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# Bioreduction of Cr(VI) by Indigenously Isolated Bacterial Strains from Stream Sediment Contaminated with Tannery Waste

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## Abstract

The potential of indigenously isolated bacteria from the Estância Velha stream to reduce Cr(VI) was evaluated and also the chromium contamination over the past ten years was verified in one of the most important industrial centers of Brazil, the "Brazilian Capital of Tanneries," Estância Velha municipality in the Rio Grande do Sul State, South Brazil. Samples were collected from the Estância Velha stream at the source (P1), as well as at upstream (P2) and downstream (P3) of the most demographically area. The bacterial strains reduced between 52.5 and 61.6% of 250 mg $L^{-1}$ Cr(VI) in 48 h. The genus *Acinetobacter* was the most abundant and could efficiently reduce 500 mg $L^{-1}$ of Cr(VI); for example, P2.8 and P2.9 strains of *Acinetobacter ursingii* reduced 21.3 and 24.5% of 500 mg $L^{-1}$ of Cr(VI), respectively, after 48 h. Moreover, an analysis of Cr levels in the stream sediment reported up to 3594 mg. $L^{-1}$ of total Cr and up to 138 mg. $L^{-1}$ of Cr(VI) in 2009. *Acinetobacter* strains were identified as the most abundant and efficient in reducing Cr(VI), makes them an ideal candidate for cleaning environments contaminated with tannery effluents, an approach that is more cost-effective than the traditional methods.

## Introduction

The leather sector is considered to be one of the 10 industrial areas that majorly impact the environment owing to several types of waste generated during leather processing [1]. In this branch of industry, residual chromium (Cr), especially in its hexavalent form (CrVI), is one of the most harmful contaminants to the environment, owing to its high toxicity [2], with proven carcinogenic, mutagenic, and allergenic properties [2–4]. Cr(VI) is approximately a thousand times more toxic than its trivalent form (CrIII) that is attributed to high oxidative power, especially in the form of the chromate anion ($CrO_4^{-2}$). Moreover, Cr(VI) is transported rapidly by a membrane transport system through cell membranes and interacts with proteins and nucleic acids [1, 5]. Many bacterial species have been reported to reduce Cr(VI) to Cr(III) with an exceptional ability to adapt to and colonize the Cr-polluted environments, which are otherwise uninhabitable by higher organisms [6]. These microorganisms have developed protective mechanisms against heavy metal toxicity, such as adsorption, uptake, methylation, oxidation, and reduction of the heavy metal [6].

Microorganisms involved in bioremediation could be indigenous to a contaminated area or could be isolated from elsewhere and brought to the contaminated site [7–12]. The characteristic of such microorganisms includes accelerated growth, tolerance to extreme environmental conditions, and low cost of cultivation [11]. Bioreduction of heavy metals is mediated by reducing the potentially more harmful, toxic, and unstable oxidation state of the metal to a more stable and less harmful form [13]. In this context, detoxification of

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Cr by naturally occurring microorganisms provides a viable option to protect the environment from Cr toxicity [14].

The Estância Velha stream is located in the metropolitan region of Porto Alegre municipality, Rio Grande do Sul (RS) State, South Brazil. The stream originates in the municipality with the same name, also known as the "Brazilian Capital of Tanneries." It directly receives the effluents from the tannery and leather footwear industries. Estância Velha stream integrates the Sinos River Basin, which supplies water to approximately 1.6 Million people [15]. Contamination of the Sinos River Basin was identified in 2001 [16] and also the mutagenic activity through the presence of large amounts of metals, including iron, manganese, Cr, lead, copper, zinc, and nickel, in its sediments. Therefore, the present work evaluated the potential of bioreduction of Cr(VI) by bacteria isolated from the Estância Velha stream sediment. In addition, analysis performed in the stream during the last 10 years was used to verify the profiles of Cr(VI) and Cr total contamination.

## Materials and Methods

### Water and Sediment Sampling

All sample points in Estância Velha stream were defined according to their geographical characteristics (Fig. S1): point 1 (P1), the source (29º 38′ 32.5″ S, 51º 09′ 29.1″ W), is located in a region that is still poorly urbanized, with rural properties in the surroundings. The region upstream of the area with the most highly populated that allows residential, commercial, and industrial activities (29º 38′ 53.6″ S, 51º 10′ 20.0″ W) was defined as point 2 (P2). The point 3 (P3) (29º 39′ 51.2″ S, 51º 12′ 07.5″ W) referred to the area downstream and close to the Portão stream (river basin of the Sinos River) within the industrial zone of Estância Velha, having large industries with medium- to high-polluting potential.

