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Bioremediation of Cr(VI) using indigenous bacterial strains isolated from a common industrial effluent treatment plant in Vishakhapatnam

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ABSTRACT

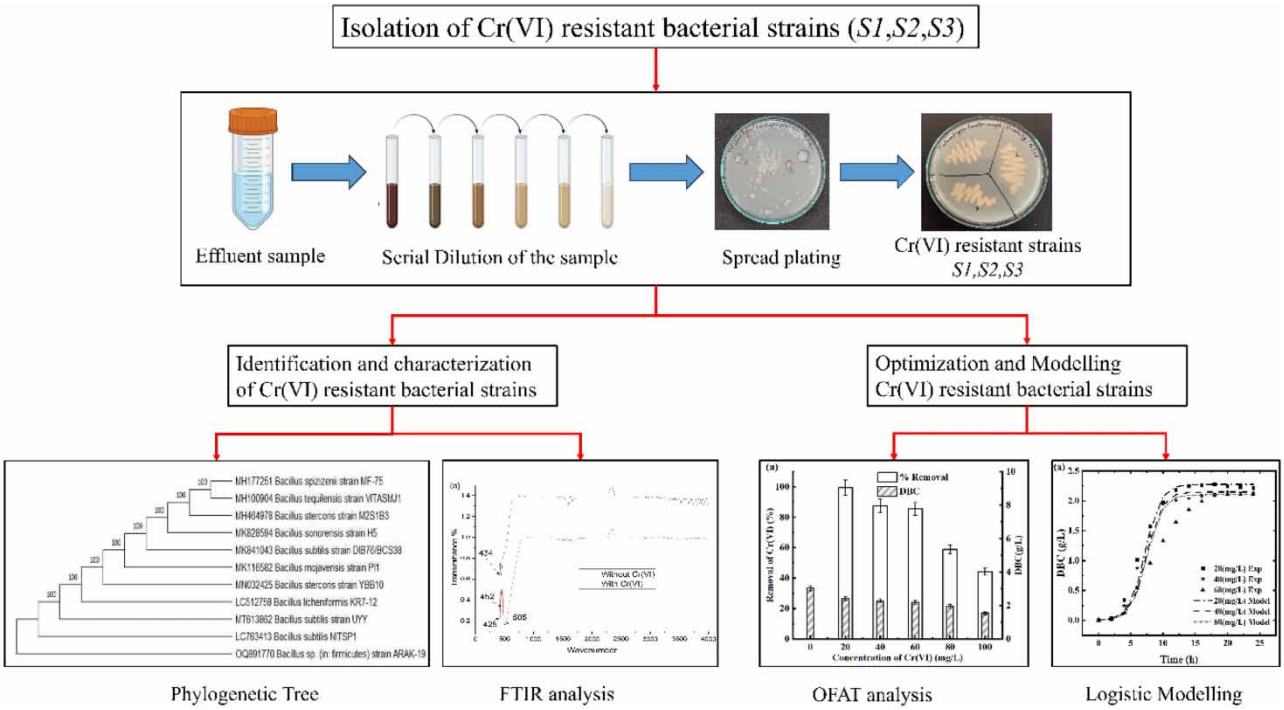
The present study focuses on removing hexavalent chromium (Cr(VI)) using indigenous metal-resistant bacterial strains isolated from a common industrial effluent treatment plant, a contaminated site in Vishakhapatnam. Three high metal-resistant isolates were screened by growing them in nutrient agar media containing different Cr(VI) concentrations for 24 h at 35 ± 2 °C. The three strains' minimum inhibitory concentrations of Cr(VI) were examined at neutral pH and 35 ± 2 °C temperature. Morphological, biochemical, and molecular characterizations were carried out, and the strains were identified as *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3. Elemental composition and functional group analysis of the native and metal-loaded cells were done using energy-dispersive spectroscopy and Fourier-transform infrared spectroscopy, respectively. The operating conditions were optimized using a one-factor-at-a-time analysis. When compared with three bacterial isolates, maximum Cr(VI) removal (80.194 ± 4.0%) was observed with *Bacillus subtilis* NITSP1 with an initial Cr(VI) concentration of 60 mg/L, pH 7.0, an inoculum size of 2% (v/v), and an incubation period of 24 h. The logistic model was used to predict the variation of biomass growth with time. The present study can be extended to remove heavy metals from industrial wastewater in an environmental-friendly manner.

Key words: *Bacillus* sp., bacteria, bioremediation, chromium(VI), logistic model, *Pseudomonas* sp. and *Rhizobium* sp

HIGHLIGHTS

- Cr(VI), being ranked 17th in the Agency for Toxic Substances and Disease Registry's list, was eliminated via a microbial route.
- Three metal-resistant bacterial strains that were isolated from a common industrial effluent treatment plant in Vishakhapatnam, India, effectively removed Cr(VI).
- Energy-dispersive spectroscopy and Fourier-transform infrared spectroscopy confirmed the metal-binding capability.
- The logistic model predicted biomass growth variations over time. *Bacillus subtilis* NITSP1 exhibited the highest Cr(VI) removal.

GRAPHICAL ABSTRACT



ABBREVIATIONS

AARD	average absolute relative deviation
DBC	dry biomass concentration
EDS	energy-dispersive spectroscopy
FTIR	Fourier-transform infrared
IC	initial concentration
LB	Luria broth
MIC	minimum inhibitory concentration
OFAT	one-factor-at-a-time
RMSE	root mean square error

INTRODUCTION

Heavy metal pollution from wastewater poses a severe environmental and health concern, as the discharge of industrial and domestic sewage can introduce toxic heavy metals like chromium (Cr), lead (Pb), zinc (Zn), cadmium (Cd), nickel (Ni), copper (Cu), mercury (Hg), and arsenic (As) into water bodies, endangering ecosystems and human well-being (Mitra *et al.* 2022). According to the Agency for Toxic Substances and Disease Registry (ATSDR), hexavalent chromium (Cr(VI)) holds the 17th position among the most hazardous chemicals posing a severe threat to human health (ATSDR 2022). Electroplating, textile dyeing and printing, leather tanning, metal finishing, and paint manufacturing industries are some that are responsible for releasing Cr(VI) into wastewater (Kerur *et al.* 2021). India, contributing to 13% of global leather production, is becoming one of the largest leather goods exporting countries, and it is noteworthy to mention that leather industry wastewater is one of the significant contributors of Cr(VI) to the environment (Princy *et al.* 2020; Venkatesan *et al.* 2021). Due to its specific chemical properties, including solubility and reactivity, Cr(VI) has carcinogenic effects, causing oxidative stress and targeting multiple organs through bioaccumulation (Wani *et al.* 2019). The World Health Organization (WHO) has set a maximum permissible limit of 0.05 mg/L for Cr(VI) in treated wastewater (Prabhakaran *et al.* 2019). Conventional methods such as membrane filtration, photocatalysis, adsorption, precipitation, and ion exchange have been employed for Cr(VI) removal (Tang *et al.* 2021). However, these methods are associated with several disadvantages, such as high operational

costs, energy consumption, the generation of secondary pollutants, and reduced efficiency, when targeting low concentrations of Cr(VI) (Diep *et al.* 2018). Green technologies employing microorganisms for removing or converting toxic heavy metals into nontoxic substances from the contaminated environment are considered more advantageous and sustainable than conventional methods.

Microorganisms, including bacteria, algae, fungi, and yeast, can efficiently bioaccumulate heavy metals in their living cells. Bioremediation is a process that utilizes different biological mechanisms to convert pollutants into nontoxic forms using live microorganisms (Sharma & Kumar 2021). In recent years, bioremediation has been perceived to be a promising technology for removing Cr(VI) in an efficient, eco-friendly, and cost-effective manner owing to several advantages of microorganisms, such as ease of handling, culture, and implementation (Kholisa *et al.* 2022, 2023). The performance of living biomass in binding metal ions depends on the nutrient and environmental status and the cell age. Over the last few years, bacteria have been widely researched and employed due to their ability to adsorb (biosorption), solubilize (bioaccumulation), and precipitate (bioprecipitation) heavy metals (Fernández *et al.* 2018). Some reported works on the bioremediation of Cr(VI) using bacteria are as follows: *Escherichia* sp., *Acinetobacter* sp., etc., could remediate Cr(VI) by biosorption; *Bacillus* sp. could remove Cr(VI) by bioreduction; and *Bacillus* sp. and *Streptomyces* sp. could abate Cr(VI) by biotransformation (Marzan *et al.* 2017; Kumaresan Sarankumar *et al.* 2020; Sanjay *et al.* 2020). Since tannery waste, chromium mines, etc., are rich in Cr(VI), the organisms isolated from such effluents exhibited high resistance toward Cr(VI). Several studies have primarily concentrated on isolating and identifying chromium-removing bacteria from tannery effluents, chromium mines, etc. (Yaashikaa *et al.* 2019; Sanjay *et al.* 2020; Elahi *et al.* 2022).

Being one among the developing industrial cities, Vishakhapatnam is facing severe pollutant discharge problems. Common industrial effluent treatment plants are used to treat the effluents from different industries in Vishakhapatnam. The pollutants being treated in such plants vary in wide ranges both in concentration and nature. Therefore, microbes isolated from such effluent water tend to have high resistance to various contaminants ranging from heavy metals to organic pollutants. Considering the toxicity of Cr(VI) and the role of bacteria in its removal, the current work has been designed based on the following three main objectives, such as the isolation and identification of suitable bacterial strain(s), a parametric study on the removal of Cr(VI), and modeling of bioremediation. High Cr(VI)-resistant bacterial strains were initially isolated and identified. The parametric study was done using a one-factor-at-a-time (OFAT) analysis to achieve the maximum removal of Cr(VI). Finally, the logistic model of population growth was used to predict the variation of biomass growth with time. The novelty of the current study lies in utilizing high Cr(VI)-resistant indigenous bacterial strains isolated from a common industrial effluent treatment plant in Vishakhapatnam for the bioremediation of Cr(VI). This approach aims to contribute toward a greener world.

