ARTÍCULO DE INVESTIGACIÓN

Hexavalent chromium-reducing bacteria on biosolids from the San Fernando Wastewater Treatment Plant in Medellín (Colombia)

Bacterias aisladas de biosólidos de la PTAR San Fernando en Medellín-Colombia con capacidad para reducir cromo hexavalente

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RESUMEN

En las últimas décadas se ha trabajado activamente para reducir el impacto ambiental generado por las actividades antrópicas que constantemente liberan componentes tóxicos al ambiente generando inestabilidad y daños en la salud de las comunidades biológicas. Entre los diferentes contaminantes, los metales pesados revisten importancia en virtud de sus propiedades, que dificultan su degradación o transformación en otros compuestos menos tóxicos. El cromo es uno de los metales de mayor interés a nivel global por su uso en múltiples industrias. Los métodos convencionales que utilizan materiales cromados en sus procesos, no sólo arrojan cantidades considerables de residuos al ambiente, sino que dan poca cuenta de la fracción de Cr^{6+} presente en determinados ecosistemas. La biorremediación se ha propuesto como una alternativa económicamente viable y ambientalmente sostenible. El propósito del presente trabajo fue evaluar la capacidad de reducción de cromo por bacterias, aisladas de una matriz de biosólidos de la Planta de tratamiento de aguas residuales (PTAR) San Fernando en la ciudad de Medellín-Colombia. Muestras de biosólidos se cultivaron en Agar Nutritivo enriquecido con diferentes concentraciones de Cr^{6+}. Las cepas que presentaron mayor tolerancia al cromo fueron aisladas para realizar ensayos de reducción por triplicado, monitoreando la concentración del metal en el tiempo. Se obtuvieron siete especies bacterianas diferentes dentro de las cuales se destacaron *Staphylococcus saprophyticus*, *Ochrobactrum anthropi* y *Bacillus cereus* por la capacidad de reducir Cr^{6+} a 96 h con eficiencias de 29.0%, 61.1% y 100%, respectivamente.

Palabras clave: Biorremediación, Reducción, Bacterias, Biosólido, Metales Pesados, Cromo hexavalente.

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ABSTRACT

During the most recent decades, advances have been made to reduce the environmental impact by anthropogenic activities that constantly release toxic components into the environment, generating instability and damage to the health of biological communities. Among the different pollutants, heavy metals are important by virtue of their properties, which hinder their degradation or transformation into other less toxic compounds. Chromium is one of the metals of greatest global interest due to its use in multiple industries. Conventional methods using chromed materials in their processes, not only throw considerable amounts of waste into the environment, but also give little account of the fraction of hexavalent chromium (Cr^{6+}) present in certain ecosystems. Bioremediation has been proposed as an economically viable and environmentally sustainable alternative. This work aimed to evaluate the chromium reduction capacity by bacteria isolated from a biosolids matrix obtained at the San Fernando Wastewater Treatment Plant (WWTP), located in Medellín (Colombia). Biosolids samples were grown in a nutrient agar enriched with different concentrations of Cr^{6+}. The strains presenting the greater tolerance to chromium were isolated to perform reduction tests by triplicate, monitoring the concentration of the metal over time. Seven different bacterial species were obtained, among which *Staphylococcus saprophyticus*, *Ochrobactrum anthropic*, and *Bacillus cereus* showed the greatest ability to reduce Cr^{6+} (29.0%, 61.1 and 100%, at 96 h) respectively.

Keywords: bacteria, bioremediation, biosolids, chromium, heavy metals, hexavalent, reduction.

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INTRODUCTION

Chromium is a heavy metal that can be found in six oxidation states in nature, being the Cr^{+3} and Cr^{+6} species the most stable ones, known as trivalent (Cr^{3+}) and hexavalent (Cr^{6+}). The Cr^{3+} form is considered essential and is involved in the metabolism of glucose and insulin, regulating cholesterol and triglyceride levels (Higdon, Drake, and Delage, 2003). However, some studies indicate that, under certain conditions, Cr^{3+} can cause genomic instability and it has been suggested that it is not essential, since it does not participate in the structural stabilization of enzymes or in nutrient uptake (Eastmond, MacGregor, and Slesinski, 2008; Wise and Wise, 2012). From the chemical point of view, Cr^{3+} is poorly soluble and of low mobility when it is complexed with the organic matter of the soil (Gutiérrez Corona *et al*., 2010). The Cr^{6+} form is mobile, soluble, and permeable through the cell membrane, causing DNA and some protein damage, therefore considered as mutagenic, teratogenic, and carcinogenic. In addition, it is reported to cause skin damage when a direct contact is presented, to induce respiratory tract cancer when inhaled, and if ingested, it can cause stomach ulcers with complications that could lead to death (Horel, 2017; Nayak, 2017).

Different studies have been carried out on bacteria isolated from chromium-contaminated matrices in order to stabilize or capture the metal. *Bacillus cereus* is a cosmopolitan bacterium of environmental interest and has been investigated in the health and food industries (Banerjee and Ghoshal, 2016; Iranzo, *et al*., 2018; Sánchez, *et al*., 2016). This bacterial species has been reported to present the ability to reduce Cr^{6+}. In 2009, in Medellín (Colombia) *B. cereus* was obtained (among other bacteria of the *Pseudomonas* genus) from runoff biofilms in a tannery industry. It was found that it reduced Cr^{6+} by 99.8% to an initial concentration of 28 ppm in 100 h (Martínez Yepes, 2009). In 2016, *B. cereus* was isolated from electroplating wastewater in Cali (Colombia), in a concentration of 10 ppm of Cr^{6+}, accomplishing a reduction of 100% in 10 h (Mora Collazos, 2016). This same bacterium has been also reported to be tolerant to variations in pH, temperature, salinity, and other conditions, which could support its use in the bioremediation field, due to its capacity for resilience and adaptation to adverse conditions (Singh, *et al*., 2013).