Water and sediment samples (approximately 500 g) for Cr analysis and isolating and analyzing Cr(VI)-reducing bacteria were collected at a depth of around 20 cm in 2000 mL and 1000 mL sterilized plastic bottles. After collection, the bottles were sealed, refrigerated at 4 ± 2 °C, and kept in dark until analysis.

### Chromium Analysis

The analysis of total Cr and Cr(VI) was performed on samples collected over a period of 10 years using the inductively coupled plasma optical emission spectroscopy (ICP-OES) and were performed in the Green Lab Laboratory (Porto Alegre/RS). The adopted analytical method was based in Standard Methods for the Examination of Water and Wastewater [17].

### Prospective Bacterial Cr(VI) Bioreducers

The Cr(VI)-reducing bacteria were selected according to the criteria of Camargo et al. [18], with some modifications. Samples of 10 g of the sediment were added to 90 mL of saline solution (0.85% NaCl) and incubated overnight with shaking. After this, serial dilutions were prepared up to $10^{-3}$, and from each dilution, a 0.1-mL aliquot was inoculated onto nutrient agar plates (3 g $L^{-1}$ meat extract, 5 g $L^{-1}$ peptone, and 15 g $L^{-1}$ agar) containing 2 mg $L^{-1}$ of Cr(VI) in the form of $K_2Cr_2O_7$ in triplicate. Pure colonies were then isolated.

The growth potential of bacterial isolates was evaluated using 30 mL of nutrient broth (3 g $L^{-1}$ of meat extract, 5 g $L^{-1}$ of peptone, and 15 g $L^{-1}$ of agar) containing different concentrations of $K_2Cr_2O_7$: 2, 250, and 500 mg $L^{-1}$ in triplicates. The broths, after inoculation with bacterial isolates, were incubated for 24 h and 48 h at 150 rpm and at 30 °C. The inoculum was standardized for absorbance of 0.3 in a spectrophotometer at 600 nm. The final Cr(VI) concentration in the nutrient broth was determined using diphenylcarbazide reagent in a spectrophotometer at 540 nm [17]. The extent of bioreduction was calculated considering the difference between the initial and final Cr(VI) concentrations in the medium, expressed as a percentage [18]. The statistical analysis was performed using the Scott–Knott test with a probability of 5%.

### Total DNA Extraction and Amplification of the 16S rRNA Gene Fragment

Bacterial cultures for DNA extractions were prepared and performed using the phenol/chloroform method, followed by precipitation with ethanol, as described by Sambrook and Russel [19]. A fragment of the 16S rRNA gene was amplified from the DNA of the bioreductive bacterial isolates in a Veriti 96-Well Thermal Cycler (Applied Biosystems) in a 25-µL reaction containing 0.1 mM of each primer, 1 mM $MgCl_2$ (Invitrogen), 10 mM of each dNTP (Amersham Biosciences), and 1U Taq DNA polymerase (Invitrogen). The sequence of 16S rRNA partial gene (1000 bp) was amplified using primers 5′ AGA GTT TGA TCC TGG CTC AG 3′ [20] and 5′ AGA AAG GAG GTG ATC CAG CC 3′ [21]. The reactions were performed with an initial denaturation cycle at 94 °C for 5 min, followed by 30 cycles of amplification: one denaturation step with duration of 1 min at 94 °C, one annealing phase of 1 min at 49 °C, and one extension phase of 1 min at 72 °C. The final extension was performed at 72 °C for 5 min. The obtained fragments were sequenced in both directions at the ACTGene laboratory of the Biotechnology Center (UFRGS/RS, Brazil) using the ABI-PRISM

3500 Genetic Analyzer automatic sequencer (Applied Biosystems). The sequences obtained were compared to those available in the GenBank database using the BLASTN program (https://www.ncbi.nlm.nih.gov/BLAST/). Sequences of the 14 bacterial isolates with the highest efficiency of reducing Cr(VI) were deposited in the database under accession numbers from MK144407 to MK144420 (Table S1).