MATERIALS AND METHODS

All experiments were replicated thrice, and the results were statistically analyzed using mean and standard deviation.

Preparation of medium

The Luria broth (LB) (Himedia, India) medium comprising of tryptone (10 g/L), yeast extract (5 g/L), and sodium chloride (10 g/L) was used as a liquid medium for bacterial cultivation. Solid agar plates, essential for bacterial growth, were prepared by supplementing the LB medium with 12 g/L agar. In order to prepare a stock solution of Cr(VI) with a concentration of 1,000 mg/L, 2.82 g of $K_2Cr_2O_7$ (Merck, India) was dissolved in 1,000 mL of sterile distilled water. The indigenous bacterial strains were cultured in the LB medium. When assessing bacterial growth in the presence of Cr(VI), the required dilution of the Cr(VI) solution was achieved by adding an appropriate volume of the sterile LB medium to the Cr(VI) solution.

Screening, isolation, and culture of Cr(VI)-resistant bacterial strains

An effluent sample was obtained from an industrial effluent treatment plant situated at a contaminated site in Vishakhapatnam (N 17.6707°, E 83.0930°). This treatment plant manages wastewater from multiple small pharmaceutical industrial units. Initially, the wastewater is gathered and stored in a tank. An API (American Petroleum Institute) separator then separates oil and grease molecules from the wastewater. The resulting treated effluent is then directed into an equalization tank. The sample was collected from this equalization tank. The temperature of the effluent sample was kept at 4 °C to preserve its chemical and biological characteristics (Nokman *et al.* 2019). The sample was serially diluted (10^{-10} times), and the diluted sample was spread on the surface of the agar plates and incubated at 35 ± 2 °C for 24 h. Colonies exhibiting distinct

morphological characteristics were identified and chosen for further experiments. These selected colonies were subcultured using the same media (Seragadam *et al.* 2021). To prepare the stock solution of Cr(VI) of 1,000 mg/L concentration, 2.82 g of $K_2Cr_2O_7$ (Merck, India) was dissolved in 1,000 mL of sterile distilled water. The required dilution of the Cr(VI) solution was achieved by adding an appropriate volume of sterile distilled water. To isolate Cr(VI)-resistant bacterial strains, a nutrient agar medium was supplemented with the Cr(VI) solution having a 50 mg/L concentration. The nutrient agar was sterilized at 121 °C for 15 min and cooled to room temperature. Three bacterial strains with high resistance to Cr(VI) concentration were isolated and termed *S1*, *S2*, and *S3*, and such strains were selected for further studies.

Morphological, biochemical, and molecular characterization of the isolated strains

The isolated metal-resistant bacterial strains were analyzed based on colony characterization, biochemical characteristics, and carbohydrate fermentation as described in Bergey's Manual for identifying the species of the bacteria (Bergey & Holt 2000). As per the standard protocol to determine the genus of the isolated bacterial strains, biochemical characterization tests like Gram stain, oxidase, catalase, methyl red, indole production, and citrate tests were done. The analysis also involved studying the utilization of carbohydrates such as glucose, sucrose, maltose, xylose, and lactose by the isolates (Marzan *et al.* 2017). Selected strains were sent to Cytogene (Lucknow, India) for molecular identification through 16S rRNA gene sequencing. The resulting nucleotide sequence was subjected to BLAST analysis using the online tool provided by the NCBI. Subsequently, the obtained sequences were submitted to the NCBI to acquire accession numbers for the isolated strains (Sahoo & Chaudhuri 2021).

Fourier-transform infrared spectroscopy and energy-dispersive spectroscopy analyses of bacterial biomass

To characterize the dry biomass of both bacterial biomasses of each strain, one grown with Cr(VI) and the other without Cr(VI), Fourier-transform infrared spectroscopy (FTIR) studies were conducted. The analysis was performed using a BRUKER Alpha II FTIR Spectrometer. For the FTIR studies, the dry biomass sample was finely ground and thoroughly mixed with potassium bromide (Merck, India) to prepare transparent pellets. These pellets were utilized to determine functional groups (Rai *et al.* 2021). An energy-dispersive spectroscopy (EDS) study was conducted to examine the elemental mapping on the surface of both bacterial biomasses of each strain, one with Cr(VI) and one without Cr(VI), using a TESCAN-VEGA 3 LMU instrument, which was operated at an accelerating voltage of 25 kV. The Cr(VI)-loaded bacterial biomass was prepared as follows: LB medium containing 50 mg/L Cr(VI) was prepared by diluting the stock solution of Cr(VI) with the necessary amount of LB medium. The bacterial isolates were then inoculated into such a solution and incubated in a BOD incubator (Modern Instruments, Kolkata, India) at 35 $\pm$ 2 °C for 24 h. Finally, the biomass was harvested by centrifugation (Eltek TC 8100F centrifuge) at 5,000 rpm for 15 min. The harvested biomass was subsequently washed three times with distilled water. The resulting pellet was dried in a Universal Hot Air Oven (Kolkata, India) at 50 $\pm$ 1 °C for 12 h and was used for characterization later. To get the native bacterial biomass without Cr(VI), each bacterial strain was incubated separately in the LB medium (Himedia, India) at 35 $\pm$ 2 °C for 24 h.

Determination of minimum inhibitory concentration

Isolated bacterial strains were grown in Cr(VI)-laden LB agar plates to check the minimum inhibitory concentration (MIC) of the metal. To prepare LB agar plates, initially agar (Himedia, India) was added to simulated the LB medium containing a definite amount of Cr(VI) solution. The mixture was then poured into a number of Petri dishes, and the standard protocol was followed to prepare agar plates (Marzan *et al.* 2017). Cr(VI) concentrations in the LB agar plates were increased gradually from 50 to 200 mg/L, and bacterial isolates were streaked on the plates. For each transfer, the culture from the lower concentration was streaked onto an agar plate containing a higher concentration of the metal. In cases where the isolates did not exhibit growth on the Petri plate, the MIC was determined following the standard protocol (Al-Ansari *et al.* 2021). The experiments were conducted individually with each of the three isolated Cr(VI)-resistant bacterial strains.

Removal of Cr(VI) from wastewater using isolated strains

Three isolated bacterial strains were grown individually in a 25-mL Cr(VI)-laden LB medium having an initial concentration (IC) of 50 mg/L to test whether the bacteria could remove the metal from the medium. A loop full of inoculum of each strain was added separately at a pH value of 7 and kept in a BOD incubator at 35 $\pm$ 2 °C for 24 h. After the incubation period, the sample was collected and subjected to centrifugation at 5,000 rpm for 15 min. The resulting supernatant was then collected

and analyzed for Cr(VI) concentration. The concentration of Cr(VI) was determined spectrophotometrically using 1,5-diphenyl carbazide at 540 nm using a UV–VIS spectrophotometer (Thermo Fisher Scientific, Genesys 20, India) (Sen *et al.* 2018).

Effect of microenvironmental factors on the removal of Cr(VI) using isolated bacterial strains

The microenvironmental factors, such as the concentration of inoculum, ICs of Cr(VI), and pH, affect the removal efficiency of Cr(VI) and the growth of microorganisms (Rai *et al.* 2020). Therefore, the effects of such factors for the removal of Cr(VI) using the test strains (*S1*, *S2*, and *S3*) were analyzed using the OFAT method to maximize the removal of Cr(VI). For the present study, pH (5–9), the IC of Cr(VI) (20–100 mg/L) and the concentration of inoculum (1–4%) of *S1*, *S2*, and *S3* were varied separately. The IC of Cr(VI) of simulated wastewater was varied first, keeping both pH of the medium and the concentration of inoculum constant at 7 and 5% (Bailey & Ollis 1986), respectively, for each of the three strains separately. The concentration of inoculum was varied next, keeping the initial pH at 7 and an IC of 60 mg/L for *S1* and *S3* and 80 mg/L for *S2* where maximum removal was obtained. Finally, the pH of the solution was varied, with an IC of 60 mg/L Cr(VI) and the concentration of inoculum 3% for *S1* and *S3* and 80 mg/L and 2% for *S2*. The temperature of incubation was kept constant at 35 $\pm$ 2 °C, and isolates were incubated for 24 h. Following incubation, the samples were centrifuged at 5,000 rpm for 15 min. The resulting supernatant was then analyzed to determine the residual Cr(VI) concentration. The cell mass at the bottom of the centrifuge tube was rinsed three times using distilled water. Subsequently, the rinsed cell mass was dried in a hot air oven at 50 °C for 12 h, and its dry weight was measured. The most preferred operating conditions were chosen based on the highest Cr(VI) removal and biomass growth after the OFAT studies (Seragadam *et al.* 2021).

Kinetic study for the removal of Cr(VI) using metal-resistant strains

The isolated bacterial strains were inoculated individually into a 25-mL Cr(VI)-laden LB medium (Cr(VI) solution diluted with a definite amount of LB medium) taken in several 100 mL Erlenmeyer flasks. The flasks were then incubated for 24 h at 35 $\pm$ 2 °C in an incubator. Samples were withdrawn after every 2 h to analyze the residual concentration of Cr(VI) and the dry cell biomass. The kinetics of Cr(VI) removal were investigated at different ICs ranging from 20 to 60 mg/L. As analyzed during the parametric study, the pH and inoculum concentration were maintained at their optimal values.

