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Research article

Optimizing the process conditions for the biosorption of chromium (VI) by *Bacillus subtilis* in artificial wastewater ☆

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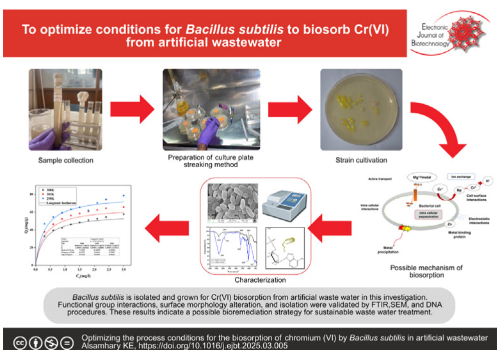
GRAPHICAL ABSTRACT

Optimizing the process conditions for the biosorption of chromium (VI) by *Bacillus subtilis* in artificial wastewater



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ABSTRACT

Background: The contamination by heavy metals, particularly hexavalent chromium Cr (VI), is a pressing environmental concern. Cr(VI) is highly toxic, non-biodegradable and carcinogenic. Traditional remediation methods are often costly, energy-intensive, or generate secondary waste. This study explores the optimal conditions for the bacterium *Bacillus subtilis* in reducing Cr(VI) from synthetic wastewater.
Results: The research employed batch experiments to simulate wastewater treatment. The removal of Cr (VI) was measured spectrophotometrically. The active functional groups were studied using Fourier Transform Infrared Spectroscopy that showed an incremental shift for alkyl halides (500.75 cm^{-1}) and OH-groups (3347 cm^{-1}) were observed. Scanning Electron Microscopy images demonstrated that the surface morphology of the biosorbent was more homogenous before than after adsorption. The biosorbent's structure was confirmed by a prominent peak in X-ray Diffraction at 290.04°. The highest adsorption was observed at the adsorbent dose of 0.5 g/L, the contact time 60 min, pH 6 and temperature of 40°C. The thermodynamic parameters validated the process's feasibility and spontaneity. Several models for biosorption kinetics and isotherm were tested. The pseudo-second-order was more suitable than the pseudo-first-order model. Langmuir isotherm model had the best fit compared to Freundlich, Dubinin–Radushkevich, and Temkin models.
Conclusions: *B. subtilis* appeared to be resistant to chromium and reduce Cr(VI) efficiently. This study shows the potential of *B. subtilis* as a viable bioremediation agent for Cr(VI) contamination in wastewater and should be studied further using real wastewater with different pollutants.

☆ Audio abstract available in Supplementary material.

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1. Introduction

The discharge of heavy metals into the environment, along with industrial advancement and population expansion, is a significant environmental issue in numerous countries [1,2]. These metals are non-biodegradable, and their hazardous effects persist within the bodies of living species. Many strategies have been employed by the researchers over the years for the removal of these pollutants from the water bodies including microbial bioremediation [3]. Studies have explored an environmentally friendly approach to utilizing algae waste by creating an adsorbent from a mixture of algae waste and bentonite to remove Pb^{2+} from aqueous solutions. Furthermore, an environmentally beneficial approach to utilizing bagasse waste by developing an adsorbent from a bagasse-bentonite mixture to remove Cd^{2+} from aqueous solutions has been developed [4]. Researchers have also investigated the adsorption of Cu, Ni, and Zn ions by *Archontophoenix alexandrae* strain FAKSA 10, in single, bi-metal, and tri-metal systems using advanced experimental designs, including factorial design, response surface methodology, and mixture design. Results showed maximum adsorption capacity for Cu, with its presence suppressing Ni and Zn adsorption, and confirmed the potential of *A. alexandrae* FAKSA 10 as an efficient biosorbent for industrial effluent treatment [5].

Chromium is a notable heavy metal contaminant in the environment due to its classification among heavy metals [6]. The most prevalent and substantial sources of chromium are alloy reparation, metal cleaning and processing, leather tanning, timber preservation, ore processing, and petroleum refining operations and its compounds in industry [7]. Chromium in the effluents from these industries predominantly exists as hexavalent chromium, Cr(VI) and trivalent chromium, Cr(III) [8]. However, the hexavalent version is five hundred times more perilous than the trivalent in nature. The International Agency for Research on Cancer (IARC) classifies chromium (VI) as a Group I human carcinogen, while the U.S. Environmental Protection Agency (EPA) classifies it as a Group A inhalation carcinogen [9]. Chromium (VI) is recognized as a prevalent teratogen, mutagen, and carcinogen, and its presence in the environment presents significant challenges and raises substantial public concern, therefore prompting the research and development of innovative and improved materials to address these concerns. Furthermore, the effects of protracted exposure to Cr(VI) on humans may result in significant health ailments, including dermatitis and dysfunction of the liver, kidneys, circulatory, and neurological system problems.

A variety of removal strategies have proved to be crafted and implemented to tackle this issue, such as reverse osmosis, ionic exchange, catalytic reduction, electrodialysis, biological denitrification, and the chemical or biological materials as the adsorbent. Electrodialysis presents difficulties due to the delicate circumstances under which it is conducted [10]. The chemical processes and expenses related to biological denitrification render it impractical for large-scale use. Reverse osmosis is an expensive process because of its substantial energy demands. In addressing the aforementioned issues and managing operations, adsorption emerges as the optimal solution due to its convenience, ease of use, straightforward execution, and cost efficiency, particularly when utilizing low-cost adsorbents with facile regeneration capabilities [11].

Chemical approaches, in particular reduction and precipitation, are frequently employed for the elimination of Cr(VI), utilizing reducing agents such as sodium bisulfite or zero-valent iron to transmute Cr(VI) to less hazardous Cr(III), forms that precipitate as chromium hydroxide. These technologies provide swift removal rates and high efficiency, rendering them appropriate for urgent remediation requirements; however, they may produce toxic by-products necessitating further management, can result in secondary contamination, and may incur substantial costs related to reagents and disposal [12]. Physical techniques, including adsorption using activated carbon or ion exchange, are direct and enable the regeneration and reuse of adsorbents; however, they often entail significant initial material costs, and their effectiveness may diminish over time, necessitating periodic replacement or regeneration [13]. However, there are certain drawbacks in these studies using composites such as laboratory-scale limitations. Many studies on synthetic composites focus on lab-scale experiments under controlled conditions. Scaling up to real-world applications often faces challenges in efficiency, cost, and consistency. Furthermore, some composites especially those involving metal oxides or nanoparticles, risk releasing toxic by-products during use or disposal. Also, composite materials often require multiple steps for functionalization, making the production process lengthy and less economically viable. Many composites include rare metals, complex polymers, or advanced nanomaterials, which can limit widespread adoption.

