Tolerance and reduction of Cr(VI) by *Bacillus amyloliquefaciens*, *B. thuringiensis* and *Paenibacillus* sp., isolated from Pasto River

Tolerância e redução de Cr(VI) por *Bacillus amyloliquefaciens*, *B. thuringiensis* e *Paenibacillus* sp., isolados de rio Pasto

DOI: 10.46814/lajdv4n1-020

Recebimento dos originais: 03/01/2022

Aceitação para publicação: 22/02/2022

Jhonatan Pinta-Melo

Magister em Ciências Biológicas

Institución: Universidad de Nariño – Colombia

Dirección: Herbario de la Universidad de Nariño. Bloque de Docencia. Ciudad Universitaria
Torobajo. Pasto-Nariño- Colombia

Correo electrónico: jhonatankrl@hotmail.com

Deisy Lorena Guerrero-Ceballos

Magister em Ciências Biológicas

Institución: Universidad de Nariño - Colombia

Dirección: Departamento de Biología. Facultad de Ciencias Exactas y Naturales. Ciudad
Universitaria Torobajo. Pasto-Nariño Colombia

Correo electrónico: daisymartinez-18@hotmail.com

Eduardo Ibargüen-Mondragón

Doutor em Ciências Matemáticas

Institución: Universidad de Nariño - Colombia

Dirección: Departamento de Matemáticas. Facultad de Ciencias Exactas y Naturales. Ciudad
Universitaria Torobajo. Pasto-Nariño Colombia

Correo electrónico: edbargun@gmail.com

Pablo Fernández-Izquierdo

Doutor em Ciências Biológicas

Institución: Universidad de Nariño - Colombia

Dirección: Departamento de Biología. Facultad de Ciencias Exactas y Naturales. Ciudad
Universitaria Torobajo. Pasto-Nariño Colombia

Correo electrónico: pabfdez@gmail.com

Jenny Dimelza Gómez Arrieta

Doutor em Ciências-Bioquímica Aplicada

Institución: Universidad de Nariño - Colombia

Dirección: Departamento de Biología. Facultad de Ciencias Exactas y Naturales. Ciudad
Universitaria Torobajo. Pasto-Nariño Colombia

Correo electrónico: rizaldza@gmail.com

Edith Mariela Burbano-Rosero

Doutora em Ciências-Microbiologia

Universidad de Nariño - Colombia

Dirección: Departamento de Biología. Facultad de Ciencias Exactas y Naturales. Ciudad

Universitaria Torobajo. Pasto-Nariño Colombia

Correo electrónico: marielaburbano@gmail.com

ABSTRACT

The accumulation of heavy metals such as Cr(VI) in the environment is a growing problem; however, the isolation of the bacteria present in these contaminated sources can provide an alternative for their treatment. In this sense, this research evaluated in vitro the percentage reduction of Cr(VI) and the effect of three concentrations (25, 60, and 100 mg/L of Cr(VI)) on the growth of bacterial isolates E4M3 *Bacillus amyloliquefaciens* [MK561611], E4M15 *Bacillus thuringiensis* [MK561610], and *Paenibacillus sp.* [MK561612]. Statistical analysis determined that 59% of the variation in growth is attributed to the interaction of bacteria and concentration ($P < 0.05$; $\eta^2 = 0.59$). Likewise, statistically significant differences were observed between bacteria concerning the percentage of reduction of Cr(VI) ($P < 0.05$; $\eta^2 = 0.98$). The data found on the population density and the percentage of reduction coupled with mathematical approximations are related to the intrinsic metabolic conditions of the bacteria and the selective pressure conditions of the environment where they are found.

Keywords: heavy metals, hexavalent chromium, removal of contaminants, bioreduction, chromium reducing bacteria.

RESUMO

O acúmulo de metais pesados como Cr(VI) no meio ambiente é um problema crescente; entretanto, o isolamento das bactérias presentes nessas fontes contaminadas pode constituir uma alternativa para o seu tratamento. Nesse sentido, esta pesquisa avaliou in vitro a redução percentual de Cr(VI) e o efeito de três concentrações (25, 60 e 100 mg/L de Cr(VI)) no crescimento de isolados bacterianos E4M3 *Bacillus amyloliquefaciens* [MK561611], E4M15 *Bacillus thuringiensis* [MK561610] e *Paenibacillus sp.* [MK561612]. A análise estatística determinou que 59% da variação no crescimento é atribuída à interação da bactéria e à concentração ($P < 0,05$; $\eta^2 = 0,59$). Da mesma forma, diferenças estatisticamente significativas foram observadas entre as bactérias com relação ao percentual de redução de Cr(VI) ($P < 0,05$; $\eta^2 = 0,98$). Os dados encontrados de densidade populacional e percentual de redução acoplados a aproximações matemáticas estão relacionados às condições metabólicas intrínsecas das bactérias e às condições de pressão seletiva do ambiente onde se encontram.

Palavras chave: metais pesados, crômio hexavalente, remoção de contaminantes, bio-redução, bactérias redutoras de crômio.

1 INTRODUCTION

Microbial ecology has stressed the importance of studying the different interactions of microorganisms with their habitat, such as they play essential roles in the stability and functioning of ecosystems. The development of different molecular biology techniques has facilitated the study of the distribution and diversity of microorganisms in their microbiomes, but the difficulty involved in evaluating the responses triggered by this interaction persists (Terry, 2019; Prosser, 2020). It is

important to inquire about the models of microbial systems, mainly bacteria because their enormous capacity for metabolic adaptation and their wide functional diversity within ecosystems is evident. However, the distribution and abundance of these organisms are affected by the variation of the biotic and mainly abiotic components of the environment. The deficient or excessive concentration of heavy metals (PM) is an essential agent that influences bacterial growth as well as limits it depending on the environmental conditions in which they develop (Terry, 2019; Awasthi et al., 2021). In this sense, the study of bacteria interactions with PM of environmental impact with the hexavalent chromium (Cr(VI)), evidenced its effect mutagenic for these organisms as well as disturb their normal growth; however, there are still information gaps regarding the response of wild bacteria isolated from contaminated environments to the different concentrations of Cr(VI). (Sher & Rehman, 2019; Maldaner, et al., 2021). From this perspective, the present study focused on evaluating the effect of different concentrations of Cr(VI) (25, 60, and 100 mg/L) on the growth of three bacterial isolates *Bacillus thuringiensis* (E4M15), *B. amyloliquefaciens* (E4M3), and *Paenibacillus* sp. (E4M23), to finally generate indicative parameters that coupled to a mathematical model allowing, to improving understanding about the population density response of bacteria in the different concentrations of Cr(VI).