## Results

An analysis of the results of Cr(VI) content in the waters of the Estância Velha stream in the last 10 years revealed that in 2013, the sampled point P3 reported a Cr(VI) content of 0.11 mg $L^{-1}$, P2 0.01 mg $L^{-1}$, and at the source (P1) Cr(VI) was not detected during this period (Fig. 1a, Table S2). The P2 location had the highest total Cr levels in 2008 (0.1 mg $L^{-1}$), whereas these values were not higher than 0.02 mg $L^{-1}$ in other years and P3 in 2012 (0.2 mg $L^{-1}$). The P1 location was the only location reported total Cr level only in 2016 (0.02 mg $L^{-1}$), according to Fig. 1b and Table S2. An analysis of the content of Cr(VI) in the sediments (Fig. 2a, Table S2) revealed P3 to have high levels in 2007 (29.4 mg $kg^{-1}$), 2011 (2.78 mg $kg^{-1}$), and the highest value reported in 2009 (138 mg $kg^{-1}$). In P2, the total Cr(VI) levels (Fig. 2b, Table S2) were reported in 2007 (1.28 mg $kg^{-1}$) and 2011 (1.32 mg $kg^{-1}$), with the highest values observed in 2009 (8.06 mg $kg^{-1}$). The highest content was found in 2009 in P1 (6.67 mg $kg^{-1}$), as well as in P2 and P3. Considering the results of total Cr in sediments obtained between 2007 and 2017 (Fig. 2b, Table S2), it was found that the highest values were found in 2007 (2145 mg $kg^{-1}$) and 2009 (3594 mg $kg^{-1}$) for P3, whereas in 2010 and 2011, P2 reported the highest values (503 and 772 mg $kg^{-1}$, respectively). In 2014, the maximum value of 314 mg $kg^{-1}$ was found at P1. The total Cr values of the Estância Velha stream sediments were higher for 2017, P1: 60.8 mg $kg^{-1}$, P2: 36 mg $kg^{-1}$ , and P3: 17.1 mg $kg^{-1}$ (Fig. 2b, Table S2).

Of the sediment samples collected along the Estância Velha stream at P1, P2, and P3, approximately 87 morphologically distinct bacterial colonies were isolated. Of these, 14 isolates were found to exhibit the highest growth potential in a medium supplemented with 2 mg Cr(VI) $L^{-1}$. To identify the most promising isolates with a high potential for reducing Cr(VI), the following species were verified: *Acinetobacter septicus, A. modestus, A. ursingii, Bacillus amyloliquefaciens, B. cereus, Exiguobacterium artemiae, Microbacterium mangrovi*, and *Ochrobactrum grignonense*.

The bacterial isolates were first checked for Cr(VI) bioreduction at an initial Cr concentration of 2 mg $L^{-1}$ after 24 h and 48 h of incubation (Table 1). It was observed that all isolates grew after the first 24 h of incubation, reducing between 96.5 and 98.5% of 2 mg $L^{-1}$ Cr(VI). After 48 h of incubation, all isolates exhibited a reduction between 99.0 and 100%. At a concentration of 250 mg $L^{-1}$ of Cr(VI) in the culture medium, a reduction between 31.8 and 50.2% after 24 h of incubation