In addition to chemical approaches, some microorganisms, including *Pseudomonas* sp. and certain fungi, have been studied for their potential in Cr(VI) bioremediation [14]. However, many methods demonstrate slow reaction rates and inconsistent efficiency depending on environmental conditions [15], and therefore, more microorganisms need to be studied.

Our investigation employs the bacterium *Bacillus subtilis*, which is broadly recognized as innocuous and poses minimal peril to human wellness and the environment. Studies on *B. subtilis* demonstrate its feasibility due to its easy production and handling. It is a naturally abundant, non-pathogenic, and environmentally friendly microorganism with high metabolic activity. Furthermore, the biomass of *B. subtilis* is low-cost and readily available. Its intrinsic adaptability enables it to thrive in various situations. Its unique biochemical traits, such as the ability to form biofilms and secrete extracellular polymeric substances, might make it efficient in binding and sequestering Cr(VI) ions. Therefore, *B. subtilis* is worth studying as a Cr(IV) adsorbent. Although *B. subtilis* has been studied for many applications and aspects, it has not been widely used in adsorption studies. The recent review presents many microorganisms, mostly fungi, to adsorb Cr(VI) and two articles using *B. subtilis* [16,17,18]. In addition, some Cr-adsorbing *B. subtilis* strains isolated from different environments have been presented. *B. subtilis* can be presumed to offer several advantages as a biosorbent for the removal of hexavalent chromium, enabling efficient bioreduction of Cr(VI) to the less toxic Cr(III) [19]. Its cell wall structure, rich in functional groups like carboxyl, hydroxyl, and amine groups, can enhance its biosorption capacity. Additionally, *B. subtilis* can thrive under diverse environmental conditions, making it suitable for real-world wastewater treatment scenarios. Compared to chemical adsorbents, it is cost-effective and sustainable, while it often outperforms other microbial biosorbents in terms of adsorption efficiency and regeneration potential [20].

In the techno-economic analysis to compare chemical and biological adsorbents such as bacterial biomass, several aspects must be considered, while the adsorption efficiency is about the same for biomass and synthetic adsorbents the costs of biomass are lower [21]. Sustainability of the biological treatment is high because the production and disposal of biological materials are eco-friendly. Chemical adsorbents are non-biodegradable and persist in the environment. The energy-intensive production of chemical adsorbents is further reducing their sustainability. Environmental impact is low for biomass and high for chemical adsorbents. Using biological materials, the reliance on chemical synthesis and secondary pollution is reduced. Biomass is ideal for low-cost, localized wastewater treatment where biomass production and disposal can be managed efficiently. For large-scale or industrial applications, synthetic adsorbents may be preferable due to their scalability, higher adsorption efficiency, and reusability [22]. In summary, biological treatments are economically and environmentally viable for small-scale or localized applications, especially in developing regions.

Biosorption, an environmentally sustainable and economical technique utilizing microorganisms to eliminate or neutralize environmental contaminants, has lately experienced notable progress through the implementation of genetic engineering, synthetic biology, and advanced microbial consortia [23]. Genetically engineered bacterial strains exhibiting increased tolerance to heavy metals, including chromium, have been created, resulting in improved biosorption through the optimization of metabolic pathways [24]. Moreover, microbial consortia have demonstrated the capacity to enhance pollutant degradation rates via synergistic interactions and improved nutrient cycling. Nonetheless, other hurdles persist, especially in converting laboratory-scale results to practical, real-world applications, where environmental conditions such as temperature, pH, and the inclusion of additional pollutants can influence microbial effectiveness [13]. The prolonged resilience and ecological impacts of these engineered strains in natural ecosystems remain little understood, and the lack of defined protocols for assessing bioremediation effectiveness obstructs cross-study comparisons and the formulation of best practices. Our research addresses these gaps by investigating *B. subtilis* and its capacity to improve chromium biosorption and tolerance, specifically examining the influence of several environmental conditions on its efficacy. This work seeks to connect laboratory research with practical applications, providing insights to enhance bioremediation procedures for chromium-contaminated ecosystems.

The bioremediation of Cr(VI) with *B. subtilis* is an effective and compliant approach for mitigating wastewater contamination in accordance with necessary regulatory standards [25]. This strategy complies with all relevant laws, such as the Toxic Substances Control Act, the Comprehensive Environmental Response, Compensation, and Liability Act, and the Resource Conservation and Recovery Act of the EPA, so ensuring comprehensive assessment and management of any risks. Moreover, adherence to European Union regulations and the Water Framework Directive highlights the method's dedication to upholding superior water quality standards and environmental conservation [26]. This method effectively remediates Cr(VI) while protecting public health and the ecosystem, rendering it a prudent option for sustainable wastewater treatment. In conclusion, addressing regulatory factors is essential for the effective implementation of *B. subtilis* in bioremediation and wastewater treatment. By conforming our proposed procedures to established norms and guidelines, we can guarantee the safety, efficacy, and environmental integrity of our bioremediation tactics. This dedication to regulatory compliance not only bolsters the credibility of our study but also aids in the sustainable control of chromium contamination and the safeguarding of ecosystems. Considering the benefits of *B. subtilis* biomass as a biosorbent of Cr, it has been studied scarcely. Cr was shown to be adsorbed by *B. subtilis* but the optimization of the conditions still needs development [27].

The primary focus of this work was to assess the efficacy of bioremediation in eliminating the heavy metal Cr(VI) from synthetic wastewater using the bacterium *B. subtilis*. The study attempts to optimize bioremediation conditions to enhance pollutant removal efficiency and assess the influence of microbial strains on this process. The study used a batch experimental approach that isolates and exploits effective microbial strains for bioremediation. The microcosm system was utilized to simulate wastewater treatment settings, while pollutants were evaluated utilizing analytical techniques such as spectrophotometry.