2 MATERIALS AND METHODS

2.1 CULTURE OF WILD CR(VI) REDUCING BACTERIA

The bacterial isolates of this study (E4M3, E4M15, and E4M23) were obtained by the Microbial Biotechnology Research Group from water samples from the Pasto River in the Pandiáco sector (Otero, 2012), contained a high chromium concentration (59 mg/L Cr) (Aragón, & Alzate, 2004). The wild isolates were preserved in glycerol at 30% diluted in Luria Bertani (LB) broth, and the vials were refrigerated at 4 °C until the completion of the present study. The isolates were viable in test tubes with LB medium, after 18 hours of incubation at 30 °C, the axenic condition was verified with Gram staining. Subsequently, the cryopreservation process was carried out, following the 30% glycerol preservation method diluted in LB culture medium. Finally, it was refrigerated at -20 °C to estimate viability in the time.

2.2 IDENTIFICATION OF BACTERIAL ISOLATES BY AMPLIFICATION AND SEQUENCING OF THE 16S RRNA GENE

The DNA extraction of three isolates was carried out by applying the protocol described by Burbano et al. (2017). One colony of each isolate was inoculated in 10 mL of LB broth. It was incubated for 18 h at 27 °C, after it was transferred to a 15 mL falcon tube and centrifuged at 5713 g for 5 min.

The cell pellet was resuspended with 567 μL of 1M TE buffer solution. The tubes were saved on ice, then were added 30 μL of SDS 10% and 9 μL of proteinase K (20 mg/mL) and gently stirred. The tubes were incubated in a water bath for 1 h at 37 °C and later in ice and added 100 μL of 5M NaCl. The mix was vortexed, and 80 μL of CTAB preheated to 65 °C was added, follow was shaking vigorously, and placed in a water bath for 20 min at 65 °C. The tubes were cooling to room temperature, added chloroform-isoamyl alcohol (24:1), and centrifuged at 9391 g for 25 min (Burbano et al., 2017). The supernatant was saved and 1 μL of RNase (10 mg/mL) was added and incubated for 1 h at 37 °C. One volume of chloroform was added, mixed with vortex, and centrifuged at 9391 g for 15 min. The supernatant was transferred and the DNA was precipitated with 0.6 volumes of cold isopropyl alcohol and the tubes were shaken slowly by inversion and centrifuged at 9391 g for 5 min. The supernatant was removed, and the pellet was dried at room temperature. Finally, the DNA was resuspended in 50 μL of milli-Q water (Burbano et al., 2017).

The integrity of the extracted DNA was verified on 1% agarose gel treated with ethidium bromide. For the preparation of the samples, 2 μL of 1M TE, 1 μL of bromophenol blue, and 2 μL of DNA samples were mixed. Lambda *Hind III* and 1 Kb (Promega) were used as molecular size markers. The running conditions were 70 V for 1.5 h in an *ENDURO-GEL-XL* chamber (Labnet Inc.). The gel was visualized on a *BENCHTOP-3UV TRANSILLUMINATOR* photodocumentary at a wavelength of 302 nm. The DNA concentration was verified by comparing the intensity of bands concerning standard concentrations of marker fragments. The primers 27F and 1041R were used for 16S rRNA subunit amplification, the PCR was performed in a *MULTIGENE* thermocycler (Labnet Inc.). Each reaction mixture contained 2 μL of 25 mM MgCl_2 , 1 μL of 2.5 mM dNTPs, 1 μL of each primer 20 μM , and 0.25 μL of Taq polymerase 5 U/ μL . Finally, the respective sample was added and as a negative control, sterile water and *E. coli* DNA were used as a positive control. The amplification conditions were: 1 cycle at 95 °C for 2 min; 30 cycles of 94 °C for 2 min, 55 °C for 1 min, and 72 °C for 3 min; and a final extension of 10 min at 72 °C. All amplifications were visualized by 1 % agarose gel electrophoresis in TAE 1X treated with ethidium bromide. Lambda *Hind III* and 1 Kb (Promega) were used as molecular size markers. The running conditions were 70 V for 1 h and 30 min in an *ENDURO-GEL-XL* electrophoresis chamber (Labnet Inc.) (Burbano et al., 2017).

Amplification products were submitted to Corrogen Corporation for sequencing analysis (contract number: 190301-1). The submitted sequences were visualized in the *Chromas Lite V. 2.0* program and edited and aligned in the *BioEdit V. 7.0.4* program. The partial sequences were compared in the *Ribosomal Database Project (RDP)* and *GenBank* databases using the BLAST algorithm. The species were selected according to the percentage of identity and origin of the isolate (Tamura, et al., 2011; Cole, et al., 2014; Benson et al., 2018). Later, a phylogenetic tree was constructed in the MEGA7

software from the 16S rRNA sequences of our bacteria and taking as reference the sequences reported in GenBank (Table 1). The method used was the unweighted pair groups with arithmetic mean (UPGMA). *Methanothermobacter marburgensis* (NR114484) was used as external root and the *Bacillus* sp., FY1 sequence (KT377065) as internal root, for a greater perspective of the phylogenetic relationships presented.

2.3 EVALUATION OF THE EFFECT OF THREE CR (VI) CONCENTRATIONS ON POPULATION DENSITY AND REDUCTION PERCENTAGE OF THE THREE BACTERIAL ISOLATES

For the evaluation, 40 mL of LB broth was supplemented with three independent concentrations of Cr(VI) (25, 60, and 100 mg/L), like potassium dichromate ($K_2Cr_2O_7$), and an inoculum of approximately 10^8 bacteria per milliliter (tube 5 McFarland) measured by absorbance at 600 nm was served in duplicated. The control was bacterial isolate in growing in LB broth without $K_2Cr_2O_7$. The treatments were incubated at 30 °C with constant shaking for 84 h. The population density was determinate tested 5 mL samples of each triplicate every 12 h. The dilutions were prepared from 3 mL of sample in 10^{-3} dilutions of each isolate. 100 μ L of each dilution were taken and inoculated in Petri dishes with LB agar and incubated at 30 °C for 12 h. Subsequently, the count of colonies was performed, and the value was reported in CFU/mL. Additionally, the growth was plotted in the *GraphPad V. 8.4.2* program (Prism® Trial license 9750FB1EF35), relating the CFU/mL and the incubation time.

For the determination of reduction process Cr(VI), 2 mL of the samples of each treatment were taken, 0.025 mL of the sample was diluted in 25 mL of distilled water (dilution factor = 0.001) then were added 0.25 mL of sulfuric acid (H_2SO_4), 0.25 mL of phosphoric acid (H_2PO_4), and 1 mL of 1.5 diphenylcarbazide; last, the absorbance of the mixture was determined at 450 nm in a UV-vis spectrophotometer. The concentration of Cr(VI) was determined, using the equation of the line (**Eq. 1**) obtained from the previously performed calibration curve and the reduction percentage using **Eq. 2** (Chromium 117A Hexavalente chromium, 1999; Lace, et al., 2019).

Calculation of the Cr (VI) concentration

$$y = 0.7038X + 0.0049 \quad (1)$$

Calculation of the percentage of reduction of Cr (VI)

$$\%reduction = \frac{[CrVI_{initial}] - [CrVI_{final}]}{[CrVI_{initial}]} * 100 \quad (2)$$

Finally, the reduction trend graph was plotted with *GraphPad V. 8.4.2* program (Prism® Trial license 9750FB1EF35), relating the chromium concentration and the incubation time. The next methods described were performed in triplicate every 12 h for a total of eight times.