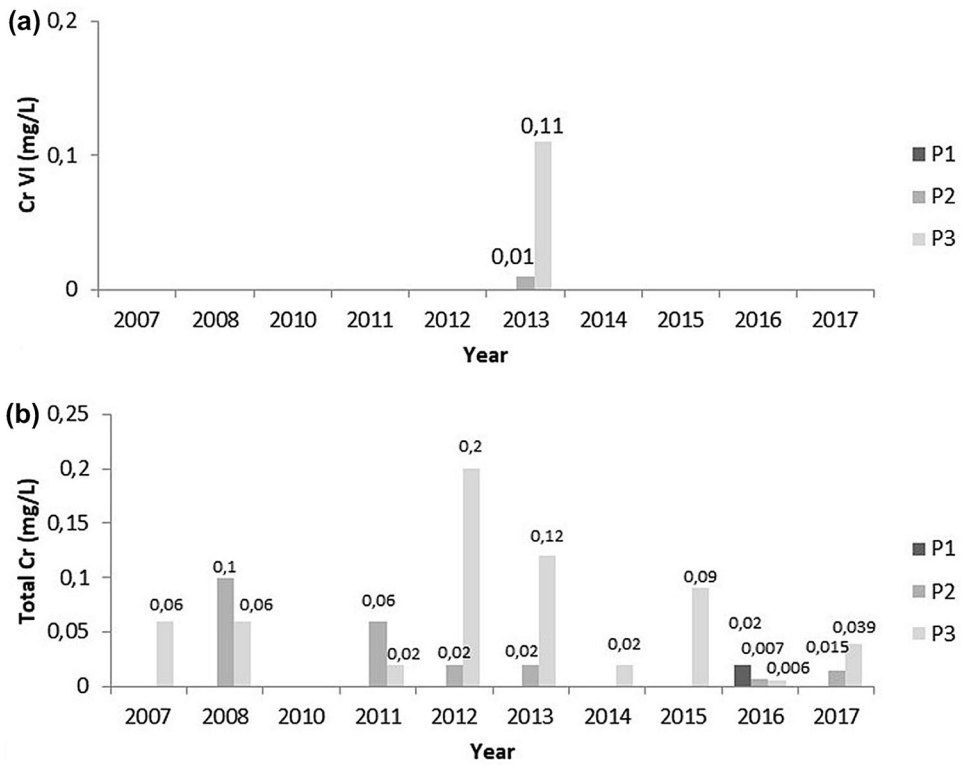


**Fig. 1** Analysis of the content found in the water of the Estância Velha stream at the sampled points 1 (P1), 2 (P2), and 3 (P3) for the period from 2007 to 2017, except for 2009: **a** Cr(VI) and **b** Total Cr

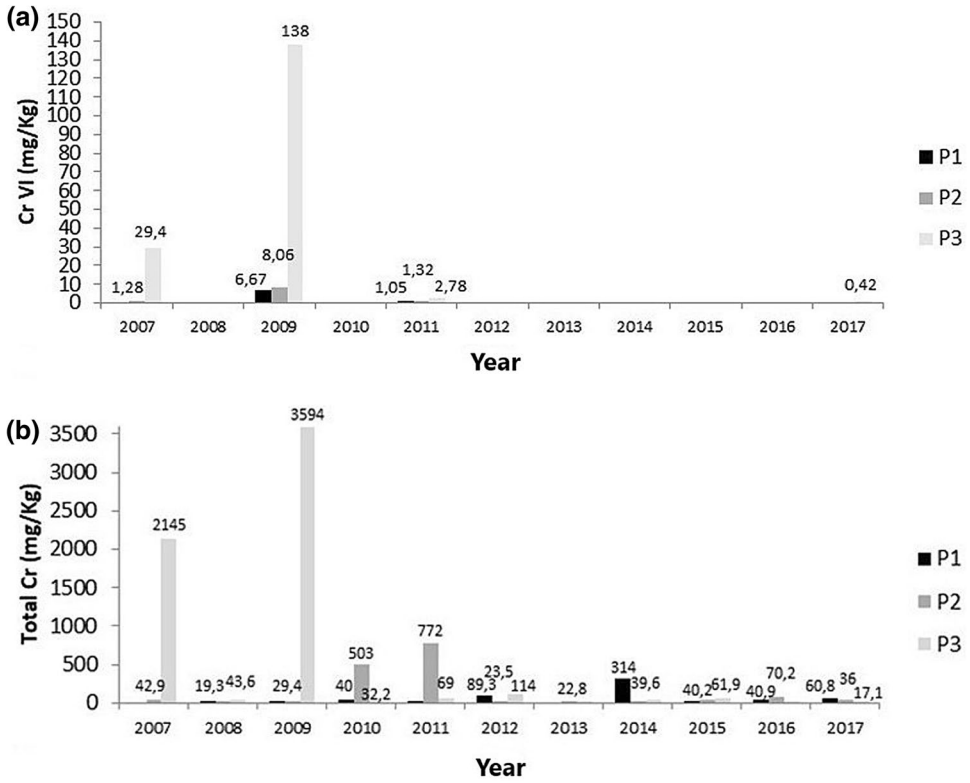


**Fig. 2** Analysis of the content found in the sediment of the Estância Velha stream in sampled points 1 (P1), 2 (P2), and 3 (P3), during the period from 2007 to 2017: **a** Cr(VI) and **b** Total Cr

