Research article

Contrast between a mathematical model and the bioreduction process of Cr(VI) by consortia of bacteria isolated from wastewater of the Pasto River

Contraste entre un modelo matemático y el proceso de biorreducción de Cr(VI) por consorcios de bacterias aisladas de agua residual del Rio Pasto

Jhonatan Pinta-Melo[1], Deisy Lorena Guerrero-Ceballos[2], Miller Cerón Gómez[3], Eduardo Ibargüen-Mondragón[4], Pablo Fernández-Izquierdo[5], Edith Mariela Burbano-Rosero[6]

Abstract

Introduction. The versatile metabolism of microorganisms allows biodegradation of a wide variety of contaminants; however, the increasing scale of the industry exceeds its pollutant processing capacity. Due to its high toxicity, hexavalent chromium Cr(VI) is one of the heavy metals with the greatest global dissemination and concern. There are numerous investigations focused on proposing treatments to purify the different ecosystems affected with this metal; however, knowledge about various microorganisms from the same source that could help solve this problem is restricted. **Objective.** To evaluate the efficiency in the reduction of Cr(VI) of bacterial consortia *(B. thuringiensis, B. amyloliquefaciens* and *Paenibacillus* sp.*)* in a Batch type treatment, using municipal wastewater from Río Pasto as a substrate. **Methodology.** A mathematical model was formulated that reliably predicted the behavior of the consortia, in relation to their growth and reduction percentage, the results of the simulations were compared with experimental data to select the consortium with the best Cr(VI) reduction results). Subsequently, its efficiency in the reduction of Cr(VI) was determined, using unsterilized residual river water as a substrate. **Results.** The statistical analyzes highlighted the absence of statistically significant differences in the

1. Departamento de Biología, Universidad de Nariño, Campus Universitario Torobajo, Pasto, Colombia.
ORCID: https://orcid.org/0000-0003-0347-2559

2. Departamento de Biología, Universidad de Nariño, Campus Universitario Torobajo, Pasto, Colombia.

3. Departamento de Matemáticas y Estadística, Universidad de Nariño, Campus Universitario Torobajo, Pasto, Colombia.

4. Departamento de Matemáticas y Estadística, Universidad de Nariño, Campus Universitario Torobajo, Pasto, Colombia.

5. Departamento de Biología, Universidad de Nariño, Campus Universitario Torobajo, Pasto, Colombia.

6. Departamento de Biología, Universidad de Nariño, Campus Universitario Torobajo, Pasto, Colombia.

Correspondence: jpinta@udenar.edu.co

DOI: https://doi.org/10.22490/24629448.6921

Recibido: 22/11/2022
Aceptado: 26/01/2023

reduction percentages between the consortia. However, with the selected culture, a reduction percentage of 91% was presented in 156 hours. **Conclusion.** The results found in this research are promising for their application in the improvement of treatment practices for Cr(VI) bioremediation.

Keywords: Metals heavy, Chromium, bacteria, decontamination, wastewater, Batch Cell Culture. DeCS

Resumen

Introducción. El metabolismo versátil de los microorganismos permite la biodegradación de una amplia variedad de contaminantes, sin embargo, la creciente ampliación de la industria supera su capacidad de procesamiento de poluentes. El cromo hexavalente Cr(VI) debido a su alta toxicidad es uno de los metales pesados de mayor difusión y preocupación a nivel global. Existen numerosas investigaciones enfocadas en proponer tratamientos para depurar los diferentes ecosistemas afectados con este metal; no obstante, el conocimiento sobre diversos microorganismos de una misma fuente que podrían ayudar a solucionar esta problemática se ve restringido. **Objetivo.** Evaluar la eficiencia en la reducción de Cr(VI) de consorcios bacterianos *(B. thuringiensis, B. amyloliquefaciens* y *Paenibacillus* sp.*)* en un tratamiento tipo Batch, utilizando como sustrato agua residual municipal del Río Pasto. **Metodología.** Se formuló un modelo matemático que predijo de manera confiable el comportamiento de los consorcios, con relación a su crecimiento y el porcentaje de reducción, los resultados de las simulaciones fueron comparados con datos experimentales para seleccionar el consorcio con mejores resultados de reducción de Cr(VI). Posteriormente, se determinó su eficiencia en la reducción de Cr(VI), usando como sustrato agua residual del rio sin esterilizar. **Resultados.** Los análisis estadísticos resaltaron la ausencia de diferencias estadísticamente significativas en los porcentajes de reducción entre los consorcios. No obstante, con el cultivo seleccionado se presentó un porcentaje de reducción de 91% en 156 horas. **Conclusión.** Los resultados encontrados en esta investigación son promisorios para su aplicación en el mejoramiento de las prácticas de tratamiento para la biorremediación de Cr(VI).

Palabras clave: Metales pesados, cromo, bacteria, descontaminación, agua residual, cultivo por lotes. DeCS

Introduction

In recent years, there is an evident interest in the evaluation of biological systems for the detoxification of effluents contaminated with Cr(VI), these offer efficient and low-cost alternatives compared to conventional technologies used for removal; one of the biological systems to highlight is bioremediation mediated by bacteria, because they present a wide functional diversity (1). There are efficient alternatives focused on the use of bacteria present in soils and water sources of environments contaminated with chromium, these organisms by adaptation have acquired mechanisms that allow them to survive in this type of media and present greater efficiency and profitability when implemented in treatment systems of effluents contaminated with toxic agents (2-6).

Chromium is found in high concentrations in most of the waste generated in industrial activities, such as: galvanizing, alloys, tanning processes, and textile dyeing (7-10). In relation to this, it has been determined that the effects of chromium for the environment and human health depend on the oxidation state, the most stable are the trivalent and hexavalent forms, although both forms are harmful, the latter is considered the most toxic; due to characteristics such as high solubility in water, high degree of oxidation and ability to cross biological membranes, causing alterations in the cellular structure, including DNA (11,12,9).

Cr in the department of Nariño, as in other regions is widely used in the leather tanning industry, a process that allows obtaining good quality leather, rational and economical production, for these reasons it is difficult for this method to lose its leadership within the microenterprises that employ it (13). Inconveniently, the waste generated in the tanning process is discharged directly and indirectly into the main water sources, permanently affecting the life forms present in the effluent, causing loss of biodiversity and a significant alteration in the ecosystem. According to the investigation of Alzate Tejada (2004), for the Rio Pasto a concentration of 59 mg L-1 of total chromium was observed in the dumping points of solid and liquid waste (Barrio Pandíaco - San Juan de Pasto), also, great amount of organic matter and low oxygen concentration (14,5).

Interested in delving into this problem, the Research Groups in Microbial Biotechnology and Mathematical Biology and Applied Mathematics (GIBIMMA), together with the microbial processes laboratory of the University of Nariño, evaluated the efficiency in the reduction of Cr(VI) from three bacterial isolates identified as *B. thuringiensis* [MK561610], *B. amyloliquefaciens* [MK561611], and *Paenibacillus sp.* [MK561612], in a Batch-type treatment using water from the Pasto River as a substrate, the results indicated reduction percentages of 82%; 80% and 79%, respectively (15). In relation to the research in question, it is indisputable that the results regarding the reduction of Cr(VI) are promising; however, gaps were accentuated on the hypothesis "a consortium made up of the combination of two or three of these bacterial isolates presents greater efficiency in the reduction of the metal in the effluents". Through the execution of this work, is possible to know at a laboratory scale the efficiency that a mixed bacterial culture could have in the reduction of Cr(VI), with the expectation of developing in the future a biological strategy for the removal or reduction of Cr(VI). In additionally, the formulation of a mathematical model will allow to generate predictions and adjustments on this reduction process, with the aim of contributing to the management of these pollutants and improving the safety of the environment.

Materials and methods

Viability of bacterial isolates

The source bacterial isolates for this study *(B. amyloliquefaciens, B. thuringiensis* and *Paenibacillus sp.)* were provided by the Microbial Biotechnology group of the University of Nariño. They were obtained from samples of water from the river near the tanneries located in the Pandíaco neighborhood (16) (Framework permit for collection for scientific research purposes Corponariño-Resolution 126 of February 19, 2015). Wild isolates were preserved in glycerol at 30% diluted in Luria Bertani (LB) broth and the vials were refrigerated at 4 °C. Additionally, the bacteria in this study are already registered in the Microbiological Collection of the University of Nariño with the Single National Registry of Collections No. 256 and are in the process of being deposited.