The examination of the kinetics and biosorption of chromium by the *B. subtilis* strain ON358108, sourced from polluted waterways, has considerable environmental and public health ramifications. This research underscores a possible eco-friendly method for remediating eutrophicated waterways by assessing the biosorption ability of *B. subtilis*. The kinetic study offers essential information into the pace and efficacy of chromium elimination, facilitating the optimization of remediation procedures. Elevated chromium levels in water present significant hazards to human health, including respiratory complications, ocular and dermal irritation, and even chronic impairment of organ function. Mitigating heavy metal pollution, namely chromium contamination, using bioremediation improves environmental health and reduces the hazards linked to high chromium concentrations, hence promoting the sustainability of water resources and public health safety.

B. subtilis was extracted from wastewater for this research. This study examined the resistance of bacteria to chromium and other compounds, as well as their efficacy in reducing Cr(VI). The specific objectives were (i) facilitating bacterial absorption and degradation of Cr(VI), (ii) elucidating and simulating the underlying mechanisms, and (iii) recording microbial proliferation before and after Cr(VI) elimination.

2. Materials and methods

2.1. Chemicals and reagents

The chemical substances and laboratory chemicals harnessed in this research had been of high purity acquired from Sigma and Merck Ltd. The hexavalent Cr, a solution of stock, was made utilizing ultrapure water that had been distilled, and successive dilutions were performed to achieve the desired practical concentrations. The adsorption procedure utilized premium analytical-grade chemicals obtained from the company Sigma-A. The substances comprised potassium dichromate, hydrochloric acid (HCl, 37% v/v), and sodium hydroxide (NaOH), exhibiting a purity range of 98–99%. Furthermore, the compounds were utilized in the current work without any additional purification being performed. Together with potassium dichromate salt, a stock solution of Cr(VI) with a concentration of 1000 mg/L was made. The stock solutions were suitably diluted to formulate the working solutions of the prescribed concentrations. The pH of the chromium solution was modified using either 0.1 M HCl (dilute acid) or 0.1 M NaOH (dilute base) [28].

2.2. Separation of microorganisms and colony formation

Water samples were obtained from various sites and thereafter held at 4°C before analysis to maintain their integrity. An appropriate medium was produced utilizing high-quality reagents from media vendors, including Sigma Aldrich, to encourage microbial growth. The bacterial strains were isolated from the effluent samples by culturing them in Luria Bertani (LB) medium, a commonly utilized nutrient-dense medium. LB broth was formulated by dissolving 10 g of casein enzymatic hydrolysate, 5 g of yeast extract, 5 g of sodium chloride, and 1.5 g of agar in 100 mL of the medium. Effluent samples were diluted with the conventional pour plate method and subsequently plated onto LB agar plates. The plates were then incubated at 37°C for 48 h. Following the incubation period, specific bacterial colonies were chosen from each plate for additional analysis. The chosen colonies underwent thorough studies, encompassing morphological, biochemical, and DNA characterization, to ascertain their qualities and possible significance to the study.

A metal-tolerant bacteria was obtained originating from water suspension by using the technique outlined by existing literature [29]. In order to determine whether or not a variety of microorganisms were present in the water sample that was collected in the form of a suspension and stored in the laboratory at a temperature ranging from 20 to 45°C, the water sample was contaminated as a result of industrial pollution. For the purpose of facilitating the cultivation of microorganisms, culture plates with basic nutrient agar were utilized. Subsequently, 100 μL of this diluted product was dropped onto the plates made from agar and incubated at 35°C over a period of 24 to 48 h. The remaining portion of the sample was used for molecular identification, while a subset of colonies that could tolerate metals were chosen and grown as the mother culture.

2.3. Batch sorption studies

An experiment was conducted utilizing a 100 ml sample containing Cr(VI) at a concentration of 100 ppm. Employing 0.1 M HCl and 0.1 M NaOH to attain the desired pH [30]. A mass of 0.1–0.7 g of bacterial biomaterial was employed as a sorbent in the biosorption of Cr(VI). A magnetic stirrer was utilized for mixing at 250 rpm. Centrifugation was conducted at 4000 rpm for 25 min to isolate the solid biosorbent. The concentration of Cr(VI) ions in the residual solution was quantified using a spectrophotometer at a wavelength of 540 nm. The adsorption of Cr(VI) was examined at pH levels from 2 to 8, contact durations of 20 to 80 min, adsorbent quantities between 0.1 and 0.7 g, a Cr(VI) concentration of 100 ppm, and temperatures ranging from 20 to 45°C. The trials have been carried out three times, and the results obtained were computed as the mean, and the removal percentage was calculated by **[Equation 1]**, **[Equation 2]** and **[Equation 3]**. The data values displayed below were acquired with the specified biosorption technique.

$$\text{Removal}\,\% = \frac{C_i - C_f}{C_i} \times 100 \tag{1}$$

$$q_t = (c_i - c_t) \times \frac{V}{m} \tag{2}$$

$$q_e = (c_i - c_t) \times \frac{V}{m} \tag{3}$$

where c_i (mg L^{-1}) denotes the initial concentration of Cr(VI), c_f (mg L^{-1}) and c_t (mg L^{-1}) indicate the final supernatant concentrations at time t, and c_e (mg L^{-1}) represents the equilibrium supernatant concentration. Additionally, q_e(mg/g) signifies the adsorption capacity, where q_t and q_e represent the process of adsorption quantities at time (t) and at a state of equilibrium accordingly.

2.4. Molecular identification of Cr(VI) resistant bacteria

A molecular approach was implemented to ascertain the identity of the bacterial strain, which involved the 16S ribosomal messenger RNA genetic sequence matches and molecular amplification. The transcription of a DNA segment was facilitated by the primer-directed combination (25F,5′-CCAGTGG GATCGTGGT-3′ and 5′GGTTACCTTGTTACGACGG-3′) used in the PCR reaction [31]. A total of 100 ng of the forward as well as the reverse primers were included in the PCR reaction mixture that was used for the procedure of amplification, 100 μM of each deoxyribonucleotide triphosphate (dNTP), 1 unit of Taq polymerase, 3.5 μL of 10X PCR buffer, and 1.5 mM of magnesium chloride ($MgCl_2$), culminating in a total volume of 25 μL. The mixture was first denatured at 94°C for 5 min. Subsequently, a total of 35 PCR cycles were carried out, regarding the process, it involves decomposition at a temperature of 94°C across a time frame of 30 s, then further heating at 55°C for 30 s, and then elongation at 72°C for 20 s. After the conclusion of the preceding cycle, the resultant mixture was put through an incubation process that lasted for 10 min at a temperature of 72°C [32]. We aligned the resultant sequences with those existing in the database's records. The BLAST NR 2.9.0+ program utilized was sourced from the National Centre for Biotechnology Information (NCBI).

