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Bioremediation of Cr(VI) using bacterial biofilms supported on rice husk: A sustainable agroresidue-based bioprocess

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Abstract

In search of a Cr(VI) removal bioprocess, within a circular economy perspective, we combined two autochthonous bacterial consortia obtained from industrial residues and an agricultural waste (rice husk of *Oryza sativa*). After characterizing the rice husk (*rh*) properties, the bacterial growth and biofilm formation, we studied the taxonomical profile of the bacterial consortia (C55, C33, and C21, the last is a combination 2:1 of the previous ones). By using SEM analysis, we confirmed the presence of a smooth layer of exopolysaccharides (EPS) over the rice husk surface. Total bacterial biodiversity, assessed through MiSeq analysis, showed that the addition of Cr(VI) stimulated the growth of two main populations (*Cellulosimicrobium* and *Siminovitchia*) while inhibiting *Peptoniphilus*, *Cutibacterium* and *Rhodococcus*. We also evaluated chromium depletion, in batch experiments, with particular emphasis on its sorption to the agroresidue and its biotransformation by the biomass. When exposed to 200 mg.l⁻¹ Cr(VI), the system containing both *rh* and the bacterial consortium completely removed Cr(VI) from the effluent with half-times between 53 and 67 h. The combination of Cr(VI) biosorption by *rh* and its biotransformation to Cr(III) by bacterial consortia confirmed an effective synergy capable of removing 92% of Cr(VI). This work supports the potential of an innovative bioprocess based on bacterial biofilms grown on *rh* for the mitigation of Cr(VI) contamination.

1. Introduction

Chromium is the 21st most abundant element in the Earth's crust, with an average concentration of 185 mg.kg⁻¹ (Shekhawat *et al* 2015). Although it exists in various oxidation states (from -2 to +6), its trivalent (Cr (III)) and hexavalent (Cr(VI)) species are the most stable and environmentally relevant.

Cr(III) typically forms complexes with organic and inorganic substances, whereas Cr(VI) exists as a strong oxidizing agent (CrO_3 , CrO_4^{2-} , and $\text{Cr}_2\text{O}_7^{2-}$) (Elahi *et al* 2020). In contrast to Cr(III), Cr(VI) is highly soluble in water and permeable through cell membranes. Once absorbed, Cr(VI) is reduced to different ionic forms, eventually forming Cr(III), which accumulates inside the cells. Through this mechanism, chromium ions can interact with biological molecules, inducing oxidative stress, mutations, and irreversible DNA damage that may lead to carcinogenesis. Accordingly, chromium is classified as a mutagen and carcinogen by the International Agency for Research on Cancer (IARC) (Kerur *et al* 2021, Hossini *et al* 2022).

Due to its chemical and physical properties, chromium—particularly in the form of chromium salts—is widely used in industries such as leather tanning, electroplating, and wood preservation. Although several technologies exist for treating chromium-contaminated effluents (Elahi *et al* 2020), these wastewaters are often discharged without proper treatment, releasing harmful concentrations of chromium salts into the environment and posing significant ecological and public health risks (Ukhurebor *et al* 2021). The main challenges for the application of these methods include their high operational costs (e.g., electrochemical treatment, ion exchange, and membrane filtration), technical issues related to sludge management (coagulation), and low process efficiency (e.g., photocatalysis, electrodialysis, membrane filtration, and adsorption) (Kerur *et al* 2021). In contrast, biological treatment processes offer the advantages of low cost and environmental sustainability. However, their limitation lies in the often-unpredictable behavior of microorganism communities.

Microbial Cr(VI) remediation is carried out by biosorption, bioaccumulation, and/or biotransformation (Ramli *et al* 2023). In natural environments, bacteria adopt two primary lifestyles: planktonic (free-living) and sessile. The transition to a sessile lifestyle is mediated by the production of extracellular polymeric substances (EPS), which promote the formation of complex, three-dimensional communities known as biofilms (Maurya *et al* 2022). These structured microbial consortia confer significant advantages for biotechnological applications. Key benefits include enhanced water retention, the establishment of localized chemical gradients and microenvironments, and the retention of extracellular enzymes. Furthermore, biofilms act as reservoirs for biological compounds by accumulating cellular products and debris. They also foster synergistic interactions among microbial species, promote horizontal gene transfer, and exhibit increased tolerance to chemicals and antimicrobial agents—a characteristic that enables continuous biofilm regeneration (Flemming *et al* 2021). Collectively, these attributes make biofilms a highly effective strategy for the environmental remediation of Cr(VI) (Long *et al* 2015, Li *et al* 2017, Attanayake *et al* 2025). Specifically, biofilm-based reactors offer the advantages of maintaining high biomass density within a compact unit, producing less sludge, avoiding clogging issues, and exhibiting high resilience to unpredictable environmental stresses (Hede and Khandeparker 2020, Maurya *et al* 2022).

Another environmentally friendly strategy for metal mitigation is biosorption using agricultural residues (ARs). These materials often exhibit high metal recovery rates through passive binding to functional groups on their surface, a process whose efficacy depends on both the biosorbent's properties and the experimental conditions (Saravanan *et al* 2021). Numerous studies have highlighted the technological potential of ARs, including sugar beet pulp, wheat stalks, corn cobs, rice husks, grape stalks, and various fruit peels (Annadurai *et al* 2002, Inbaraj and Sulochana, 2004, Hashem *et al* 2005, Wesley *et al* 2007). While most studies utilize ARs converted into biochar (Zou *et al* 2023, Saad *et al* 2024, Hinsene *et al* 2024), Khalil *et al* 2021 specifically investigated rice husks (*rh*) in their natural form and identified them as an economically feasible biosorbent for Cr(VI). Their findings indicate that the Cr(VI)-*rh* interaction occurs through physical, monolayer sorption processes, with $-\text{NH}_2$, alkyl, $-\text{OH}$, and $\text{COO}-$ functional groups serving as the primary sites responsible for Cr(VI) removal. Further advantages of *rh* include its abundance, low cost, and lignocellulosic composition, which is rich in cellulose, hemicellulose, and lignin (De Bhowmick *et al* 2018, Šelo *et al* 2021).

In contrast to previous studies focused solely on biosorption or single strains, we propose a first-of-its-kind approach that combines ARs with autochthonous microorganisms isolated from a Cr(VI)-contaminated site. In this configuration, the AR acts not only as an adsorbent but also as a support matrix for microbial biofilm development, thereby enhancing chromium removal efficiency through synergistic mechanisms.

Regardless of microbial metabolic strategy, bioprocess can use isolated strains or consortia (Karthik & Arulselvi, 2017, Zheng *et al* 2019). Bacterial consortia (C55, C33, and C21) enriched from chromite ore processing sites have exhibited high tolerance to Cr(VI) and the ability to effectively transform it into a less toxic form. This study presents an applied proposal for using these microorganisms within a circular economic framework to address the Cr(VI) contamination. The primary objective of this work is to investigate the performance of the consortia C55, C33, and C21 in reducing Cr(VI) using *rh* as a support. Specifically, we aim to: (a) analyze biofilm formation on the *rh* support using scanning electron microscopy (SEM); (b) evaluate the bacterial diversity of the consortia exposed to Cr(VI); and (c) determine Cr(VI) removal ranges in the effluent.

2. Materials and methods

2.1. Experimental design

Four batch experiments (table 1) were conducted to evaluate: (B0) the effect of Cr(VI) on the *rh*; (B1) the biofilm growth, using only the three consortia and the *rh* compacted into a large sphere (see figure 1(a)); (B2) the microbial growth and biodiversity in the presence of chromium, using integral and loose *rh* in the liquid culture medium (figure 1(b)); and (B3) the Cr(VI) diminution by using *rh* compacted into small spheres to test a reactor prototype with gentle aeration (figure 1(c)). Also, control configurations were designed respectively for: (C0) *rh* characterization, (C1) microbial growth, (C2) Cr(VI) depletion by microorganisms alone, (C3) interaction of microorganisms with *rh*, (C4) adsorption onto the *rh* support, and (C5) chemical reduction. Systems containing microorganisms were inoculated with 1×10^7 cells·ml⁻¹ of consortium C33, C55, or C21. For systems with chromium, potassium dichromate (K₂Cr₂O₇) was added to achieve an initial Cr(VI) concentration of 200 mg·l⁻¹. To prevent potential biofilms disruption, no agitation was applied in any system. All systems were performed in triplicated.

2.1.1. Cr(VI) solution preparation

A stock Cr(VI) solution (2000 mg·l⁻¹) was prepared by weighing 0.56 g of K₂Cr₂O₇ and diluting it to a final volume of 100 ml. The correct volume of this stock solution was calculated using the formula $V_1 \cdot C_1 = V_2 \cdot C_2$. Here, V_1 is the volume of the stock K₂Cr₂O₇ solution, C_1 is its concentration (2000 mg·l⁻¹), V_2 is the reactor volume (50 ml or 100 ml), and C_2 is the target initial Cr(VI) concentration in the reactors (200 mg·l⁻¹ of Cr(VI)).