The isolates were made viable in test tubes with LB broth (Tryptone 10 g L^{-1}, yeast extract 5 g L^{-1} and NaCl 10 g L^{-1}), after 24 h of incubation at 35 °C, they were propagated in petri dishes with LB agar to subsequently verify their axenic condition by Gram staining; consecutively, the cryopreservation process was carried out, following the method of conservation in glycerol at 30% (diluted in LB broth) and the vials were refrigerated at -20 °C.

Cr(VI) tolerance assay

A pilot test was carried out to confirm the tolerance to different concentrations of Cr(VI) of each of the bacteria used in the study. Cr(VI)-reducing bacteria were seeded using the spread plate technique on LB agar (Luria Bertani), immediately four discs of filter paper (Whatman type 3) impregnated with 7 µL of different concentrations of Cr(VI) were placed (1200, 1500, 2000 and 2500 mg L^{-1}) in the form of potassium dichromate ($K_2Cr_2O_7$), an antibiotic sensidisc was also deposited as a positive control

(ATM Aztreonam 30 µg). Assays were incubated for 36 hours at 35°C; Cr(VI) tolerance was established by the absence of inhibition halos around each disk. All assays were performed in triplicate.

Cross-antagonism tests

To use mixed cultures as initiators of the bioremediation process, the compatibility verification between the isolates was carried out by means of the crossed streak test. The preparation of the culture was carried out by inoculating a colony of a culture previously grown in two tubes with 10 mL of LB broth, corresponding to each microorganism, when the liquid cultures reached a turbidity equivalent to the McFarland scale 5 at a wavelength of 600 nm, the corresponding bacteria were seeded in LB agar, for which the petri dish was divided into three sections, in each of the angles *B. thuringiensis, B. amyloliquefaciens* and *Paenibacillus sp.*

Subsequently, at a 90° angle, the sowing of the two additional isolates was carried out, that is, in the line of *B. thuringiensis, B. amyloliquefaciens* was sown transversally and 1,5 cm below Paenibacillus sp., in the same way we proceeded for the isolates remaining, the process was carried out in triplicate. The plates were incubated at 35 °C for 24 hours, after this period, the antagonistic activity was corroborated by the presence of zones of inhibition (2).

Determination of Cr(VI) concentration

A standard curve was made in order to quantify the Cr(VI) reduction of the cultures in the treatments. From a standard solution of Cr(VI) (100 mg L^{-1}) in the form of $K_2Cr_2O_7$, six dilutions of concentrations between 0,1 and 1 mg L^{-1} of Cr(VI) were prepared with deionized water. Sequentially, 5 mL of each solution were chemically analyzed using the 1,5 diphenylcarbazide colorimetric method, which consists of adding 0,25 mL of sulfuric acid (0,5M H_2SO_4), 0,25 mL of phosphoric acid (0,5M H_2PO_4) and 1 mL of 1,5 diphenylcarbazide solution dissolved in acetone, homogenized, and allowed to stand until the development of the characteristic color of the reaction was observed. The absorbance of all solutions was determined at a wavelength of 540 nm in a UV-1800 spectrophotometer (SHIMADZU ©) (15,17,5). Finally, the calibration curve that relates the chromium concentration and the absorbance was plotted using GraphPad Prism version 8.4.2 for Windows, GraphPad Software, San Diego, California USA. (Prism® Trial license 9750FB1EF35).

Proposal for the composition of the consortia

Pure cultures of each bacterial isolate were incubated at 35°C until they reached a turbidity with an approximate optical intensity of tube number five on the McFarland scale at a wavelength of 600 nm; after the incubation period, they were combined in 1:1 proportion for the cultures made up of two isolates and 1:1:1 for the culture containing the three bacteria. The volume of the cultures depended on the inoculum that was used for each experimental phase (10% of the volume of the medium), (Modified from Chandar, Singh, & Yadav, 2012).

Mathematical approximation

Characteristics of the analytical method

The estimation of the behavior over time of the mixed cultures was carried out, considering the reduction of the Cr(VI) concentration and the percentage of reduction. For this, ordinary differential equations based on logistic growth, the law of mass action and Holling-type competition models were used, equations that have been used in different studies at the biological level; allowing to describe the cause-effect relationships in the evaluated systems (18-20).

Applicable parameters for growth in mixed culture and Cr(VI) reduction

The formulation of the mathematical model for bacterial growth and Cr(VI) reduction was carried out under the following considerations: The bacterial population at time t was denoted as (**B**), assuming that bacteria reproduce under logistic growth with a reproduction rate (**ϱ**) dependent on the amount of substrate (**S**) present in the experimental unit; additionally, the effect of intra and interspecific competition of bacteria for substrate was denoted (**ac**) and added to the growth-limiting parameters. Chromium (**C**) affects bacterial growth following the law of mass action; since the interaction between Cr and bacteria is not necessarily one-to-one, that is, several moles of Cr can interact with a bacterium; Similarly, its action depends on the rate of bacterial elimination (**α**) due to the concentration of Cr(VI). On the other hand, this law is associated with the rate of Cr reduction (**r**), since it will depend on both the Cr concentration in the medium and the bacterial population, (**kc**) represented the rate of substrate consumption. Finally, (**γ**) symbolized the rate of natural death.

With these considerations, the following system of ordinary differential equations was obtained:

Equation 1. **Modeling of the effect of Cr on the growth of the bacterial population in the culture with three bacteria.**

The suffix on Bi varies from i to k where **i** represents *B. thuringiensis*, **j** represents *B. amyloliquefaciens* and **k** represents *Paenibacillus sp.*

Thus, the equation for ***B. thuringiensis:***

$$\frac{dB_i}{dt} = \rho_i B_i \left(1 - ac_i \frac{B_i + B_j + B_k}{S}\right) - \alpha_i C B_i + \gamma_i S B_i,$$

and for ***B. amyloliquefaciens*** it was:

$$\frac{dB_j}{dt} = \rho_j B_j \left(1 - ac_j \frac{B_i + B_j + B_k}{S}\right) - \alpha_j C B_j + \gamma_j S B_j,$$

Finally, the equation for ***Paenibacillus*** was:

$$\frac{dB_k}{dt} = \rho_k B_k \left(1 - ac_k \frac{B_i + B_j + B_k}{S}\right) - \alpha_k C B_k + \gamma_k S B_k.$$

(1)

Equation 2. **Modeling of the reduction in Cr(VI) concentration by the bacterial population.**

$$\frac{dC}{dt} = -C\left(r_i B_i + r_j B_j + r_k B_k\right)$$

(2)

Equation 3. **Modeling of substrate consumption during the growth of the bacterial population.**

$$\frac{dS}{dt} = -S\left(kc_i B_i + kc_j B_j + kc_k B_k\right)$$

(3)

The adjusted values for the mentioned kinetic parameters were estimated mathematically based on previous experimental data through growth kinetics of each bacterium in a synthetic medium (5). Finally, numerical simulations were carried out with the previously calculated parameters through the optimization of the values using the genetic algorithm method (AG1) in the MATLAB 8.5 version R2015a for windows, The MathWorks software, Inc., Natick, Massachusetts, United States. (License: 40904757), which allowed predicting different behavior scenarios between the different bacterial cultures and the concentration of Cr.

Experimental design

Percentage reduction of Cr(VI) of the four consortia in sterile wastewater from the Pasto River

The experimental system consisted of fifteen 500 mL flasks with a working volume of 250 mL, made up of previously sterilized municipal wastewater (SMWW) from the Pasto River, the corresponding bacterial inoculum, and the supplementation of 50 mg L^{-1} of $K_2Cr_2O_7$. The MWW sample was collected at the Juan 23 sewage and domestic water collector located in the Pandíaco neighborhood of the city of Pasto (N: 01°13’ 55.9”, W: 77°17’ 25.4”) between the month March and April 2019. It should be noted that, to avoid interference with the measurement methods, the water was filtered and subsequently diluted in buffered water in a 1:1 ratio. It was sterilized in an autoclave at 121 °C for 15 minutes.

We worked from a 10% bacterial inoculum that contained approximately 10^8 bacteria per milliliter, considering the different bacterial cultures. The above procedure was standardized to 10^8 bacteria per milliliter, at an absorbance of 600 nm; in order to have inoculate with a similar population density before starting the process. The abiotic control treatment consisted of 250 mL of sterile municipal wastewater (SMWW). The process lasted 156 hours, during this time the temperature was kept constant at 23 °C, pH 7 and agitation 0,2 vvm.

In this instance, the following clarifications should be made: the final concentration of Cr(VI) in the medium was calculated considering that only a fraction of the molecular weight of $K_2Cr_2O_7$ corresponds to Cr(VI), therefore, it must be considered count the purity of the reagent. The conditions in which the Cr reduction process was evaluated approximate the environment in which this bioremediation process would be carried out, where the growth and, in general, the metabolism of microorganisms is limited by different factors such as temperature, pH and bioavailability of minerals and nutrients.