Bacteria play a vital role in the removal of pollutants during secondary treatment of wastewater. They consume pollutants as substrates, and, hence, pollutant concentrations decrease during the growth of bacterial cells. During the log phase, the kinetics of bacterial growth can be expressed by the following equation:

$$\frac{dC_d}{dt} = \mu C_d \tag{1}$$

where μ is the specific growth rate of bacteria (h^{-1}), C_d is the concentration of biomass (g/L), and t is the time of incubation (h).

Several kinetic growth models are present in the literature to describe the specific growth rates of bacteria. These models can be categorized into structured and unstructured kinetic growth models. Structured kinetic growth models consider genetic, morphological, or biochemical characteristics that collectively determine the physiological state of the biomass. While these models can accurately describe the specific growth rate of bacteria, they are complex and often require the solution of multiple equations. On the other hand, unstructured growth models treat bacteria as independent organisms that interact with their environment. Unstructured kinetic growth models describe the growth rates of bacteria based on the concentration of the biomass and the substrate (Muloiwa *et al.* 2020). The relationship between the specific growth rate (μ) and the substrate concentration (C_A) is a valuable tool in predictive biotechnology. The Monod equation is extensively employed to describe the growth-linked utilization rate of substrates. The unrealistic nature of Monod's model arises from the assumption of exponential growth, which would ultimately result in either a population explosion of the microorganism or its extinction. The logistic model of biomass growth incorporates this factor and provides a more accurate representation. This model says that every process has its carrying capacity, which is the maximum sustainable bacteria in that environmental condition (Gwala *et al.* 2021). The logistic model of bacterial biomass growth can be expressed as follows (Equation (2)):

$$\frac{dC_d}{dt} = \mu_m C_d \left(1 - \frac{C_d}{C_{dm}}\right) \tag{2}$$

where μ_m is the maximum specific growth rate (h^{-1}), C_d is the concentration of biomass (g/L), C_{dm} is the carrying capacity (g/L), and t is the time of incubation (h). Integration of the preceding differential equation between $t = 0$ and $t = t$ gives:

$$C_d = \frac{C_{d0}e^{\mu_m t}}{1 - \left(\frac{C_{d0}}{C_{dm}}\right)(1 - e^{\mu_m t})}. \quad (3)$$

RESULTS AND DISCUSSION

Screening and isolation of Cr(VI)-resistant bacteria

After the initial screening of the collected effluent samples, three isolates were identified, namely, *S1*, *S2*, and *S3* (Figure 1). Upon visual examination, it was observed that all three isolated bacteria exhibited growth in the agar medium supplemented with Cr(VI) at a concentration of 50 mg/L. This growth was observed after incubating the samples at 35 ± 2 °C for 24 h. Notably, these isolates demonstrated the ability to grow in the Cr(VI)-laden environment.

Morphological, biochemical, and molecular characterization of the bacterial isolates

The morphological and biochemical characteristics of the three potential metal-resistant isolates (*S1*, *S2*, and *S3*) are shown in Table 1.

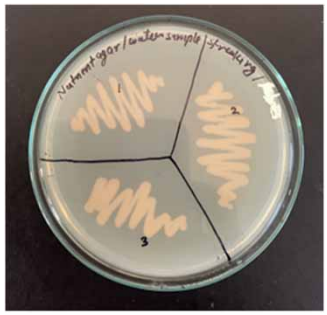


Figure 1 | Pure cultures of three (*S1*, *S2,* and *S3*) bacterial isolates.

Table 1 | Morphological and biochemical characteristics and results of carbohydrate utilization tests

Bacterial strains		*S1*	*S2*	*S3*
Morphological characteristics	Colony shape	Circular diffused	Circular	Flat
	Colony size	Medium and small	Small	Large
	Colony opacity	Translucent	Thick	Opaque
	Colony texture	Glossy	Creamy	Glossy
	Colony color	White	Off white	Greenish
	Colony surface morphology	Smooth	Smooth	Smooth
	Gram stain test	+ve	−ve	−ve
Biochemical test results	Indole test	−ve	−ve	−ve
	Catalase test	+ve	−ve	−ve
	Methyl red test	−ve	−ve	−ve
	Citrate rest	−ve	−ve	+ve
	Oxidase rest	+ve	−ve	−ve
Utilization of carbohydrate	Glucose	−ve	−ve	+ve
	Lactose	−ve	−ve	−ve
	Sucrose	+ve	+ve	−ve
	Maltose	−ve	+ve	−ve
	Xylose	−ve	−ve	−ve

Through 16S rRNA gene sequencing, the isolated bacterial samples were identified as *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3. The sequences of these bacterial strains were submitted to GenBank and accession numbers were assigned as LC763413, LC763414, and LC763415, respectively. The phylogenetic tree construction for the isolated strains was performed using MEGA-11, and the resulting trees are shown in Figure 2.

FTIR spectroscopy and EDS analyses of bacterial biomass

FTIR spectra of the dry biomass of *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa NITSP3* grown in the LB medium, with and without Cr(VI), are shown in Figure 3(a)–3(c), respectively. Prior to treatment with Cr(VI), distinct peaks at 414 and 430 cm^{-1} were observed for *Bacillus subtilis* NITSP1, while for *Rhizobium pusense*

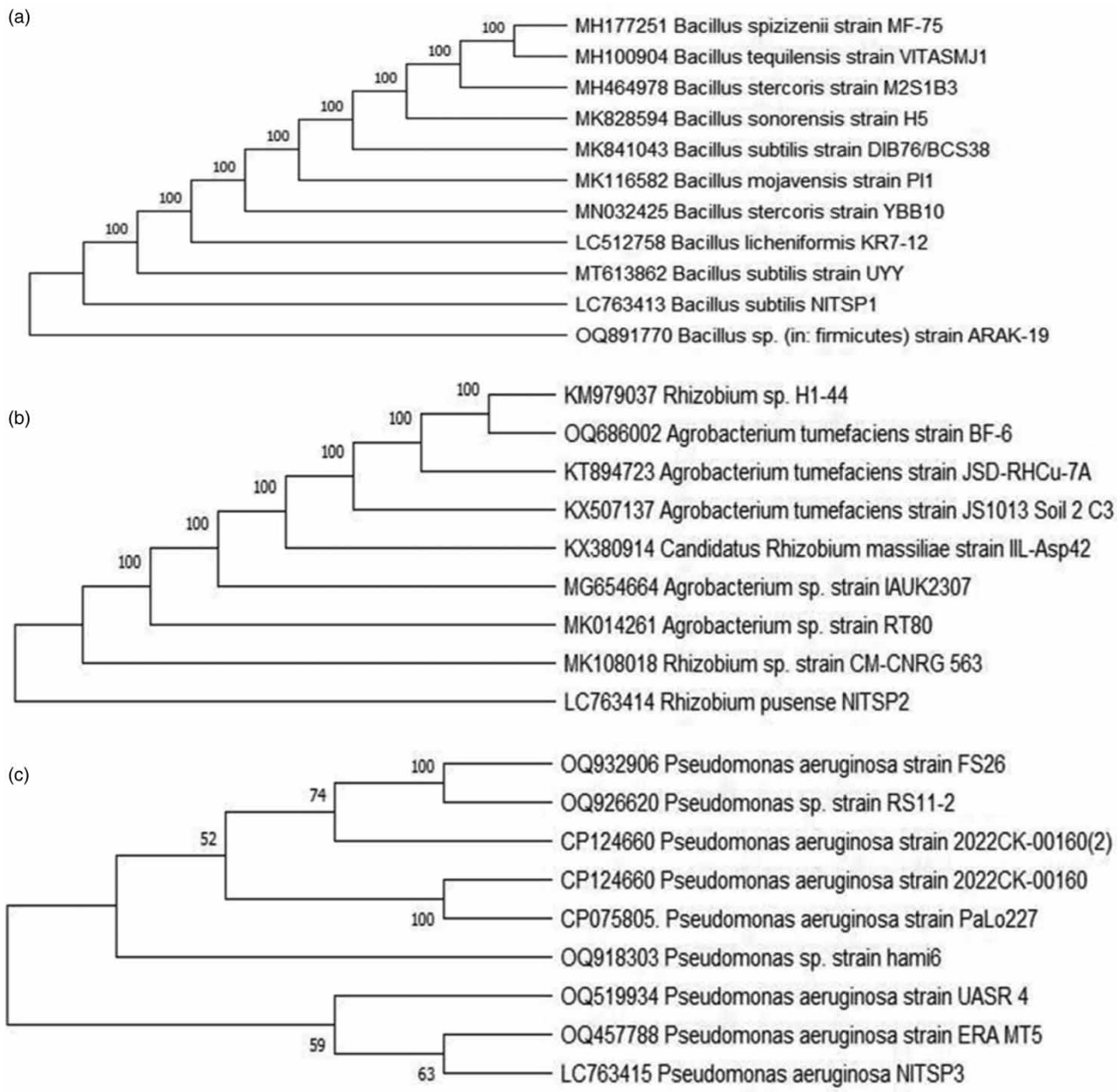


Figure 2 | Phylogenetic trees for identified bacteria: (a) *Bacillus subtilis* NITSP1, (b) *Rhizobium pusense* NITSP2, and (c) *Pseudomonas aeruginosa* NITSP3.