Prior to reactor assembly, the final working volumes of 50 or 100 ml were marked on each reactor. To begin the experiment, the corresponding volume of culture medium was removed from each reactor and replaced with the calculated volume of the stock K₂Cr₂O₇ solution. Immediately after adding the Cr(VI), an aliquot was collected as the time-zero reference sample.

2.1.2. Bacterial consortia culture

The C33 and C55 consortia were originally recovered from leach channel sediments of chromite ore tailings (located in Guanajuato state, Mexico –21°02'32"N, 101°47'29"W) (Brito *et al* 2013). The C21 consortium was prepared by mixing the C33 and C55 consortia at a ratio of 1:2 (Martínez-Aldape *et al* 2024, Brito *et al* 2025).

The C55 and C33 consortia were cultured in modified LB (mlB) medium (g·l⁻¹: peptone 10, yeast extract 5, NaCl 40, with pH controlled at 8) and incubated at 37 °C. After growth, the cells were stained with methylene blue (0.08 M) and counted at 400x magnification using a Neubauer chamber under an optical microscope (Primo Star Zeiss). The inoculum volume was calculated using the equation $V_1 \cdot C_1 = V_2 \cdot C_2$, where V_1 is the inoculum volume, C_1 is the cell concentration (cells·ml⁻¹) of the culture, V_2 is the reactor volume (50 ml, 100 ml or 500 ml), and C_2 is the desired cell concentration in the reactors (10⁷ cells·ml⁻¹). For consortium C21, the inoculum was prepared by combining two-thirds of V_1 calculated for consortium C55 and one-third of the V_1 for consortium C33. The calculated inoculum was then centrifuged (8000 rpm for 3 min), and the resulting pellet was added to the reactors.

Preliminary studies with the C33 and C55 consortia revealed complementary functional traits. The C33 consortium demonstrated a high capacity for biofilm formation but exhibited low resistance to Cr(VI) (up to 200 mg·l⁻¹). In contrast, the consortium C55, produced minimal biofilm (evidenced only by scant EPS production in SEM analysis), yet showed exceptional Cr(VI)-reducing activity, achieving complete removal of 100 mg·l⁻¹ Cr(VI) within three days. This efficacy, although diminished, persisted even at concentrations exceeding 300 mg·l⁻¹. Based on these complementary characteristics, we hypothesized that combining the two consortia would result in a synergistic system, leveraging the robust biofilm matrix of C33 to enhance the survival and performance of the highly resistant C55 consortium at elevated Cr(VI) concentrations. This synergic behavior is observed in consortium C21.

2.1.3. Evaluation of culture media and biofilm formation

Bacterial growth and biofilm-forming ability were evaluated using microcultures in 96-well microtiter plates (200 µl). Bacterial growth was quantified by measuring the increase in turbidity at 600 nm (OD₆₀₀). Initial bacterial growth was tested in minimal mineral medium (MM) supplemented with glucose (1% and 2%) and compared with growth in mlB medium. Additionally, mlB medium was supplemented with Cr(VI) (50, 100, 200, 300, and 400 mg·l⁻¹) to determine its potential inhibitory or stimulatory effect on biofilm formation. After 4 h of incubation at 35 °C, planktonic cells were removed from the wells using a micropipette. Subsequently, 100 µl of 99% methanol was added to each well, and the plate was incubated for 15 min to fix the adhered cell. Following fixation, 100 µl of crystal violet (0.2%–0.5%) was added to each well. After 30 min, the plates were washed with distilled water and air-dried at room temperature in an inverted position. The stained biofilm was

Table 1. Operating conditions of the experimental systems. The systems containing microorganisms were inoculated with 1×10^7 cells·ml⁻¹ of C33, C55, or C21 consortium. The initial Cr(VI) concentration was 200 mg·l⁻¹. All systems were conducted in triplicate. *rh*: rice husk.

	Systems	System description	Addition of Consortium (C33, C55, C21)	Addition of <i>rh</i> (loose/compacted)	Addition of Cr(VI)	Acronym
B0	Batch 0	Characterization of the effluent of Cr(VI) on <i>rh</i>	—	✓	✓	RC
C0	Rice husk alone	Characterization of <i>rh</i>	—	✓	—	R
B1	Batch 1	Biofilm development over the <i>rh</i> in the absence of Cr(VI)	✓✓✓	✓	—	BR
C1	Biological control	Biofilm formation without <i>rh</i> and Cr(VI)	✓✓✓	—	—	B
B2	Batch 2	Complete experiment	✓✓✓	✓	✓	BRC
C2	Cr(VI) transformation control	Cr(VI) transformation by microorganisms only	✓✓✓	—	✓	BC
C3	Organic control	Bacterial growth over <i>rh</i>	✓✓✓	✓	—	BR
B3	Batch 3	Reactor Prototype	✓✓✓	✓	✓	BRC
C4	Rice husk control	Cr(VI) adsorption by support	—	✓	✓	RC
C5	Chemical control	Cr(VI) reduction independent of <i>rh</i> and microorganisms	—	—	✓	C

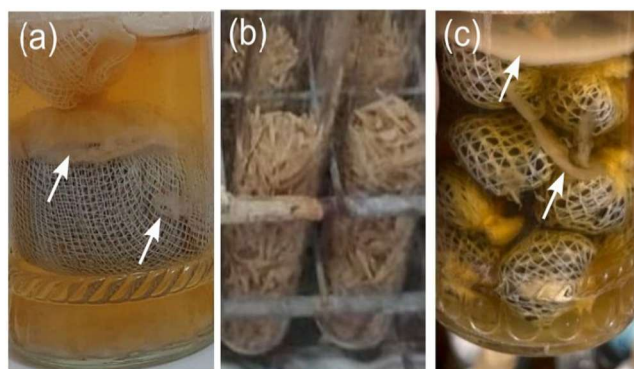


Figure 1. Experiment systems containing *rh* inoculated with microorganisms: (a) B1 (BR), (b) B2 (BRC), and (c) B3 (BRC). Biofilm is indicated by white arrows.

then solubilized with 150 μl of 33% acetic acid. Finally, the optical density of each well was measured at a wavelength of 630 nm (OD_{630}) using an ELISA microplate reader (HBS-1101). All assays were performed in triplicate.

The MM medium contained (g.L^{-1}): Na_2HPO_4 (6), KH_2PO_4 (3), NH_4Cl (10), MgSO_4 (2 mM), CaCl_2 (1 mM), pH 8. After autoclaving (121°C for 20 min), 1 ml of trace elements and 1 ml of vitamins solutions were added. Oligoelement solution (Overmann *et al* 1992) contained (mg.L^{-1}): $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (1500), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (190), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (100), ZnCl_2 (70), H_3BO_3 (62), $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (36), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (24), and $\text{CuCl}_2 \cdot \text{H}_2\text{O}$ (17), pH 7.2. The vitamin solution contained (mg.L^{-1}): biotin (0.2), nicotinic acid (10), thiamine (50), riboflavin (50), and inositol (50).

2.1.4. B0: characterization of *rh* and its interaction with Cr(VI)

Rice husks (*Oryza sativa*) were purchased from a local market and thoroughly washed with distilled water to remove impurities. They were then sterilized by autoclaving at 121°C and 15 psi (103.4 kPa) for 20 min, with the process repeated three times at 24 h intervals to ensure complete sterility. After sterilization, the *rh* were dried in an oven at 35°C . The physical and chemical properties were characterized using Fourier-transform infrared spectroscopy (FTIR; Spectrum Two, Perkin Elmer) and scanning electron microscopy (SEM-EDX; Jeol JSM 7401F microscope).

2.1.5. B1: biofilm growth

To evaluate biofilm formation and development, 250 ml reactors containing a single spherical bag (3-cm diameter) filled with *rh* were used. The reactors were inoculated with mLB liquid medium and incubated at room temperature (30°C – 33°C) for 45 days. Biofilm formation was observed both on the surface of the spherical bag and within its interior. Both components were subsequently analyzed using SEM. The mLB medium contained (grams per liter) tryptone (10), yeast extract (5), and NaCl (40), adjusted to pH 8.

2.1.6. B2: small batches

Small 50 ml reactors containing 2 g of loose *rh* in liquid MM medium with Cr(VI) were inoculated and then incubated at 36°C . Sub-samples from supernatants were periodically collected to monitor Cr(VI) concentration. These systems were used to evaluate total bacterial biodiversity before and after the addition of Cr(VI). In this experiment, microorganisms and 200 mg.L^{-1} Cr(VI) were simultaneously introduced in liquid MM containing *rh*, allowing the observation of their immediate interaction (that is, without waiting for any biofilm formation).