Operational procedure

For the determination of the Cr(VI) reduction process, samples (0,4 mL) were taken every 12 hours in triplicate, which were diluted in 25 mL of deionized water and consecutively to 5 mL of this dilution, 0,25 mL of 0,5 M H_2SO_4, 0,25 mL of 0,5 M H_2PO_4 and 1 mL of 1,5 diphenylcarbazide, were added, the mixture was homogenized to determine the absorbance at a wavelength of 540 nm. Immediately afterwards, the concentration of Cr(VI) and the reduction percentage were determined considering the previously standardized calibration curve and equation 4 (21).

Equation 4. **Percentage reduction of Cr(VI).** Ratio between the initial concentration minus the final concentration, over the initial concentration of Cr(VI).

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

(4)

The determination of bacterial biomass was carried out using the plate extension technique. Initially, 1 mL was used for the preparation of serial dilutions. Finally, 100 μL of the 10^{-3} and 10^{-5} dilutions of the sample were seeded by spread plate in petri dishes with LB agar and incubated at 35 °C for approximately 24 hours; After this time, the colony-forming units (CFU) were counted. The report was made in CFU mL^{-1} using equation 5 (22).

Equation 5. **Colony-forming units per milliliter.** Relationship between the number of colonies by the dilution factor.

$$CFUmL^{-1} = Colonies\ on\ plate\ *\ dilution\ factor$$

(5)

Percentage reduction of Cr(VI) of the selected consortium in unsterilized municipal wastewater from the Pasto River (MWWs)

In the second phase of the research, PVC bioreactors with a total capacity of 12 L and a working volume of 7,4 L of unsterilized municipal wastewater (UMWW) were used, supplemented with 50 mg L-1 of potassium dichromate. The water sample was collected considering the same specifications as in the previous phase, except for its sterilization. Two control treatments were implemented, biotic and abiotic control that consisted of 7,4 L of unsterilized municipal wastewater (UMWW) and sterile municipal wastewater (SMWW), respectively. In the same way, the bioreactors were operated for 156 hours, the verification processes of the reduction percentage in the treatments and the growth determination were carried out following the same operational procedures.

Statistical data analysis

All observations were carried out in triplicate (n = 3) to improve the analytical precision of the experiment. To confirm the variability of the data obtained and the validity of the growth results and the Cr(VI) reduction potential, a General Linear Model (GLM) was run due to the characteristics of the data obtained. All analyzes were performed with a significance level of 95% ($p<0,05$) using STATGRAPHICS® Centurion XVIII Version 18.1.06, StatPoint Technologies software. (UDENAR Business Academic License).

Ethical considerations

This research was evaluated and approved by the Research Ethics Committee of the University of Nariño, who according to the categories established in article 11 of Resolution No. 008430 of October 4, 1993, of the Ministry of Health, classified as "No risk" to this study (Minutes No. 001 of May 15, 2018).

The microorganisms handled in the study correspond to risk type I or II. The waste generated in the development of this project was delivered to the Metropolitan Cleaning Company of Pasto (EMAS Pasto) under the agreement to provide services for the collection, transportation, treatment, and final disposal of hazardous waste (Code 5016540).

Results

Cr(VI) tolerance

The three bacterial isolates grew massively when subjected to four solutions with different concentrations of Cr(VI), *B. thuringiensis* and *Paenibacillus sp.*, showed tolerance below 2000 mg L-1, *B. amyloliquefaciens* presented a halo of weak inhibition from 1500 mg L^{-1}. This made it possible to confirm that after the cryopreservation process, the isolates maintained the ability to tolerate the metal.

Antagonism assay

The seeded plates were examined by the cross-streak method in search of zones of inhibition, which would indicate antagonism between the isolates. When observing the plates with the combinations, it was noted that they did not show antagonism; therefore, it was determined that the three isolates could be compatible with each other for the formation of effective consortia in the reduction of Cr(VI) (2).

Cr(VI) quantification

The 1,5 diphenylcarbazide colorimetric method used in this study for Cr(VI) quantification is used as a standard method due to its success in determining Cr(VI) at trace levels in tap, river, and wastewater samples and groundwater (23). For this study, a coefficient of determination (R2) of 0,9958 (Abs = 0,5992 [Cr]-0.02465) was obtained and a detection limit of 0,09 mg L-1 was reached when a sample volume of 400 μL was used.

Table 1 reports the average values of the absorbance data obtained for the standards of known concentration, as well as the coefficient of variation and the standard deviation of the data, values that support the precision and reliability of the calibration curve. The coefficient of variation indicates the homogeneity of the absorbance data obtained for each concentration, while the standard deviation allows us to observe the concentration of the data around the mean, these two values ensure the quality and reproducibility of the data. In addition, the calibration curve (see figure 1) shows good linearity, with a fit R2 = 0,9958, which represents reliability of the experimental fit.

Table 1. Data used in the calibration curve.

Concentration Cr(VI) mg L^{-1}	Absorbance (540 nm)			Arithmetic average	SD	%VC
	M1	M2	M3			
0,1	0,037	0,035	0,035	0,036	0,001	1,619
0,2	0,106	0,089	0,098	0,098	0,009	8,708
0,4	0,226	0,201	0,214	0,214	0,013	5,852
0,6	0,31	0,298	0,304	0,304	0,006	1,974
0,8	0,459	0,479	0,469	0,469	0,01	2,132
1	0,619	0,544	0,582	0,582	0,038	6,447

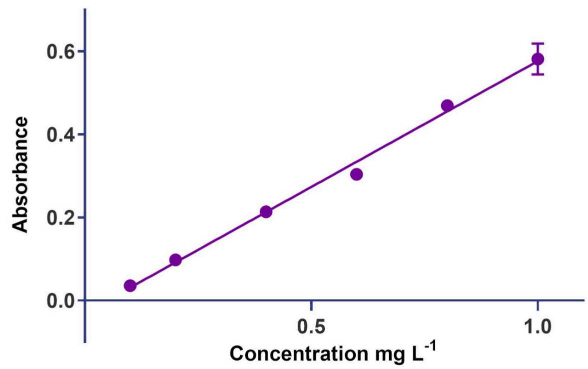

Figure 1. Calibration curve. Concentration vs. absorbance relationship by colorimetric analysis.

Mathematical approximation

Mathematical prediction of the behavior of mixed crops

Table 2 reports the values of the parameters present in the model; It should be noted that, although the values of the parameters were postulated from data on the kinetics of growth and reduction of each of the bacterial isolates; an absolute similarity between the prediction and the experimental data of the present work cannot be expected, since these parameters were adjusted under optimal culture conditions for bacterial growth.

Table 2. Kinetic parameters obtained using the genetic algorithm.

Model parameters	B. thuringiensis	B. amyloliquefaciens	Paenibacillus sp.
Reproduction rate (ρ)	0,0867	0,2359	0,1354
Substrate consumption rate (**kc**)	8,0350e-9	1,0158e-8	1,2018e-8
Adjustment parameter–competence (**ac**)	6,3300e-9	4,0490e-9	9,9900e-9
Cr(VI) reduction rate (**r**)	1,4900e-9	2,6700e-9	1,8690e-9
Natural death rate (γ)	0,0580	0,0188	0,0040
Death rate by Cr action (α)	0,00038	0,0031	0,00068

Figures 2 to 5 show the curves resulting from the simulation process of the consortia, the growth curves are located on the right and the Cr(VI) reduction graphs on the left. For the first mixed culture made up of *B. thuringiensis* and *Paenibacillus sp.* (Bt + Ps); based on the simulations, a reduction percentage of 24 % was observed when using this culture. In the bacterial growth curves, two growth peaks are observed, they can be caused by differences in the metabolism of the bacteria, which is reflected in variations in the phases of adaptation to the medium with Cr, dissimilar growth rates and inequality in the metabolic efficiency against Cr(VI) (Figure 2).