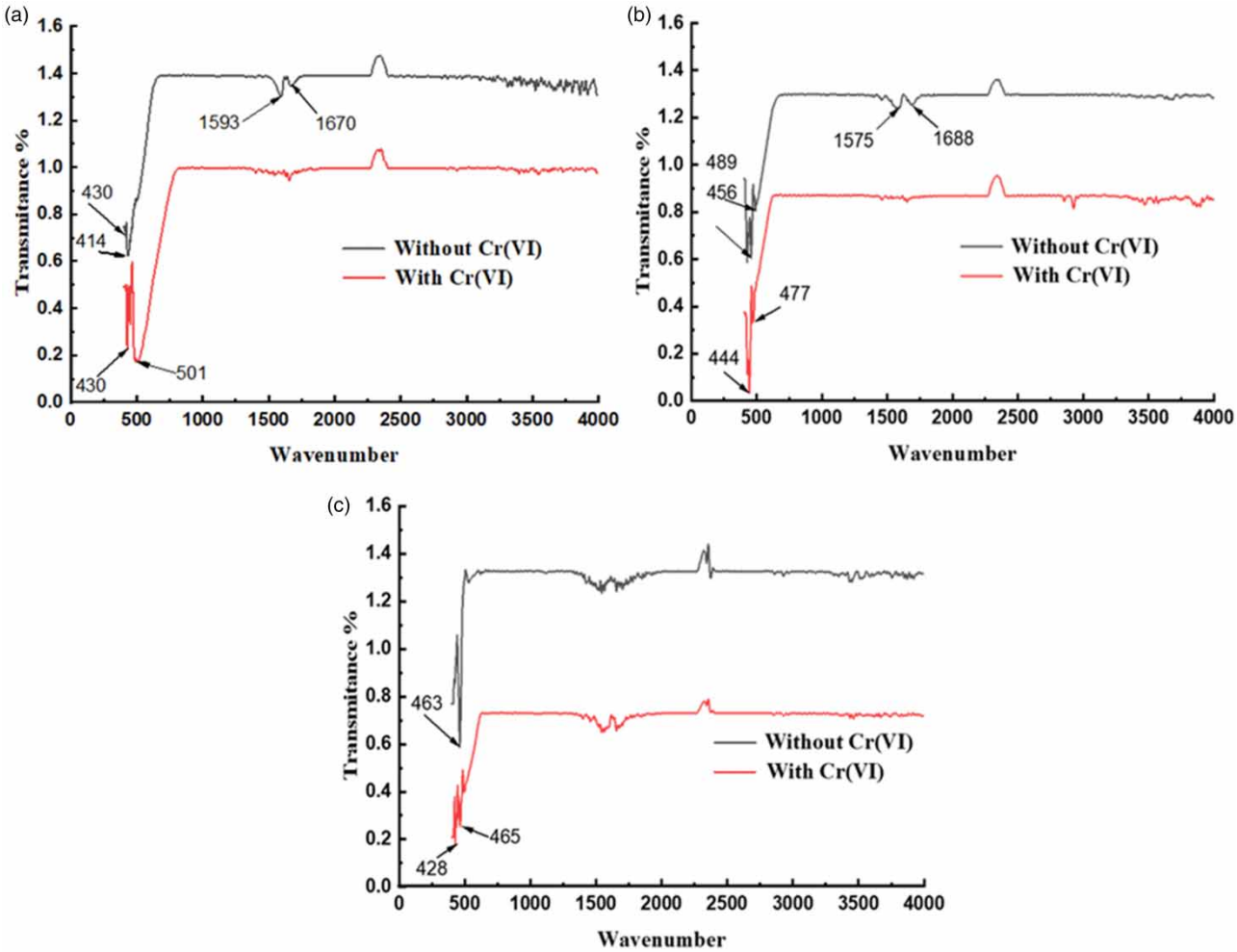


Figure 3 | FTIR spectra of bacterial biomass before treatment and after treatment with Cr(VI) ions: (a) *Bacillus subtilis* NITSP1, (b) *Rhizobium pusense* NITSP2, and (c) *Pseudomonas aeruginosa* NITSP3.

NITSP2, the peaks were observed at 456 and 489 cm^{-1}. In the case of *Pseudomonas aeruginosa* NITSP3, the peak was observed at 463 cm^{-1}. After treatment with the Cr(VI)-laden LB medium, the sharp peaks of *Bacillus subtilis* NITSP1 shifted to 430 and 501 cm^{-1}. Similarly, the peaks of *Rhizobium pusense* NITSP2 were observed at 444 and 477 cm^{-1}, and the peaks of *Pseudomonas aeruginosa* NITSP3 were observed at 428 and 465 cm^{-1} after treatment with the Cr(VI)-laden LB medium. The weak bands observed between 3,000 and 4,000 cm^{-1} in both the Cr(VI)-treated and untreated samples of the three bacterial strains are attributed to O–H stretching vibrations. *Bacillus subtilis* NITSP1 without Cr(VI) exerted peaks at 1,593 and 1,670 cm^{-1}, corresponding to N–H and C = C bonds, respectively. Similarly, the biomass of native *Rhizobium pusense* NITSP2 showed peaks at 1,575 and 1,688 cm^{-1}, corresponding to N–H and C = C bonds, respectively (Nandiyanto *et al.* 2019). However, FTIR spectra of Cr(VI)-treated biomass of *Bacillus subtilis* NITSP1 and *Rhizobium pusense* NITSP2 showed no N–H and C = C bonds. The results are in agreement with the EDS reports in which the presence of Cr(VI) was observed in the biomass of *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3 after treatment with Cr(VI).

Table 2 presents the elemental composition of the biomass of three bacterial isolates, such as *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3, grown in the LB medium (with and without Cr(VI)). The weight percent of chromium after treatment with Cr(VI) laden LB medium was 0.27% for *Bacillus subtilis* NITSP1, 0.17% for *Rhizobium pusense* NITSP2, and 0.01% for *Pseudomonas aeruginosa* NITSP3, whereas for treatment with LB medium only, the concentration of Cr(VI) was found as 'zero'. Comparing the elemental composition data, it is evident that

Table 2 | The elemental composition of the bacterial biomass grown in the LB medium (with and without Cr(VI))

Weight %	*Bacillus subtilis* NITSP1 without Cr(VI)	*Bacillus subtilis* NITSP1 with Cr(VI)	*Rhizobium pusense* NITSP2 without Cr(VI)	*Rhizobium pusense* NITSP2 with Cr(VI)	*Pseudomonas aeruginosa* NITSP3 without Cr(VI)	*Pseudomonas aeruginosa* NITSP3 with Cr(VI)
Carbon	54.03	47.5	63.81	54.99	55.43	56.25
Nitrogen	6.68	9.11	0	10.65	8.75	7.20
Oxygen	24.56	29.92	26.17	25.18	26.85	27.77
Sodium	5.18	4.59	3.61	3.39	2.8	3.37
Phosphorous	1.72	3.50	1.33	1.95	1.76	1.80
Potassium	0.47	1.87	0.19	1.22	0.14	0.40
Sulphur	0.09	0.34	0.17	0.53	0.25	0.31
Magnesium	0	0	0.06	0	0	0
Silicon	0	0	0.16	0	0	0
Chlorine	7.25	2.92	4.50	1.92	4.01	2.88
Chromium	0	0.27	0	0.17	0	0.01

Bacillus subtilis NITSP1 displayed the highest uptake capacity of chromium. Similar results were obtained by Elahi *et al.* (2022) in their studies involving the *Bacillus cereus* strain b-525k. Gram-positive bacteria, such as *Bacillus subtilis* and *Bacillus cereus*, lack an outer membrane, and cell walls are characterized by multiple layers of peptidoglycan. This peptidoglycan-rich cell wall is the primary interaction site with metal ions. It plays a crucial role in determining the ability of bacteria to bind metal ions.

Determination of MIC and the removal of Cr(VI) from Cr(VI)-laden LB medium using isolated strains

The studies on MIC were done to examine the maximum Cr(VI) concentration up to which such strains can resist the toxic effect of the metal and can grow. The three bacterial isolates, namely, *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3, were found to have resistance to the toxic effect of Cr(VI). The growth of *Bacillus subtilis* NITSP1 and *Rhizobium pusense* NITSP2 was observed up to 200 mg/L of Cr(VI) at 35 ± 2 °C and a neutral pH 24 h incubation period, while the growth of *Pseudomonas aeruginosa* NITSP3 was only observed up to 100 mg/L. Metal-tolerant organisms use various mechanisms to survive in a toxic environment, such as cations transporter and iron carriers (Al-Ansari *et al.* 2021). While studying the removal of Cr(VI) using isolated three strains at the IC of 50 mg/L, *Bacillus subtilis* NITSP1 and *Rhizobium pusense* NITSP2 showed 100% removal, but *Pseudomonas aeruginosa* NITSP3 only achieved 31.62% removal (Figure 4).

Effect of microenvironmental factors on the removal of Cr(VI) with isolated bacterial strains

Effect of initial Cr(VI) concentration

The effect of the IC of Cr(VI) on its removal efficiency and dry biomass production is essential to assess the potential of isolated strains to be used to treat Cr(VI)-laden wastewater. Studies were carried out at 35 ± 2 °C, pH 7, with an inoculum concentration of 5%. The results are shown in Figure 5. For *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3, the removal of Cr(VI) decreased from 99.71 ± 4.98 to 44.315 ± 2.21%, from 95.81 ± 4.79 to 55.33 ± 2.76%, and from 88 ± 4.4 to 30.41 ± 1.52%, respectively, with an increase in initial Cr(VI) concentration from 20 to 100 mg/L. Based on Figure 5, the highest removal was achieved at an IC of 20 mg/L for all three bacterial strains (*Bacillus subtilis* NITSP1, *Pseudomonas aeruginosa* NITSP3, and *Rhizobium pusense* NITSP2). When the IC was increased to 60 mg/L for *Bacillus subtilis* NITSP1 and *Pseudomonas aeruginosa* NITSP3 and to 80 mg/L for *Rhizobium pusense* NITSP2, there was a slight reduction in the percentage of Cr(VI) removal. However, when the IC exceeded these threshold levels, a notable decrease in Cr(VI) removal was observed. As a result, the optimal ICs of Cr(VI) were selected for the respective strains as 60 mg/L for *Bacillus subtilis* NITSP1, 80 mg/L for *Rhizobium pusense* NITSP2, and 60 mg/L for *Pseudomonas aeruginosa* NITSP3. Seragadam *et al.* (2021) observed that *Bacillus* sp. exhibited a similar trend at 40 mg/L IC of Cr(VI), achieving a removal efficiency of 95% within 24 h.