2.1.7. B3: reactor prototypes

250 ml reactors were used, each containing 14 small spherical bags (1.6 cm diameter) filled with 0.5 g of *rh* (figure 2). The reactors were initially inoculated and incubated in mLB medium at 36°C until biofilm formation was observed. Subsequently, the mLB medium was replaced with MM supplemented with Cr(VI). Aliquots of the supernatant from Cr(VI) experiments were periodically collected for Cr(VI) analysis.

2.2. Scanning electron microscopy (SEM) analysis

The cell morphology of the consortia and biofilms was examined by SEM, following the protocol described by Maldonado *et al* (2011) and Concha-Guerrero *et al* (2014). Fresh cultures were centrifuged at 3000 rpm for



Figure 2. Reactor prototype of 250 ml. (a) schematic picture, (b) photo of the systems, (c) details of biofilm.

5 min, washed twice with Millonig's phosphate buffer (0.1 M, pH 7.3), and subsequently fixed in 3% glutaraldehyde prepared in the same buffer for 4 h. The samples were then washed four times with the buffer. Thereafter, the samples were filtered through a polycarbonate membrane (0.22 μm , Millipore[®]), dehydrated through a graded ethanol series (30, 50, 70, 90, and 100%), and dried using a critical point drying method. The dehydrated samples were mounted on metal stubs and sent to a specialized laboratory (Center for Research in Advanced Materials, Chihuahua, Mexico) for analysis. There, the samples were coated with gold using a Denton Vacuum Desk II sputter coater. SEM imaging was performed using a Jeol JSM 7401F microscope.

2.3. Microbial community analysis

Total DNA was extracted from the effluent samples using the DNeasy PowerSoil Pro Kit[®] (QIAGEN), following the manufacturer's protocol. DNA concentration was determined using a Qubit 1X dsDNA BR assay kit (ThermoFisher Scientific, USA).

16S rRNA gene fragments were amplified from 1 μl DNA template with AmpliTaq Gold[™] 360 Master Mix (Applied Biosystems) and 0.5 μM of each primer, in a final volume of 50 μl . The amplification conditions included initial denaturation at 95 $^{\circ}\text{C}$ for 10 min, followed by 30 cycles of denaturation at 95 $^{\circ}\text{C}$ for 30 s, annealing at 65 $^{\circ}\text{C}$ for 30 s, and elongation at 72 $^{\circ}\text{C}$ for 40 s, and a final extension step at 72 $^{\circ}\text{C}$ for 10 min. PCR products quality was verified by UV-vis spectroscopy (NanoDrop ND-1000, Peq-lab, Erlangen, Germany), Illumina MiSeq 250 bp paired-end sequencing was performed by the Get-PlaGe sequencing service (INRA, Toulouse, France).

The V4–V5 hypervariable region of the bacteria 16S rRNA gene targeting bacteria was amplified using the primers 515F, 5'-GTGYCAGCMGCCGCGGTA-3' and 928R, 5'-CCCGYCAATTCMTTTRAGT-3'. Forward and reverse primers contained the adapter sequences (CTTCCCTACACGACGCTCTTCCGATCT) and (GGAGTTCAGACGTGTGCTCTTCCGAT), respectively, required for Illumina sequencing.

Preprocessing included primers removal, read trimming (removal of reads <260bp), and paired-end assembly (minimum overlap of 10 nt and up to 0.1% mismatches). Sequences were clustered using Swarm (Mahé *et al* 2014) with an aggregation distance of 3 and an additional denoising step.

Operational taxonomic units (OTU) with relative abundance <0.005% were removed (Bokulich *et al* 2012). The SILVA database 138 (release date 16.12.2019) was used to perform the OTU affiliations (Quast *et al* 2012). The number of sequences per sample was normalized to 30,053 reads. The raw sequence data have been deposited in the Sequence Read Archive (SRA) database under BioProject PRJNA1121098.

2.4. Cr(VI) and CrT determination

Cr(VI) concentration was determined spectrophotometrically using S-diphenylcarbazide (DPC) at 5 $\text{mg}\cdot\text{l}^{-1}$ in acetone, as recommended by Mexican regulations (NMX-AA-044-SCFI-2014). Briefly, 100 μl of the sample was added to a mixture of 3950 μl of distilled water and 1000 μl of 2N H_2SO_4 . Then, 50 μl of DPC solution was added, and the mixture was incubated in the dark for 15 min. The absorbance was measured at 540 nm, and the Cr(VI) concentration was determined using a standard curve. A blank was prepared by replacing the sample with distilled water. All measurements were performed in triplicate.

A standard curve was generated using $\text{K}_2\text{Cr}_2\text{O}_7$. The $\text{K}_2\text{Cr}_2\text{O}_7$ was dried in an oven at 45 $^{\circ}\text{C}$ for 24 h prior to preparing a 2000 $\text{mg}\cdot\text{l}^{-1}$ Cr(VI) stock solution. Working solutions with Cr(VI) concentrations of 1, 2.5, 5, 10, 50, 80, and 100 $\text{mg}\cdot\text{l}^{-1}$ was prepared in triplicate. The Cr(VI) content of these solutions was determined following the procedure, and the absorbance was measured at 540 nm. A linear regression analysis of the absorbance data yielded the equation $y = 0.0052x + 0.0113$ with an R^2 value of 0.9994. The minimum and maximum detection limits were established as 0.25 and 80 $\text{mg}\cdot\text{l}^{-1}$ Cr(VI), respectively, corresponding to absorbance values of 0.025 ± 0.006 and 0.428 ± 0.010 . The quantification limit (0.004 ± 0.002 ; $n = 8$) was determined using distilled water.

At the end of the experiment, once Cr(VI) was no longer detected in the effluent, the reactor was dismantled and its components were separated. The support material (rice husk) was removed from the effluent by filtration (micron rating approximately 10–20 μm), and the effluent was centrifuged (2000 rpm for 8 min) to separate the bacterial biomass. The rice husk and the bacterial biomass were dried at 40 $^{\circ}\text{C}$, and then the rice husk samples were ground using a mechanical grinder. Subsequently, between 0.1 and 0.5 g of each sample (powdered rice husk or bacterial biomass) was digested with 2 ml of concentrated HNO_3 (Merck[®] P.A.) and 2 ml of concentrated HCl (Merck[®] P.A.) at 100 $^{\circ}\text{C}$ for 2 h using a temperature-controlled block digester (Hach DRB 200). All digested samples, as well as the effluent, were then filtered (0.45 μm), diluted to a final volume of 10 ml with 0.1 $\text{mol}\cdot\text{L}^{-1}$ HCl , and analyzed for total chromium content by atomic absorption spectroscopy (Perkin Elmer PinAAcle 900F). Detection limits were calculated using the formula: $(3\cdot S_b)/X_b$, where S_b is the standard deviation of six blank measurements and X_b is the mean slope of the calibration curve. The obtained results were employed to calculate the mass balance, which was expressed in percentage terms.

2.4.1. Analytical and experimental controls

To account for instrumental and analytical variability, a 500 μl aliquot was collected from each reactor and immediately subdivided into three 100 μl technical replicates. The average of these three determinations was used for data analysis. This procedure enabled real-time monitoring of data quality, as significant deviations between replicates would prompt immediate re-analysis.

2.4.2. Assessment of environmental variability

To assess environmental variability and ensure reproducibility, all reactors were set up in triplicate. Means values and standard deviations were calculated. Potential sources of variation included chemical (e.g., the xenobiotic), physical factors (e.g., pH, temperature), or biological factors.