A search sequence can be compared to thousands of sequences in a database using the online program BLAST (Basic Local Alignment Search Tool), which displays the top matches. This tool efficiently examines extensive databases in a short amount of time. BLAST, which was created by Altschul et al. [33], aligns each position of the search sequence with those in the database, assigning a positive score for matching nucleotides and accommodating gap insertions during the alignment process. The alignment score is adversely affected by the introduction of a gap; however, the negative effect is mitigated by the presence of an adequate number of nucleotide matches, which allows the gap to be incorporated into the alignment. Subsequently, these scores are employed to calculate the alignment score in "bits", which is then converted into the statistical E-value. The most similar sequence is displayed as the main result, indicating a higher degree of similarity between the sequences in the database and the query sequence. A lower E-value represents this. Furthermore, MEGA version 5 was employed to construct a phylogenetic tree, as depicted in Fig. 1, which was based on 16S rRNA sequences of bacteria [34].

2.5. Kinetic and adsorption isotherms

The pseudo-first-order (PFO) and pseudo-second-order (PSO) models are commonly employed to investigate the rate and nature of liquid–solid interactions, specifically physisorption or chemisorption [35]. Adsorption isotherms provide insight into the relationship between the amount concentrated of the substance being studied and the surface area of the absorbing material at optimum. When analyzing the isotherms associated with the absorbent, several models are utilized for the purpose of analysis. We have utilized the Langmuir, Freundlich, Dubinin–Radushkevich and Temkin, conceptual frameworks.

2.6. Analysis of biomass properties

The *B. subtilis* biomass was characterized by scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX), XRD, Brunauer-Emmett-Teller (BET) analysis, transmission electron microscopy (TEM), and Fourier transform infrared (FTIR) anal-a

Description	Max.score	Total score	Query cover	E.valve	Per.ident	Accession
Bacillus subtilis strain UYY	2182	2182	100%	0.0	99.17%	MT613862.1
Bacillus rugosus strain SPB7	2182	2182	100%	0.0	99.17%	MT554518.1
Bacillus subtilis strain ZIM3	2182	2182	100%	0.0	99.17%	MT539995.1
Bacillus subtilis strain 2-2	2182	2182	100%	0.0	99.17%	MT525227.1
Bacillus subtilis strain HYS21	2182	2182	100%	0.0	99.17%	MT513998.1
Bacillus subtilis strain EB54	2182	2182	100%	0.0	99.17%	MT509757.1
Bacillus subtilis strain KA9	2182	2182	100%	0.0	99.17%	MT491101.1
Bacillus subtilis strain R31	2182	2182	100%	0.0	99.17%	MT478568.1
Bacillus subtilis sub.sp. inaquorosom T1	2182	2182	100%	0.0	99.17%	MT339257.1
Bacillus subtilis LSRBMOFPIKRGCFTRI39	2182	2182	100%	0.0	99.17%	MT133346.1

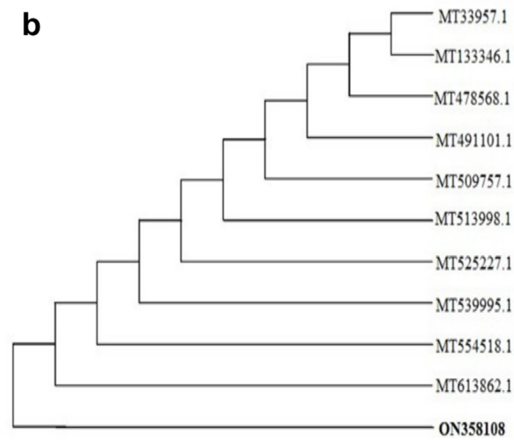

Fig. 1. (a) Similarity homology for the *B. subtilis* bacteria; (b) Phylogenetic tree based on 16S rRNA sequences.

ysis. The cells cultivated in the medium of LB broth supplemented with Cr (VI) at a concentration of 100 mg/L, together with cells cultivated without Cr (VI), were isolated using centrifugation at a speed of 7000 rpm for a duration of 20 min at an ambient temperature of 5°C. After being rinsed twice with sterile phosphate buffer, the pellet was then spread out on a glass slide surface and left to dry overnight at a temperature of 50°C [36]. The assortment of wave numbers that were utilized ranged from 4000 to 400 cm^{-1} to capture infrared spectra of biomass. At first, biosorption investigations were carried out in their original, unaltered state. The quantity of Cr(VI) in the suspensions was measured utilizing a spectrophotometer (PG T80 model) both before and after the adsorption process. For the purpose of calibration, a stock solution of Cr(VI) was utilized, and the initial concentration of the solution was set at 100 mg/l. Deionized water was used to dilute the residues, which allowed for the subsequent determination of the concentrations.

The FE-SEM technique was utilized to investigate the morphological characteristics of the biosorbent. The Merlin Carl Zeiss Gemini SEM 500 model was utilized for this analysis, as depicted in Figs. 2b,c. We also analyzed the biomass through TEM analysis and observed internal morphology, where we found compact structure has been completely changed into irregularities due to metal ion adsorption as depicted in Fig. 2d. The outermost portion of the bacterial isolate was analyzed using FTIR analysis (FTIR-VERTEX 70-BRUKER) to ascertain the reactive groups present and understand their involvement in Cr(VI) biosorption Fig. 2e. The scrutiny was accomplished both prior to and following the adsorption. X-ray diffraction (XRD) with Cu Kα energy discharge was leveraged to analyze the adsorbent derived from *B. subtilis*, as illustrated in Fig. 2f. The crystal structure of the object was elucidated by these investigations, which is crucial for understanding its functionality. In addition, we performed the BET technique to determine the surface area of the sample. The information provided indicated an overall surface area of 2.3650 g/m^2, the typical width of the pores is 43.06 A° along with a pore size of 0.0080 g/cm^2 Fig. 2g. This information provides insights into the adsorbent's porous characteristics and enhances our comprehension of its efficacy in capturing and interacting with various chemicals. The porous structure of biosorbents is a critical factor in the removal process of Cr(VI). It ensures a high surface area, efficient ion diffusion, and better accessibility to functional groups, all of which contribute to improved adsorption performance.