and between 52.5% and 62.4% after 48 h of incubation was noted (Table 1). Thus, the isolates that achieved a more efficient bioreduction included P2.25 (*Bacillus cereus*), P2.18 (*B. amyloliquefaciens*), P3.3 (*Exiguobacterium artemiae*), and P2.9 (*Acinetobacter ursingii*), with 50.2, 49.8, 49.4, and 49.3% reduction, respectively, after 24 h of incubation. Moreover, P3.26 (*Acinetobacter modestus*), P3.22 (*Ochrobactrum grignonense*), P2.18 and P1.10 (*B. amyloliquefaciens*), P3.3 (*Exiguobacterium artemiae*), and P2.25 (*B. cereus*) reported Cr(VI) reduction between 61.3% and 62.4% after 48 h of incubation. After 24 h, all microorganisms could reduce only a small percentage of 500 mg $L^{-1}$ of Cr(VI), ranging from 0.2 to 9.7%. The isolates exhibiting bioreduction after 24 h of incubation included P2.4 (*A. septicus*), P2.9 (*A. ursingii*), P3.25 (*A. modestus*), and P3.3 (*Exiguobacterium artemiae*) with values of 9.7, 5.8, 5.0, and 4.6%, respectively. On the contrary, all isolates after 48 h of incubation exhibited increased reduction indexes of the 500 mg $L^{-1}$ of Cr(VI) medium, ranging from 9.4% to 24.5% (Table 1). It should be noted that isolates P2.9, P2.8 (*A. ursingii*), P2.1 (*A. septicus*) and P3.25 (*A. modestus*) could reduce the highest amounts of Cr(VI), between 14.7% and 24.5% (Table 1).

## Discussion

Estância Velha stream receives tannery waste as well as the domestic sewage of the local area. Tannery effluents are among the most hazardous pollutants released by leather industries, as these are extremely complex in their composition and characterized by high contents of toxic nitrogen compounds, Cr, copper, cadmium, and sulfides, among others [22]. These pollutants in the environment are used by several microorganisms that degrade metals, such as Cr(VI), thereby converting them into less toxic products [23]. These microbial degraders can be isolated from several kinds of environments, especially from those with a history of contamination [23], such as the Estância Velha stream. The municipality of Estância Velha is known to be a hub of the leather and footwear industry, and this industry uses salts of Cr that contaminate the water bodies into which these wastes are dumped. Currently, the municipality has only 10 licensed industries to discharge effluents into the stream as compared with 40 licensed industries in 2006 [24]. As per the guidelines

**Table 1** Reduction of Cr(VI) by the bacterial isolates obtained from Estância Velha stream after 24 h and 48 h of incubation in medium containing 2250 and 500 mg Cr(VI).$L^{-1}$