2.5. Kinetic and isothermal adsorption analysis

The kinetics of Cr(VI) reduction were considered to follow a pseudo-first-order reaction model (Zheng *et al* 2019, Gautam and Dubey 2022). The kinetic parameters were determined using equation (1):

$$\frac{C_t}{C_0} = e^{-kt} \quad (1)$$

where C_t is the Cr(VI) concentration at time t , C_0 is the initial concentration, and k is the constant decay rate, calculated as the slope of the line obtained by plotting $\ln(C_t/C_0)$ against time (t). The half-life ($t_{1/2}$) of the Cr(VI) was calculated using equation (2):

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k} \quad (2)$$

For the Isothermal adsorption models, the calculations were the following:

Langmuir model:

$$K_L = \frac{1}{bq_m} \quad (3)$$

Freundlich model:

$$K_F = 10^b \quad (4)$$

Temkin model:

$$B = m \cdot A = e^{(b/B)} \quad (5)$$

3. Results and discussion

3.1. Characterization of rice husk (B0 experiment)

SEM images of *rh* (C0) showed lemma-like morphology, characterized by a smooth surface with globular structures. Epidermal cells were arranged in linear grooves (lg) with dotted ridges (Dr) and globular protrusions (gp) (figure 3(a)). These results suggest that the organic compounds in the *rh*, such as lignin, cellulose, and hemicellulose, as well as compounds containing silicon, are recalcitrant to biodegradation, in agreement with Ciannamea *et al* (2010), who also reported that regions between protrusions and adjoining sloping areas have high levels of amorphous silica. Such property may limit the use of *rh* as a carbon source for microorganisms. In fact, the presence of Si in the *rh* has previously detected to inhibit biodegradation (Pereira *et al* 2003). On the

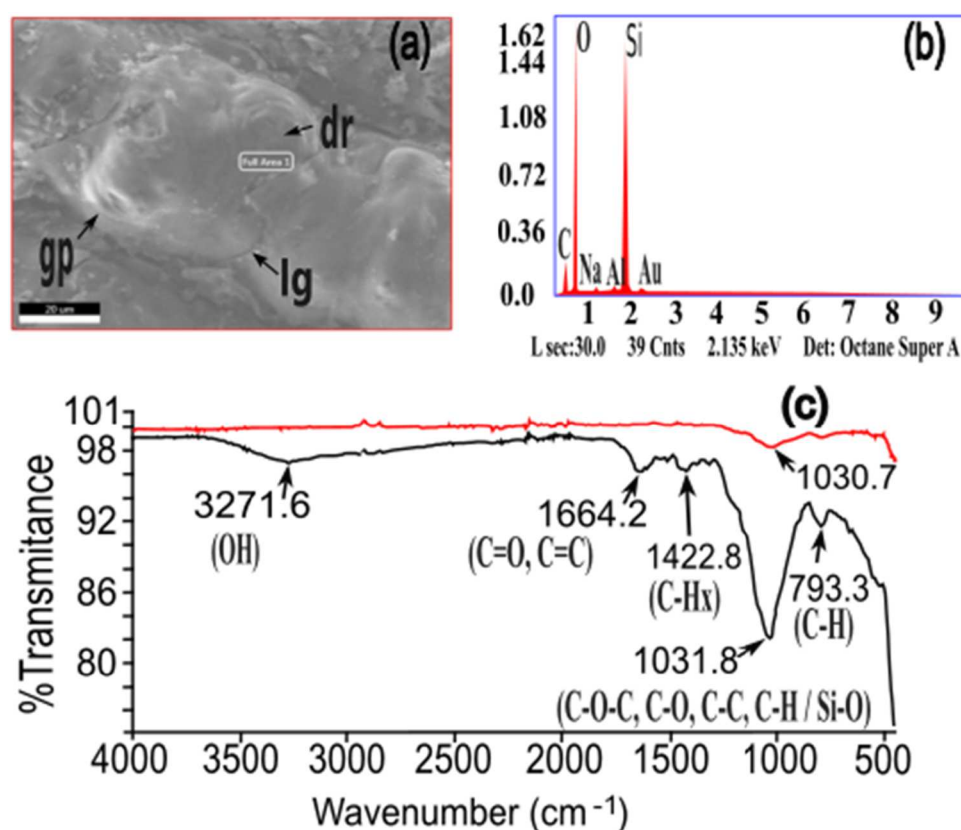


Figure 3. Characterization of rice husk (*rh*). (a) SEM images showing details of the epithelial cells of *rh*, including linear grooves {lg}, dotted ridges {dr}, and globular protrusions {gp}. Scale bar = 20 μm. (b) EDX analysis of *rh*. (c) FTIR spectra of *rh* exposed to 200 mg·l⁻¹ Cr(VI) (black line) compared to *rh* not exposed to Cr(VI) (red line).

other hand, it is beneficial for a biomaterial intended as a support or packing filler in reactors, as it will decompose more slowly and cause less clogging in the system.

The EDX-Xray analysis of this material revealed high levels of oxygen, silicon, and carbon, along with trace amounts of sodium, aluminum, and gold (figure 3(b)). The gold (Au) signal in the EDX spectrum is attributed to the gold-coated sample supports used during microscopy observation. This peak is therefore considered an instrumental artifact, rather than an element present in the sample itself. This elemental composition is consistent with previous reports (e.g., Valverde *et al* 2007, Ciannamea *et al* 2010, Chen *et al* 2017). No chromium was detected in the analysis of *rh* alone.

The spectrogram of *rh* samples (B0) exposed to Cr(VI) showed an increase in the dominant absorbance compared to the unexposed samples (figure 3(c), black and red lines, respectively). Exposure to Cr(VI) resulted in a noticeable decrease in the continuum and an increase in the intensity of absorption bands. The band between 3000 and 3600 cm⁻¹ corresponds to the -OH stretching vibrations attributed to alcohol groups in cellulose structures (El-Sheikh *et al* 2009a, 2009b, Singha *et al* 2011, Malik *et al* 2023). The band at 1664.19 cm⁻¹ is associated with unsaturated functional groups such as alkene and/or aromatic carbonyl/carboxyl (C=C & C=O) (Nakbanpote *et al* 2007, Singha *et al* 2011, Singh *et al* 2020). The bands corresponding to C=O, C=C, and C-O stretching vibrations are characteristic of organic materials such as lignin, cellulose, and hemicellulose (Singha *et al* 2011, Malik *et al* 2023). The band observed at 1032 cm⁻¹ may be attributed to the asymmetric stretching vibrations of the C-O-C bonds in cellulose (Singh *et al* 2020) and/or to Si-O stretching (Malik *et al* 2023), similar to the bands at 793.34 cm⁻¹, and 657 cm⁻¹, which are also assigned Si-O stretching. The presence of these functional groups on the *rh* surface indicates that this AR can act as an affective adsorbent for Cr(VI) (Malik *et al* 2023).

3.2. Evaluation of biofilm formation

The capacity for biofilm formation and general bacterial growth was evaluated via microculture in microtiter plates. Cultures were grown in an mLB medium and an MM medium supplemented with glucose and chromium. No detectable growth was observed in the MM medium. However, all three consortia exhibited growth in the mLB medium supplemented with Cr(VI) at concentrations ranging from 50 to 400 mg·l⁻¹ (figure 4).

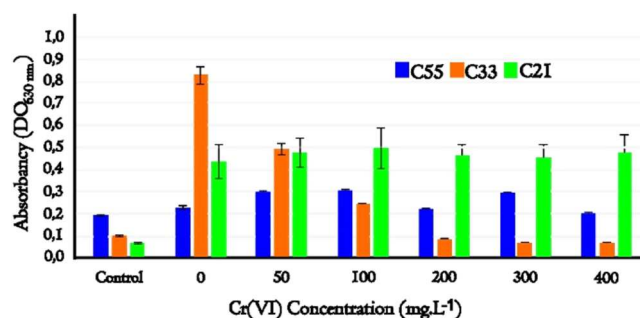


Figure 4. Impact of Cr(VI) on biofilm formation. Bacterial consortia were cultured in a modified LB medium supplemented with varying concentrations of Cr(VI) (50–400 mg·L⁻¹). After a 4 h incubation, biofilm formation was assessed by crystal violet staining and measuring the optical density at 630 nm. The control well contained medium only. For each consortium and concentration, data represent the mean ($\pm$ SD) of 30 replicate wells.

Consortium C21 exhibited the highest tolerance to the xenobiotic, maintaining consistent biofilm formation across all Cr(VI) concentrations (mean OD₆₃₀ = 0.466 $\pm$ 0.020). Consortium C55 showed a similar pattern of stable, though less robust, biofilm production with minimal variation between treatments (mean OD₆₃₀ = 0.258 $\pm$ 0.046). In contrast, consortium C33 exhibited a clear concentration-dependent inhibition of biofilm formation. Although strong biofilm growth in the absence of Cr(VI) (OD₆₃₀ = 0.828 $\pm$ 0.038), a 41% reduction was observed at 50 mg·L⁻¹ (OD₆₃₀ = 0.490 $\pm$ 0.026), and a 70% reduction at 100 mg·L⁻¹ (OD₆₃₀ 0.244 $\pm$ 0.001). At higher concentrations ($\geq$ 200 mg·L⁻¹), very little biofilm growth was detected.

3.3. Biofilm observation (B1 experiment)

The time required to form consolidated biofilm was consortium-dependent. Biofilm development was markedly delayed in the C55-inoculated system. To ensure a consistent starting point across all reactors, the incubation period was extended until a consolidated biofilm was confirmed in every reactor; in the B1 batch experiment, this occurred after approximately 35 days (figure 5). In the C1 control systems (with only the consortia), biofilm formation occurred mostly at the bottom of the glass bottle (figure 5(a)). In contrast, systems containing *rh* exhibited biofilm formation both around and within the microfiber bags, referred to as macro-biofilm (MaB, figure 5(e)) and micro-biofilm (MiB; figure 5(i)). Planktonic cells (figure 5(b)–(d)), respectively for C33, C55, and C21 inocula and both MaB (figure 5(f)–(h)) and MiB biofilms (figure 5(j)–(l)) were analyzed using SEM. Various cell morphologies — including cocci, coccobacilli, bacilli, and diplobacilli — were identified across all systems. A smooth layer covering *rh* surface was consistently observed. At higher magnifications, EPS were visible surrounding groups of cells and forming aggregates in some regions. The EPS layer appeared more developed and homogeneous in the MaB compared to the MiB. Additionally, spherical starch granules with diameters ranging from 2–8 μ m were observed in the MiB, consistent with those described by Kassem *et al* (2011).