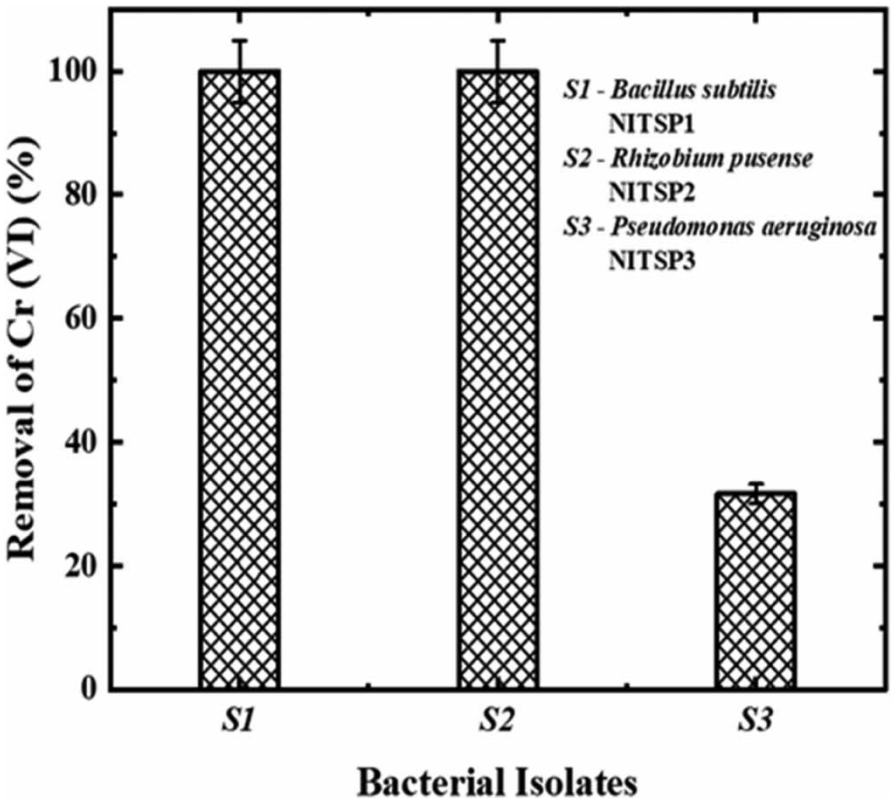


Figure 4 | Removal of Cr(VI) using isolated strains: *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3 (temperature = 35 ± 2 °C and pH = 7).

Effect of inoculum concentration

The variation in the removal of Cr(VI) and dry biomass concentration (DBC) using three bacterial isolates at different inoculum concentrations is shown in Figure 6. Other variables, such as pH and IC of Cr(VI), were kept at 7 and 60 mg/L for *Bacillus subtilis* NITSP1 and *Pseudomonas aeruginosa* NITSP3 and 7 and 80 mg/L for *Rhizobium pusense* NITSP2, respectively. For *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3, the removal of Cr(VI) increased from 50.307 ± 2.515 to 76.615 ± 3.830%, from 50.909 ± 2.545 to 76.907 ± 3.845%, and from 38.88 ± 1.944 to 56.52 ± 2.82%, respectively, with an increase in inoculum concentration from 1 to 4%. As the inoculum concentration increases, the number of cells increases, resulting in enhanced cell growth and ultimately leading to the higher removal of Cr(VI). A higher inoculum concentration indicates a greater cell density, resulting in enhanced pollutant removal and increased biomass production (Rai *et al.* 2020). *Bacillus subtilis* NITSP1 exhibited DBC values of 3.76 ± 0.188 g/L and Cr(VI) removal of 72.77 ± 3.64% at 2% inoculum concentration, which are quite similar to the results obtained at 3% inoculum concentration. Similarly, *Pseudomonas aeruginosa* NITSP3 demonstrated DBC values of 3.79 ± 0.189 g/L and Cr(VI) removal of 51.77 ± 2.59% at 2% inoculum concentration, which are comparable to those obtained at 3% inoculum concentration. In case of *Rhizobium pusense* NITSP2, DBC values of 3.11 ± 0.155 g/L and Cr(VI) removal of 73.22 ± 3.66% were observed at 3% inoculum concentration, which closely resemble the results obtained at 4% inoculum concentration. Hence, from the economic point of view, 2% inoculum concentration was selected judiciously *for Bacillus subtilis* NITSP1 and *Pseudomonas aeruginosa* NITSP3. However, for *Rhizobium pusense* NITSP2, an inoculum concentration of 3% was chosen as a suitable one.

Effect of pH

The removal of Cr(VI) was observed as 39.66 ± 1.983, 80.194 ± 4.00, 51.186 ± 2.55, and 54.57 ± 2.72% at initial pH values of 5, 7, 8, and 9, respectively, with *Bacillus subtilis* NITSP1 in 24 h (Figure 7(a)). Figure 7(b) shows that *Rhizobium pusense*

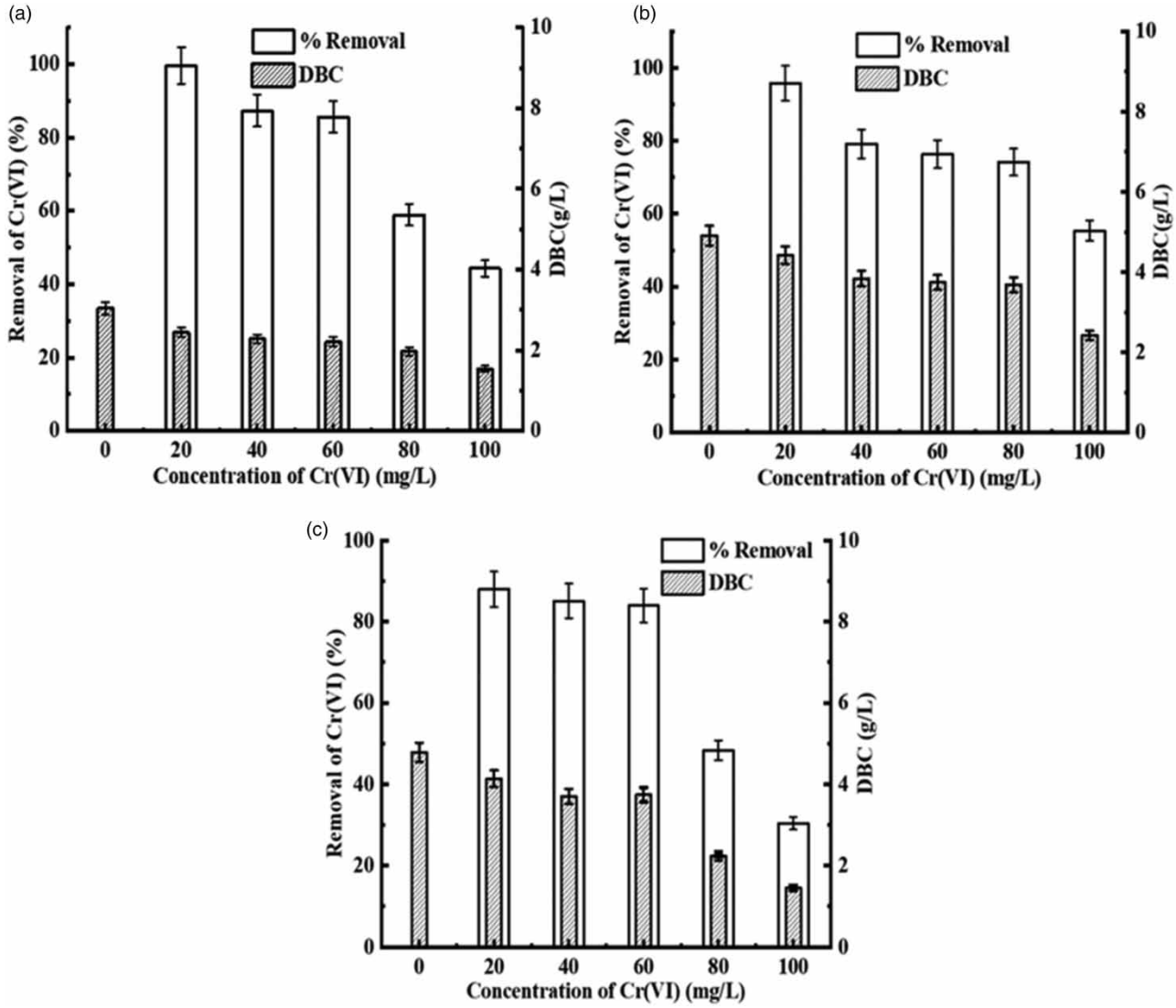


Figure 5 | Removal of Cr(VI) and DBC using (a) *Bacillus subtilis* NITSP1, (b) *Rhizobium pusense* NITSP2, and (c) *Pseudomonas aeruginosa* NITSP3 during OFAT analysis (temperature = 35 ± 2 °C, pH = 7, and inoculum concentration = 5%).