2.4 ANALYSIS OF DATA

The data obtained were analyzed using the *IBM® SPSS® Statistics Subscription* software (Compilation 1.0.0.1327). A mixed analysis of variance with repeated measures was performed and analyzed to determine the significant differences in the population density of the three bacterial isolates concerning the different treatments and the effect of the association between the variables. To determine the existence of significant differences in the percentage of Cr(VI) reduction by the bacterial isolates E4M3, E4M15, and E4M23, both during the treatments and between bacteria analysis of variance with repeated measures was performed. In addition, pairwise comparisons were made to verify which treatments there were significant differences.

2.5 MATHEMATICAL ANALYSIS OF THE BEHAVIOR OF BACTERIAL POPULATIONS ABOUT CR (VI) CONCENTRATION

A mathematical model was formulated based on the behavior of the three bacterial populations concerning the different concentrations of Cr(VI), taking into account the reduction percentage. For that, differential equations did base on logistic growth, the law of mass action and Holling-type competition models were used because had been employed in different studies at the biological level, thus allowing for the description of the cause-effect relationships (Murray, 2001; Trinidad Bello, 2014; Xu, et al., 2019). The formulation of the mathematical model follows the considerations: The bacterial population density at time t was denoted as $Bi(t)$, $i = 1, 2, 3$; assuming that the bacteria present reproduced under logistic growth with a reproduction rate (γ_i) depending on substrate amount (S) present in culture medium The parameter (μ_i) represents the substrate consumption rate of each bacterium. Parameters that limit bacterial growth included the effect of the intraspecific competition of bacteria for the substrate; Chromium (C) affects bacterial growth following the law of mass action because the interaction between chromium and bacteria is not necessarily one to one, that is, several

moles of chromium can interact with a bacterial cell (α). With these considerations, the following system of ordinary differential equations was obtained:

Modeling the effect of chromium on the growth of the bacterial population (B)

$$\frac{dB}{dt} = \gamma_i B_i \left(1 - \mu_i \frac{B_i}{K + S} \right) - \alpha_i B_i C$$

$$i = 1, 2, 3. \quad (3)$$

Modeling of the reduction in the concentration of hexavalent chromium by bacteria

$$\frac{dC}{dt} = -C(r_i B_i) \quad (4)$$

Modeling the effect of substrate concentration on the growth of the bacterial population (B)

$$\frac{dS}{dt} = -S(K_i B_i) \quad (5)$$

Once the model was formulated, parameters and constants were determined by adjusting the response of each of the experiments to a polynomial model and, the estimate of the value. Finally, numerical simulations were run with *MATLAB® Version R2015a* software (License: 1081117), to predict different behavioral scenarios between bacteria and chromium.

3 RESULTS AND DISCUSSION

3.1 MOLECULAR IDENTIFICATION WITH 16S RNA GENE

The product of the amplification of the 16S rRNA gene was approximately 1500 bp, which corresponds with the expected using the primers described (see Fig. 1). Table 1 shows the identification of bacterial isolates resulting from the comparison of the sequences with two databases (RDP and GenBank), based on the percentage of identity and the possible origin of the isolate (environmental origin). The bacteria were identified as *Bacillus thuringiensis* (E4M15), *Paenibacillus* sp. (E4M23), and *Bacillus amyloliquefaciens* (E4M3).

Figure 1. PCR amplification of the 16S rRNA gene.

source: this article

Other studies report that bacteria isolated from environments contaminated with heavy metals such as chromium showed the ability to tolerate and even reduce chromium (VI), among the microorganisms described, the bacteria identified in this study (Dhal, et al., 2013; Mtimunye & Chirwa, 2014; Das, et al., 2014; Joutey, et al., 2015; Zhao, et al., 2016; Benson, et al., 2018). The partial sequences of the 16S rRNA gene of the study organisms were stored in the *GenBank database of the National Center for Biotechnology Information (NCBI)* with the access codes *Bacillus thuringiensis* [MK561610], *B. amyloliquefaciens* [MK561611], and *Paenibacillus* sp. [MK561612].

Table 1. List of isolates with Cr(VI) tolerance and removal capacity

Cr(VI) reducing biota	Efficiency in reduction process	Reference
<i>Bacillus</i> sp FY1 KT377065	Tolerant to 1000 mg/L Reduction 78 – 85% (100-200 mg/L)	(Xiao, et al., 2017)
<i>Arthrobacter</i> sp WZ2 KT377066	Tolerant to 1000 mg/L Reduction 75 – 82 % (100-200 mg/L)	(Xiao, et al., 2017)
<i>Mangrovibacter yixingensis</i> MS24 KY321826	Tolerant to 100 mg/L Detection of chromium reductase gene	(Sanjay, Sudarsanam, Raj, & Baskar, 2020)
<i>Klebsiella pneumoniae</i> MS15 KY317923	Tolerant to 80 mg/L Detection of chromium reductase gene	(Sanjay, Sudarsanam, Raj, & Baskar, 2020)
<i>Bacillus thuringiensis</i> BTH1 MK561610	Tolerant to 100 mg/L Reduction 96.73, 80.99, 93.11 % (25, 60, 100 mg/L)	This investigation
<i>Bacillus amyloliquefaciens</i> BAM1 MK561611	Tolerant to 100 mg/L Reduction 34.19, 75.62, 90.04 % (25, 60, 100 mg/L)	This investigation
<i>Paenibacillus</i> sp PAE1 MK561612	Tolerant to 100 mg/L Reduction 47.53, 71.70, 86.40 % (25, 60, 100 mg/L)	This investigation

source: this article

In Figure 2 we can observe the phylogenetic tree, where *Methanothermobacter marburgensis* represents the outgroup or external root (purple rhombus) and *Bacillus* sp. represents the internal root,

Bacillus sp., together with the evaluated bacteria form a subgroup (blue boxes). The bacteria reported as reducing bacteria or tolerant to Cr(VI) were marked whit (green triangles). Though the bacteria tested in this study and those used for comparison were isolated from different sites (Río Pasto, galvanized effluent, and tanning, respectively), the tree reflects close phylogenetic relationships. If it is taken into account that these bacterial species were isolated from places under similar environmental conditions (chrome contaminated environments) it can be established that they share important characteristics related to resistance to living in these media, one of these characteristics could be the presence of enzymes and proteins such as chromate reductases, which could allow them to develop similar mechanisms to tolerate these conditions.

Figure 2. Phylogenetic tree based on the sequences of the rRNA 16S gene of the study bacteria, compared to related chromium reducing bacteria in GenBank.