3.4. Bacterial growth (B2 experiment)

Microbial growth was monitored in the B2 batch experiment at λ = 630 nm for 24 h intervals (table 2). The C2 systems, which contained only Cr(VI) and the inoculum, showed slow bacterial growth (growth time, t_g > 1000 h). Conversely, C3 systems, containing only *rh* and the inoculum, exhibited higher growth rates (330 < t_g < 770 h). In B2 systems, which included the microorganisms, the xenobiotic Cr(VI) and *rh*, bacterial growth was unexpectedly more efficient even than C3 systems, with t_g values ranging from 217 to 330 h. These results suggest that *rh* plays a critical role in the Cr(VI) bioprocess, enhancing significantly the bacterial growth even in the presence of the xenobiotic. This enhancement may result from *rh* stimulating biofilm formation, and/or partial adsorption of Cr(VI) onto *rh*, and/or the release of *rh*-derived compounds capable of interacting with and reducing Cr(VI).

3.5. Taxonomic composition (B2 experiment)

High-throughput sequencing of original consortium samples and reactors inoculated with microorganisms (C2 and B2) yielded 240,224 reads of approximately 400 bp. Nine OTUs were detected in nearly all samples, predominantly belonging to the phyla *Actinomycetota* and *Bacillota*. Both phyla are recognized as potential bioremediation agents for the Cr(VI) environmental mitigation (Mawang *et al* 2021). The dominant OTUs, representing 95% of total abundance, included the genera *Cellulosimicrobium* (44%), *Siminovitchia* (27%), *Enterococcus* (9%), *Peptoniphilus* (8%), and *Oceanobacillus* (6%), with relative proportions varying across the

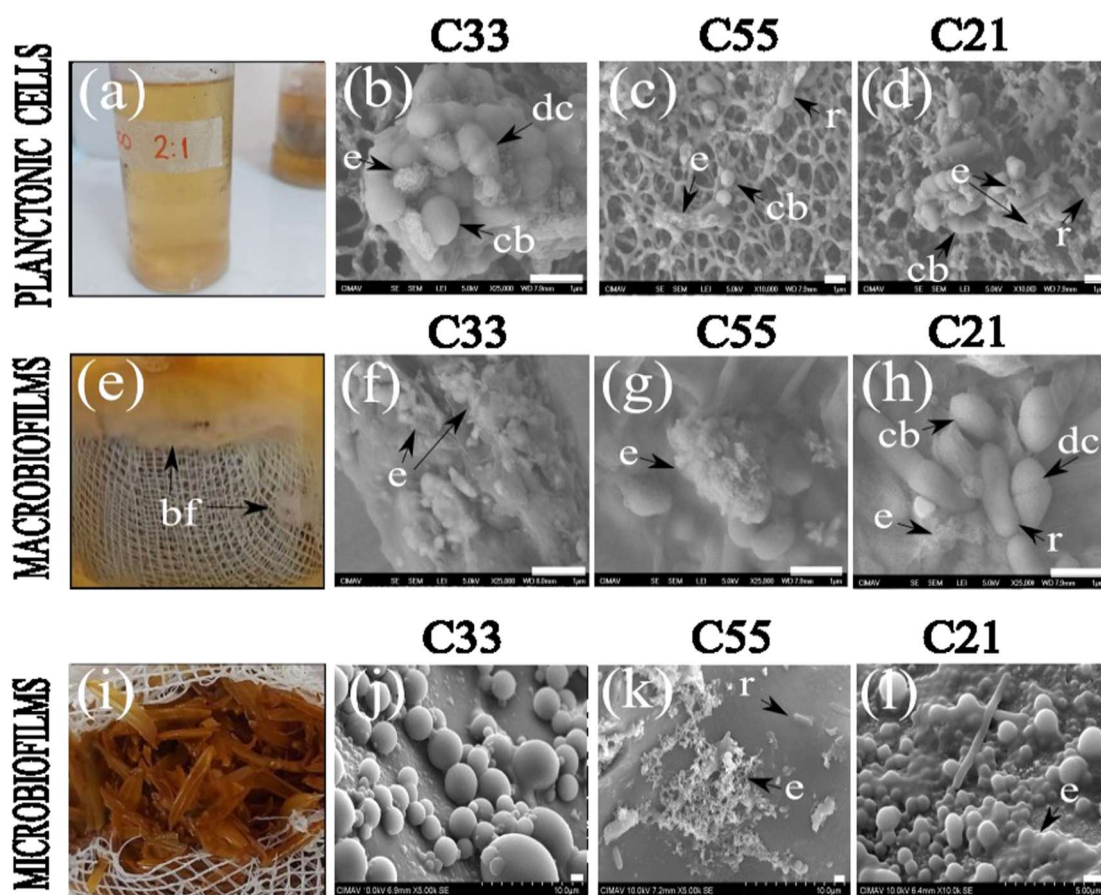


Figure 5. Biofilm formation and microbial morphology observed through SEM. (a) Overview of the biological control (C1) reactor, with biofilms developing at the air-culture media interface. (b)–(d) SEM micrographs illustrating the morphology of planktonic cells. (e) Large sphere of *rh* with macrobiofilm (MaB) formation around its surface. (f)–(h) SEM micrographs of MaB. (i) Interior of the large sphere showing *rh* with collected microbiofilms (MiB). (j)–(l) SEM micrographs of MiB. Reactors inoculated with C33 (b), (f), (j), C55 (c), (g), (k), and C21 (d), (h), (l). Observed microbial morphologies include: *r*—rods, *cb*—coccobacilli, *dc*—diplococci. Additionally, polymeric substances (*e*) and biofilms (*bf*) are highlighted. Scale bar: 1 μm .

Table 2. Bacterial growth rate (μ) during the exponential phase on MM liquid media for the small reactors (B2 experiment and its C2 and C3 controls). Growth rates were measured at $\lambda = 630 \text{ nm}$ on intervals of 24 h. The μ values were used to calculate the generation time (t_g) required for the population to double its size ($t_g = \ln 2 / \mu$).

Consortia and mixtures	Systems	μ	R^2	t_g (h)
C55	C2	0.0006	0.602	1155
	C3	0.0017	0.092	408
	B2	0.0032	0.978	217
C33	C2	0.0002	0.896	3466
	C3	0.0021	0.839	330
	B2	0.0021	0.884	330
C21	C2	0.0001	0.025	7702
	C3	0.0009	0.436	770
	B2	0.0022	0.913	315

studied systems (figure 6). Minority OTUs (each comprising $<3\%$ of the total biodiversity) included the genera *Cutibacterium*, *Rhodococcus*, *Staphylococcus*, *Bacillus*, *Listeria*, and *Virgibacillus*.

The bacterial community structure of systems inoculated with the C21 consortium was similar to that of C55, consistent with the fact that the C21 was generated using two parts of C55 for one part of C33.

The addition of Cr(VI), in C2 systems, stimulated the growth of *Cellulosimicrobium* and *Siminovitchia* populations, while inhibiting the populations of *Peptoniphilus*, *Cutibacterium*, and *Rhodococcus*. *Oceanobacillus*