| Bacterial isolate | 2 mg Cr(VI) $L^{-1}$ | | | | 250 mg Cr(VI) $L^{-1}$ | | | | 500 mg Cr(VI) $L^{-1}$ | | | | Identification (Genbank accession number) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cr(VI) after 24 h (mg $L^{-1}$) | Reduction after 24 h (%) | Cr(VI) after 48 h (mg $L^{-1}$) | Reduction after 48 h (%) | Cr(VI) after 24 h (mg $L^{-1}$) | Reduction after 24 h (%) | Cr(VI) after 48 h (mg $L^{-1}$) | Reduction after 48 h (%) | Cr(VI) after 24 h (mg $L^{-1}$) | Reduction after 24 h (%) | Cr(VI) after 48 h (mg $L^{-1}$) | Reduction after 48 h (%) | |
| P1.5 | 0.03a | 98.5 | 0.01a | 99.5 | 128.5f | 48.6 | 118.7m | 52.5 | 490.6i | 1.9 | 435.5g | 12.9 | *Microbacterium mangrovi* (MN686584) |
| P1.10 | 0.03a | 98.5 | 0.00a | 100 | 165.7n | 33.7 | 96.0b | 61.6 | 499.1n | 0.2 | 444.1m | 11.2 | *Bacillus amyloliquefaciens* (MN686583) |
| P2.1 | 0.05a | 97.5 | 0.00a | 100 | 131.7l | 47.3 | 97.3f | 61.2 | 492.3l | 1.5 | 419.7c | 16.1 | *Acinetobacter septicus* (MN686582) |
| P2.4 | 0.05a | 97.5 | 0.00a | 100 | 131.5j | 47.4 | 102.8h | 58.9 | 451.4a | 9.7 | 438.2i | 12.4 | *Acinetobacter septicus* (MN686581) |
| P2.5 | 0.04a | 98.0 | 0.00a | 100 | 130.2i | 47.9 | 97.2e | 61.1 | 482.2 g | 3.6 | 440.9l | 11.8 | *Bacillus amyloliquefaciens* (MN686580) |
| P2.7 | 0.04a | 98.0 | 0.00a | 100 | 128.2e | 48.7 | 107.2i | 57.1 | 491.9j | 1.6 | 452.9n | 9.4 | *Acinetobacter ursingii* (MN686579) |
| P2.8 | 0.05a | 97.5 | 0.00a | 100 | 129.5g | 48.2 | 97.9g | 60.8 | 486.9h | 2.6 | 393.5b | 21.3 | *Acinetobacter ursingii* (MN686578) |
| P2.9 | 0.04a | 98.0 | 0.00a | 100 | 126.7d | 49.3 | 112.0 l | 55.2 | 470.9b | 5.8 | 377.5a | 24.5 | *Acinetobacter ursingii* (MN686577) |
| P2.18 | 0.03a | 98.5 | 0.00a | 100 | 125.4b | 49.8 | 96.0b | 61.6 | 492.5m | 1.5 | 419.7c | 16.1 | *Bacillus amyloliquefaciens* (MN686576) |
| P2.25 | 0.06a | 97.0 | 0.00a | 100 | 124.6a | 50.2 | 96.8d | 61.3 | 478.3e | 4.3 | 438.4j | 12.3 | *Bacillus cereus* (MN686575) |
| P3.3 | 0.04a | 98.0 | 0.00a | 100 | 126.4c | 49.4 | 96.3c | 61.5 | 476.8d | 4.6 | 428.3e | 14.3 | *Exiguobacterium artemiae* (MN686574) |

**Table 1** (continued)

| Bacterial isolate | 2 mg Cr(VI) $L^{-1}$ | | | | 250 mg Cr(VI) $L^{-1}$ | | | | 500 mg Cr(VI) $L^{-1}$ | | | | Identification (Genbank accession number) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cr(VI) after 24 h (mg $L^{-1}$) | Reduction after 24 h (%) | Cr(VI) after 48 h (mg $L^{-1}$) | Reduction after 48 h (%) | Cr(VI) after 24 h (mg $L^{-1}$) | Reduction after 24 h (%) | Cr(VI) after 48 h (mg $L^{-1}$) | Reduction after 48 h (%) | Cr(VI) after 24 h (mg $L^{-1}$) | Reduction after 24 h (%) | Cr(VI) after 48 h (mg $L^{-1}$) | Reduction after 48 h (%) | |
| P3.22 | 0.05a | 97.5 | 0.00a | 100 | 170.5o | 31.8 | 96.0b | 61.6 | 478.3e | 4.3 | 431.4f | 13.7 | *Ochrobactrum grignonense* (MN686573) |
| P3.25 | 0.07a | 96.5 | 0.00a | 100 | 129.7h | 48.1 | 108.1j | 56.8 | 474.9c | 5.0 | 426.3d | 14.7 | *Acinetobacter modestus* (MN686572) |
| P3.26 | 0.04a | 98.0 | 0.02a | 99.0 | 152.6m | 39.0 | 93.9a | 62.4 | 480.2f | 4.0 | 437.7 h | 12.5 | *Acinetobacter modestus* (MN686571) |

Means followed by the same letter do not differ by Scott–Knott test at 5% error probability

of the FEPAM (State Foundation for the Protection of the Environment, RS/Brazil), all tanneries in the Rio Grande do Sul (RS) state (Brazil) have effluent treatment systems installed.