The *Ochrobactrum anthropi* has been identified as a nosocomial pathogen, causing infections that are difficult to treat, due to its resistance to most antibiotic principles (Chudasama and Thaker, 2017; Haviari, *et al*., 2016; Henderson, *et al*., 2016). However, its potential in bioremediation has exceeded expectations, attesting its ability to metabolize different aromatic compounds and hydrocarbons, as a source of carbon and energy (Chudasama and Thaker, 2017). It has also been reported as an alternative in fuel-related research, due to its ability to produce biosurfactants (Ibrahim, 2011). Some authors report reductions of Cr^{6+} in concentrations of 200 ppm, from 95 to 100% in 24 h on a dead biomass (Cheng, *et al*., 2010; Francisco, *et al*., 2002).

The *Staphylococcus saprophyticus* has been reported in clinical cases in humans mainly associated to urinary tract infections, with few studies in the environmental area. However, it has the capacity to tolerate Cr^{6+} in concentrations up to 3,000 ppm (Alekhya and Subbaiah, 2016), without reducing it.

This work aimed to evaluate the chromium reduction capacity by bacteria isolated from a biosolids matrix ob-

tained at the San Fernando Wastewater Treatment Plant (WWTP), located in Medellín (Colombia).

Materials and methods

Isolation of bacteria from biosolids

Biosolids samples were randomly collected from the dehydrators of the San Fernando WWTP, located on the South of the city of Medellín, Province of Antioquia (Colombia). A physicochemical characterization of the samples was performed. The concentration of heavy metals was determined by the atomic emission method —using Inductively Coupled Plasma (ICP), and the cold vapor method was used to determine chromium. The critical values —regulated by the Decree #1287 of 2014 of the Ministry of Housing, City, and Territory of Colombia (in Spanish, *Ministerio de Vivienda Ciudad y Territorio*), were considered as a reference (1,200 mg K^{-1}). Eight aliquots were randomly collected (for a total of 10 g of wet base) and suspended in 100 ml of 0.1% w/v sterile peptone water for 15 min at 15 psi and pH of 5.5. This suspension was stirred for 1 h, and then, 100 µL were used for culture on a nutritive agar enriched with Cr^{6+} ($K_2Cr_2O_7$) at 400, 500, 600, 800, and 1000 ppm, and three replications each, using the surface exhaustion method. Culture media were incubated at 35°C for 7 days (US-EPA, 1992). Bacterial colonies were obtained from a nutrient agar under the same incubation conditions. The morphological of the isolates was based on their shape, size, consistency, brilliance, translucency, among others features. Isolates were then cryopreserved at -20°C on a nutrient broth with 20% v/v glycerol.

Determination of the bacterial capacity to reduce Cr^{6+}

The cryopreserved isolates were directly activated on a nutrient agar and incubated at 37°C for 40 h. Subsequently, three colonies were transferred to a Luria Bertani (LB) broth, enriched with 100 ppm of Cr^{6+}, and then incubated at 37°C for 48 h. Aliquots of 1.5 mL were taken at 0, 24, and 48 h of culture. The samples were centrifuged at 10,000 centrifugal force (g) for 20 min, and the supernatant was separated and refrigerated at 4°C to perform the chromium measurements at the end of the assay. Simultaneously, one the samples was incubated for 96 h following the same protocol detailed above. The final Cr^{6+} concentration was determined by the diphenylcarbazide colorimetric method, following EPA protocol 7196A. From a stock of 100 ppm of Cr^{6+}, serial dilutions were made until a concentration of 1 ppm was reached. The volume of the EPA 7196A method reaction mixture was changed to 9.5 mL of diluted sample, 0.1 mL of 1N sulfuric acid, with 0.2 mL of a diphenylcarbazide solution (250 mg of 1.5 diphenylcarbazide in 50 mL of acetone) and completed with distilled water to a final volume of 10 mL. Ten minutes later, the absorbance was determined at a wavelength of 540 nm. For the estimation of the Cr^{6+} concentrations of the samples, a calibration curve was prepared using 0.25, 0.5, 1, and 2-ppm concentration solutions (US EPA, OSWER, ORCR, 1992).

Total chromium measurement

Samples collected at 0, 24, 48, and 96 h, with Cr^{6+} concentrations of 100 ppm diluted until 1 ppm, and a pH reduced to a value of 1.0 (with nitric acid 0.1 mL at 65%, v/v), were subjected to total chromium measurement, using an Agilent Technologies 4100 MP-AES atomic emission equipment. Data processing was carried out with the MP Expert program (vers.1.5.1.6821).

Determination of the microbial growth curve in the presence of Cr^{6+}

A growth curve was performed by spectrophotometry. The LB-bacteria were inoculated on a 96-well ELISA dish containing 100 ppm of Cr^{6+} for 48 h at 37°C. For the identification of the bacterial isolates, the absorbance at 600 nm was determined every hour (after shaking for 30 seconds), using a Multiskan go TM spectrophotometer, Thermo SCIENTIFIC.

Identification of bacterial isolates

The isolates obtained were biochemically identified using the Biolog Microstation ID System OmniLog®, this system provides 96 microplates which have different means for the biochemical characterization of each strain, the appendix 1- 4 show the biochemical rection made in each well in the microplate; the equipment provides a specific kit for the bacteria cultivation and distribution among the wells. The bacteria were cultivated in the BUG agar, after 24 h and distributed in the 96 wells equally and finally taken to the equipment to do the measures, the results for the bacteria grow and the biochemical reactions it takes 24 h.

Statistical analysis

Differences on the ability of the bacterial strains to reduce Cr^{6+} at different concentrations were estimated. Normality and homogeneity estimations were performed using the Shapiro Wilk (Chacon Montalvan, 2014), and Bartlett tests (Mellado, 2013), respectively. Then, a one-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the means (Sokal and Rohlf, 1995). Finally, treatment averages were analyzed by the Tukey's honestly significant difference (HDS) and multiple comparisons tests, using the R-student software (R-core team 2017).