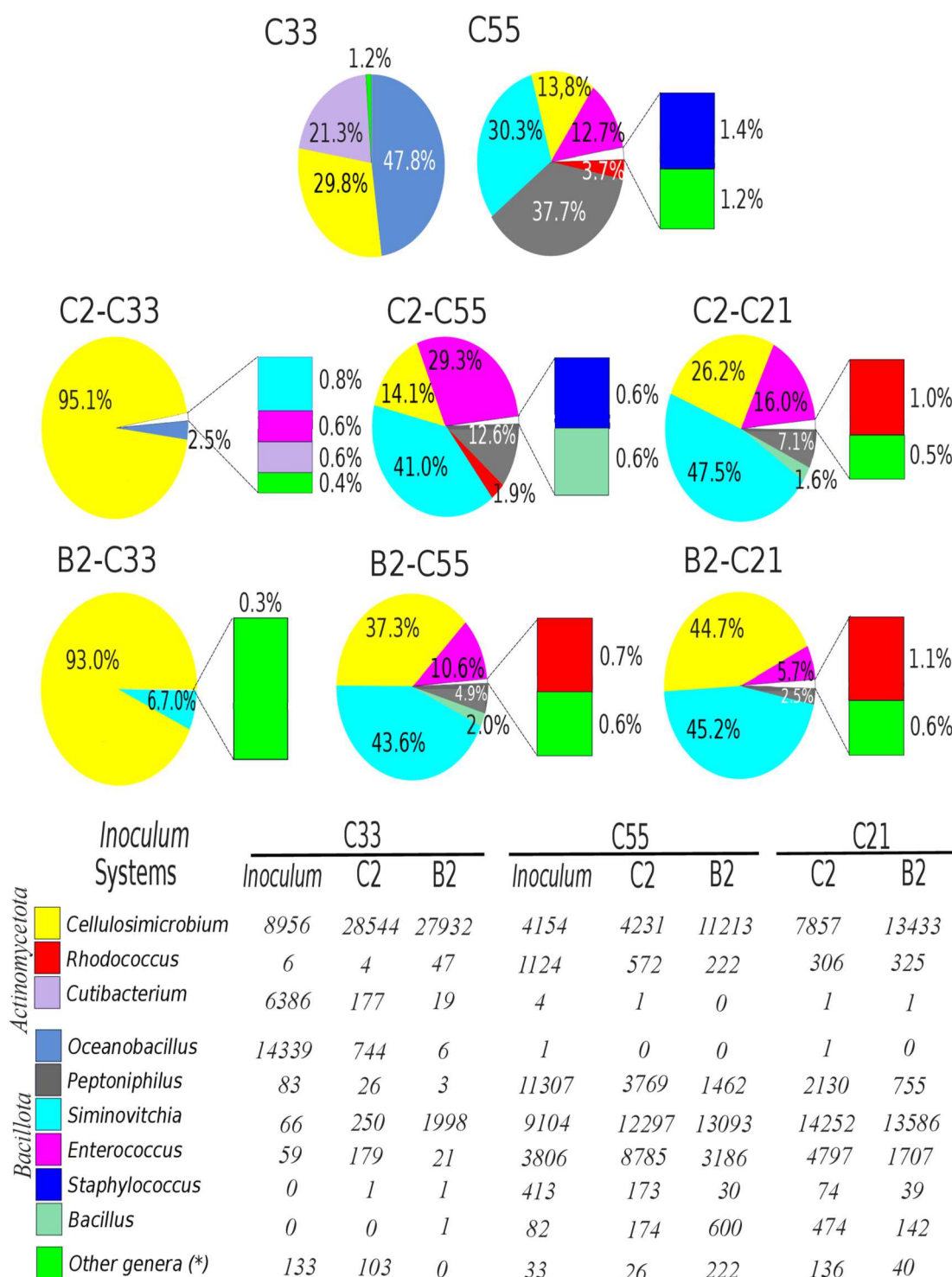


Figure 6. Bacterial diversity based on the V4-V5 region of 16S rRNA. Pie diagrams show the relative abundance of OTUs at the genus level for the reactors inoculated with C33, C55, or C21 consortia. According to the experimental design (table 1), C2 represents the control systems with only bacteria and Cr(VI), while B2 represents the complete systems with chromium, rice husk, and microorganisms. The comparison between these systems was used to understand the variables that could favor chromium reduction. (*) OTUs with less than 0.5% of relative abundance.

and *Cutibacterium*, typical genera of C33, did not benefit from either the xenobiotic or the *rh*, while *Cellulosimicrobium*, present in both C33 and C55 (also in C21), were stimulated by both Cr(VI) and the *rh*.

The case of *Oceanobacillus* suggests a lower tolerance to Cr(VI), in contrast with some previous studies that demonstrated that strains such as *O. profundus* QRePLB33E exhibited high Cr(VI) resistance and removal capabilities when grown in axenic culture (Martínez-Aldape *et al* 2024, Brito *et al* 2025).

Cellulosimicrobium populations evidently deserve attention. Previous studies have shown the metal resistance of *Cellulosimicrobium* species, including Cr(VI) tolerance (Karthik and Arulselvi 2017 Bharagava and

Mishra 2018). Strains such as *C. funkei* QRePRA55 and *C. aquatile* QReMLB55A have demonstrated high Cr(VI) resistance and reduction capabilities (Martínez-Aldape *et al* 2024).

The conclusion that growth is stimulated by both Cr(VI) and *rh* also applies to the *Siminovitchia* population. This genus, which was common in C55 and present at a marginal abundance in C33, comes from a reclassification of certain *Bacillus species* (Gupta *et al* 2020). It comprises aerobic, rod-shaped bacteria typically isolated from soil environments.

Notably, our study provides the first evidence of an interaction between *Siminovitchia* and Cr(VI) (Brito *et al* 2025). Additionally, the reported ability of some species in this genus to produce biofilms (Iungin *et al* 2024) suggests that the observed population increase after Cr(VI) addition may be associated with enhanced biofilm formation in the reactors. This potential mechanism is supported by the presence of other genera with biofilm-forming capabilities, such as *Peptoniphilus* and *Enterococcus* (Tsegahun 2019), despite their decline in abundance post-Cr(VI) addition and their potential pathogenicity. Collectively, the biofilm-forming potential of this microbial community likely contributes significantly to the bioremediation process. This synergy could play an important role in enhancing overall biofilm stability and function performance in bioremediation applications.

3.6. Chromate reductase genes

Few studies have focused on molecular biology or proteomic approaches to identify the specific existence of chromate reductase genes, especially for *Cellulosimicrobium* genus. Karthik *et al* (2021) studied *C. funkei* AR6 strain as a plant growth-promoting rhizobacteria capable of down-regulating the major metal transporter NRAMP genes. NRAMPs constitute a large family of proteins that function as proton-coupled metal ion transporters and facilitate metal compartmentalization (Ding and Cai 2017). The complete genome of the strain *C. funkei* RVMD1, isolated from a rock varnish in the Ma'an desert, was recently sequenced (Alnaimat *et al* 2024). Using a bioinformatic approach, the authors identified 46 genes related to metal resistance but did not provide a detailed description of them.

Recently, we obtained a *Cellulosimicrobium* strain in axenic culture (QRePLB55C). The genomic DNA was extracted, fully sequenced and analyzed (Martínez-Aldape 2025). In the first annotation, 38 genes related to metals were identified using Prokka. BLAST analysis revealed 81 proteins associated with metal transporter genes, of which 35 were linked to chromium, including the *ChrC* gene. The *ChrC* gene is part of a chromium resistance system that includes other genes, such as *ChrA*, *ChrB* and *ChrF*. The *ChrC* gene encodes for an enzyme that belongs to the superoxide dismutase (SOD) family, specifically a manganese-dependent SOD. This enzyme converts the superoxide anion into molecular oxygen and hydrogen peroxide, reducing the oxidative stress inside the cells, so it is a crucial metabolic mechanism for cell protection from chromium oxidative stress. This mechanism may explain the increase in *Cellulosimicrobium* populations in the studied systems: a better physiological adaptation to Cr(VI) is probably allowing this population to occupy an ecological niche rich in metals highly toxic to other microorganisms, taking advantage of the decline of competing populations.

3.7. Chromium depletion systems (B3 experiment)

For the B3 experiment, Cr(VI) was monitored at 24 h intervals until it became undetectable (figure 7). In these systems, no change in Cr(VI) concentration was observed for the C5 control essay.

Consolidated biofilms were observed after 45 days of incubation, forming a smooth, homogeneous MaB layer around the pellets and on the medium surface. Clearly, B3 (BRC) systems diminished the Cr(VI) much more rapidly than C4 (RC) systems.

The results above are better shown in table 3. As hypothesized, the best performance in Cr(VI) diminution was observed in BRC systems, with similar half-life ($t_{1/2}$) values for different *inocula*: between 53 and 67 h compared to *rh* systems (with C21 and C55 consortia showing slightly better performance than C33). The behavior of RC systems was not bad: presenting a $t_{1/2}$ of 165 h for compacted *rh*, confirming that the *rh* alone is also capable of adsorbing Cr(VI) or reducing Cr(VI) to Cr(III).