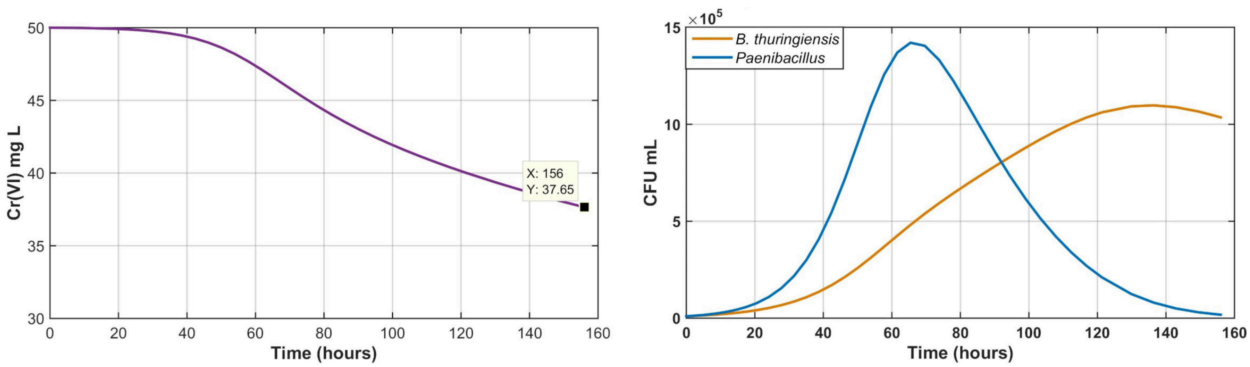

Figure 2. Prediction behavior of conformed culture by *B. thuringiensis* and *Paenibacillus sp.* (Bt + Ps), (left) Reduction of Cr(VI), (right) Bacterial growth.

Figure 3 represents the postulated reduction curve for the mixed culture made up of *B. thuringiensis* and *B. amyloliquefaciens.* (Bt + Ba), which indicates that the reduction percentage for this crop is likely to be 28%. When introducing the values of the bacteria parameters in the equations of the proposed mathematical model, right side graph was obtained, in which a lower growth was observed for *B. thuringiensis*, almost parallel to the growth of *B. amyloliquefaciens*, with dissimilarities in the phase of cell death, since rate of cell elimination by the action of Cr seems to be lower in the case of *B. thuringiensis*.

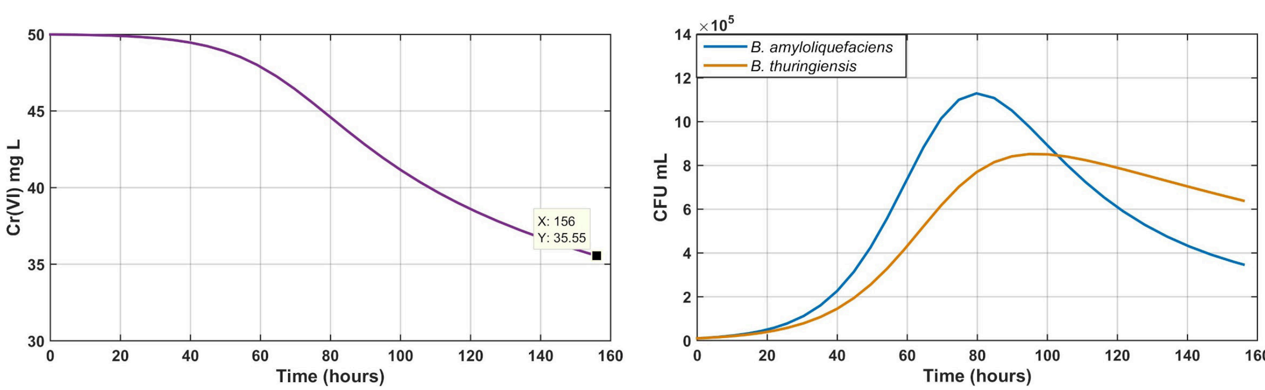

Figure 3. Prediction behavior of conformed culture by *B. thuringiensis* and *B. amyloliquefaciens.* (Bt + Ba), (left) Cr(VI) reduction, (right) Bacterial growth.

The reduction percentage for the culture composed of *B. amyloliquefaciens* and *Paenibacillus sp.*, was 32% in the period of 156 hours. Unlike the two previous models, in this case a simile of the stationary phase of the theoretical growth curves is observed, most of the reduction could be carried out by the bacterium *B. amyloliquefaciens*; since, up to 60 hours, the percentage of reduction is around 6%, later the *Paenibacillus* bacterium begins its death phase and *B. amyloliquefaciens* increases its growth, which overlaps with the increase in the metal reduction process (see figure 4).

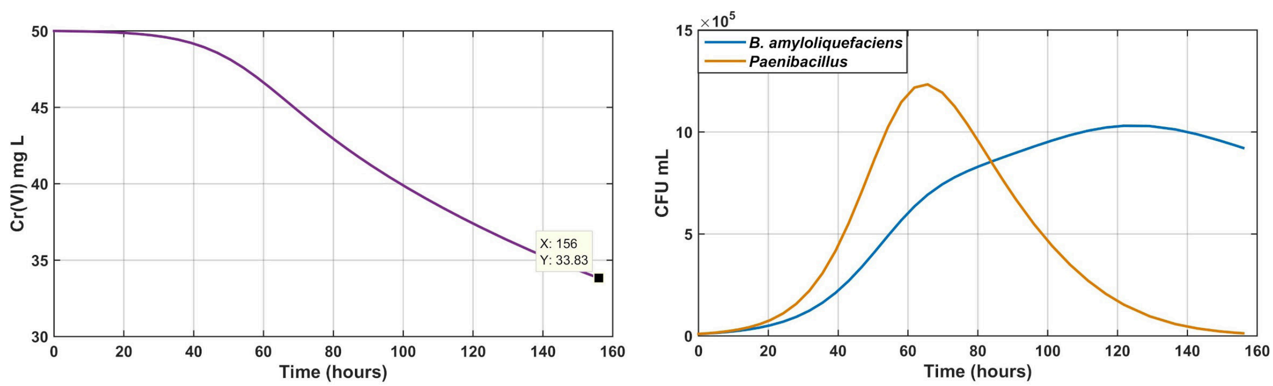

Figure 4. Prediction behavior of conformed culture by *B. amyloliquefaciens* and *Paenibacillus sp.* (Ba + Ps), (left) Cr(VI) reduction, (right) Bacterial growth.

Figure 5 indicates the result of the simulation of the mathematical modeling for the culture made up of the three bacteria *B.thuringiensis, B. amyloliquefaciens* and *Paenibacillus sp.*, this consortium reached a reduction percentage of 30%. The greatest reduction activity occurred in the interval from 60 to 120 hours, a period in which the bacteria *B. thuringiensis* and *B. amyloliquefaciens* seem to reach the exponential and stationary phases. On the other hand, the bacterium *Paenibacillus sp.*, presented a similar growth curve in each of the predictions, this is probably due to the fact that, according to the data used in the estimation of the model parameters, this bacterium presented a good growth in different concentrations. of Cr (data not supplied). A similar phenomenon occurred with the bacterium *Pseudomonas putida* APRRJVITS11, which despite indicating a high tolerance to Cr (1250 ppm) showed better growth at concentrations of 100 ppm Cr(VI) (24).

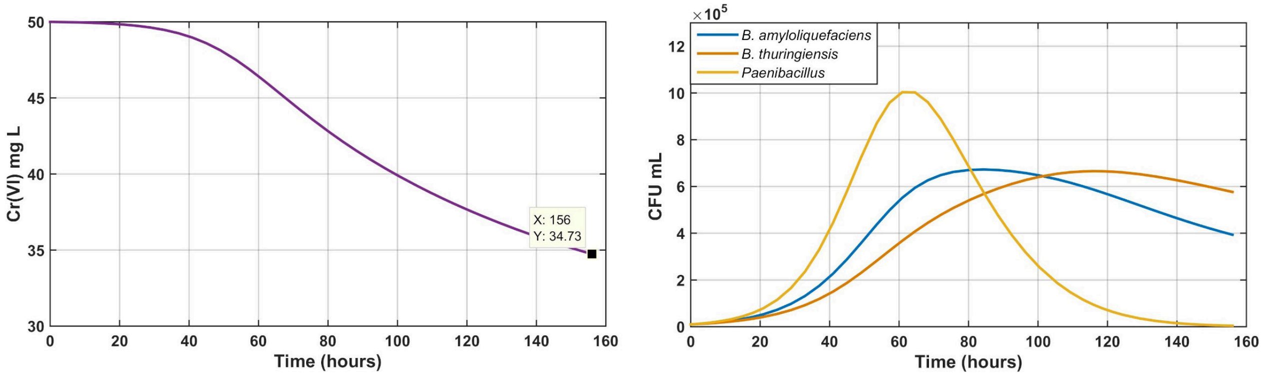

Figure 5. Prediction behavior of conformed culture by *B. thuringiensis, B. amyloliquefaciens* and *Paenibacillus sp.* (Bt + Ba + Ps), (left) Reduction of Cr(VI), (right) Bacterial growth.