2.7. Statistical analysis

A comprehensive statistical analysis of the data was conducted following an experiment conducted under optimal medium conditions to systematically validate the Response Surface Methodology (RSM) mathematical model. The results of an analysis of variance (ANOVA) have been illustrated through the use of a statistical *t*-test and the assessment of critical statistical indicators. In statistics, df (degrees of freedom) refers to the number of values that can vary, the *F* value indicates the ratio of variances used to assess group differences, and the *P* value shows the probability that the observed result occurred by chance. The coefficient of correlation (R^2), adjusted R^2 (R^2 adj.), and predicted R^2 were all included in these measurements.

By conducting an experiment under optimal conditions and subsequently conducting a comprehensive statistical analysis of the results, this investigation employed a statistical *t*-test to evaluate critical statistical metrics, including the coefficient of determination (R^2), altered R^2 (R^2 adj), and anticipated R^2 (R^2 pred). Table 1 provides an overview of the analysis of variance (ANOVA) results, while Table 2 presents the model statistics.

3. Results and discussion

3.1. Biomass characterization

FE-SEM images demonstrated that the surface morphology of the biosorbents was more homogenous before the adsorption than post-adsorption. The external architecture got degraded and became uneven during the process. Fig. 2b depicts the surface of the porous biosorbent, which physically adsorbs Cr(IV), whereas Fig. 2c shows that these pores have become saturated with Cr(IV) following adsorption [37]. Transmission Electron Microscopy (TEM) analysis was also performed in this study to examine the ultrastructural changes in the microbial cells confirming the successful biosorption and internalization mechanisms Fig. 2d.

The protein peptide component of the microbial cell wall exhibited a robust affinity for Cr(VI) ions. The sequestration of these reduced Cr(IV) ions is primarily influenced by the configuration of active surface-bound chemical functional groups, which were comprehended using FTIR analysis. Fig. 2e displays the FTIR investigation acquired prior to and during the intervention of *B. subtilis*

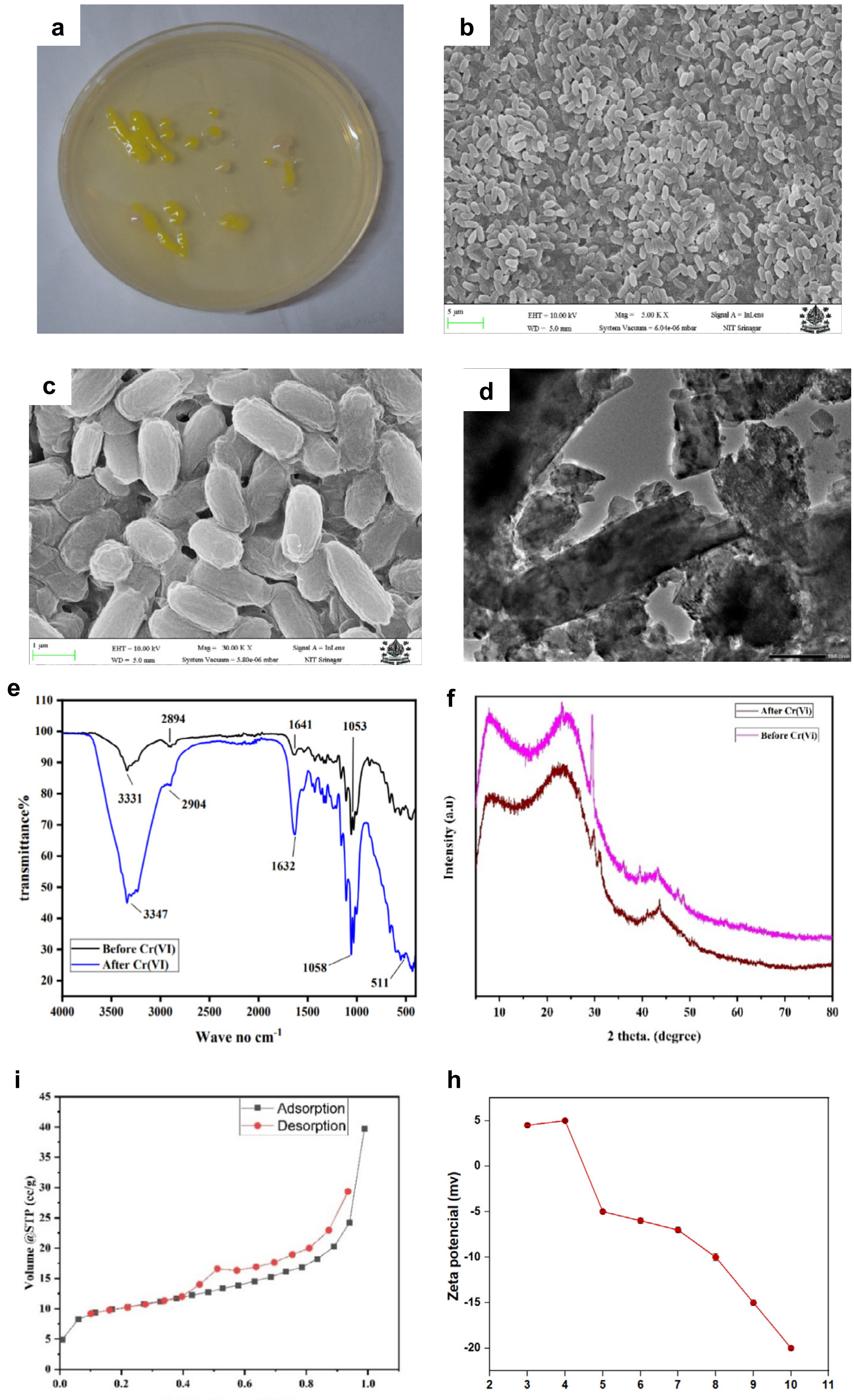

Fig. 2. (a) Bacterial culture formation; (b,c) scanning electron microscopy (SEM) analysis of Cr(IV) before and after adsorption; (d) transmission electron microscopy (TEM) analysis of Cr(IV) after adsorption; (e) FTIR analysis of *B. subtilis* before and after adsorption; (f) XRD analysis; (g) adsorption and desorption studies; (h) zeta potential studies.