After Cr(VI) level became undetectable in the effluent, the systems (B3, C4 and C2) were disassembled and CrT was measured in all their parts (figure 8). The fractions of CrT retained in the effluents were $15.1 \pm 1.9\%$, $33.6 \pm 5.9\%$ and $59.3 \pm 5.1\%$, in the B3, C4 and C2 respectively. This residual chromium corresponds to Cr(III) since no Cr(VI) was detected in the final effluents. Thus, both the bacteria (C2) and the *rh* (C4) alone could transform Cr(VI) to Cr(III) externally, being the bacteria more efficient in doing it. Concerning CrT adsorbed in *rh* fraction, values of $66.4 \pm 10.8\%$ for C4 and $73.4 \pm 19.2\%$ for B3 were measured. These high fractions, about 70%, confirm previous results of *rh* ability to adsorb chromium (e.g., Kiran *et al* 2025, Haroon *et al*, 2025). However, the challenge of regeneration and reuse this biosorbent is maintained. On the other hand, the fractions of CrT absorbed by the biomass were 40.7 and $11.5 \pm 0.2\%$, for C2 and B3 respectively. This chromium is expected to be transformed to Cr(III) inside the biological cells, but since the technique used (AAS) is

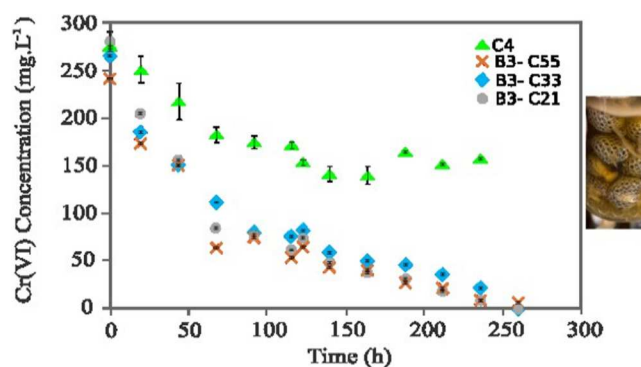


Figure 7. Hexavalent chromium removal. Diminution of Cr(VI) concentration in reactors with *rh* compacted in 1.2 cm small balls, and an illustrative photo of the experiment. C4 represents the control systems with only rice husk, and Cr(VI), while B3 represents the complete systems with chromium, rice husk and microorganisms.

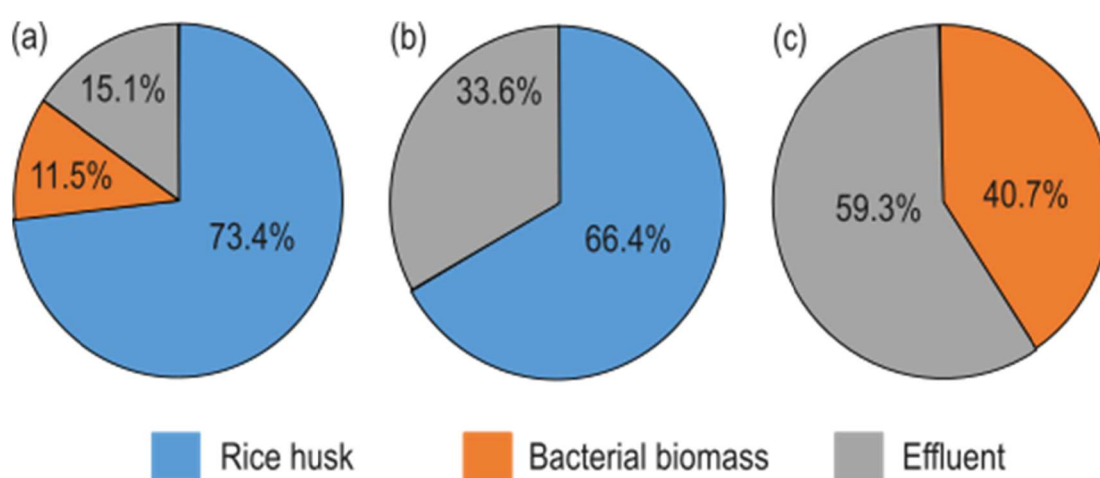


Figure 8. Proportion of total chromium retained in the reactor components (rice husk support, bacterial biomass, including biofilms, and the effluent) at the end of Cr(VI) diminution in the effluent. (a) complete system (B3), (b) system containing only rice husk without bacterial inoculum (C4), and (c) system with only bacterial inoculation (C2).

Table 3. Decrease of Cr(VI) in reactors with *rh* compacted into small pellets (B3). Kinetic parameters of Cr(VI) diminishing reactions, where: (*k*) is the first-order kinetic constant calculated from logarithmic decay regression based on data from figure 5; (*t*_{1/2}) is the half-life in hours, and (*R*²) is the regression fit accuracy.

Consortia and mixtures	Systems	Compacted <i>rh</i> (B3)		
		<i>k</i>	<i>R</i> ²	<i>t</i> _{1/2}
C55	C4	0.0042	0.9616	165
	C2	0.0017	0.8298	408
	B3	0.0128	0.9441	54
C33	C2	0.0026	0.8257	267
	B3	0.0103	0.9454	67
C21	C2	0.0043	0.9209	161
	B3	0.0131	0.9607	53

unable to detect Cr(III), this remains a supposition. Finally, when both components entered the ‘competition’ for chromium (B3), the adsorptive capacity of the *rh* is maintained (about 70%), as well as the absorptive capacity of the bacteria (about 40% of the remaining chromium). It is also worth noting that an advantage of B3 system is its configuration as a fixed bed reactor, easier to monitor than a suspended biomass one.

In table 4, we provide a comparison of our results on biosorption and results from the literature. Note the high initial concentration (*C*₀) in our experiments and the alkaline pH.

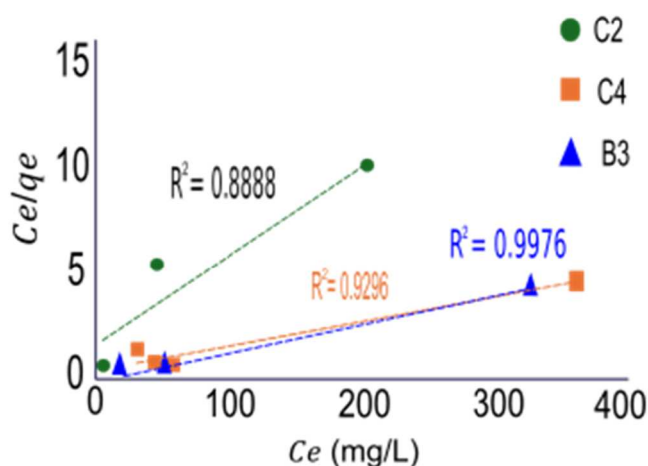


Figure 9. linearized Langmuir model for Cr(VI) diminution in the effluent for the following systems: (B3) complete system, (C4) system containing only rice husk without bacterial inoculum, and (C2) system with only bacterial inoculation.

Table 4. Removal rates of Cr(VI) from this study and the literature.

Adsorbent	Cr(VI) removal rate (%)	Specific experimental conditions		References
		C_o (mg.g ⁻¹)	pH	
Bacterial biofilm (C2 systems)	100	200	8.5	This Study
<i>Rh</i> (C4 systems)	100	200	8.5	This Study
B3 Systems (<i>rh</i> + biofilm)	100	200	8.5	This Study
Rice husk carbon activated	95	20	2	Zhang <i>et al</i> 2023
Tea leaf	100	10	2	Ahalya <i>et al</i> 2005
Biochar-poly(m-phenylenediamine)	93	200		Yang <i>et al</i> 2024
Jute stick charcoal	99	20	2	Zaman <i>et al</i> 2022
Fungal biofilm of <i>Aspergillus niger</i>	92	30	3	Chatterjee <i>et al</i> 2022
Algae biomass carbon	90	80	2	Ranwala <i>et al</i> 2025
Alginate-encapsulated bacterial beads	91	5.4	7	Srivastava <i>et al</i> 2025

Cr(VI) adsorption may involve various mechanisms (Islam *et al* 2019), including electrostatic attraction, ion exchange, or surface complexation. Furthermore, a portion of adsorbed Cr(VI) can be reduced to Cr(III) species. This newly formed Cr(III) can then either adsorb onto the substrate via ion-exchange or surface complexation, or it may be released into the solution due to electrostatic repulsion (Islam *et al* 2019). Consequently, both the elemental composition of the AR surface and the nature of the chromium ionic species are of vital importance to the Cr(VI) adsorption mechanism. In aqueous systems, Cr(VI) can exist as five main species: H_2CrO_4 ; $HCrO_4^-$; CrO_4^{2-} ; $HCr_2O_7^-$; $Cr_2O_7^{2-}$, whose distribution is dependent on pH and total chromium concentration (Miretzky and Cirelli 2010).

Since we worked with alkaline concentrations (final pH between 8.5–8–7), $Cr(OH)_3$ is predominant (Hu *et al* 2004). $Cr(OH)_3$ may be physically deposited or adsorbed onto the *rh* surface following the chemical reduction of Cr(VI) to Cr(III) (Hu *et al* 2004). Thus, at alkaline pH, little to no Cr(VI) adsorption was expected to occur. Despite the unfavorable conditions for direct Cr(VI) adsorption at this pH, a significant reduction in Cr(VI) concentration was observed.