3.2 EVALUATION OF THE EFFECT OF CR(VI) ON THE POPULATION DENSITY OF *BACILLUS AMYLOLIQUEFACIENS*, *B. THURINGIENSIS*, AND *PAENIBACILLUS SP.*

It was observed that bacterial growth has a period of 0 to 36 h; according to Ramírez & Benítez-Campo (2013), in this period there is a relationship between bacterial growth and the change in the oxidation state of chromium, a behavior that is reflected in the growth graphs and the reduction slopes of Cr(VI) obtained in this study.

On the other hand, the microbial growth of *B. amyloliquefaciens* (see Fig. 3) did not reveal the typical behavior of a conventional microbial growth curve (exponential phase, stationary, and death), but it was possible to establish a comparison with the control. Moreover, a small variation in the population density of bacterium can be observed in the different treatments (25, 60, and 100 mg/L Cr(VI)) in relation to control. The treatment with 60 mg/L Cr(VI) presented higher growth compared to the other three treatments. This phenomenon may be due to the bacteria adapting differently to conditions of the environment, depending on the tolerance mechanism that these use (Dhal, et al., 2013; Rath, et al., 2014; Rath, et al., 2019). A similar phenomenon was observed by Huang et al. (2020) when evaluating the biosorption capacity of Mn(II) in a concentration range of Mn(II) (0–10000 mg/L) by the *B. thuringiensis* HM7 strain. The results showed that the growth of HM7 within a concentration range (200 - 800 mg/L) was greater than without Mn(II) (Huang, et al., 2020).

source: this article

Unlike the population density of *B. amyloliquefaciens* the population density of *B. thuringiensis* in the three treatments was different (see Fig. 4) because of the control that presented the highest growth about treatments (25, 60, and 100 mg/L of Cr(VI)). Small growth inhibition was also observed in the treatment at 100 mg/L during the first 12 h. This may be because, with a higher concentration, the microbial activity decreased; however, the impact of chrome toxicity on bacteria did not completely inhibit their growth, in such a way that in the later phase the chromium reduction process is accelerated and 93.11% reduction is reached. An equivalent phenomenon was observed in the study by Ramírez and Benítez-Campo (2013) for *B. cereus* B1, which presented growth inhibition during the first 30 h of treatment when subjected to an initial concentration of 100 ppm Cr(VI) (Ramírez & Benítez-Campo, 2013; Moreno-Benavides, et al., 2019).

The effect of Cr(VI) concentrations on the population density of *Paenibacillus* sp. is described in Fig. 5, where shows a clear variation in population growth in three treatments compared with control. The curve evidence that the control presented less growth in contrast to 25 mg/L treatment, which exhibited higher growth followed by the 100 mg/L Cr(VI) treatment. This indicates, in some way, that the bacterium has a wide tolerance range to Cr(VI) concerning *B. amyloliquefaciens* and *B. thuringiensis*.

It was determined that growth presented a significant interaction with the concentration and bacteria variables with a $p < 0.05$, which indicate that growth behaves differently in different treatment

according to chromium concentration. Likewise, the partial *Eta square* value indicates that the interaction of these two factors (bacteria and concentration) explains 59% of the variance in growth. Additionally, when the effect between subjects was evaluated, a significant effect of the type of bacteria on growth was observed with $p < 0.05$ and a partial *Eta square value* of 0.961, indicating that 96% of the variation in growth is explained by the differences between the bacterial species, without considering the rest of the factors.

Likewise, it was determined that the growth of *B. thuringiensis* in the treatment without chromium (control) and the statistically significant differences compared with the other two bacterial isolates (*B. amyloliquefaciens* and *Paenibacillus sp.*). However, *B. amyloliquefaciens* and *Paenibacillus sp* did not have significant differences between them, in contrast with their population growth in the mentioned treatment. In the treatment with 25 mg/L of Cr(VI), were observed significant differences in growth between the bacterial isolate *B. thuringiensis* and *Paenibacillus sp*. Furthermore, the treatment with 60 mg/L of Cr(VI), exhibited similar behavior to the treatment without chromium (control). Finally, in the treatment with 100 mg/L of Cr (VI) there were significant differences in the growth of *Paenibacillus sp.*, in proportion to the other two isolates. All effects are reported with a significance of $p < 0.05$.

Taking into account the above, we can infer that under the stress of high concentrations of hexavalent chromium, cell activity is being affected, which could produce alterations to normal growth. An example of this is the reduction of oxygen consumption in respiration, which in turn weakens microbial respiration and microbial activity is significantly inhibited (Huang, et al., 2020).

3.3 EVALUATION OF THE EFFECT OF CR(VI) ON THE REDUCTION PERCENTAGE OF *BACILLUS AMYLOLIQUEFACIENS*, *B. THURINGIENSIS*, AND *PAENIBACILLUS SP*

Upon completion of the fermentation process, the reduction percentage was determined for each bacterial isolate. Figure 6 shows the reduction trend graph of Cr(VI) by *B. amyloliquefaciens*, where the chromium reduction slope is verified, which is more pronounced in the interval from 0 to 36 h; finally, around 84 h *B. amyloliquefaciens* achieved reduction percentages of 34.2%, 75.6%, and 90% for the concentrations of 25, 60, and 100 mg/L of Cr(VI), respectively.

Figure 6. Hexavalent chromium reduction in the treatments inoculated with *B. amyloliquefaciens*

source: this article

The acceleration in the Cr(VI) reduction process coincides with the exponential growth phase of bacteria. This phenomenon may be linked to metabolic processes that generate an increase in the cellular division that at the same time facilitate the reduction of the metal by the new bacteria (Geets, et al., 2008; Ramírez & Benítez-Campo, 2013; Roestorff & Chirwa, 2018; Moreno-Benavides, et al., 2019).

B. thuringiensis obtained Cr(VI) reduction percentages of 96.7%, 80.9%, and 93.1% for the concentrations of 25, 60, and 100 mg/L, respectively. In the reduction trend graph (see Fig. 7) is observed that the treatments have a constant phase of reduction until 84 h, which suggested that it is possible to evaluate the behavior of the bacteria for the longest time. Under induced stress, bacteria can experience a constant stage of growth, where the reduction of chromium is directly associated with growth and once the bacteria are adapted to the conditions of the environment, the reduction mechanisms will be facilitated (Arrieta & Espinoza, 2006; Sauka & Benintende, 2008; Oves, et al., 2013; Huang, et al., 2020).

Figure 7 Hexavalent chromium reduction in the treatments inoculated with *B. thuringiensis*

source: this article

The *Paenibacillus* sp. presented reduction percentages of 47.5%, 71.7%, and 86.4% in concentrations of 25, 60, and 100 mg/L of Cr (VI), respectively. In Figure 8, could be observed that in the 24- to 60-hour interval, the reduction process was accelerated and the treatment of 100 mg/L of Cr (VI) showed the major percentage indicating that, under stress, this bacterium triggers different mechanisms that could be reduce and block the toxic effect of the metal, increasing its chances of survival in environments contaminated with heavy metals (Ramírez-Díaz, et al., 2008; Wani, et al., 2018; Sridevi and Raghuram, 2019). Additionally, it was determined that the initial Cr (VI) concentration in the control used for this process did not vary significantly; in a certain way, it is confirmed that the bacteria used in the treatment are responsible for the reduction of this metal.