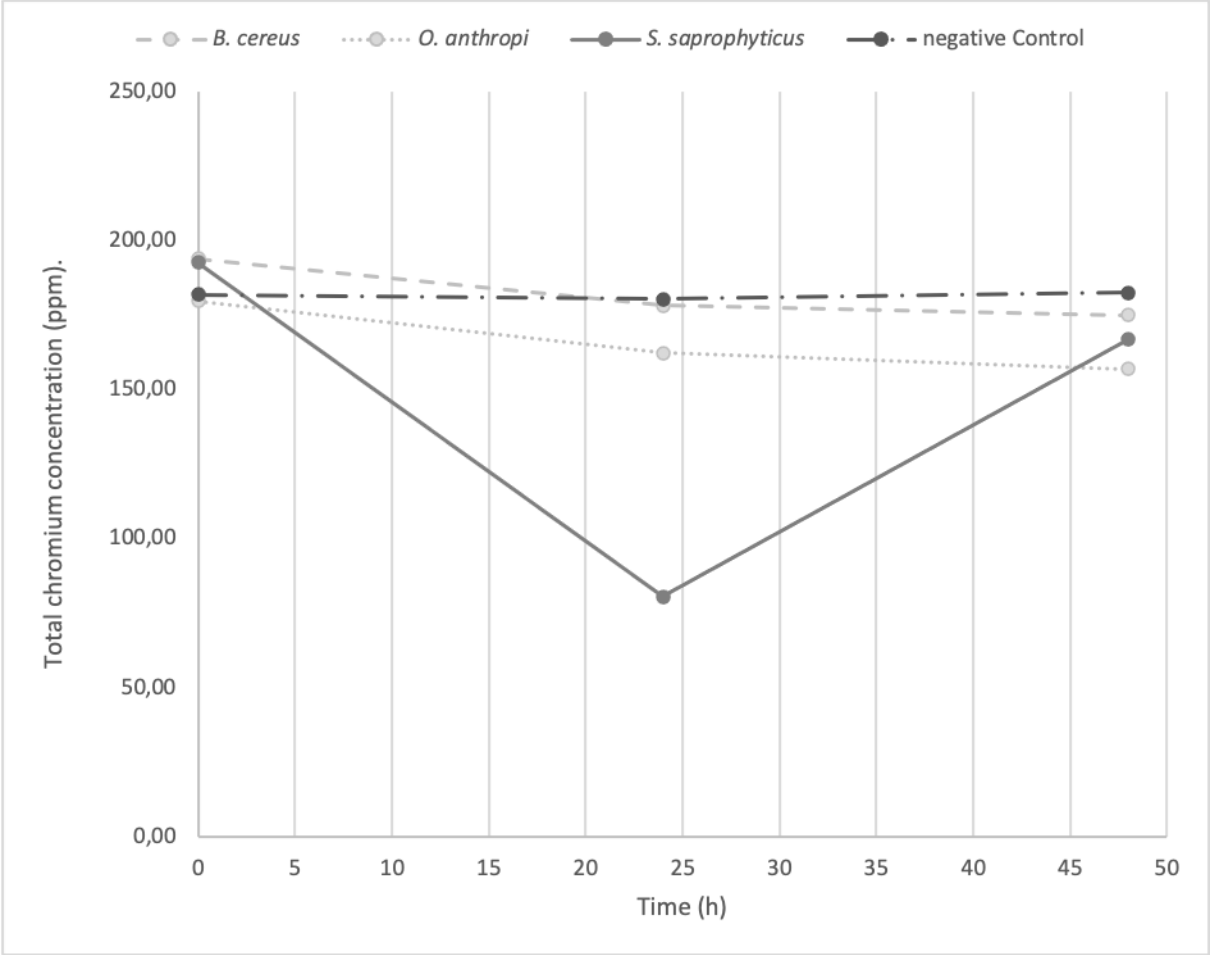

Figure 1. Total chromium reduction kinetics at 48 h, pH 5.5 by *Ochrobactrum anthropi*, *Staphylococcus saprophyticus*, *Bacillus cereus* species and Negative Control. Three replications each, obtained from biosolids of the San Fernando Wastewater Treatment Plant (WWTP), in Medellín (Colombia)

RESULTS

Bacterial isolates and Bacterial identification

Thirty-two bacterial biotypes, from seven different species, were obtained from media with concentrations of 400 to 600 ppm of Cr^{6+}). The three strains of greatest interest were *O. anthropic*, *B. cereus* and *S. saprophyticus*. In addition, four other strains of bacilli were found with some interest to remove chromium, *Bacillus megaterium, Bacillus subtilis, Bacillus firmus, Bacillus lentus.*

The morphological identification of the colonies allowed the separation of microorganisms such as Gram positive and Gram-negative, bacilli and cocci. *S. saprophyticus* is a creamy, rounded, yellow and smooth-edged spore, Gram positive cocci. *B. cereus* is a Gram-positive bacillus with round, white rough, dry consistency spores. *O. anthropic* is a Gram-negative bacillus, round and smooth, with a juicy texture and a translucent white color. The characterization was carried out with Biolog Microstation ID System OmniLog®. The values show each biochemical reaction and the isolated name with its respective probability.

Hexavalent chromium-reduction assays

The *O. antrhopi* was the most tolerant to the metal, showing growth in concentrations of 300 to 600 ppm of Cr^{6+}, and presenting the highest reduction (63% at 48 h), followed by *B. cereus* and *S. saprophyticus* with 62% and 49%, respectively. Figure 1 shows the bacterial behavior on the reduction of total chromium during the test carried out during 48 h (without significant changes for the three bacterial species of the study). During the first 24 h, the curve reached its lowest point, obtaining a significant decrease of 58.2% on the total chromium. Figures 2 and 3 show the Cr^{6+} reduction kinetics of the three mentioned bacterial strains and a negative control, during 48 and 96 h, respectively.