with chromium ions. The FTIR spectra of *B. subtilis* post-Cr(VI) adsorption (wavelength range of 400–4000 cm^{-1}) exhibited new and developing changes relative to pure bacteria, indicating alterations in the behavior of some membrane-bound functional groups upon interaction with metallic elements. In Fig. 2e, an incremental shift at 500.75 cm^{-1} (alkyl halide) upon adsorption was ascribed to Cr(VI) [38]. A signal at 3347 cm^{-1} indicated the presence of a hydroxyl (OH) stretch bond in bacteria. A methyl group was identified at 2904 cm^{-1}. Alkenes accounted for the mild signal at 1632 cm^{-1} noted in the spectral data. CO-stretching and aliphatic amines can be differentiated by peaks at 1258 cm^{-1} and 1058 cm^{-1}, respectively. Shift rotations suggest that alkyl halide, aliphatic amine, carboxylate, and hydroxyl groups significantly influence the binding of Cr(VI) metallic ions and ligands in the

Table 1
The analysis of variance (ANOVA) for the quadratic response surface model.

Source	Sum of squares	df	Mean square	F value	P value	
Model	5831.87	14	304.55	57.89	<0.0001	Significant
A: Bacterial dose	150.00	1	150.00	31.06	0.0004	
B: Cr(VI) concentration	6.17	1	6.17	1.15	0.3012	
C: Temperature	1086.50	1	1086.50	153.53	<0.0001	
D: pH	1266.00	1	1266.00	304.12	<0.0001	
AB	4.52	1	4.52	0.8665	0.3637	
AC	20.25	1	20.25	3.25	0.0571	
AD	25.00	1	25.00	3.31	0.0704	
BC	22.25	1	22.25	2.84	0.1124	
BD	1.0000	1	1.0000	0.1404	0.7131	
CD	576.00	1	576.00	92.91	<0.0001	
A^2	27.43	1	27.43	3.85	0.0685	
B^2	23.71	1	23.71	3.92	0.0433	
C^2	1365.86	1	1365.86	208.47	<0.0001	
D^2	4.67	1	4.67	0.5516	0.3731	
Residual	102.93	15	5.12			
Lack of fit	102.93	10	10.68			
Pure error	0.0000	4	0.0000			
Cor total	5788.70	27				

Table 2
Statistical parameters of the developed model.

Statistical Parameters	Values of developed model
R^2	0.9815
Adjusted R^2	0.9742
Predicted R^2	0. 9237
Adeq. precision	44.4289
Standard deviation	2.67

aforementioned biosorption process. FTIR graphs related to the mentioned functional groups were much similar when the biomasses of *B. megaterium* [39] or *Halomonas* sp., or bacterial cellulose were used as adsorbents [40]. However, one difference was observed, hydroxyl groups being the most pronounced in our study for *B. subtilis* biomass.

The crystal orientations of the *B. subtilis* adsorbent were investigated using Cu Kα radiation in X-ray diffraction (XRD) within a scanning angle range of 10° to 80°, as illustrated in Fig. 2f**.** The adsorbent's physical and chemical properties were understood through the elucidation of its crystal structure through XRD analysis. A Bruker D8 Advance X-ray diffractometer was employed to execute X-ray diffraction (XRD) in Bragg-Brentano geometry (40 kV, 40 mA). Through an enclosed X-ray tube (CuKα1, λ = 1.5406 Å) and a LYNXEYE XE-T detector, the instrument model that has been specified is equipped with the necessary components. With a step size of 0.02° and a time interval of 2 s per step, the diffraction patterns were obtained within the angular range of $5 < 2\theta < 50°$. For the purpose of improving peak profiles for analysis, the samples were rotated while the measurement was being taken to reduce the impact of preferred orientation. As a result, the broadening of peaks at 2θ values of 21.584° and 29.004°, respectively, correlate to the (111) and (222) planes, the (111) and (222) planes in XRD represent specific atomic layers in a crystal structure, typically indicating a face-centered cubic (FCC) arrangement and it was proposed that the biosorbent did not contain any major inorganic stages. This may be attributed to the concealing effect of the organic compounds in bacterial biomass. The biosorbent's crystalline structure was confirmed by a prominent peak in XRD at 290.04. The biosorbent's face-centered cubic crystal structure provides sufficient unoccupied porosity sites for the adsorption of Cr(VI) ions on its surface.

The BET technique was utilized to ascertain the outermost area of the biosorbent material, as illustrated in Fig. 2g, by scrutinizing nitrogen adsorption–desorption isotherms at 77 K. The BET investigation results indicated a pore volume of 0.0050 cm^3/g, an average pore width of 36.03 Å, and a surface area of 2.1540 m^2/g. These results clarify the porous characteristics of the adsorbent, imparting crucial details for the assessment of its absorption capacity and relationship to the compound of interest. BET analysis observations with bacterial biomass for Cr adsorption are limited. The bacterial cellulose-derived sorbents demonstrated a porous structure with a significant surface area, contributing to their high Cr adsorption capacity [41].

The biosorption capacity of the biosorbent is substantially influenced by surface features, including pore volume and surface area. Our study's BET surface area analysis indicated a surface area of 2.3650 m^2/g, which was directly associated with a biosorption capacity of [95 ± 1.5] mg/g for Cr(VI). An increased surface area elevates the quantity of active binding sites for Cr(VI) ions, consequently improving the adsorption process. Pore volume is essential for enabling the passage of Cr(VI) ions into the interior structure of the biosorbent. The measured pore size of 0.0080 g/cm^2 facilitated efficient ion penetration, hence enhancing the total adsorption efficacy. Pore size and shape were identified as the most significant physical factors influencing Cr(VI) removal effectiveness. The biosorbent demonstrated a pore size ideal for the accommodation of Cr(VI) ions (~0.4 nm). This facilitated effective ion diffusion and engagement with the biosorbent surface. SEM morphological analysis indicated a coarse surface characterized by distinct pores and fissures, which offer supplementary binding sites and augment the effective surface area. Additionally, the existence of functional groups, including hydroxyl and carboxyl groups, as shown by FTIR analysis, facilitated Cr(VI) binding via electrostatic interactions and complexation. These findings emphasize that an optimal mix of pore size, elevated surface area, and appropriate morphological characteristics is essential for attaining high Cr(VI) removal efficiency.