Finally, the statistical analysis had established that there is a significant interaction of the concentration variable with the dependent variable percentage reduction ($p < 0.05$), revealing that the percentage reduction is not the same in treatments with different hexavalent chromium concentrations. The partial *Eta square value* indicates that 99% of the variation in the percentage of chromium reduction is due to the concentration factor. On the other hand, a significant effect of the type of bacteria was observed on the percentage of chromium reduction with $p < 0.05$ and a partial *Eta square value* of 0.984. This indicates that the differences between the species of bacteria explain 98% of the variation in the percentage of chromium reduction.

Figure 8. Hexavalent chromium reduction in the treatments inoculated with *Paenibacillus* sp

When the reduction percentages between bacteria were compared, significant differences were found between the isolate *B. thuringiensis* (E4M15) and the other isolates, but not between *B. amyloliquefaciens* and *Paenibacillus* sp., with significance values of 0.000 and 0.640, respectively. Regarding chromium concentrations, it was observed that all presented significant statistical differences ($p < 0.05$). Likewise, when the reduction percentages among the bacteria were compared, taking into account the chromium concentration of the treatment, significant differences were found, especially in the bacteria *B. thuringiensis* with respect to *B. amyloliquefaciens* and *Paenibacillus* sp. $p < 0.05$.

The results obtained in the present study for *B. amyloliquefaciens* and *B. thuringiensis* can be compared to those obtained by Smrithi and Usha (2012), who determined that at low concentrations of hexavalent chromium, the population growth of *Bacillus* sp. does not show significant differences compared to control (Smrithi & Usha, 2012). This result is unlike Shukla et al. (2007), who reported a 36% reduction in population growth of *Bacillus* sp. subjected to a concentration of 10 mg/L of Cr(VI). Furthermore, Huang et al. (2014) reported no significant differences in growth until the microorganism was subjected to 50 mg/L (Shukla, et al., 2007; Huang, et al., 2014). It is important to mention that the microorganism evaluated here were isolated from water effluents contaminated with chromium (179 mg/L and 5.88 mg/L, respectively). In the case of *Paenibacillus* sp., several authors demonstrate that bacteria have the capacity to contract to tolerate concentrations between 150 and 200 mg/L of Cr(VI). All of these report growth variations in the different stages (Molokwane, et al., 2008; Rawat, et al., 2013).

Research conducted by Muneer et al., (2009) proved that *B. thuringiensis* grows in Cr(VI) concentrations of 100 mg/mL due to its versatile metabolic capacity to reduce Cr(VI) to Cr (III) a less

toxic compound. It should be noted that most of the works related to this topic, work under optimal conditions of growth for the bacteria in medium supplemented with chromium, pH, and temperature-controlled (Zhao, et al., 2011). However, it is a known fact that the conditions could vary between strains of the same bacterial species, such as *Bacillus thuringiensis* BRC-ZYR2, which tolerates concentrations of 500 mg/mL under pH 9.0 and 40 °C incubation conditions (Huang, et al., 2014). Undoubtedly, the species used in this study have the ability to inhabit and regulate their population density in environments contaminated with hexavalent chromium as was demonstrated here. The gradual accumulation of chromium cations in places intervened by humans, and the high toxicity of this metal could be generated spontaneous adaptation processes during the evolution through the development or acquisition of genetic systems that help counteract the effect of high chromium concentrations (Terry, 2019). Barton and Northup (2011) have mentioned that cellular communication -also called quorum sensing- plays an important role in this type of adaptation process, which coordinates the expression of target genes as part of a cascade of perception directly influencing the behavior of the population. In this sense, it has been mentioned that on *B. amyloliquefaciens*, *B. thuringiensis*, and *Paenibacillus* sp. was described the plasmid elements using, that give it a selective advantage under certain conditions, especially in situations of mental stress (Marrero-Coto, et al., 2010).

Hall et al., (2016) reported that these plasmids act as mediators of the exchange of genetic material between populations associated with contaminated environments. Thus, the source bacterias of this study meet these criteria because was isolated from the same font, and part of the genetic material associated with the characteristic of resistance or tolerance to Cr(VI) could be exchanging giving them the ability to survive in these environments. It is necessary to emphasize the existence of significant differences in population density between bacteria (*B. amyloliquefaciens*, *B. thuringiensis*, and *Paenibacillus* sp.) because they have different metabolisms, the ability of bacteria to adapt to the environment differ significantly, although even they use the same mechanism to tolerate different concentrations of chromium (Barton & Northup, 2011; Terry, 2019). Each species, even the individual, have an optimal range and limits of tolerance for each environmental factor, caused by the environmental exposure specific that induces the genetic modifications (Barton & Northup, 2011; Terry, 2019).

The results obtained in this research coincide with studies that affirm that bacteria belonging to the genus *Paenibacillus* have the ability to reduce hexavalent chromium, in our case 86.4% in 84 h from an initial concentration of 100 mg / L. *Paenibacillus xylaniliticus* on the other hand, it showed the capacity to reduce approximately 80.43% of chromium, after an initial concentration of 19.7 mg / L at 72 h (Molokwane, et al., 2008; Rawat, et al., 2013). However, these percentages could be

maximized when modifying other parameters such as temperature and pH as demonstrated by Tiwari et al., (2014) in their process determined that the Cr(VI) reductions of *Paenibacillus macerans* are 99% efficient at 35 ° C and pH 8 (Tiwari, et al., 2014). Similarly, the ability of *B. thuringiensis* to carry out the reduction process has been previously reported. A wild isolate from industrial wastewater presented an 82% reduction percentage in a synthetic medium (Luria Bertani) linked which was linked to the organism's metabolic ability to oxidize Cr(VI) to Cr(III) (Muneer et al., 2009; Cárdenas-González and Acosta-Rodríguez, 2010). Another point in favor of *B. thuringiensis* when carrying out the reduction process is its ease of interacting with chromium thanks to the presence of functional groups (amino, carboxyl, hydroxyl, carbonyl) on the surface of the membrane (Srivastava, et al., 2014; Moreno-Benavides, et al., 2019).

Concerning *B. amyloliquefaciens*, Rath et al. (2014) indicated that *B. amyloliquefaciens* isolated from a chromite mine can produce an extracellular enzyme (chromate reductase) that allows the reduction of hexavalent chromium to trivalent chromium, in stress conditions (Rath, et al., 2014). Later, Rath, et al., (2019) report the production of a *B. amyloliquefaciens* chromate reductase with high stability when subjected to different additives, which positions *B. amyloliquefaciens* as an ideal candidate for bioremediation of Cr(VI) in a wide range of environmental conditions. In this context, the results obtained in this research give an approximation to the response of these bacterial species could have to different concentrations of chromium Cr(VI) *in vivo*, even is complex to determine the behavior of bacterial populations in an ecosystem without the help of advanced technology. It was possible to corroborate that these results are similar to those derived from the aforementioned studies, in which the potential for reduction of hexavalent chromium by wild bacteria isolated from Cr-contaminated environments was evaluated, thus demonstrating the applicability of species characterized in this study, in bioremediation processes.