According to the analysis of last 10 years in Estância Velha stream, the concentrations of total Cr were found to be above the limit in the waters (< 0.05 mg $L^{-1}$), according to CONAMA Resolution no 357 [25] with P2 reaching its highest value in 2012 (0.20 mg $L^{-1}$), P2 and P3 reported total Cr levels to be 0.01 mg $L^{-1}$ (P2 in 2015) and 0.11 mg $L^{-1}$ (P3 in 2013). Considerable levels of Cr(VI) were found in the sediments, mainly in 2009, with the maximum value (138 mg $kg^{-1}$) obtained from P3, where the concentration of industries along the stream was higher. According to Dong et al. [26], tannery is one of the major sources of Cr pollution in sediments. Additionally, the analysis of the total Cr in the sediments revealed the three sampled points reported high values, with P3 reporting the highest levels in 2007 (2145 mg $kg^{-1}$) and 2009 (3594 mg $kg^{-1}$), and P2 in 2010 (503 mg $kg^{-1}$) and 2011 (772 mg $kg^{-1}$). In this way, all values were detected to be above the reference value of Cr in soils (< 400 mg $kg^{-1}$), according to CONAMA Resolution no 420 [27]. In Brazil, no parameters and legislation for sediment samples exist. This was also verified by the analysis of Cr contamination of this water body in the past years confirmed the isolation and presence of highly efficient Cr(VI)-reducing microorganisms, mainly isolated from P2 and P3 locations. According to Rosales et al. [28], the presence and concentration of Cr in sediments represent a high environmental risk, because Cr may be redissolved in water, remain suspended, or be transported downstream of the river. In fact, the levels of Cr far exceeded its turnover by the natural cycle, thus triggering serious health and environmental problems in countries involved in tanning. Rosales et al. [28] found high Cr levels (11,099 mg $kg^{-1}$) at a depth of 1 m in the sediments of the Guadalentin River (Spain), into which tanning effluents are discarded.

The use of bacteria for reducing metals is not as usual as their use in the biodegradation of polluting organic compounds. However, some authors have used known bacteria to reduce Cr(VI) [14, 23, 29–32]. In the present study, 11 bacterial isolates P1.5 (*Microbacterium mangrovi*), P2.5 (*Bacillus amyloliquefaciens*), P2.1, and P2.4 (*Acinetobacter septicus*), P2.7, P2.8, and P2.9 (*A. ursingii*), P2.18 (*B. amyloliquefaciens*), P2.25 (*B. cereus*), P3.3 (*Exiguobacterium artemiae*), and P3.25 (*A. modestus*) were reported to reduce close to 50% of 250 mg $L^{-1}$ Cr(VI). We obtained the highest percentages of Cr(VI) reduction using these bacterial isolates: between 61.3% (P2.25, *B. cereus*) and 62.4% (P3.26, *Acinetobacter modestus*) after 48 h of incubation in 250 mg $L^{-1}$ Cr(VI). Additionally, it was evidenced by our work that longer incubation times (48 h) resulted in greater bioreduction of Cr(VI) by all bacterial isolates at all concentrations

tested. It is also worth mentioning that *A. ursingii* P2.8 and P2.9 exhibited the highest Cr(VI) bioreductions after 48 h of incubation at the highest concentration (500 mg $L^{-1}$ Cr(VI)), i.e., 21.3% and 24.5%, respectively. The isolation of indigenously bacterial strains with the potential to efficiently reduce Cr(VI) from contaminated environments has been reported by several authors [33–35]. *Bacillus* isolated from Cr-contaminated soils in India reduced up to 90% of 100 µg $L^{-1}$ Cr(VI) after 120 h of incubation [35]. Thus, despite a greater percentage reduction obtained than that attained in the present work by *Bacillus* species (about 61% bioreduction after 48 h, 250 mg $L^{-1}$ Cr(VI), isolates P1.10, P2.5, P2.18, and P2 0.25), it should be noted that the initial concentration was lower and the incubation time was five times higher, which might have contributed to a greater activity obtained by Wani et al. [35].