NITSP2 could remove Cr(VI) by 24.87 ± 1.24, 65.53 ± 3.28, 68.11 ± 3.40, and 67.21 ± 3.36% at initial pH values of 5, 7, 8, and 9, respectively, in 24 h. Similarly, Figure 7(c) demonstrates that *Pseudomonas aeruginosa* NITSP3 could remediate Cr(VI) by 30.44 ± 1.53, 62.12 ± 3.1, 61.89 ± 3.095, and 59.84 ± 2.99% at initial pH values of 5, 7, 8, and 9, respectively, in 24 h. Hence, the neutral pH was found to be most favorable for the removal of Cr(VI) with *Bacillus subtilis* NITSP1 and *Pseudomonas aeruginosa* NITSP3. For *Rhizobium pusense* NITSP2, a pH value of 8 was found to be the most suitable one. Since the pH value of industrial wastewater lies in the range of 6.8–7.8, these isolated strains could safely be used for industrial applications. Similar results were observed by other scientists (Sun *et al.* 2020). Karthik *et al.* (2017) and Seragadam *et al.* (2021) also found the pH value of 7 as the most suitable one for the maximum removal of Cr(VI). The Cr(VI) removal rates are different for different bacterial strains. This may be due to their distinct properties owing to their different genus. In a series of experiments conducted during OFAT analysis, the effects of various initial Cr(VI) concentrations were examined on the three strains. *Bacillus subtilis* NITSP1 and *Pseudomonas aeruginosa* NITSP3 exhibited optimal removal at an initial Cr(VI) concentration of 60 mg/L. In contrast, *Rhizobium pusense* NITSP2 displayed the highest removal efficiency at an initial Cr(VI) concentration of 80 mg/L. Utilizing the OFAT approach, the optimal concentrations for each strain were established, and a comparative analysis of these bacterial strains was performed.

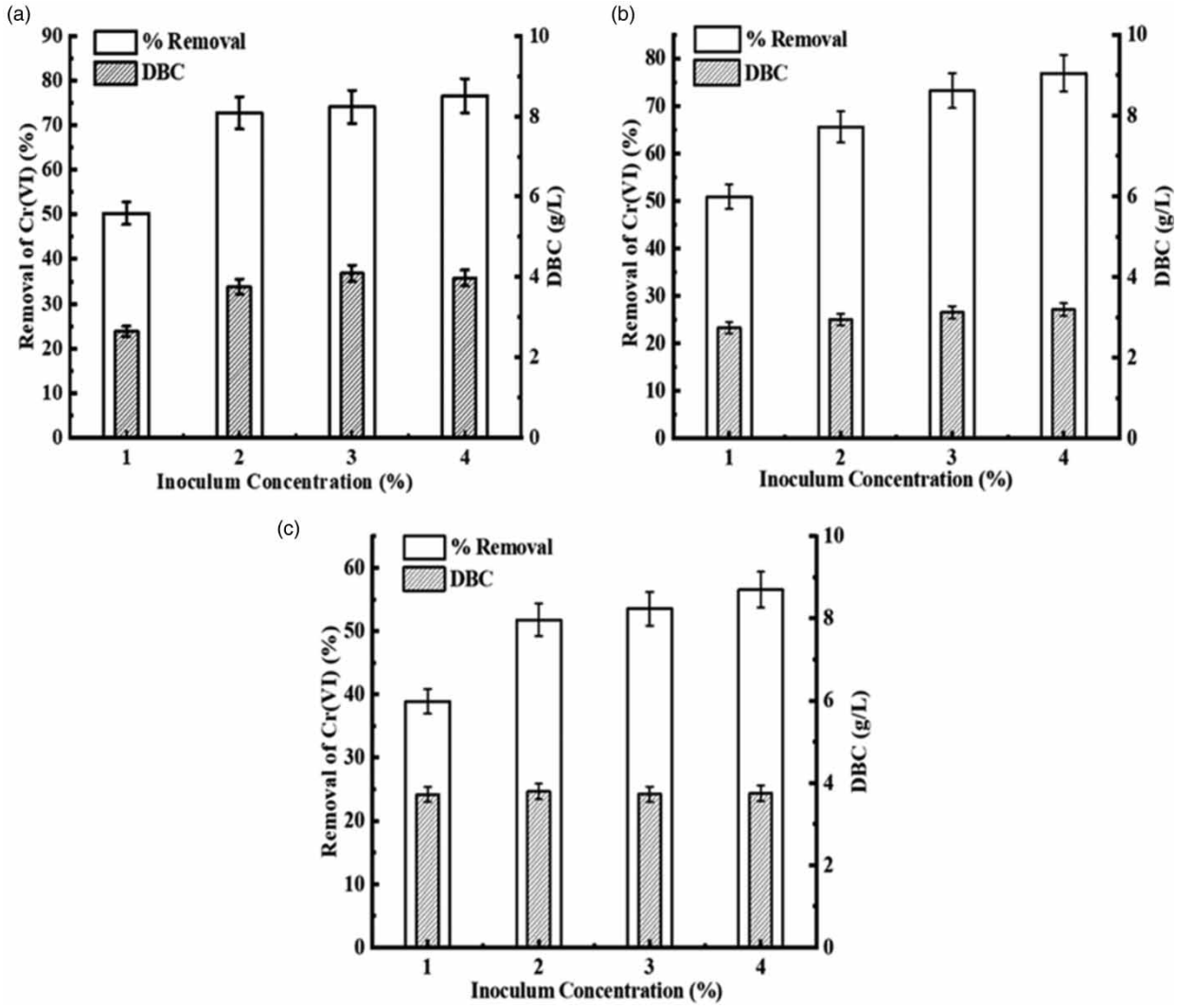


Figure 6 | Removal of Cr(VI) using (a) *Bacillus subtilis* NITSP1, (b) *Rhizobium pusense* NITSP2, and (c) *Pseudomonas aeruginosa* NITSP3 during OFAT analysis at different inoculum concentrations (temperature = 35 ± 2 °C and pH = 7).

Table 3 shows that *Bacillus subtilis* NITSP1 is most efficient in removing Cr(VI) from the synthetic wastewater at specific conditions. It is well known that Gram-positive bacteria are more efficient in removing hexavalent chromium (Banerjee *et al.* 2019). Hence, *Bacillus subtilis* NITSP1 showed the same. A key factor contributing to this is the composition of the peptidoglycan layer in Gram-positive bacteria. Such bacteria possess multiple layers of peptidoglycan enriched with unique teichoic acids, amino acids like alanine and glutamate, and meso-diaminopimelic acid. This layer consists of enzymes, glycoproteins, lipopolysaccharides, and phospholipids. These molecules act as ligands and offer active sites for metal binding. Teichoic acid and other acidic groups in the cell wall are sources of carboxyl groups that play a crucial role in metal uptake (Nanda *et al.* 2019). The EDS study also proved the highest capability in removing Cr(VI) using *Bacillus subtilis* NITSP1 (Table 1).

Kinetic study for the removal of Cr(VI) using metal-resistant strains

Figure 8 illustrates the time-dependent variation of DBC during the removal of Cr(VI) using three indigenous bacterial strains at various Cr(VI) concentrations, ranging from 20 to 60 mg/L. The maximum specific growth rates (μ_m) for *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3 were found to be 0.8439, 0.406, and 0.499 h^{-1}, respectively. The highest value of μ_m for *Bacillus subtilis* NITSP1 reconfirmed its superiority in removing Cr(VI)