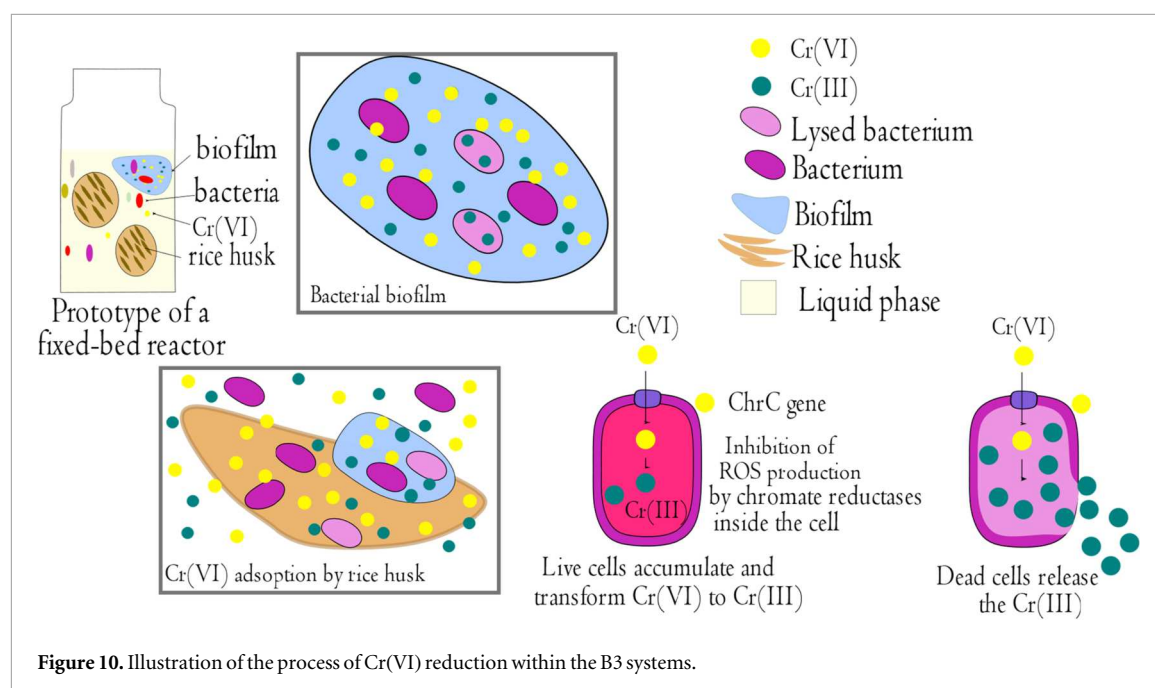
3.8. Isothermal adsorption models analysis

To a first approximation, equilibrium data were analyzed through linearized forms of the models by Langmuir, Freundlich and Temkin models (Islam *et al* 2022 and Dos Santos *et al* 2025; Supplementary Materials S1). According to R^2 statistics, the Langmuir model provided the best linear fit for the B3, C2 and C4 systems ($R^2 = 0.998$, 0.889 and 0.930, respectively; figure 9). This result is consistent with different studies of Cr(VI) adsorption over biomaterials (table 5).

For C2 system, composed by the bacterial consortium alone, this is consistent with a process of surface adsorption with relatively homogeneous sites distributed in the cellular wall, forming a monolayer through its

Table 5. Adsorption parameters obtained by fitting linear models to bacterial, lignocellulosic and composite systems for the Cr(VI) removal.

System	Model	R ² (lineal)	q _m (mg/g)/B	KL/KF/A	n (Freundlich)	Interpretation/affinity	Notes
B3	Langmuir	0.998	72.686	-7.205×10^{-2}	—	negative KL	High R ² but model does not describe the real curve
B3	Freundlich	0.632	—	2.163×10^2	−5.962	negative n	High KF but n < 0 indicates unphysical behavior
B3	Temkin	0.558	−16.377	1.740×10^{-5}	—	negative B	Low representativity linear model
C2	Langmuir	0.889	23.84	2.515×10^{-2}	—		
C2	Freundlich	0.494	—	6.22	5.62		
C2	Temkin	0.551	2.57	3.97	—		
C4	Langmuir	0.930	84.875	3.328×10^{-2}	—	moderate positive KL	Reasonable linear model
C4	Freundlich	0.208	—	1.748×10^1	3.477	n≈3.5, favorable adsorption in heterogeneous surface	Low R ² indicating that model is not ideal
C4	Temkin	0.146	11.915	4.571	—	positive B, adsorption	Low R ² indicating that model is not ideal



functional groups ($-\text{COOH}$, $-\text{NH}_3$, $-\text{PO}_4$). On the other hand, *rh* system (C4) exhibited a behavior typical of lignocellulose biomass, with a high R^2 due to saturation of its functional sites. Finally, the mixed B3 system generated negative physical parameters (KL, n and B), showing a superposition of metabolic, competitive and chemical mechanisms, which bias the classical form of the isotherms, as have been reported in bacteria-biomass based systems. Several authors have pointed out that the linearization of isotherms may lead to wrong parameters and interpretations, especially in heterogeneous surfaces and biosorbent systems (Murphy *et al* 2023, El Jery *et al* 2024, Pieczykolan *et al* 2025). Therefore, for the physicochemical interpretation, we had to use more general no-linear models, like Dubinin–Radushkevich para B3 y Sips para C4, which showed more consistent numerical fits and parameters more coherent with the heterogeneous and biological nature of the applied materials.

3.9. Final remarks

Numerous studies have highlighted significant metabolic and physiological traits in various microorganisms that enable the biotransformation of Cr(VI) to Cr(III) (see the review by D. Pradhan *et al*, 2017). However, *Cellulosimicrobium* and *Siminovitchia*, the two microbial populations that became dominant in our systems following Cr(VI) addition, are not included in this list. The presence of *Siminovitchia* is particularly noteworthy, as this is the first report suggesting its potential involvement in Cr(VI) reduction processes. Moreover, for the genus *Cellulosimicrobium*, no chromate reductase gene has been identified to date.

It is plausible that, similar to other aerobic bacteria, Cr(VI) reduction in these genera may occur via non-specific enzymatic electron transfer systems, NADH-dependent soluble reductases, extracellular reduction mediated by secreted metabolites, or efflux pumps encoded by homologous *Chr* operons (Rahman and Thomas 2021, Xie *et al* 2025). Of these mechanisms, we were only able to verify the latter through genomic analyzes of *Cellulosimicrobium*.

However, research investigating the synergistic effect of the two components –natural rice husk and Cr(VI)-resistant microorganisms– remains largely unexplored. This is precisely the focus of the present study, particularly within our BRC systems.

In our systems, microorganisms reduce Cr(VI) through active and passive processes, including transport, accumulation, biosorption, and both enzymatic and non-enzymatic transformation into Cr(III) (Viti *et al* 2014, Gutiérrez-Corona *et al* 2016). Additionally, it should be considered that chromium can also be adsorbed and/or transformed by the biofilm itself. For example, components such as tryptophane present in the polysaccharide matrix of the biofilm may promote Cr(VI) reduction (Luo *et al* 2022). The process model is illustrated on figure 10.

4. Conclusions

No chromium was detected in the *rh* alone, but the presence of Si compounds may inhibit its biodegradation. Although not directly useful for the microorganisms, it can act as a resilient biomaterial.

Members of the *Actinomycetota* and *Bacillota* phyla were found by the metagenomic analysis of the consortia. Populations of *Cellulosimicrobium* genus were favored by both the presence of *rh* and the xenobiotic, while populations of *Siminovitchia* were stimulated by the addition of Cr(VI), and populations of *Peptoniphilus* and *Rhodococcus* were inhibited. Finally, *Oceanobacillus*, *Cutibacterium* and *Enterococcus* populations were not significantly affected in the experiment.

While the ability of C33 consortium to produce biofilm is the highest in the absence of Cr(VI), when present a concentration-dependent inhibition occurs. The opposite happens with C55, maintaining small biofilm formation in all the Cr(VI) concentrations probed. C21 benefits from the best part of both: higher biofilm formation than C55 and tolerance along all concentrations.

It seems that *rh* does not interfere in the biofilm formation, but its presence enhances the Cr(VI) depletion due to its adsorption capability. This may also induce a better growth of the microorganisms in a less toxic environment. This suggests its potential to enhance the Cr(VI) bioprocessing.

From the Cr(VI) depletion analysis we found that the complete experiment (inoculum + *rh* + Cr(VI)) was the most effective, compared to the consortia and *rh* alone with Cr(VI). This means that our hypothesis for proposing a low-cost, scalable and eco-friendly bioprocess was confirmed. However, further studies are necessary to optimize the conditions for effective Cr(VI) removal using real leachate from chromite industrial residue.

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Conflict of interest

The authors declare that they have no conflict of interest and have read and approved the final version of the manuscript.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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