Growth in the presence of Cr^{6+}

Figure 4 shows the bacterial growth in cultures enriched with Cr^{6+}. A higher absorbance reflects a greater growth

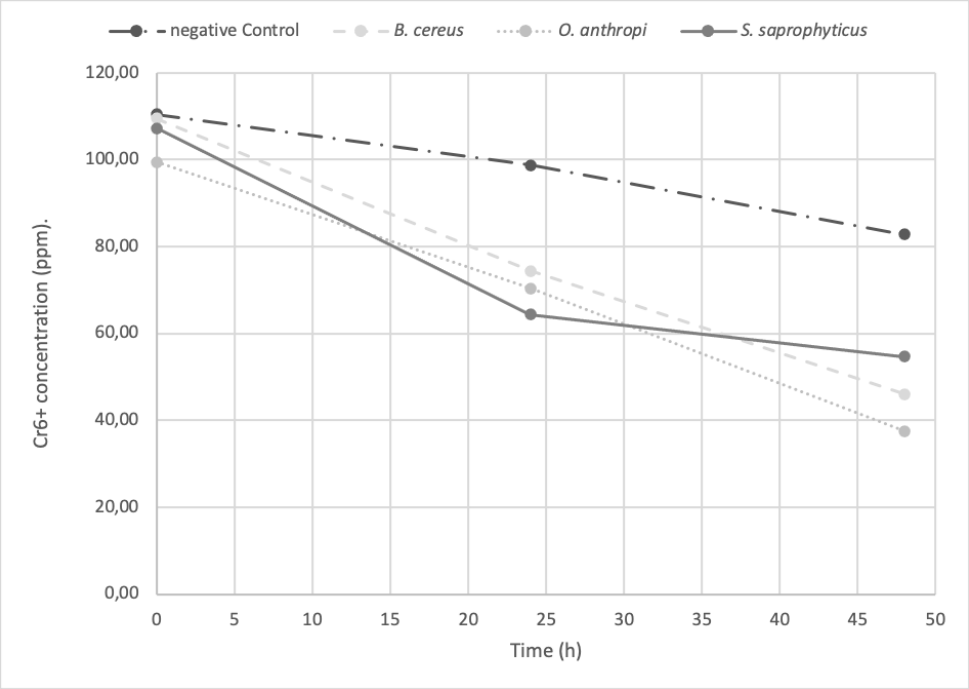

Figure 2. The Cr^{6+} reduction kinetics at 48 h, pH 5.5 by *Ochrobactrum anthropi*, *Staphylococcus saprophyticus*, *Bacillus cereus* species and Negative Control. Three replications each, obtained from biosolids of the San Fernando Wastewater Treatment Plant (WWTP), in Medellín (Colombia).

Table 1 Cr^{6+} reduction (%) from 0 to 96 h of culture caused by the three bacterial species in the test.

	Times				
	0 h	24 h	48 h	72 h	96 h
Species	Reduction %*	Reduction %*	Reduction %*	Reduction %*	Reduction %*
Staphylococcus saprophyticus	0^{a}	39.9^{bc}	49.0^{b}	47.9^{b}	29.0^{b}
Ochrobactrum anthropi	0^{a}	22.6^{ab}	57.9^{b}	59.2^{c}	61.1^{c}
Bacillus cereus	0^{a}	31.9^{c}	62.3^{c}	100^{d}	100^{d}
Negative control	0^{a}	7.4^{a}	3.5^{a}	8.9^{a}	12.7^{a}

*The same letter in the same column indicates no significant difference between levels. Different letters indicate significant differences between groups (according to the Tukey´s HDS test).

of the colonies as a function of time. At 48 h, *B. cereus* and *O. anthropi* did not completed their exponential growth phase, while *S. saprophyticus* reached it around 24 h later.

Statistical analysis

Normal and homogeneous results were obtained [W = 0.82641 (p = 0.01901); Chi square = [15.318 (p = 0.001564)]. Table 1 presents a summary of the behavior and the percentage reduction of Cr^{6+} from 0 to 96 h. For the initial time (0), all the treatments were equal; therefore, there were no differences between them. At 24 h, differences were observed, although there was a tendency to present common results among microorganisms. At 48 h, the only strain that showed a significant difference was *B. cereus*; however, at 72 and 96 h, all strains showed different reduction levels of Cr^{6+}.

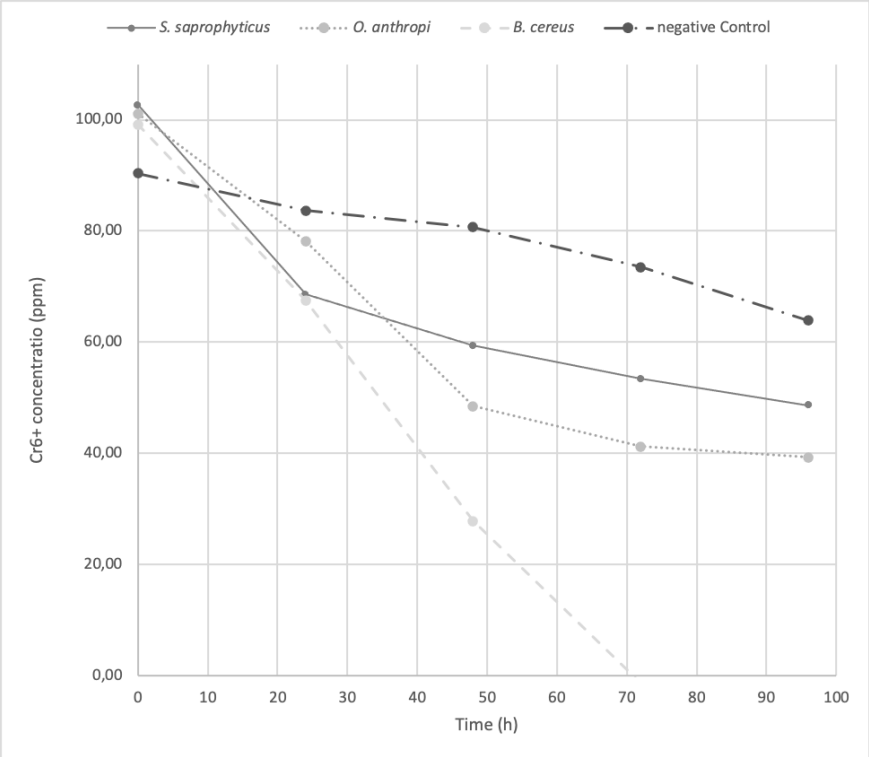

Figure 3. The Cr^{6+} reduction kinetics at 96 h, pH 5.5 by *Ochrobactrum anthropi*, *Staphylococcus saprophyticus*, *Bacillus cereus* species and Negative Control. Three replications each, obtained from biosolids of the San Fernando Wastewater Treatment Plant (WWTP), in Medellín (Colombia).