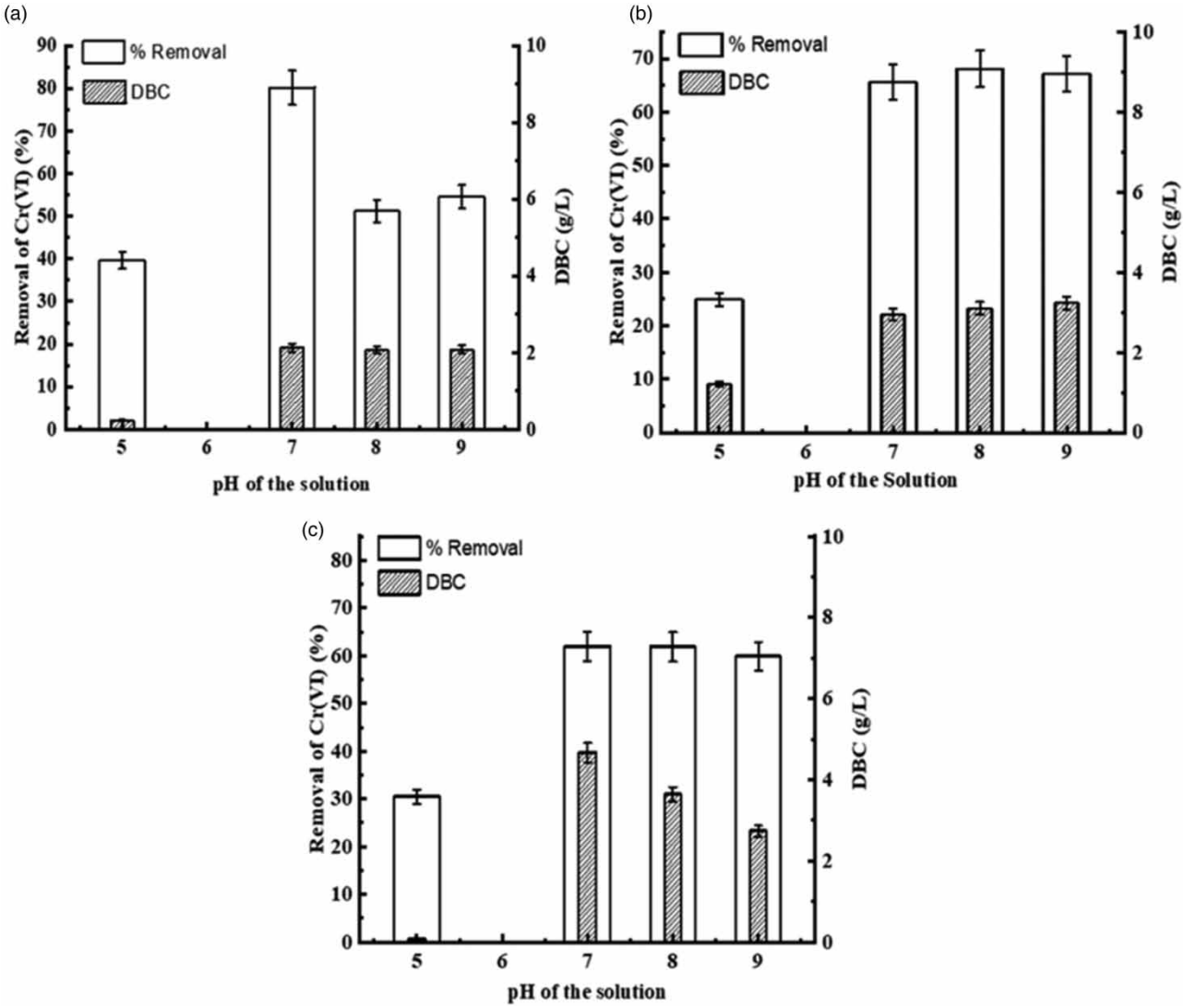


Figure 7 | Removal of Cr(VI) using (a) *Bacillus subtilis* NITSP1, (b) *Rhizobium pusense* NITSP2, and (c) *Pseudomonas aeruginosa* NITSP3 during OFAT analysis at different pH values of the solution (temperature = 35 ± 2 °C).

Table 3 | Most suitable condition for maximum removal of Cr(VI) for three bacterial isolates

Bacteria	Initial Cr(VI) concentration (mg/L)	Concentration of inoculum (%)	pH of the solution	Maximum removal of Cr(VI) (%)
Bacillus subtilis NITSP1	60	2	7	80.194 ± 4.0
Rhizobium pusense NITSP2	80	3	8	68.11 ± 3.40
Pseudomonas aeruginosa NITSP3	60	2	7	62.12 ± 3.1

from simulated wastewater. For calculating the model parameters (μ_m; X_m), the least absolute residuals curve-fitting method available in MATLAB version R2023a was used, and the average absolute relative deviation (AARD) between experimental (N is the number of data points) and predicted data was minimized (Gwala *et al.* 2021). AARD is mathematically represented as follows:

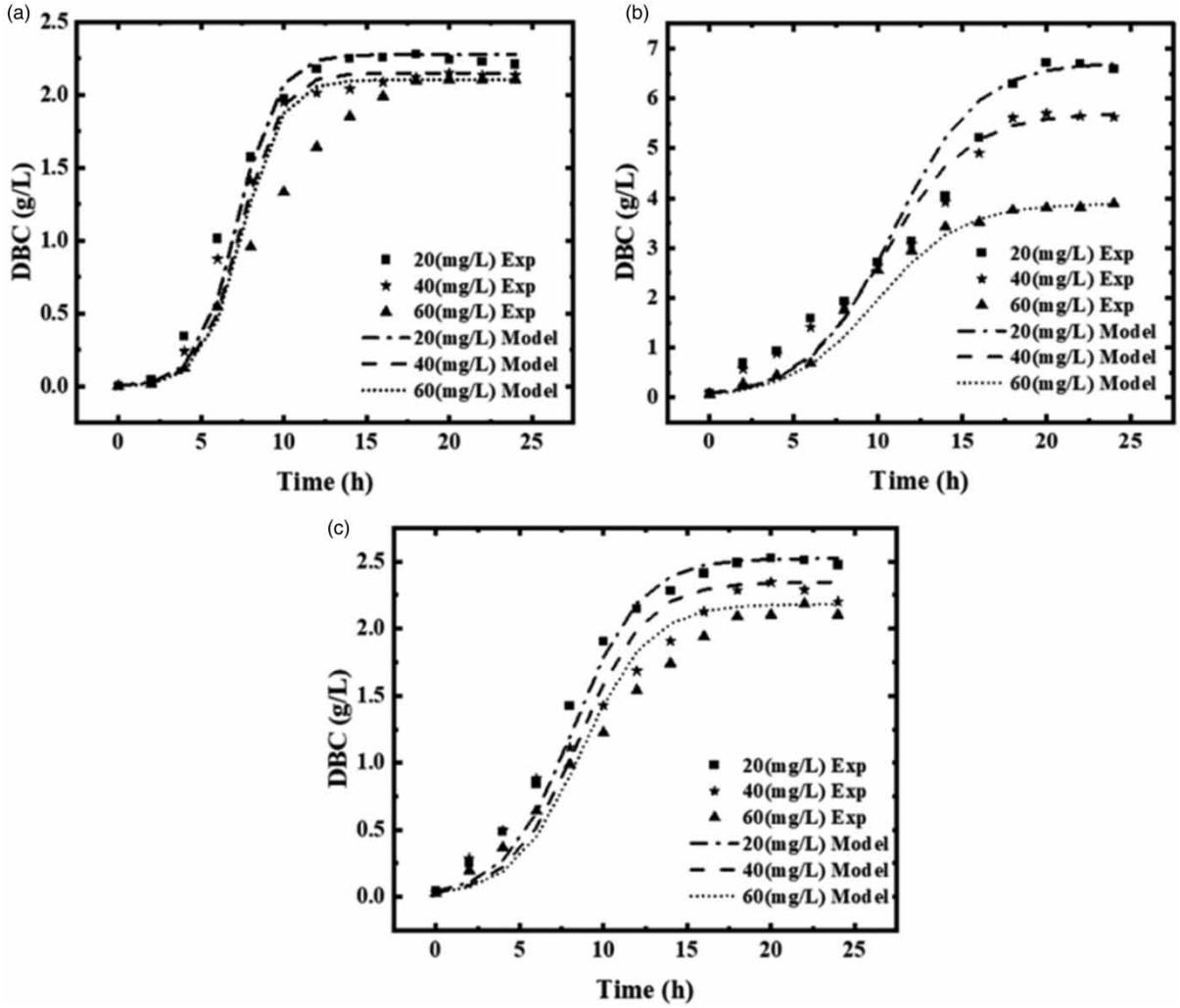


Figure 8 | Comparison between experimental and predicted DBC at different ICs of Cr(VI) using (a) *Bacillus subtilis* NITSP1, (b) *Rhizobium pusense* NITSP2, and (c) *Pseudomonas aeruginosa* NITSP3 (temperature = 35 ± 2 °C and pH = 7).

$$\text{AARD} = \frac{1}{N}\sum_{n=1}^{n=N}\left|\frac{(\text{Experimental data}) - (\text{Predicted data})}{\text{Experimental data}}\right| \times 100\%. \quad (4)$$

The predicted DBC calculated using the logistic model was superimposed on the corresponding plots of the variation of experimental data with time. The values of kinetic parameters μ_m , X_m, and R^2 and root mean square error (RMSE) are shown in Table 4.

Table 4 | Kinetic parameters, R^2, RMSE, and AARD of the logistic model

Bacteria	*Bacillus subtilis* NITSP1			*Rhizobium pusense* NITSP2			*Pseudomonas aeruginosa* NITSP3		
Concentration (mg/L)	20	40	60	20	40	60	20	40	60
μ_m (h^{-1})	0.8439			0.406			0.499		
X_m (g/L)	2.27	2.149	2.104	6.87	5.697	3.887	2.524	2.347	2.184
R^2	0.9836	0.987	0.9685	0.9622	0.9693	0.9667	0.9648	0.9401	0.9632
RMSE	0.116	0.099	0.153	0.483	0.372	0.254	0.171	0.202	0.153
AARD	7.6	5.89	5.64	1.94	3.96	9.44	7.34	2.165	1.64

With an increase in initial Cr(VI) concentration from 20 to 60 mg/L, the carrying capacity, X_m, decreased from 2.27 to 2.104, 6.87 to 3.887, and 2.524 to 2.184 g/L for *Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3, respectively. For each initial Cr(VI) concentration, the R^2 values between experimental and simulated data of DBC were in the range of 0.94–0.987, whereas the RMSE values were in the range of 0.099–0.483. The high values of R^2 and low values of RMSE proved that the proposed logistic model holds good in predicting the variation of biomass growth with time. Using the logistic model, Sen *et al.* (2018) found the maximum specific growth rate (μ_m) and maximum carrying capacity of 0.31 day^{-1} and 0.857 g/L, respectively, for the removal of Cr(VI) at an IC of 20 mg/L using the cyanobacterial strain *Limnococcus* sp.

CONCLUSION

Three bacterial strains (*Bacillus subtilis* NITSP1, *Rhizobium pusense* NITSP2, and *Pseudomonas aeruginosa* NITSP3) having high resistance to Cr(VI) were isolated from a common industrial effluent treatment plant, a contaminated site in Vishakhapatnam. *Bacillus subtilis* NITSP1 and *Rhizobium pusense* NITSP2 exhibited growth at 200 mg/L Cr(VI) within 24 h at 35 ± 2 °C and neutral pH. The growth of *Pseudomonas aeruginosa* NITSP3 was observed at 100 mg/L Cr(VI) for 24 h at 35 ± 2 °C at neutral pH. Among the three isolates, *Bacillus subtilis* NITSP1 was found to be the most efficient in removing Cr(VI) (80.194 ± 4.0%) with an initial Cr(VI) concentration of 60 mg/L, a pH value of 7.0, an inoculum size of 2% [(v/v)], and an incubation period of 24 h. The present study suggests that the logistic model of population growth could adequately describe the variation of DBC over time, indicating the potential of the current strains to remove Cr(VI) from synthetic wastewater. However, further research is needed to explore the applicability of these findings for the treatment of real industrial wastewater, taking into account all relevant parameters in a continuous reactor setup.