Combining FTIR, SEM, XRD, and BET analysis results gives us the confidence that the biomass of *B. subtilis* is an efficient biosorbent of Cr(VI). Using FTIR, SEM, XRD, and BET together offers a synergistic approach to understanding and optimizing Cr adsorption by microbial biomass or other adsorbents. These techniques provide complementary data on chemical, physical, and structural properties, enabling a detailed assessment of adsorption mechanisms and efficiency [22,42]. FTIR identifies chemical interactions, while SEM examines surface morphology. XRD provides crystallinity information, and BET measures porosity and surface area. Combining these methods clarifies how Cr is adsorbed, including chemical bonding, physical trapping, or precipitation. The changes in functional groups, surface structure, and surface area collectively demonstrated the adsorption efficiency of *B. subtilis* biomass. High surface area, increased pore size, porous and crystalline structure and homogeneous morphology together with the functional groups observed enhance the biosorption process. The high surface area provides more active sites for metal ion binding, leading to increased adsorption capacity. It allows for better interaction between the biosorbent and the adsorbate, improving the efficiency of the biosorption process. Larger pore sizes facilitate the diffusion of metal ions into the biosorbent structure, enhancing adsorption, especially for larger molecules or ions. Uniform morphology ensures consistent distribution of active sites, leading to predictable and uniform adsorption behavior. This homogeneity improves reproducibility and reliability in the biosorption process, making the material more effective for practical applications. It ensures effective utilization of internal adsorption sites, contributing to higher overall adsorption efficiency. These properties collectively optimize the interaction between the biosorbent and the target metal ions, improving adsorption kinetics, capacity, and overall performance in removing pollutants from aqueous solutions.

3.2. Zeta potential study

The Zeta potential measurements of *B. subtilis* revealed a surface charge that varied from 10.63 mV to −19.6 mV across a range of pH values (pH 2–8), as illustrated in Fig. 2h. Zeta potential, representing the surface charge of bacterial biomass, varies with pH and significantly influences the adsorption mechanisms of Cr species, particularly Cr(VI). Understanding this relationship is crucial for optimizing bioremediation strategies. At low pH levels, bacterial surfaces often exhibit a positive charge, enhancing attraction to negatively charged Cr(VI) species. As pH increases, the surface charge becomes negative, potentially leading to the repulsion of Cr(VI) anions [40]. Adsorption efficiency was shown to be decreased as pH increased from 2 to 9, highlighting the preference for acidic environments in Cr(VI) biosorption [43]. However, in this study, the highest adsorption capacity was observed at pH 6 and the zero-point charge (ZPC) was detected at a pH of 4.1, while the outermost charge remained positive at pH values that were lower than the ZPC. The observation that the highest Cr adsorption by bacterial biomass was at pH 6, even though the zeta potential was negative above pH 4.1, can be explained through several complex mechanisms. The surface chemistry of bacterial biomass is complex. The observed net positive charge, which is lower than the ZPC value, may be attributed to the protonation of active groups on the bacterium's surface as the pH increases [44]. This was suggested previously when the highest adsorption of Cd was observed at pH 6 [45]. Bacterial surfaces are rich in carboxyl, amine, and hydroxyl groups, whose ionization states change with pH. At pH 6, these groups may be partially ionized, which could help form bonds with Cr ions even if the net surface charge of the biomass is negative [44]. Moreover, amine groups can form complexes with Cr(VI), and carboxyl groups can act as ligands to bind chromium ions. Bacterial biomass surfaces are often heterogeneous, meaning that different regions of the surface may have different surface charges or functional groups that vary in their ionization states with pH. Even though the overall zeta potential is negative at pH 6, localized areas on the bacterial surface may have positive charges or provide specific adsorption sites that promote Cr(VI) binding through hydrogen bonding or coordination bonds, independent of the overall surface charge [19].

3.3. Effect of initial concentration

The preliminary proportion of Cr(VI) has a substantial impact on the biosorption process, and it was noticed that the process of biosorption decreases as the initial level of Cr(VI) in the solution raises. The Cr(VI) concentration in our study varied from 1 to 10 mg/l. Over the course of the experiments, the temperature was consistently maintained at 30°C, while all other variables remained constant. The outcomes of the studies reveal how the concentrations of Cr(VI) affect the biosorption process are depicted in Fig. 3a. The way biosorption works evidently escalates in proportion to the initial concentration of Cr(VI), reaching a maximum at about 8 mg/l, as demonstrated. Each subsequent increase in the initial Cr(VI) concentration leads to a decrease in the rate of Cr(VI) elimination beyond this concentration threshold. This decrease can be attributed to the suppressive effect of elevated Cr(VI) concentrations. In contrast, the biosorption rate is anticipated to decrease at lower Cr(VI) concentrations as a result of mass transfer limitations, which results in a decreased availability of Cr(VI) to the biomass. The biosorption of low-concentration heavy metals has been shown to be significantly impacted by mass transfer, which has been proposed as a significant contributor [46].

3.4. Effect of other coexisting ions

Additional pollutants in wastewater can have a detrimental impact on bacterial activity, thereby limiting its biosorption capacity. The presence of other metals and sulfate in wastewater introduces competition for binding sites and changes in ionic strength, which can reduce the Cr(VI) adsorption efficiency of microbial biosorbents [14]. When other metal ions (e.g., Pb^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, etc.) are present in wastewater, they compete with Cr(VI)