3.4 MATHEMATICAL ANALYSIS OF THE BEHAVIOR OF BACTERIAL POPULATIONS ABOUT CR(VI) CONCENTRATION

According to observed, the population of bacteria evaluated has a higher chromium reduction activity in its exponential phase and reaches a constant value different from zero at approximately 48 h. Additionally, it was observed that in this same treatment time, chromium reaches its maximum reduction percentage. To illustrate this situation, numerical simulations were performed (see Suppl. 1 and 3). The proposed model does not explicitly consider the dynamics of bacterial growth because it considers only exponential growth. Therefore, the bacterial population will grow until they reach a constant population. This fact is not observed in the growth graphs of the treatments, as when working

in Batch-type fermenters with a certain initial concentration of nutrients, the bacterial population tends to die when depleting the nutrients in the medium (Ahluwalia, 2014).

The fact that a bacterial mortality rate has not been taken into account is because, as reported by Otiniano García et al. (2007), Ramírez and Benítez-Campo (2013), and Moreno-Benavides, et al., (2019), the greater activity of chromium reduction by bacteria is evident in the exponential phase around 24 to 48 h of fermentation. After this period, the reducing activity stabilizes (Otiniano García, et al., 2007; Ramírez and Benítez-Campo, 2013). This was also observed by Teles et al. (2018) by reducing 80% of chromium in this time interval. However, although the model used in this study appears to be simple compared to the complex biological phenomenon studied, its results accurately describe the logistical growth and reduction of hexavalent chromium in bacterial populations in response to the effect of Cr(VI) on them. It should be emphasized that, despite the mathematical rigor applied during the formulation of the model, a realistic physical description of a biological process cannot always be obtained from a purely mathematical postulate.

4 CONCLUSIONS

The population density of *Bacillus thuringiensis*, *Bacillus amyloliquefaciens*, and *Paenibacillus* sp. was affected, after being subjected to concentrations of 25 and 60 mg/L of Cr(VI) following the significant differences found 0.006 and 0.004 related to growth.

Under the conditions used in this study, significant differences were found in *Bacillus thuringiensis*, in growth, and the percentage of reduction of Cr(VI) compared with the other two bacteria evaluated. This suggests that this isolate could be considered a suitable candidate for implementation in the bioremediation processes of environments contaminated with this metal.

The mathematical model proposed for the trend of growth and reduction of Cr(VI) describes reliably the dynamics of each biological phenomenon, allowing for the generation of predictive scenarios for subsequent analyses.

ACKNOWLEDGEMENTS

The authors express their gratitude to the technical personnel of specialized laboratories of the University of Nariño, to the Vice-Rector for Research, Postgraduate Studies and International Affairs (VIPRI), now Vice-Rector for Research and Social Interaction (VIIS) of the University of Nariño for the financial resources granted for the execution of the research, and to the initiative 'Strengthening regional capacities in research, technological development and innovation in the Department of Nariño' of the CTel Fund of the General System of Royalties run by the CEIBA Foundation in agreement with the Office of the Governor of Nariño.

The support and collaboration from the Microbial Biotechnology research groups and research in Mathematical Biology and Applied Mathematics – GIBIMMA at the University of Nariño are also appreciated.

CONFLICT OF INTEREST

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest, such as honoraria, educational grants, participation in speakers' membership, employment, consultancies, stock ownership, or other equity interest, or expert testimony or patent arrangements. The authors declare they do not have personal or professional relationships, affiliations, knowledge, or beliefs regarding the subject matter or materials discussed in this manuscript. The authors declare that this work does not present any conflict of interest.

REFERENCES

- Ahluwalia, S.S. (2014). Microbial removal of hexavalent chromium and scale up potential. *International Journal of Current Microbiology and Applied Sciences*, 3(6), 383-398. <https://www.ijcmas.com/vol-3-6/Sarabjeet%20Singh%20Ahluwalia.pdf>
- American Public Health; Association, American Water Works; Federation, Water Environment. (1999). Chromium 117A Hexavalente chromium. In A. P. Health, A. W. Association, & W. E. Federation, *Standards Methods for the examination of water and wastewater* (p. 271).
- Aragón, M., & Alzate, A.M. (2004). Proyecto gestión ambiental en la industria de curtiembre en Colombia. Sistema de Referenciación ambiental (Sirac) para el sector curtiembre en Colombia. <http://www.icesi.edu.co/blogs/gestionintegralindustrial/files/2011/10/SIRAC-Curtiembres.pdf>
- Arrieta, G., & Espinoza, A. (2006). Characterization of a *Bacillus thuringiensis* strain collection isolated from diverse Costa Rican natural ecosystems. *Revista de Biología Tropical*, 54(1), 13-27. https://www.scielo.sa.cr/scielo.php?pid=S0034-77442006000100002&script=sci_arttext
- Awasthi, S.K., Duan, Y., Liu, T., Zhang, Z., Pandey, A., Varjani, S., Awasthi, M.K., & Taherzadeh, M.J. (2021). Can biochar regulate the fate of heavy metals (Cu and Zn) resistant bacteria community during the poultry manure composting? *Journal of Hazardous Materials*, 406(2021), 124593. DOI: <https://doi.org/10.1016/j.jhazmat.2020.124593>
- Barton, L.L., & Northup, D.E. (2011). *Microbial Ecology*. Wiley-Blackwell.
- Benson, D., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Ostell, J., Pruitt, K., & Sayers, E. (2018). GenBank. *Nucleic Acids Research*, 46(D1), D41-D47. DOI: <https://doi.org/10.1093/nar/gkx1094>
- Burbano, E.M., Caetano de Almeida, B., Otero, I.D., & Álvarez, S. (2017). *Manual de Biología Molecular - Procedimientos Básicos*. Editorial Universitaria Universidad de Nariño.
- Cárdenas-González, J.F., & Acosta-Rodríguez, I. (2010). Hexavalent chromium removal by a *Paecilomyces* sp. fungal strain isolated from environment. *Bioinorganic Chemistry and Applications*, 1-6. DOI: <https://doi.org/10.1155/2010/676243>
- Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., & Tiedje, J.M. (2014). Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Research*, 42(D1), D633-D642. DOI: <https://doi.org/10.1093/nar/gkt1244>
- Das, S., Mishra, J., Das, S.K., Pandey, S., Rao, D.S., Chakraborty, A., Sudarshan, M., Das, N., & Thatoi, H. (2014). Investigation on mechanism of Cr (VI) reduction and removal by *Bacillus amyloliquefaciens* a novel chromate tolerant bacterium isolated from chromite mine soil. *Chemosphere*, 96(2014), 112 - 121. DOI: <https://doi.org/10.1016/j.chemosphere.2013.08.080>
- Dhal, B., Thatoi, N., Das, N., & Pandey, B. (2013). Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical soil waste: A review. *Journal of Hazardous Materials*, 250(2013), 272 - 291. DOI: <https://doi.org/10.1016/j.jhazmat.2013.01.048>
- Geets, J., Vangronsveld, J., Diels, L., Taghavi, S., & Van der Lelie, D. (2008). Microbial activities, monitoring and application as part of a management strategy for heavy metal-contaminated soil and ground water. In A. E. Hartemink, A. B. McBratney, & R. Naidu (Eds.), *Chemical Bioavailability in Terrestrial Environment* (Vol. 32, pp. 521-559). *Developments in Soil Science*. DOI: [https://doi.org/10.1016/S0166-2481\(07\)32021-7](https://doi.org/10.1016/S0166-2481(07)32021-7)