When observing the predictions in more detail, it is possible to notice that *Paenibacillus* first develops its growth on the other two bacteria involved in the models, which would corroborate the hypothesis that the theoretically evaluated Cr concentration affects its growth to a lesser extent. The results observed in the simulations indicate that there is no major difference in the percentages of Cr(VI) reduction (24, 28, 32 and 30%) when using any of the proposed cultures, since all reached concentrations close to 35 mg L^{-1} at the end of the evaluation period.

Under the conditions of the experiment, it was possible to evaluate the effect of Cr(-VI) concentration on bacterial growth and, at the same time, how it relates to Cr(VI) reduction; however, the interaction of the bacteria with another type of substrate, such as the residual water of the Pasto River, could present different perspectives to that observed in the models; therefore, it is pertinent to carry out the selection process with the four proposed treatments.

Selection of the mixed bacterial culture with the greatest reduction potential

The growth and Cr reduction curves of the cultures made up of *Bacillus thuringiensis* (**Bt**), *B. amyloliquefaciens* (**Ba**) and *Paenibacillus sp.* (**Ps**); cultured in sterile municipal wastewater in the presence of Cr(VI) at a concentration of 50 mg L^{-1} are shown in figures 6A to 6D, in general there was no adaptation or latency phase in the first hours of the trial, for In this phase, bacterial growth was not significantly influenced by Cr(VI), while two peaks of exponential growth were observed, the first from 0 to 36 hours and the second during 84 to 96 hours of fermentation.

The results of the statistical analysis allowed us to observe that there is a highly significant relationship between growth and the factors, treatment (bacterial culture) and time (p-value 0,0000). The general linear model (GLM) was significant and explained 80,4% of the variability in the data (p-value 0,0000), consequently, there is 19,6% of variation in the data that is attributed to other factors that modify the dependent variable (growth). Using Fisher's multiple comparison test (LSD), the existence of significant differences with respect to growth and the comparison between pairs of means was graphically verified (see figure 7).

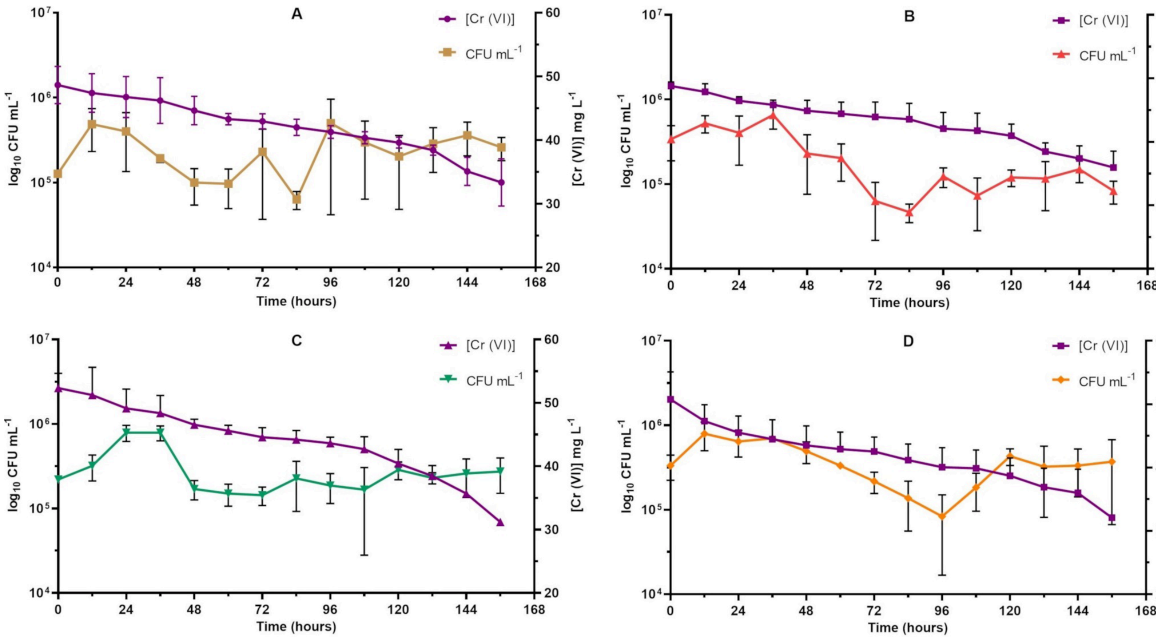

Figure 6. Evaluation of the joint action of crops and their effect on the reduction of Cr(VI) in sterile municipal wastewater. a) Growth and reduction of Cr by *B. thuringiensis* and *Paenibacillus sp.* (Bt+Ps.), b) Growth and reduction of Cr by *B. thuringiensis* and *B. amyloliquefaciens* (Bt+Ba), c) Growth and reduction of Cr by *B. amyloliquefaciens* and *Paenibacillus sp.* (Ba+Ps) and d) Growth and reduction of Cr by *B. thuringiensis, B. amyloliquefaciens* and *Paenibacillus sp.* (Bt+Ba+Ps).

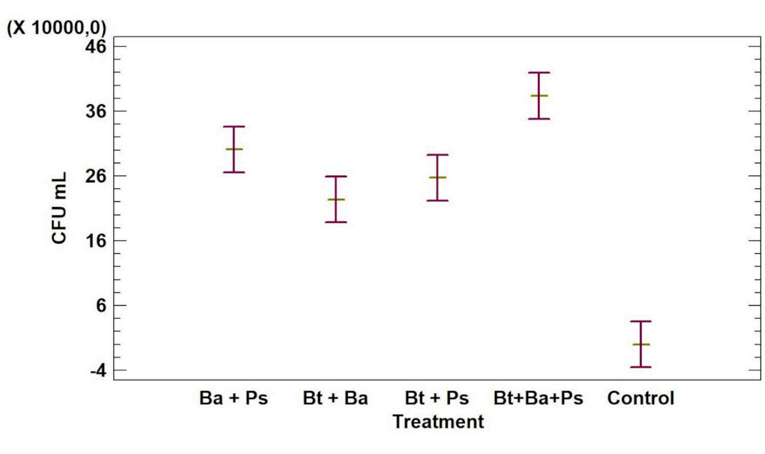

Figure 7. Fisher's multiple comparison procedure – growth phase 1, graphs that do not exceed indicate significant differences between pairs of means.

When evaluating the percentage of Cr(VI) reduction by the four mixed cultures, it was found that in sterile municipal wastewater (SMWW) supplemented with approximately 50 mg L^{-1} of Cr(VI), the isolates presented percentages of reduction of 26, 31, 36 and 40% for Bt+Ba, Bt+Ps, Bt+Ba+Ps and Ba+Ps, correspondingly, since it was determined that there are statistically significant differences in the reduction percentages of three of the four treatments (P value 0,030 α 0,05) and considering that the other cultures are less effective in percentage in metal reduction, the Ba+Ps culture was selected to further expand the study of Cr reduction in municipal wastewater without sterilizing MWW (Phase 2) for presenting a higher reduction percentage after a fermentation period of 156 hours.

Cr reduction test in unsterilized municipal wastewater

The action of the selected culture (Ba+Ps) linked to the reduction of Cr(VI) in unsterilized municipal wastewater (UMWW) was evaluated, considering that it presented a greater potential for Cr(VI) reduction. The consortium showed a reduction of 91,5% of Cr(VI) after 156 hours with a reduction rate of 0,30 mg h-1 in a medium consisting of UMWW and approximately 50 mg L^{-1} of Cr(VI); Bacterial growth in the assay continued to increase until 72 hours, when it reached the maximum value of CFU mL-1 (see figure 8). In contrast, the assay that contained SMWW without inoculum presented an almost null reduction (3%), which indicates that possibly there are some elements in the medium that induce the reduction of Cr in a very small fraction; Studies related to Cr(VI) reduction processes mention that Fe ions present in the treated solutions can reduce Cr(VI) to Cr(III) under alkaline conditions (25).

For this study, the results of the partial physicochemical and microbiological analyzes contracted with the laboratories of the University of Nariño (report No: LAQ-R-99A-19), indicated Fe concentrations lower than 0,26 mg Fe+3 L^{-1} in treatments, showing that this is possibly responsible for the reduction in the treatment without inoculation. Additionally, the COD and BOD ratios (>0.4) in the treatments indicate that the collected wastewater can be treated by biological processes. Otherwise, values less than 0,2 indicate that most of the load present in the water is not biodegradable, for this reason the chemical treatment would be adequate for its purification (26).