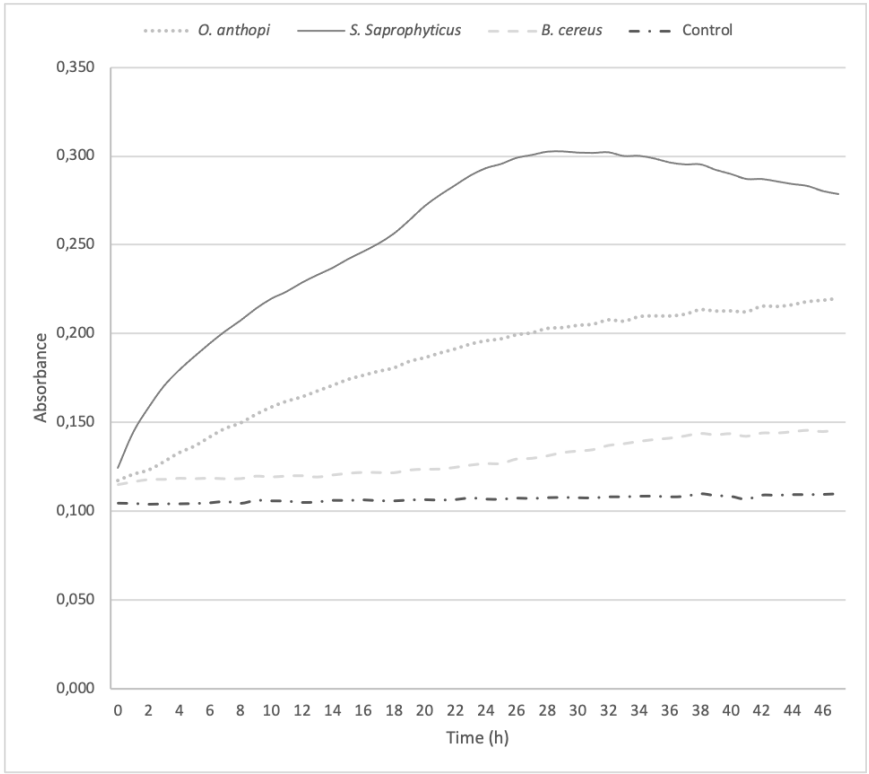

Figure 4. Bacterial growth of *Ochrobactrum anthropi*, *Staphylococcus saprophyticus*, *Bacillus cereus* species, and Negative Control, cultured in a Cr^{6+}enriched media. Three replications each, obtained from biosolids of the San Fernando Wastewater Treatment Plant (WWTP), in Medellín.

DISCUSSION

In the present study, different Cr^{6+}tolerant bacteria (in concentrations of 100 to 600 ppm) were isolated under standard aerobic culture conditions, in a nutrient medium enriched with this metal. Among the isolates obtained, *O. anthropic, B. cereus,* and *S. saprophyticus* showed the greatest ability to reduce and tolerate Cr^{6+}.

Treatments on *O. anthropi* and *B. cereus* did not achieve the maximum reduction of Cr^{6+} at 48 h, as recorded by the growth curve of these two strains (Figure 4), since both strains were in their exponential phase at that time. Actually, as *O. anthropi* continued its exponential phase at 48 h, *B. cereus* was just starting this phase. This is the reason why it was considered to extend the chromium reduction curve up to 96 h (Figure 3). The *S. saprophyticus* strain showed a different behavior, since it completed its exponential growth phase in the first 24 h, reaching its stationary phase at 48 h and finally yielding cell death. Nevertheless, significant changes in the decrease of total chromium were not observed, remaining stable over time for all the strains. In all three cases, the growth behavior at 48 h was directly related to the reduction of the metal. Como podría explicarse esta observación, qué indicaría, que implicaciones tiene?

Ochrobactrum anthropi presents high resistance to most families of antibiotics (Haviari, *et al*., 2014; Henderson, *et al*., 2017). However, studies on its potential in bioremediation exceed expectations, as its ability to reduce different aromatic compounds and hydrocarbons in order to use them as a source of carbon and energy has been proven (Chudasama and Thaker, 2017). Its utility in the alternative fuels field has been also reported, due to its ability to produce biosurfactants (Ibrahim, 2011). Previous studies have found that this bacterium can reduce Cr^{6+} in concentrations of up to 200 ppm of chromium in a 24 h period from 95 to 100%, using their dead biomass (Cheng, et al., 2011; Francisco, *et al*., 2002). To the date, no reports on the use of the live biomass of this bacterium to reduce the metal are available, and, although in the present study the Cr^{6+} reduction was of 61% at 96 h, the reduced chromium concentration remained stable over time.