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AUTHORS CONTRIBUTIONS

S.D. and K.C.G. conceptualized the study. S.D. and B.S. carried out methodology. B.S. did formal analysis. P.S. investigated the work. P.S. wrote the original draft preparation. K.C.G., S.D., and B.S. wrote the original draft and reviewed and edited the manuscript. B.S. and S.D. found resources for the study. S.D., K.C.G., and B.S. supervised the work.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Al-Ansari, M. M., Benabdelkamel, H., AlMalki, R. H., Abdel Rahman, A. M., Alnahmi, E., Masood, A., Ilavenil, S. & Choi, K. C. 2021 Effective removal of heavy metals from industrial effluent wastewater by a multi metal and drug resistant *Pseudomonas aeruginosa* strain RA-14 using integrated sequencing batch reactor. *Environmental Research* **199**, 111240.

ATSDR (Agency for Toxic Substances and Disease Registry) 2022 ATSDR's Substance Priority List. (accessed 12 June 2023).

Bailey, J. E. & Ollis, D. F. 1986 *Biochemical Engineering Fundamentals*, 2nd edn. McGraw-Hill, New York, NY, USA.

Banerjee, S., Misra, A., Chaudhury, S. & Dam, B. 2019 A *Bacillus* strain TCL isolated from Jharia coalmine with remarkable stress responses, chromium reduction capability and bioremediation potential. *Journal of Hazardous Materials* **367**, 215–223.

Bergey, D. H. & Holt, J. G. 2000 *Bergey's Manual of Determinative Bacteriology*, 9th edn. Lippincott Williams & Wilkins, Philadelphia, PA, USA.

Diep, P., Mahadevan, R. & Yakunin, A. F. 2018 Heavy metal removal by bioaccumulation using genetically engineered microorganisms. *Frontiers in Bioengineering and Biotechnology* **6**, 1–20.

Elahi, A., Rehman, A., Zajif Hussain, S., Zulfiqar, S. & Shakoori, A. R. 2022 Isolation and characterization of a highly effective bacterium *Bacillus cereus* b-525k for hexavalent chromium detoxification. *Saudi Journal of Biological Sciences* **29** (4), 2878–2885.

Fernández, P. M., Viñarta, S. C., Bernal, A. R., Cruz, E. L. & Figueroa, L. I. C. 2018 Bioremediation strategies for chromium removal: Current research, scale-up approach and future perspectives. *Chemosphere* **208**, 139–148.

Gwala, M., Rai, A., Chaudhuri, R. G., Chakrabarty, J., Mondal, B. K. & Dutta, S. 2021 Treatment of hydrocarbon-rich wastewater to enhance reusability of water using a novel indigenous microalgal-bacterial consortium isolated from Kovalam Beach, India. *Journal of Environmental Engineering* **147** (12), 4021063.

Karthik, C., Ramkumar, V. S., Pugazhendhi, A., Gopalakrishnan, K. & Arulselvi, P. I. 2017 Biosorption and biotransformation of Cr(VI) by novel *Cellulosimicrobium funkei* strain AR6. *Journal of the Taiwan Institute of Chemical Engineers* **70**, 282–290.

Kerur, S. S., Bandekar, S., Hanagadakar, M. S., Nandi, S. S., Ratnamala, G. M. & Hegde, P. G. 2021 Removal of hexavalent chromium-industry treated water and wastewater: A review. *Materials Today: Proceedings* **42**, 1112–1121.

Kholisa, B., Matsena, M. T. & Chirwa, E. M. N. 2022 Biotreatment of hexavalent chromium in a rectangular column using municipal wastewater sludge as a bioreactive barrier. *Chemical Engineering Transactions* **94**, 1405–1410.

Kholisa, B., Matsena, M. T. & Chirwa, E. M. N. 2023 In situ bioreduction of hexavalent chromium-contaminated water using a microbial culture barrier. *Chemical Engineering and Technology* **46** (6), 1307–1311.

Kumaresan Sarankumar, R., Arulprakash, A., Devanesan, S., Selvi, A., AlSalhi, M. S., Rajasekar, A. & Ahamed, A. 2020 Bioreduction of hexavalent chromium by chromium resistant alkalophilic bacteria isolated from tannery effluent. *Journal of King Saud University – Science* **32** (3), 1969–1977.

Marzan, L. W., Hossain, M., Mina, S. A., Akter, Y. & Chowdhury, A. M. M. A. 2017 Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. *The Egyptian Journal of Aquatic Research* **43** (1), 65–74.

Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A., Idris, A. M., Khandaker, M. U., Osman, H., Alhumaydhi, F. A. & Simal-Gandara, J. 2022 Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *Journal of King Saud University – Science* **34** (3), 101865.

Muloiwa, M., Nyende-Byakika, S. & Dinka, M. 2020 Comparison of unstructured kinetic bacterial growth models. *South African Journal of Chemical Engineering* **33**, 141–150.

Nanda, M., Kumar, V. & Sharma, D. K. 2019 Multimetal tolerance mechanisms in bacteria: The resistance strategies acquired by bacteria that can be exploited to 'clean-up' heavy metal contaminants from water. *Aquatic Toxicology* **212**, 1–10.

Nandiyanto, A. B. D., Oktiani, R. & Ragadhita, R. 2019 How to read and interpret FTIR spectroscope of organic material. *Indonesian Journal of Science and Technology* **4** (1), 97.

Nokman, W., Benluvankar, V., Maria Packiam, S. & Vincent, S. 2019 Screening and molecular identification of heavy metal resistant *Pseudomonas putida* S4 in tannery effluent wastewater. *Biocatalysis and Agricultural Biotechnology* **18**, 101052.

Prabhakaran, D. C., Bolaños-Benitez, V., Sivry, Y., Gelabert, A., Riotte, J. & Subramanian, S. 2019 Mechanistic studies on the bioremediation of Cr(VI) using *Sphingopyxis macrogoltabida* SUK2c, a Cr(VI) tolerant bacterial isolate. *Biochemical Engineering Journal* **150**, 107292.

Princy, S., Sathish, S. S., Cibichakravarthy, B. & Prabagaran, S. R. 2020 Hexavalent chromium reduction by *Morganella morganii* (1Ab1) isolated from tannery effluent contaminated sites of Tamil Nadu, India. *Biocatalysis and Agricultural Biotechnology* **23**, 101469.

Rai, A., Chakrabarty, J. & Dutta, S. 2020 Phycoremediation of pollutants from coke-oven wastewater using *Tetraspora* sp. NITD 18 and estimation of macromolecules from spent biomass. *Journal of Water Process Engineering* **39**, 101746–101758.

Rai, A., Gowrishetty, K. K., Singh, S., Chakrabarty, J., Bhattacharya, P. & Dutta, S. 2021 Simultaneous bioremediation of cyanide, phenol, and ammoniacal-N from synthetic coke-oven wastewater using *Bacillus* sp. NITD 19. *Journal of Environmental Engineering* **147** (1), 4020143.

Sahoo, B. & Chaudhuri, S. 2021 Removal of lindane in liquid culture using soil bacteria and toxicity assessment in human skin fibroblast and HCT116 cell lines. *Environmental Technology* **44** (9), 1213–1227.

Sanjay, M. S., Sudarsanam, D., Raj, G. A. & Baskar, K. 2020 Isolation and identification of chromium reducing bacteria from tannery effluent. *Journal of King Saud University – Science* **32** (1), 265–271.

Sen, G., Sen, S., Thakurta, S. G., Chakrabarty, J. & Dutta, S. 2018 Bioremediation of Cr(VI) using live cyanobacteria: Experimentation and kinetic modeling. *Journal of Environmental Engineering* **144** (9), 4018089.

Seragadam, P., Rai, A., Ghanta, K. C., Srinivas, B., Lahiri, S. K. & Dutta, S. 2021 Bioremediation of hexavalent chromium from wastewater using bacteria–A green technology. *Biodegradation* **32** (4), 449–466.

Sharma, P. & Kumar, S. 2021 Bioremediation of heavy metals from industrial effluents by endophytes and their metabolic activity: Recent advances. *Bioresource Technology* **339**, 125589.

Sun, Y., Lan, J., Du, Y., Guo, L., Du, D., Chen, S., Ye, H. & Zhang, T. C. 2020 Chromium(VI) bioreduction and removal by *Enterobacter* sp. SL grown with waste molasses as carbon source: Impact of operational conditions. *Bioresource Technology* **302**, 121974.

Tang, X., Huang, Y., Li, Y., Wang, L., Pei, X., Zhou, D., He, P. & Hughes, S. S. 2021 Study on detoxification and removal mechanisms of hexavalent chromium by microorganisms. *Ecotoxicology and Environmental Safety* **208**, 111699.

Venkatesan, G., Subramani, T., Sathya, U. & Karunanidhi, D. 2021 Evaluation of chromium in vegetables and groundwater aptness for crops from an industrial (leather tanning) sector of South India. *Environmental Geochemistry and Health* **43** (2), 995–1008.

Wani, P. A., Wahid, S., Khan, M. S. A., Rafi, N. & Wahid, N. 2019 Investigation of the role of chromium reductase for Cr (VI) reduction by *Pseudomonas* species isolated from Cr (VI) contaminated effluent. *Biotechnology Research and Innovation* **3** (1), 38–46.

Yaashikaa, P. R., Senthil Kumar, P. & Saravanan, A. 2019 Molecular characterization of chromium resistant gram-negative bacteria isolated from industrial effluent: Bioremedial activity. *Journal of Industrial and Engineering Chemistry* **80**, 640–646.

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