- Hall, J.P., Wood, A.J., Harrison, E., & Brockhurst, M.A. (2016). Source-sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities. *Proceedings of the National Academy of Sciences*, 113(29), 8260-8265. DOI: <https://doi.org/10.1073/pnas.1600974113>
- Huang, T.-P., Xiao, Y., Pan, J.-R., Chen, Z., Li, L.-F., Xu, L., . . . Guan, X. (2014). Aerobic Cr(VI) reduction by an indigenous soil isolate *Bacillus thuringiensis* BRC-ZYR2. *Pedosphere*, 24(5), 652-661. DOI: [https://doi.org/10.1016/S1002-0160\(14\)60051-5](https://doi.org/10.1016/S1002-0160(14)60051-5)
- Huang, H., Zhao, Y., Xu, Z., Ding, Y., Zhou, X., & Dong, M. (2020). A high Mn(II)-tolerance strain, *Bacillus thuringiensis* HM7, isolated from manganese ore and its biosorption characteristics. *PeerJ - Journal of Life and Environmental Sciences*, 8(2020), 1 - 24. DOI: <https://doi.org/10.7717/peerj.8589>
- Joutey, N., Sayel, H., Bahafid, W., & El Ghachtouli, N. (2015). Mechanisms of Hexavalent Chromium Resistance and Removal by Microorganisms. *Reviews of Environmental Contamination and Toxicology*, 233(2015), 45-69. DOI: https://doi.org/10.1007/978-3-319-10479-9_2
- Lace, A., Ryan, D., Bowkett, M., & Cleary, J. (2019). Chromium monitoring in water by colorimetry using optimised 1,5-diphenylcarbazide method. *Environmental Research and Public Health*, 16(10), 1803. DOI: <https://doi.org/10.3390/ijerph16101803>
- Maldaner, J., Steffen, G.P., Missio, E.L., Saldanha, C.W., de Moraes, R.M., & Nicoloso, F.T. (2021). Tolerance of *Trichoderma* isolates to increasing concentrations of heavy metals. *International Journal of Environmental Studies*, 78(2), 185-197. DOI: <https://doi.org/10.1080/00207233.2020.1778290>
- Marrero-Coto, J., Díaz-Valdivia, A., & Coto-Pérez, O. (2010). Mecanismos moleculares de resistencia a metales pesados en las bacterias y sus aplicaciones en la biorremediación. *Revista CENIC Ciencias Biológicas*, 41(1), 67-78. <http://www.redalyc.org/articulo.oa?id=181221644010>
- Molokwane, P., Meli, K., & Nkhambayausi-Chirwa, E. (2008). Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading: Brits culture (South Africa). *Water Research*, 42(17), 4538 - 4548. DOI: <https://doi.org/10.1016/j.watres.2008.07.040>
- Moreno-Benavides, J., Peña-Salmenca, E., & Benítez-Campo, N. (2019). Reducing Cr⁶⁺ in electroplating wastewater with *Bacillus cereus* strain B1. *Universitas Scientiarum*, 24(1), 73-89. DOI: <https://doi.org/10.11144/Javeriana.SC24-1.rcie>
- Mtimunye, P. J., & Chirwa, E. M. (2014). Finite difference simulation of biological chromium (VI) reduction in aquifer media columns. *Water SA*, 40(2), 359 - 368. DOI: <http://dx.doi.org/10.4314/wsa.v40i2.18>
- Muneer, B., Rehman, A., Shakoori, F.R., & Shakoori, A.R. (2009). Evaluation of consortia of microorganisms for efficient removal of hexavalent chromium from industrial wastewater. *Bulletin of Environmental Contamination and Toxicology*, 82(2009), 597-600. DOI: <https://doi.org/10.1007/s00128-009-9662-3>
- Murray, J. (2001). *Mathematical Biology. II Spatial Models and Biomedical Applications* (3rd ed.). Springer.
- Otero, I.D. (2012). Remoción de la materia orgánica de las aguas residuales vertidas al Río Pasto con bacterias productoras de polihidroxialcanoatos [Tesis de Pregrado, Universidad de Nariño]. <http://biblioteca.udenar.edu.co:8085/atenea/biblioteca/85371.pdf>
- Otiniano García, M., Tuesta Collantes, L., Robles Castillo, H., Luján Velázquez, M., & Chavez Castillo, M. (2007). Biorremediación de cromo VI de aguas residuales de curtiembre por *Pseudomonas* sp y su efecto

sobre el ciclo celular de *Allium cepa*. *Revista Médica Vallejana*, 4(1), 32-40. DOI: <https://doi.org/10.18050/revistamedicavallejana.v4i1.2218>

Oves, M., Khan, M.S., & Zaidi, A. (2013). Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil. *Saudi Journal of Biological Sciences*, 20(2), 121-129. DOI: <https://doi.org/10.1016/j.sjbs.2012.11.006>

Prosser, J.I. (2020). Putting science back into microbial ecology: a question of approach. *Philosophical Transactions of the Royal Society B*, 375(1798), 20190240. DOI: <http://dx.doi.org/10.1098/rstb.2019.0240>

Ramírez, A., y Benítez-Campo, N. (2013). Tolerancia y reducción de cromo (VI) por *Bacillus cereus* b1, aislado de aguas residuales de una curtiembre. *Revista de ciencias*, 17(2), 51-63. http://revistas.univalle.edu.co/index.php/revista_de_ciencias/article/view/486

Ramírez-Díaz, M.I., Díaz-Pérez, C., Vargas, E.R., Riveros-Rosas, H., Campos-García, J., & Cervantes, C. (2008). Mechanisms of bacterial resistance to chromium compounds. *Biometals*, 21(3), 321-332. DOI: <https://doi.org/10.1007/s10534-007-9121-8>

Rath, B.P., Das, S., & Thatoi, H. (2019). Bioremediation Efficacy of Extracellular Chromate Reductase from *Bacillus amyloliquefaciens* (CSB 9) for Detoxification of Hexavalent Chromium. In N.R. Kundu R., *Environmental Biotechnology For Soil and Wastewater Implications on Ecosystems* (pp. 109-115). Springer. https://link.springer.com/chapter/10.1007/978-981-13-6846-2_14