Bacillus cereus was the only strain that presented a total reduction of Cr^{6+} at 72 h (Figure 3). During this time, the hexavalent chromium became trivalent, remaining so until the end of the test; however, there was no decrease of the total chromium since the reduction percentage at 96 h was only 7%. This result reflects a clearly reducing behavior of the bacillus. Other authors have reported *B. cereus* as a cosmopolitan bacterium, used in bioremediation in the environmental research field, as well as in the health and food industry (Iranzo, 2018; Sánchez, *et al*., 2016; Banerjee and Ghoshal, 2017). Among the isolated bacteria, *B. cereus* showed the highest Cr^{6+} reducing capacity. In 2009, *B. cereus* was isolated from the runoff biofilm in a tannery industry in Medellín (Colombia). The strain reduced Cr^{6+} by 99.8% from an initial concentration of 28 ppm, over 100 h (Martínez Yepes, 2009). A study made by Mora Collazos, *et al*. (2016), reported the isolation of a *B. cereus* strain from wastewater in an electroplating company, with an initial concentration of 10 ppm of Cr^{6+}, achieving a reduction of chromium of 100% in 10 h. Recent studies found an increase in the ability of *B. cereus* in the presence of Mn (II) and Mg (II) to reduce and remove hexavalent chromium to levels of 64% in 120 h. (Xu, *et al*., 2011)

Comparing the results obtained in this study, *B. cereus* showed a greater tolerance to the metal, growing at concentrations of up to 300 ppm of Cr^{6+} and achieving a reduction of the same in concentrations higher than those reported in the aforementioned studies. Other authors have reported that this species requires approximately 20 h to start growing in a culture medium enriched with more than 100 ppm of Cr^{6+} (Singh, *et al*., 2013). Figure 4 confirms that growth starts after 20 h in a culture with 100 ppm of Cr^{6+}. Likewise, once growth begins, the chromium reduction rate accelerates, achieving reductions of Cr^{6+} to Cr^{3+} of 100% at 72 h in a shake culture. *Bacillus cereus* has been also reported as tolerant to variations in pH, temperature, salinity, and other conditions, which supports its use in the field as bioremediation due to its adaptability (Singh, *et al*., 2013).

Staphylococcus saprophyticus is an environmental bacterium, but has been also reported in clinical cases, mainly associated with urinary tract infections. It has the ability to tolerate Cr^{6+} in concentrations up to 3,000 ppm but without the ability to reduce it (Iyengar and Subbaiah Usha, 2016). The isolated strain reached its maximum reduction of Cr^{6+} from 39.9% at 24 h to 49. % at 48 h (Figure 2). The total chromium concentration over time showed an interesting behavior, since at 24 h the concentration decreased to 58.2%, but at the end of the test (48 h) the metal capture dropped to 13.5%, suggesting release of the initially captured metal.

Tahri, *et al*. (2011), reported that a microbiological cell presents different mechanisms to reduce Cr^{6+} to Cr^{3+}, including the extracellular ability to reduce Cr^{6+} to Cr^{3+} using functional groups present on the cell surface; reduction in the cell membrane, usually preceded by the adsorption of Cr^{6+} to functional groups located on the bacterial cell surface; intracellular reduction of Cr^{6+}, that when reduced to Cr^{3+}, is released from the cell, then

conserving a low cytoplasmic concentration of Cr^{6+}, also facilitating the accumulation of chromate from the extracellular medium into the cell. (Appendix 5).

In the present study, the strain of *S. saprophyticus* captured, reduced, and then released the metal to the external medium.

Other species isolated during the research belong to the group of sporulated bacilli such as *B. megaterium, B. subtilis, B. firmus*, and *B. lentus*, but they did not present significant reductions of Cr^{6+} in comparison to the three strains already mentioned. However, literature report these bacteria as tolerant and Cr^{6+} reducers, as in the case of *B. subtilis* and *B. megaterium* isolated from wastewater associated with tanneries (Pan, *et al*., 2014; Martínez Yepes, 2009). In this specific report, these bacteria presented values between 22 and 35% of Cr^{6+} reduction, although, in the aforementioned reports the reductions exceeded these values.

CONCLUSION

From biosolids of a WWTP, different species of bacteria of interest in chromium bioremediation were isolated, among other microorganisms such as fungi and yeasts. Isolated bacteria showed high adaptability and ability to reduce Cr^{6+}. The *O. anthropic* strain showed the greatest tolerance and Cr^{6+} reduction capacity. *S. saprophyticus* is a bacterium with the capacity to capture chromium and retain it for a considerable time, allowing its possible use in the design of biofilters for the purpose of co-remediation of waters contaminated with this heavy metal.

Bacillus cereus was the only strain capable of reducing the metal by 100% from its hexavalent form to the trivalent one, indicating its biotechnological potential and the possibility of being considered for environmental bioremediation programs. New concentrations of Cr^{6+} should be evaluated to observe their behavior and tolerance thresholds.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest with the information contained in this article.

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GEN III MicroPlate ™

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D-Cellobiose	A6 Gentiobiose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 pH 6	A12 pH 5
B1 D-Raffinose	B2 α-D-Lactose	B3 D-Melibiose	B4 β-Methyl-D-Glucoside	B5 D-Salicin	B6 N-Acetyl-D-Glucosamine	B7 N-Acetyl-β-D-Mannosamine	B8 N-Acetyl-D-Galactosamine	B9 N-Acetyl Neuraminic Acid	B10 1% NaCl	B11 4% NaCl	B12 8% NaCl
C1 α-D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic Acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 myo-Inositol	D5 Glycerol	D6 D-Glucose-6-PO4	D7 D-Fructose-6-PO4	D8 D-Aspartic Acid	D9 D-Serine	D10 Troleandomycin	D11 Rifamycin SV	D12 Minocycline
E1 Gelatin	E2 Glycyl-L-Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic Acid	E6 L-Glutamic Acid	E7 L-Histidine	E8 L-Pyroglutamic Acid	E9 L-Serine	E10 Lincomycin	E11 Guanidine HCl	E12 Niaproof 4
F1 Pectin	F2 D-Galacturonic Acid	F3 L-Galactonic Acid Lactone	F4 D-Gluconic Acid	F5 D-Glucuronic Acid	F6 Glucuronamide	F7 Mucic Acid	F8 Quinic Acid	F9 D-Saccharic Acid	F10 Vancomycin	F11 Tetrazolium Violet	F12 Tetrazolium Blue
G1 p-Hydroxy-Phenylacetic Acid	G2 Methyl Pyruvate	G3 D-Lactic Acid Methyl Ester	G4 L-Lactic Acid	G5 Citric Acid	G6 α-Keto-Glutaric Acid	G7 D-Malic Acid	G8 L-Malic Acid	G9 Bromo-Succinic Acid	G10 Nalidixic Acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween 40	H2 γ-Amino-Butyric Acid	H3 α-Hydroxy-Butyric Acid	H4 β-Hydroxy-D,L-Butyric Acid	H5 α-Keto-Butyric Acid	H6 Acetoacetic Acid	H7 Propionic Acid	H8 Acetic Acid	H9 Formic Acid	H10 Aztreonam	H11 Sodium Butyrate	H12 Sodium Bromate

Appendix 1. Biochemical distribution of wells microplate BioLog microorganism identification.