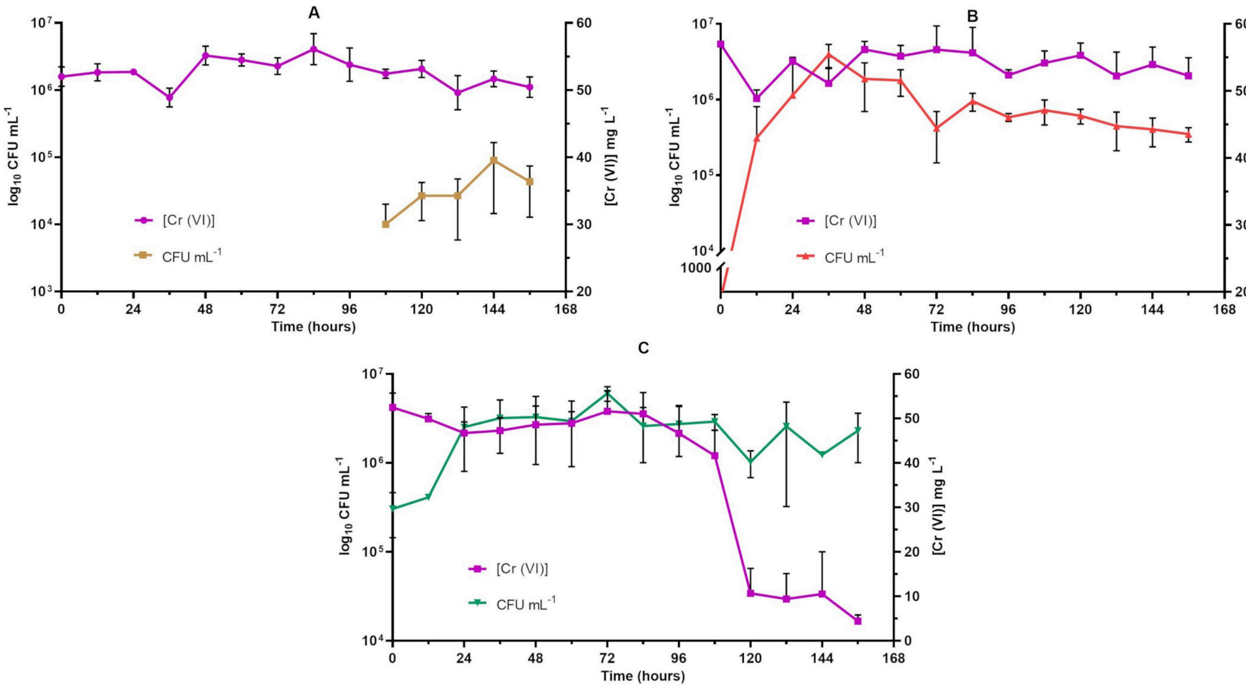

Figure 8. Evaluation of the joint action of *B. amyloliquefaciens* and *Paenibacillus sp.* (Ba+PS), in the reduction of Cr (VI) in municipal wastewater. a) Control sterile municipal wastewater (SMWW), b) Control unsterilized municipal wastewater (UMWW) and c) Municipal wastewater inoculated with Ba+Ps. Source: This study

The MLG was highly significant and explained 83% of the variability in the growth data (p-value: 0,0000); In addition, it was determined that there is a statistically significant relationship between the dependent variable *(growth)* and the independent variables *(treatment and time)*. Using Fisher's least significant difference (LSD) procedure, it was verified that there are significant differences in the growth kinetics of the inoculated treatment and the controls with sterile and non-sterile residual water (see figure 9).

In the same way, it was found that there is a highly significant interaction between the reduction of Cr(VI) and the variables treatment and time (p-value 0,0000), these two factors explained 96,7% of the variability of the data of the metal reduction. It was determined that there are statistically significant differences in the percentage reduction of the inoculated treatment compared to the controls (P value 0,0000 α 0,05); on the contrary, no differences were found regarding the reduction percentage of the control with UMWW and the control with SMWW and without inoculation (UMWW) (3 %); which indicates that the addition of the selected mixed culture was responsible for the increase in the reduction percentage.

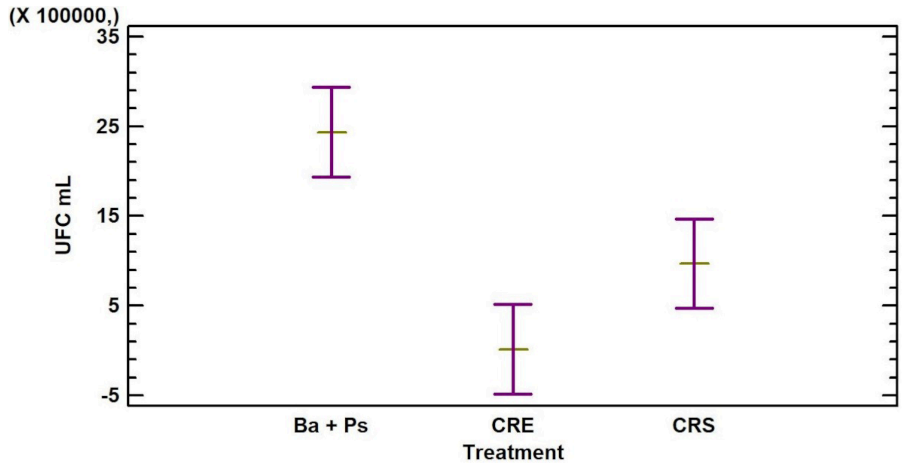

Figure 9. Fisher's multiple comparison procedure – growth phase 2, graphs that do not exceed indicate significant differences between pairs of means. Ba+Ps Municipal wastewater inoculated with Ba+Ps., SMWW Control-sterile municipal wastewater and UMWW Control-unsterilized municipal wastewater.

Discussion

Conformation of mixed cultures and their tolerance to hexavalent chromium

In accordance with the tolerance to Cr, what was reported by Banerjee, Misra, Chaudhury, & Dam, (2019), is similar to what was found in this study, they reported the isolation of *Bacillus* obtained from soil samples from an abandoned mine, which presented a high tolerance to heavy metals such as nickel, cadmium, and Cr(VI), for the latter a minimum inhibitory concentration (MIC) of 2000 mg L^{-1} is reported (27). However, as described by Castro & Marín (2018), metal tolerance depends on several factors, from the genus to which it belongs to the conditions of the site from which it was isolated. Castro and Marin reported three bacteria, one of the *Bacillus* genus that presented a MIC of 200 mg L^{-1}, *Pseudomonas* MIC of 2000 Cr(VI) and Halomonas >5000 mg L^{-1} of MIC (28).

Similarly, Kafilzadeh & Saberifard (2016) isolated bacteria from river sediments, belonging to the species *Pseudomonas aeruginosa* and *Bacillus cereus* that, despite being isolated from the same source, differ in their resistance to Cr(VI), MIC of 200 and 100 mg L^{-1} Cr(VI), respectively. Likewise, Kalsoom et al. (2021), mention that of 46 bacteria isolated from the rhizosphere soil, 100% were resistant to Ni, Pb and Zn; while only 53% showed tolerance to Cr at concentrations of 100 mg L^{-1}. Therefore, it is believed that, compared to other bacteria isolated from ecosystems contaminated with heavy metals, the bacterial isolates used in the current study have a greater resistance capacity to high concentrations of

Cr(VI) ≤2000 mg L^{-1}. Therefore, these can be effective in remediation strategies for ecosystems contaminated with Cr(VI).

Regarding the cross-streak method is a reliable and effective technique, used in detection programs for microorganisms with antagonistic activity of possible antibiotic producers, it is also used to evaluate the compatibility of bacterial strains used for the composition of consortia used as starter cultures of antibiotics. different processes. (29,2). Certainly, since there is no antagonism between these bacteria, there are other types of interactions that are possibly related to commensalism, synergism, mutualism and even a certain degree of competition. However, as can be seen later in the description of the growth of the consortia, a commensal relationship is possible where, in the early stages of the process, one of the bacteria initiates the process of transforming the medium, adapting it to the needs of the bacteria (30,31).

Evaluation of the growth and reduction of chromium by the conformed cultures in sterilized municipal wastewater

As mentioned in section 4.5, in the growth curves of the different crops, two growth peaks were observed. This phenomenon was also observed by Roestorff & Chirwa (2018), when implementing a second self-sustaining carbon source to the Cr(VI) bioremediation process with bacteria. In their study, they reported that after the cell content is expelled into the medium due to lysis caused by Cr(VI) toxicity, the persistent bacteria are capable of generating new biomass from the metabolites released (32). On the other hand, according to Mbonambi & Chirwa (2019), the absence of the latency period in this kinetics could be associated with the environment from which the bacteria used in the present study were isolated; since to survive under conditions in which they receive periodic loads of the contaminant, they must have the ability to tolerate high concentrations of Cr(VI). Considering that untreated discharge sites are common in small tanning industries and therefore these bacteria are adapted to the presence of Cr (33,9).

