	1.5	1.00:1.56	1.14×10^{-4}	7.77×10^{-5}
	2.0	1.00:2.08	1.45×10^{-4}	9.49×10^{-5}
	2.5	1.00:2.61	1.34×10^{-4}	8.71×10^{-5}

Table 3
Cr(III) precipitation with commercial flocculants (COAG-C and FLOX-A).

Initial Cr(III) solution (mol)	Flocculated Cr(III)	
	$Cr(OH)_3$ (mol)	$Cr(OH)_3(H_2O)_3$ (mol)
9.58×10^{-5}	1.48×10^{-4}	9.68×10^{-5}
	1.64×10^{-4}	1.08×10^{-5}

of precipitate formed but also the complete coagulation of the jelly like product obtained. The results, taking into account the possible chemical composition of the precipitate, are shown in Table 2.

Considering the two possible identities for the precipitate, the results were quantitative for both standards being the best ones a result of using a molar relationship cation:base of 1:2 and not the expected 1:3 for the hydroxide. Based on these results the exact pH of the mixture seemed to be more important than the molar relation between the reagents. Commercial flocculating products were used and the results are shown in Table 3. In this case the identity of the precipitate obtained is unknown due to the characteristics of the coagulant and flocculant used; if the same compositions of the product are considered, this process was quantitative as well. Preliminary experiments showed that the initial concentration of the cation is very important in order to be able to isolate the flocculated product because of its jelly consistency and exact pH of redissolution.

4. Conclusions

The five autochthonous strains present at least one tolerance/resistance mechanism because all of them are able to grow and develop in the presence of Cr(VI).The maximum decreases in Cr(VI) concentration were produced by *K. oxytoca* P2 (74.32%) in pure cultures and the combination *K. ornithinolytica* 1P, *P. veronii* 2E (72.88%) in mixed cultures. The performance of these two microorganisms is better in mixed than in the individual cultures (65.01% for *K. ornithinolytica* 1P, and 44.47% for *P. veronii* 2E) showing a synergetic effect together (72.88%).

The nucleic acid studies show that the only two microorganisms which are able to grow together in all the stages are the two last mentioned but it could not be established yet a correlation between this fact and the best percentage Cr(VI) disappearance found.All the strains showed a good Cr(VI) biotransformation efficiency but to decrease the final metal values in the treated effluents a combination of techniques must be used. In order to achieve this goal, further studies using pure and the best mixed cultures, in growing or non-growing conditions, combined with flocculation steps, should be developed.

Acknowledgements

This work was supported by the Universidad Nacional de General Sarmiento and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICTO-UNGS 36782-341).

We are grateful to Dr. María Carolina Martínez (Instituto de Biotecnología, INTA, Castelar) for DNA quantification and Dr. Marcela Ferrero (PROIMI-CONICET, Tucumán, Argentina) for the 16S r-RNA identification of P2 strain and her help in DGGE experiments. Lic. Eduardo Reciulschi provided the chromium (III) salt for the standard preparation and the commercial flocculants. Thanks to Lic. Aldo Colautti (Centro de Desarrollo Analítico, Buenos Aires, Argentina) for total chromium determinations and to Miss Leticia Rossi for the English language revision.

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