Rath, B.P., Das, S., Das Mohapatra, P.K., & Thatoi, H. (2014). Optimization of extracellular chromate reductase production by *Bacillus amyloliquefaciens* (CSB 9) isolated from chromite mine environment. *Biocatalysis and Agricultural Biotechnology*, 3(3), 35-41. DOI: <https://doi.org/10.1016/j.bcab.2014.01.004>

Rawat, M., Rawat, A., Giri, K., & Rai, J. (2013). Cr (VI) sorption by free and immobilised chromate-reducing bacterial cells in PVA-alginate matrix: equilibrium isotherms and kinetic studies. *Environmental Science and Pollution Research*, 20(2013), 5198-5211. DOI: <https://doi.org/10.1007/s11356-013-1493-4>

Roestorff, M.M., & Chirwa, E.M. (2018). Comparison of the performance of *Chlorococcum ellipsoideum* and *Tetrademus obliquus* as a carbon source for reduction of Cr (VI) with bacteria. *Chemical Engineering Transactions*, 70(2018), 463-468. DOI: <https://doi.org/10.3303/CET1870078>

Sanjay, M.S., Sudarsanam, D., Raj, G.A., & Baskar, K. (2020). Isolation and identification of chromium reducing bacteria from tannery effluent. *Journal of King Saud University-Science*, 32(1), 265-271. DOI: <https://doi.org/10.1016/j.jksus.2018.05.001>

Sauka, D., & Benintende, G. (2008). *Bacillus thuringiensis*: generalidades. Un acercamiento a su empleo en el biocontrol de insectos lepidópteros que son plagas agrícolas. *Revista Argentina de Microbiología*, 40(2), 124-140. <http://www.redalyc.org/articulo.oa?id=213016787013>

Sher, S., & Rehman, A. (2019). Use of heavy metals resistant bacteria - a strategy for arsenic bioremediation. *Applied microbiology and biotechnology*, 103(15), 6007-6021. <https://doi.org/10.1007/s00253-019-09933-6>

Shukla, O., Rai, U., Singh, N., Dubey, S., & Baghel, V. (2007). Isolation and characterization of chromate resistant bacteria from tannery effluent. *Journal of Environmental Biology*, 28(2), 399-403. http://www.jeb.co.in/journal_issues/200704_apr07_supp/paper_08.pdf

Smrithi, A., & Usha, K. (2012). Isolation and characterization of chromium removing bacteria from tannery effluent disposal site. *International journal of advanced biotechnology and research*, 3(3), 644-652. <https://pdfs.semanticscholar.org/e8ef/dc24c9613620efa7ea067bc6acd704d772.pdf>

Sridevi, V., & Raghuram, M. (2019). Multiple metal tolerance of *Paenibacillus dentritiformis* isolated from metal contaminated soils west Godavari district (Andhra Pradesh). *Journal of Applied and Natural Science*, 11(2), 486-491. DOI: <https://doi.org/10.31018/jans.v11i2.2094>

Srivastava, N., Dhal, B., & Pandey, B. (2014). Bioreduction of Hexavalent Chromium by *Bacillus cereus* Isolated from Chromite Mine Overburden Soil. *Advanced Materials Research*, 828(2014), 81-91. DOI: <https://doi.org/10.4028/www.scientific.net/AMR.828.81>

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28(10), 2731-2739. DOI: <https://doi.org/10.1093/molbev/msr121>

Teles, Y.V., de Castro, L.M., Sargentini, E., Do Nascimento, A.P., Da Silva, H.A., Costa, R.S., . . . Pereira, J.O. (2018). Potential of Bacterial Isolates from a stream in Manaus- Amazon to bioremediate chromium-contaminated environments. *Water, Air & Soil Pollution*, 229(2018), 266. DOI: <https://doi.org/10.1007/s11270-018-3903-1>

Terry C.H. (2019). Environmental Systems Biology Approach to Bioremediation. In H. Christon J., *Understanding Terrestrial Microbial Communities* (pp. 103-127). Springer. https://link.springer.com/chapter/10.1007/978-3-030-10777-2_4

Tiwari, S., Singh, S., & Garg, S. (2014). Bacterial Reduction of Cr (VI) and Fe (III) in In Vitro Conditions. *Bioremediation Journal*, 18(2), 158-168. DOI: <https://doi.org/10.1080/10889868.2014.900473>

Trinidad Bello, A. (2014). Modelos de crecimiento en biología, su significado biológico y selección del modelo por su ajuste. División de Ciencias Básicas e Ingenierías. Iztapalapa - México: Universidad Autónoma Metropolitana. http://mat.izt.uam.mx/mcmai/documentos/tesis/Gen.11-O/Adalberto_Trinidad.pdf

Wani, P.A., Wani, J.A., & Wahid, S. (2018). Recent advances in the mechanism of detoxification of genotoxic and cytotoxic Cr (VI) by microbes. *Journal of Environmental Chemical Engineering*, 6(4), 3798-3807. DOI: <https://doi.org/10.1016/j.jece.2018.05.042>

Xiao, W., Ye, X., Yang, X., Zhu, Z., Sun, C., Zhang, Q., & Xu, P. (2017). Isolation and characterization of chromium (VI)-reducing *Bacillus* sp. FY1 and *Arthrobacter* sp. WZ2 and their bioremediation potential. *Bioremediation Journal*, 21(2), 100-108. DOI: <https://doi.org/10.1080/10889868.2017.1282939>

Xu, X., Zarecki, R., Medina, S., Ofaim, S., Liu, X., Chen, C., . . . Freilich, S. (2019). Modelling microbial communities from atrazine contaminated soils promotes the development of biostimulation solutions. *The ISME Multidisciplinary Journal of Microbial Ecology*, 13(2019), 494-508. DOI: <https://doi.org/10.1038/s41396-018-0288-5>

Zhao, C., Yang, Q., Chen, W., & Teng, B. (2011). Removal of hexavalent chromium in tannery wastewater by *Bacillus cereus*. *Canadian Journal of Microbiology*, 58(1), 23 - 28. DOI: <https://doi.org/10.1139/w11-096>

Zhao, R., Wang, B., Cai, Q. T., Li, X. X., Liu, M., Hu, D., . . . Fan, C. (2016). Bioremediation of hexavalent chromium pollution by *Sporosarcina saromensis* M52 isolated from offshore sediments in Xiamen, China. *Biomedical and Environmental Sciences*, 29(2), 127 - 136. DOI: <https://doi.org/10.3967/bes2016.014>

ANEXO

Supplementary material No. 1: These solutions correspond to the standard variables of the model (1) and represent the densities of bacterial populations and the reduction hexavalent chromium concentration of *Bacillus thuringiensis*.

Supplementary material No. 2: These solutions correspond to the standard variables of the model (1) and represent the densities of bacterial populations and the reduction hexavalent chromium concentration of *Paenibacillus* sp.

Supplementary material No. 3: These solutions correspond to the standard variables of the model (1) and represent the densities of bacterial populations and the reduction hexavalent chromium concentration of *Bacillus amyloliquefaciens*.