Program	MicroLog 3/5.2.01 35
Project	ML5
File Name	Bacterias 2.D5E
User	biolog1
Instrument	MicroStation 2 Reader
Instrument S/N	1403197
Incubation Hours	29.00
Plate Number	1
Plate Type	GEN III
Protocol	A
Usuario	Lilly
Codigo muestra	4003
Origen	Biosolidos
Proyecto	
Lugar	
Date & Time of Read	Mar 24 2017 3:56 PM
Biolog ID DB	GEN-III_2.7.1.40.I5G

Result	Species ID: Ochrobactrum anthropi
Comment	
Notice	

Rank	PROB	SIM	DIST	Organism Type	Species
1	0.920	0.641	4.384	GN-Nent	Ochrobactrum anthropi
2	0.058	0.034	6.156	GN-Nent	Ochrobactrum intermedium
3	0.018	0.009	6.920	GN-Nent	Ochrobactrum tritici
4	0.004	0.002	7.833	GN-Nent	Ochrobactrum grignonense

Key: <x: positive, x: negative, <x+: mismatched positive, x+: mismatched negative, {x: borderline, -x: less than A1 well

Well Color Values

Plate	1	2	3	4	5	6	7	8	9	10	11	12
A	61	{ 177	< 193	{ 124 +	{ 140	{ 145	< 190	< 195	77	< 275	< 246	43
B	76	87	{ 110	98	88	< 221	{ 122	{ 175	103	< 269	{ 146	51
C	< 221	< 182	< 226	< 221	102	< 235	< 252	< 225	{ 163	< 271	56	56
D	{ 136	{ 156	< 232	< 212	< 204 -	{ 120	{ 159	{ 159	{ 168	< 273	< 275	57
E	104	{ 144	< 243	< 191	< 247	< 222	< 236	{ 130	< 219	< 273	{ 224	{ 206
F	< 203	{ 138	{ 172	< 249	{ 140	{ 151	70	{ 160	95	< 276	< 407	< 267
G	69	< 193	94	< 227	{ 150	< 240	< 192	< 266	{ 155	69	{ 219	{ 178
H	89	{ 131 +	106	106	80	93 +	{ 164	< 251	62	< 270	96	58

Report Date Mar 24 2017 4:12 PM

Appendix 2. *Ochrobactrum anthropi* identification.

Program	MicroLog 3/5.2.01 35
Project	ML5
File Name	Bacterias 2.D5E
User	biolog1
Instrument	MicroStation 2 Reader
Instrument S/N	1403197
Incubation Hours	29.00
Plate Number	1
Plate Type	GEN III
Protocol	A
Usuario	Lilly
Codigo muestra	4008.1
Origen	Biosolidos
Proyecto	
Lugar	
Date & Time of Read	Mar 24 2017 3:58 PM
Biolog ID DB	GEN-III_2.7.1.40.I5G

Result	Species ID: Staphylococcus saprophyticus ss saprophyticus
Comment	
Notice	

Rank	PROB	SIM	DIST	Organism Type	Species
1	0.624	0.624	5.405	GP-Coccus	Staphylococcus saprophyticus ss saprophyticus
2	0.126	0.126	6.609	GP-Coccus	Staphylococcus saprophyticus ss bovis
3	0.068	0.068	7.376	GP-Coccus	Staphylococcus pseudintermedius
4	0.059	0.059	7.535	GP-Coccus	Staphylococcus aureus ss aureus

Key: <x: positive, x: negative, <x-: mismatched positive, x+: mismatched negative, (x: borderline, -x: less than A1 well

Well Color Values

Plate	1	2	3	4	5	6	7	8	9	10	11	12
A	165	< 255	< 271	< 278	(192	(187	< 276	< 272	(215 -	< 283	< 281	108
B	172	< 273 -	(195	(192	(191	< 273	(226	(211	(196	< 250	< 250	< 264
C	< 259	168	< 264	< 270	(209	< 233	(207	(226	< 244	< 255	57	< 282
D	(220	< 270	< 249	(193	< 265	(216	(223	(209 -	< 253	< 251 -	57	59
E	(182	< 266	< 253	< 262	< 255	< 258	< 251	(225	< 260	< 255 -	(159	57
F	< 261	(194	(202	< 277	(178	(181	(189	(187	(190	60	124	93
G	144	< 261	< 236	< 270	< 240	(200	158	(191	138	< 256	< 247	< 278
H	(197	< 257	< 236	158	(180	< 253	175	< 258	< 254	< 276	< 254	(148

Report Date Mar 24 2017 4:18 PM

Appendix 3. *Staphylococcus saprophyticus* identification.

Program	MicroLog 3/5.2.01 35
Project	ML5
File Name	Segunda sesion bact y hongos mediodia 19mayo.D5E
User	biolog1
Instrument	MicroStation 2 Reader
Instrument S/N	1403197
Incubation Hours	48.00
Plate Number	1
Plate Type	GEN III
Protocol	B
Usuario	Lilly
Codigo muestra	AcD
Origen	Biosolidos
Date & Time of Read	May 19 2017 11:41 AM
Biolog ID DB	GEN-III_2.7.1.40.I5G

Result	Species ID: Bacillus cereus/thuringiensis
Comment	
Notice	

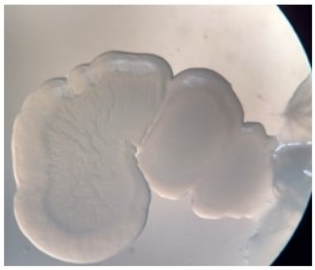
Rank	PROB	SIM	DIST	Organism Type	Species
1	0.575	0.575	6.184	GP-Rod-SB	Bacillus cereus/thuringiensis
2	0.132	0.132	6.959	GP-Rod-SB	Bacillus thuringiensis/cereus
3	0.109	0.109	7.199	GP-Rod-SB	Bacillus pseudomycoides/cereus
4	0.053	0.053	8.095	GP-Rod-SB	Bacillus firmus