In the four treatments between 48 and 120 hours, time in which the exponential and stationary growth phases are observed in the simulations, there are oscillations in the bacterial growth kinetics of the experiment; which according to Ontañon, Fernández, Agostini, & González (34) may be caused by the accumulation of Cr(III) inside the cell leading to lysis, a response that translates into the decline of bacterial density, as a response to the effect of Cr(III) into the cell, since during this time the scarcity of nutrients does not greatly limit growth. Therefore, the bacteria that have not yet been (34)affected by Cr concentrations continue with the process of cell division and transformation of the medium (35,34). Additionally, the variations in the growth patterns in the kinetics of the four treatments also depend on the metabolic efficiency of each microorganism to assimilate the nutrients present in the medium (36,37).

Ontañon, Fernández, Agostini, & González (2018) reported reduction percentages close to those observed in the present study, when submitting a strain of the *Bacillus* genus isolated from the SFC 500-1 consortium, which came from a tannery effluent (34). When evaluating this isolate at a concentration of 50 mg L^{-1} of Cr(VI) in a synthetic medium, they observed that, after more than 72 hours, the treatment reached a reduction percentage of 43%, this suggests that the treatments evaluated in this study are close to efficiencies reported by other authors; even, by not presenting optimal nutritional requirements such as those available in synthetic media. The foregoing makes these cultures and especially Ba+Ps a potential alternative for the biotreatment of effluents/wastewater, considering the ecological advantages of using wild bacteria for bioremediation.

Initially, the results of this first phase show that the bioassay with two or three of the bacterial isolates is less efficient than the individual action of these, since, as mentioned by Guerrero et al. (15), the isolates separately present percentages of 82 %; 80% and 79% for *B. thuringiensis, B. amyloliquefaciens,* and *Paenibacillus sp*, respectively. This coincides with the data obtained by Mora Collazos (8), who determined that the test with two and three additional bacteria decreases the reduction percentages and increases the process time compared to individual reduction. However, when performing an additional test with the *A. hydrophila* CrMFC5 strain and its study microorganism *Bacillus sp.* G3 found an improvement in the Cr(VI) reduction efficiency, which was reflected in the reduction percentage and time (100% at 32 hours); therefore, the hypothesis of obtaining better reduction percentages with the association of other microorganisms present in the wastewater cannot be ruled out.

Evaluation of the growth and reduction of chromium by B. amyloliquefaciens y Paenibacillus in unsterilized municipal wastewater

In accordance with the results of section 4.6, it is suggested that the Ba + Ps crop supplement in the treatment could be a suitable biological system to improve the biotreatment of effluents/wastewater contaminated with Cr(VI), because in general, most of the methods traditional remediation are unfeasible at concentrations below 100 mg L^{-1} Cr(VI) (38,37). Currently, different microorganisms are reported, especially “indigenous” bacterial species, with high resistance and reduction of Cr(-VI); however, most evaluate these percentages under optimal conditions, pH, temperature, carbon source, agitation, aeration, etc. (39,40,3), implying to increase in a certain way additional costs to the treatment processes.

In the present study, the process of Cr(VI) reduction was evaluated by simulating environmental conditions, considering that the mechanism of resistance and reduction of Cr(VI) is dependent on these factors and therefore, the strains used in bioremediation processes on a larger scale, they should be able to reach high percentages of reduction under unfavorable conditions for their growth. In this sense, as can be seen in figure 8C, the initial reducing activity is lower in the interval from 0 to 72 hours, and subsequently rises until Cr(VI) is reduced by 91%. This phenomenon may possibly be due to the different distributions of growth among the bacteria and even due to the action of other types of contaminants present in the water that could interfere in the reduction processes, causing the bioremediation of Cr to be limited in the first hours (41,8,11).

In figures 8A and 8B, fluctuations in the amount of Cr(VI) are observed during the evaluated time, this can be attributed to the portion of Cr adsorbed by the cells due to biosorption or the formation of precipitated organometallic complexes with Cr(VI). It is important to highlight that the presence of these Cr(VI) complexes in the media does not contribute to the spectrophotometric readings [Cr(VI)], since these measurement techniques involve the analysis of supernatants that quantify only soluble Cr (38). On the other hand, despite the generation of contamination in the final stages of the process in the abiotic control (SMW), no reducing activity was observed by these microorganisms, the concentration of Cr did not decrease from 49,6 mg L^{-1}, which implies and reiterates that the tolerance to Cr(VI) is independent of the reduction capacity (11,27,42).

Conclusions

The predictive mathematical models executed in the first phase of the study allowed an assertive approach to the dynamics of Cr(VI) reduction due to the joint action of the three bacterial isolates used in the study, which in addition to the results of the second phase is confirmed for this study, that the joint action of several microorganisms does not necessarily grant greater efficiencies in the reduction process.

The consortium formed by the bacteria *B. amyloliquefaciens* and *Paenibacillus sp.*, is capable of carrying out the effective reduction of Cr(VI) in bioreactors with wastewater from the sterile Pasto River, therefore, it can be used as a potential agent for effective bioremediation from sites contaminated with heavy metals.

Under unfavorable fermentation conditions for its growth, the Ba + Ps culture made up of *B. amyloliquefaciens* and *Paenibacillus sp.*, was able to reduce 91% of Cr(VI) from an initial concentration of 50 mg L-1 in a treatment with unsterilized municipal wastewater from the Pasto River.

In order to continue scaling up the bioremediation process, it is necessary to investigate the possibility of designing bioproducts from these isolates that allow their application in water treatment technologies that contribute to mitigating the effect of contaminated effluents on the ecosystems.

Consequently, it is recommended to investigate what genetic determinants bacteria *B, thuringiensis, B. amyloliquefaciens* and *Paenibacillus sp.* present, which allow them to tolerate or reduce the concentration of Cr(VI) in the medium.

Funding: This study was sponsored by the Vice-Rectory for Research, Postgraduate Studies and International Affairs (VIPRI), now Vice-Rectory for Research and Social Interaction (VIIS) of the University of Nariño.

Acknowledgements: The authors express their gratitude to the technical personnel of laboratories of the University of Nariño, to the Vice-Rectory 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 CTeI 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 no have affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript. The authors declare that this work does not present any conflict of interest.

Data Availability: The data used to support this study are available from the corresponding author upon request.

References

1. Corrales L, Sánchez L, Sánchez Cortes P, Sánchez León A, Sánchez Quintero V, Zárate D. Estudio piloto de aislamiento y fenotipificación de bacterias que participan en los procesos de biolixiviación, en las zonas mineras del Departamento de Boyacá. NOVA. 2006; 4(5).
2. Sarkar P, Chourasia R. Bioconversion of organic solid wastes into biofortified compost using a microbial consortium. International Journal of Recycling of Organic Waste in Agriculture. 2017; 6(4): p. 321 - 334.
3. Huang H, Zhao Y, Xu Z, Ding Y, Zhou X, Dong M. A high Mn(II)-toletance strain, Bacillus thuringiensis HM7, isolated from manganese ore and its biosorption characteristics. PeerJ - Journal of Life and Environmental Sciences. 2020; 8(2020): p. 1 - 24.
4. Kalsoom W, Batool A, Din G, DIn SU, Jamil J, Hasan F, et al. Isolation and screening of chromium resistant bacteria from industrial waste for bioremediation purposes. Brazilian Journal of Biology. 2021; 83(2021).

5. Pinta-Melo J, Guerrero-Ceballos L, Ibargüen-Mondragón E, Fernández-Izquierdo P, Gómez Arrieta JD, Burbano-Rosero EM. Tolerance and reduction of Cr(VI) by Bacillus amyloliquefaciens, B. thuringiensis and Paenibacillus sp., isolated from Pasto River. Latin American Journal of Development. 2022; 4(1): p. 272–297.

6. Soto C, Gutiérrez S, Rey-León A, González-Rojas E. Biotransformación de metales pesados presentes en lodos ribereños de los ríos Bogotá y Tunjuelo. NOVA. 2010; 8(14).

7. Góngora E, Cadena CD, Dussán J. Toxic metals and associated sporulated bacteria on Andean hummingbird feathers. Environmental Science and Pollution Research. 2016; 23(22): p. 22968 - 22979.

8. Mora Collazos A. Bacillus sp. G3 un microorganismo promisorio en la biorremediación de aguas industriales contaminadas con cromo hexavalente. Nova scientia. 2016; 8(17): p. 361 - 378.