The bacterial strains in the present study belonged to the species *A. ursingii* (P2.7, P2.8, and P2.9) were isolated from P2 location of the Estância Velha stream. These were found to be most efficient Cr(VI) reducers, especially P2.8 and P2.9 strains (reduced 21.3 and 24.5% of 500 mg $L^{-1}$ Cr(VI) in 24 h of incubation, respectively). In addition to the above species, isolate P3.25 (*A. modestus*) reported the fourth largest reduction (14.7%) of 500 mg $L^{-1}$ Cr(VI), P3.26 (*A. modestus*) presented the highest reduction index (62.4%) of 250 mg $L^{-1}$ of Cr(VI), both after 48 h of incubation. Many studies on the reduction of Cr(VI) by *Acinetobacter* are available: *Acinetobacter* AB1 (56.9% reduction of 200 mg $L^{-1}$ Cr(VI) after 72 h of incubation) [36], *A. haemolyticus* [37] and *Acinetobacter* sp. (100% bioreduction of 200 mg $L^{-1}$ Cr(VI) after 120 h of incubation) [38]. Therefore, it could be said that the genus *Acinetobacter* has a high Cr(VI) bioreductive potential, as also evident from the present study. Following isolates were also obtained from the P2 location of Estância Velha stream: *B. amyloliquefaciens* (P1.10, P2.5, and P2.18), and *B. cereus* (P2.25). These strains achieved a Cr(VI) reduction of more than 61% after 48 h of incubation with 250 mg $L^{-1}$ of Cr(VI), *Bacillus* being the genus with the highest potential of the reduction under these conditions. The fact that P2 location of the Estância Velha stream had more efficient bioreducers, eight isolates: P2.1 and P2.4 (*A. septicus*) P2.7, P2.8, and P2.9 (*A. ursingii)*, P2.5 and P2.18 (*B. amyloliquefaciens*), and P2.25 (*B. cereus*) could be associated with the historical contamination of the stream sediments with total Cr and Cr(VI), as evident from the analysis of the past 10 years. The P3 sample of Estância Velha stream reported four efficient Cr(VI)-reducing isolates at 250 mg $L^{-1}$ after 48 h of incubation: *Exiguobacterium artemiae* (P3.3), *Ochrobactrum grignonense* (P3.22), *A. modestus* (P3.25 and P3.26), with 61.5, 61.6, 56.8, and 62.4% Cr(VI) bioreduction, respectively. Rehman and Faisal [39] reported 39.0% Cr(VI) bioreduction from an initial concentration of 200 µg $mL^{-1}$ (after 24 h) by *Exiguobacterium* sp., lower than that found in the present study for this species. Kavita and Keharia [40] highlighted the high Cr(VI) reduction capacity (100%) of *Ochrobactrum* sp. (112 mg $L^{-1}$ of Cr(VI) after 52 h), which is isolated from Cr-contaminated industrial landfill (Gorwa, India). The bioreduction capacity of the bacterial isolates obtained was expected to be higher at P3 location of the Estância Velha stream, since this point was located at the end of the stream and, consequently, the one with the highest intake of industrial effluents. In addition, this location also presented a high concentration of Cr(VI) in the sediments in 2007, 2009, 2011, and 2017, confirming the historical contamination of this sampled point. Historically, P1 site was the least contaminated by Cr(VI), resulting in the isolation of fewer bioreducing bacterial isolates, namely, P1.5 (*Microbacterium mangrovi*) and P1.10 (*B. amyloliquefaciens*) that reduced 52.5 and 61.6% of 250 mg $L^{-1}$ Cr(VI), respectively, after 48 h of incubation. Das et al. [41] reported a bioreductive potential of *B. amyloliquefaciens*, isolated from chromite mine soil (Sukinda, India), which was 37% of 100 mg $L^{-1}$ of Cr(VI), lower than the results obtained in the present study, where the medium containing 250 mg $L^{-1}$ Cr(VI) was reduced to 61.6% by the isolate P1.10 (*B. amyloliquefaciens*). A bacterial isolate, P1.5, identified as *Microbacterium mangrovi* and the Cr(VI)-reducing potential of which was also cited by Molokwane et al. [42], reported a reduction of 38.1% of an initial Cr(VI) concentration of 100 mg $L^{-1}$ after 70 h of incubation. This percentage was also lower than that reported in the present study, where the maximum reduction was 52.5% of 250 mg $L^{-1}$ Cr(VI) by the isolate P1.5 after 48 h of incubation.

In general, all bacterial isolates obtained in the present study had the ability to reduce different concentrations of Cr(VI) used (2, 250, and 500 mg $L^{-1}$) and the fact that the Cr(VI) pollutant has been present for some years in the water and sediment of the Estância Velha stream explains the presence of microorganisms with Cr(VI) reducing ability. Moreover, the isolation of resistant microorganisms capable of reducing contaminants could be more effective when performed in areas with a history of contamination [23]. The high Cr(VI) bioreduction capacity of these bacterial isolates makes them ideal candidates for removing the tannery waste effluents. Moreover, owing to their ability to adapt to diverse complex environments, their use is potentially more cost-effective than the traditional physical or chemical methods.

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