Key: <x: positive, x: negative, <x: mismatched positive, x+: mismatched negative, (x: borderline, -x: less than A1 well

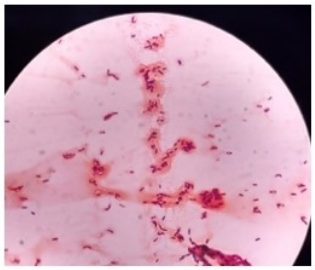
Well Color Values

Plate	1	2	3	4	5	6	7	8	9	10	11	12
A	98	< 197	< 221	111 +	110	106	< 218	119	117	< 253	< 256	50
B	95	104	105	(138	< 201	< 226	115	119	114	< 245	< 237	< 240
C	< 206	111	< 201	(123	111	118	(131	(133	< 222	< 256	71	< 264
D	94	105	101	111	(172	< 262	< 240	114	(134	67	< 255	79 +
E	< 245	(149	(155	(125	(172	< 199	< 239	(136	< 227	75	< 227	72
F	< 195	(129	(133	< 221	(130	(161 -	112	115	118	75	(118	89
G	93	(135 +	114	< 204	(137	118	104	117 +	85	(175	< 247	< 266
H	(137	109	117	110 +	108	(142	(138	(172	< 207	< 268	< 260	< 232

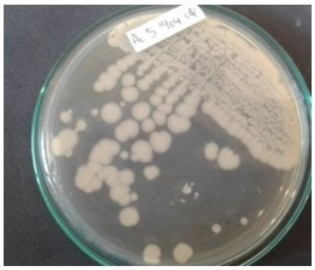
Report Date May 23 2017 12:26 PM



Morphology

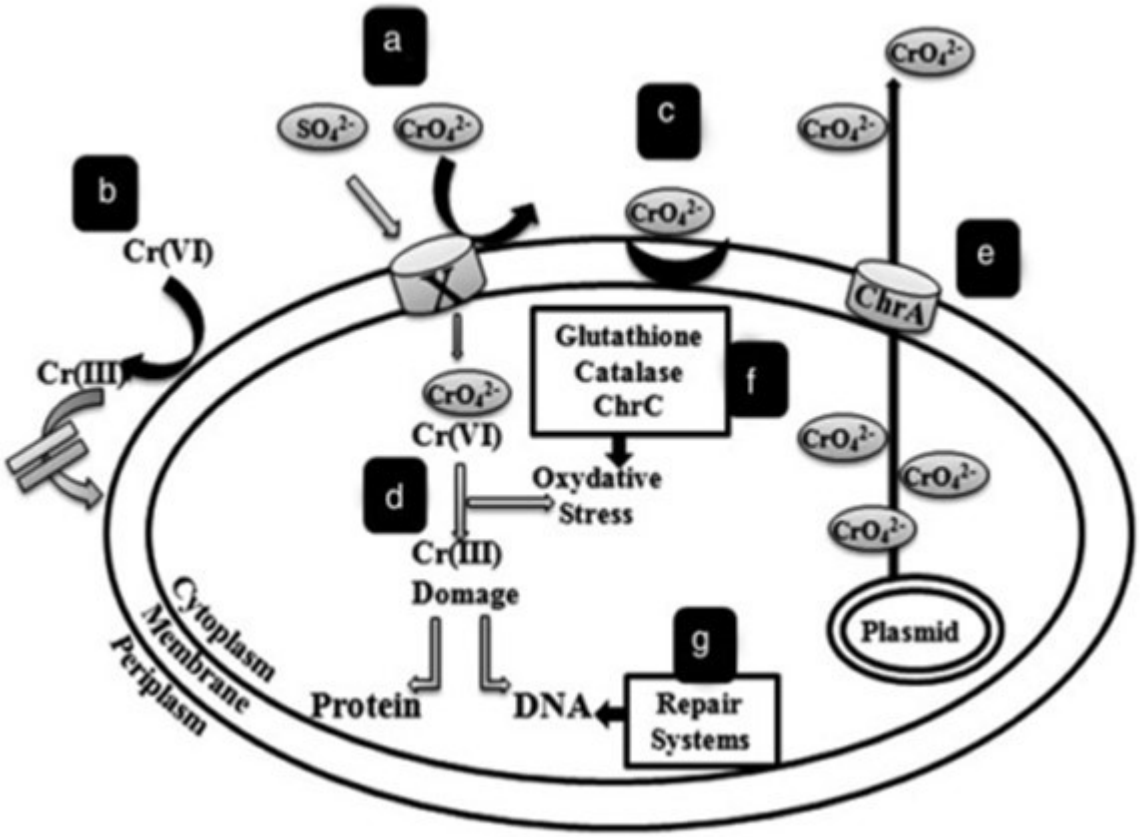


Gram Stain



Concentratión 600 Cr6+

Appendix 4. *Bacillus cereus* identification.



Appendix 5. Mechanisms of microbial chromate transport, toxicity, resistance and reduction of Cr^{6+} . Schematic depicting the mechanisms of microbial chromate transport, toxicity, resistance and reduction. (a) Sulfate uptake pathway, which is also used by chromate to enter cells. (b) Extracellular reduction of Cr (VI) to Cr (III), in which the metal forms do not cross the membrane. (c) Membrane-bound chromate reductase. (d) Intracellular Cr (VI) to Cr (III) reduction may generate reactive oxygen species (ROS) and thereby oxidative stress that causes protein and DNA damage. (e) Active efflux of chromate from the cytoplasm by means of the ChrA protein. (f) Detoxifying enzymes can be exuded to protect against oxidative stress. (g) DNA repair systems protect against damage generated by chromium derivatives (taked from Tahri, *et al*., 2011).