9. Sharma P, Singh SP, Parakh SK, Tong YW. Health hazards of hexavalent chromium (Cr (VI)) and its microbial reduction. Bioengineered. 2022; 13(3): p. 4923 - 4938.

10. Vásquez Perea Y, Villamil Poveda J, Sánchez Leal L, Lancheros Diaz A. Evaluación de un sistema de medio fijo como soporte para una película microbiana capaz de reducir Cr (VI) de lodos residuales de curtiembres. NOVA. 2014; 12(21).

11. Bharagava RN, Mishra S. Hexavalent chromium reduction potential of Cellulosimicrobium sp. isolated from common effluent treatment plant of tannery industries. Ecotoxicology and Environmental Safety. 2018; 147: p. 102 - 109.

12. Maldaner J, Steffen GP, Missio EL, Saldanha CW, de Morais RM, Nicoloso FT. Tolerance of Trichoderma isolates to increasing concentrations of heavy metals. International Journal of Environmental Studies. 2021; 78(2): p. 185 - 197.

13. Martínez Buitrago SY, Romero Coca JA. Revisión del estado actual de la industria de las curtiembres en sus procesos y productos: Un análisis de su competitividad. Revista Facultad De Ciencias Económicas. 2017; 26(1): p. 113 -124.

14. Alzate Tejada AM. Anexo1. En Diagnóstico ambiental del sector curtiembre en Colombia: Proyecto de gestión ambiental en la industria de curtiembre. Colombia: Centro Nacional de Producción más limpia y Tecnologías Ambientales.; 2004. p. 7-9.

15. Guerrero Ceballos , Pinta Melo , Fernandez Izquierdo P, Ibarguen Mondragón E, Hidalgo Bonilla P, Burbano Rosero E. Eficiencia en la reducción de cromo por una bacteria silvestre en un tratamiento tipo batch utilizando como sustrato agua residual del municipio de Pasto, Colombia. Universidad y salud. 2017; 19(1): p. 102-115.

16. Otero ID. Remoción de la materia orgánica de las aguas residuales vertidas al Río Pasto con bacterias productoras de polihidroxialcanoatos. Trabajo de grado presentado como requisito parcial para optar al título de Biólogo. Pasto: Universidad de Nariño, Colombia.

17. Lace A, Ryan D, Bowkett M, Cleary J. Chromium monitoring in water by colorimetry using optimised 1,5-diphenylcarbazide method. Environmental Research and Public Health. 2019; 16(10): p. 1803.

18. Murray JD. Mathematical Biology I: An Introduction; 2003.

19. Trinidad Bello A. Modelos de crecimiento en biología, su significado biológico y selección del modelo por su ajuste. Iztapalapa - México:, División de Ciencias Básicas e Ingenierías.

20. Xu X, Zarecki R, Medina S, Ofaim S, Liu X, Chen C, et al. Modelling microbial communities from atrazine contaminated soils promotes the development of biostimulation solutions. The ISME Multidisciplinary Journal of Microbial Ecology. 2019; 13(2019): p. 494 - 508.

21. American Public Health; Association, American Water Works; Federation, Water Environment. Chromiun 117A Hexavalente chromiun. En Health AP, Association AWW, Federation WE. Standards Methods for the examination of water and wastewater.; 1999. p. 271.

22. Niño Camacho L, Torres Sáenz R. Implementación de diferentes técnicas analíticas para la determinación de biomasa bacteriana de cepas de Pseudomonas putida biodegradadoras de fenol. Revista ION. 2010; 23(1): p. 41 - 46.

23. Sereshti H, Vasheghani Farahani M, Baghdadi M. Trace determination of chromium (VI) in environmental water samples using innovative thermally reduced graphene (TRG) modified SiO2 adsorbent for solid phase extraction and UV–vis spectrophotometry. Talanta. 2016; 146: p. 662 - 669.

24. Rinaldo J, Anand Prem R. Bioreactor level optimization of chromium(VI) reduction through Pseudomonas putida

APRRJVITS11 and sustainable remediation of pathogenic DNA in wate. Beni-Suef University Journal of Basic and Applied Sciences. 2022; 11(2022): p. 1 - 8.

25. Arroyo Núñez MG. Tratamiento de disoluciones que contienen cromo hexavalente mediante electrocoagulación con ánodos de hierro. Tesis doctoral para optar al grado de Doctor en Ingeniería química y Nuclear. Valéncia.

26. López Vázquez CM, Buitrón Méndez G, Garcia HA, Cervantes Carrillo FJ. Tratamiento biológico de aguas residuales: Principios, modelación y diseño: IWA Publishing; 2017.

27. Banerjee S, Misra A, Chaudhury S, Dam B. A Bacillus strain TCL isolated from Jharia coalmine with remarkable stress responses, chromium reduction capability and bioremediation potential. Journal of hazardous materials. 2019;: p. 215 - 223.

28. Castro Echavez FL, Marín Leal JC. Comparación de la ecotoxicidad por metales pesados sobre bacterias heterótrofas de dos sitios contrastados del lago de maracaibo (Venezuela). Revista Facultad De Ciencias Básicas. 2018; 1(1): p. 9 - 17.

29. Hossain N, Rahman M. Antagonistic activity of antibiotic producing Streptomyces sp. against fish and human pathogenic bacteria. Brazilian Archives of Biology and Technology. 2014; 57(2): p. 233 - 237.

30. Atlas RM, Bartha R. Capitulo 3: Interacciones entre poblaciones microbianas. En Ecología microbiana y microbiología ambiental. Madrid: Pearson-Addison Wesley; 2002. p. 64 - 81.

31. Ginovart M, Tutusaus A, Mas MT. Agent-based modeling: microbial canibalism. Modelling in Science Education and Learning. 2019; 12(2): p. 5 - 46.

32. Roestorff MM, Chirwa EM. Comparison of the performance of Chlorococcum ellipsoideum and Tetradesmus obliquus as a carbon source for reduction of Cr (VI) with bacteria. Chemical Engineering Transactions. 2018; 70(2018): p. 463 - 468.

33. Mbonambi NC, Chirwa EM. Biological remediation of chromium (VI) in aquifer media columns. Chemical Engineering Transactions. 2019; 76(2019): p. 1333 - 1338.

34. Ontañon OM, Fernandez M, Agostini E, González PS. Identification of the main mechanisms involved in the tolerance and bioremediation of Cr(VI) by Bacillus sp. SFC 500-1E. Environmental Science and Pollution Research. 2018; 25(16): p. 16111 – 16120.

35. Dong G, Wang Y, Gong L, Wang M, Wang H, He N, et al. Formation of soluble Cr(III) end-products and nanoparticles during Cr(VI) reduction by Bacillus cereus strain XMCr-6. Biochemical Engineering Journal. 2013; 70(2013): p. 166 - 172.

36. Kafilzadeh F, Saberifard S. Isolation and identification of chromium (VI)-resistant bacteria from soltan abad river sediments (Shiraz-Iran). Jundishapur Journal of Health Sciences. 2016; 8(1): p. 41 - 47.

37. Rahman Z, Thomas L. Chemical-assisted microbially mediated chromium (cr) (vi) reduction under the influence of various electron donors, redox mediators, and other additives: An outlook on enhanced Cr(VI) removal. Frontiers in Microbiology. 2021; 11(2021): p. 3503.

38. Narayani M, Vidya Shetty K. Reduction of hexavalent chromium by a novel Ochrobactrum sp.–microbial characteristics and reduction kinetics. Journal of Basic Microbiology. 2014; 54(4): p. 296 - 305.

39. Mohapatra RK, Parhi PK, Thatoi H, Panda CR. Bioreduction of hexavalent chromium by Exiguobacterium indicum strain MW1 isolated from marine water of Paradip Port, Odisha, India. Chemistry and Ecology. 2017; 33(2): p. 114 - 130.

40. Elahi A, Rehman A. Multiple metal resistance and Cr6+ reduction by bacterium, Staphylococcus sciuri A-HS1, isolated from untreated tannery effluent. Journal of King Saud University-Science. 2019; 31(4): p. 1005 - 1013.

41. Sandana Mala JG, Sujatha D, Rose C. Inducible chromate reductase exhibiting extracellular activity in Bacillus methylotrophicus for chromium bioremediation. Microbiological Research. 2015; 170(2015): p. 235 - 241.

42. Vélez JA, Quiroz LF, Ruiz OS, Montoya OI, Turrión MB, Ordúz S. Hexavalent chromium-reducing bacteria on biosolids from the San Fernando wastewater treatment plant in Medellín (Colombia). Revista Colombiana de Biotecnología. 2021; 23(1): p. 32 - 45.