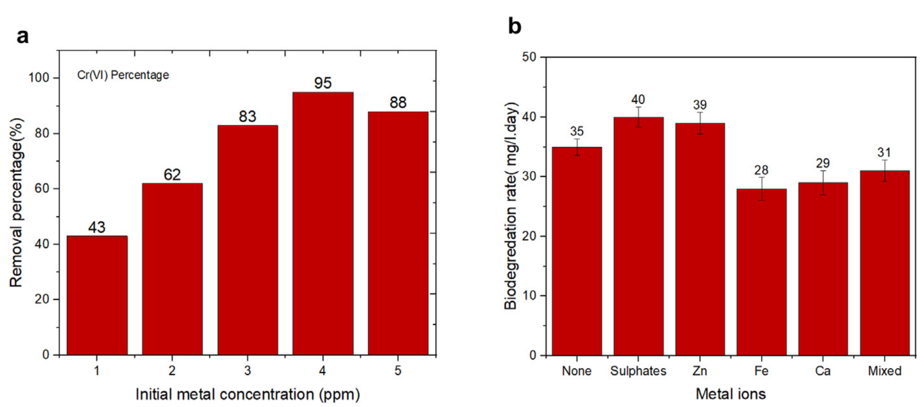

Fig. 3. Effect of various process parameters. (a) Removal percentage at different initial concentrations of Cr(VI); (b) Effect of co-existing ions.

for the limited active sites on the microbial biosorbent. Sulfate ions are negatively charged, similar to the dominant Cr(VI) species. They can compete with Cr(VI) for positively charged or neutral binding sites on the biosorbent surface. In this study, other metals or sulfates present did not affect remarkably to the adsorption. As illustrated in Fig. 3b, the experimental results unequivocally indicated that these pollutants exerted a minor influence on the removal of Cr(VI). The concentrations of these contaminants were chosen to be within or significantly above the typical levels observed in wastewater. Although iron appears to have a marginally adverse impact on the biodegradation rate Fig. 3b, this fact can be disregarded, as the biodegradation rate remains unaffected, when microorganisms are subjected to a combination concerning such metallic ions, as demonstrated in the illustration. Sodium sulfate was utilized at concentrations between 100 and 1000 mg/l to assess the influence of sulfates on the biodegradation rate. The biodegradation rate was not influenced by the incorporation of sodium sulfate, as evidenced by the experimental results, an elevated level of 1000 mg/l. Based on our results, we suggest that *B. subtilis* biomass can be studied further in real wastewater containing other pollutants. This is supported by a recent review that showed *Bacillus* species to detoxify various heavy metal ions, including Cr(VI) [47]. *Bacillus* species potential was shown also in a study where the certain *Bacillus* strains reduced metals including lead, cadmium, mercury, chromium, arsenic, or nickel in the environment [48]. This indicates that *B. subtilis* may have the capacity to adsorb multiple heavy metals simultaneously. This information improves the potential of *B. subtilis* in real wastewater applications.

3.5. Effect of contact time and adsorbent dose

The experiment took into account the duration of contact time, and it was shown that the highest level of metal absorption, amounting to 94.12 ± 1.5%, was achieved after 60 min Fig. 4a. It is important to note that most studies have indicated that the best period to achieve the greatest biosorption rate is between 40 and 60 min.

Extended exposure to *B. subtilis* may reduce chromium elimination due to the maximum capacity of docking spots, leading to decreased absorption. Moreover, prolonged exposure might diminish bacterial metabolic activity and efficiency, while alterations in ambient circumstances, such as pH and temperature, may further

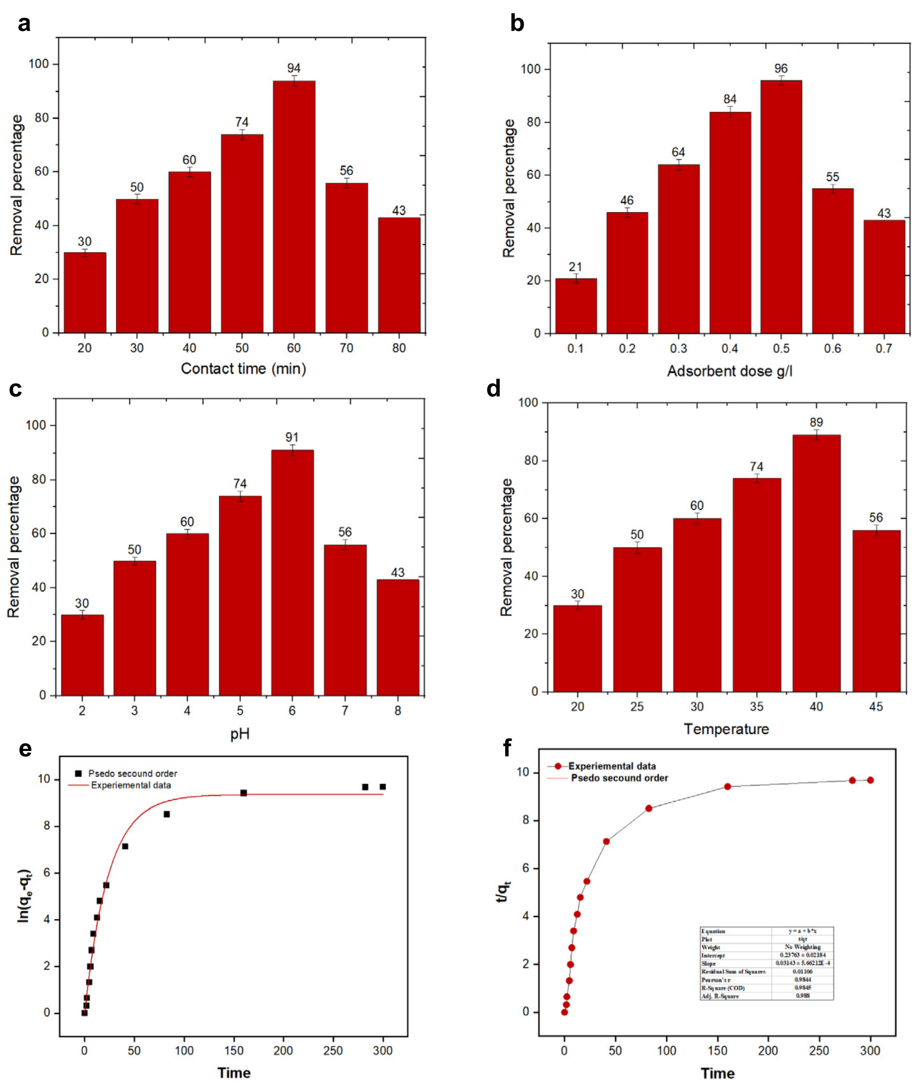

Fig. 4. (a) Removal percentage of Cr (VI) in different contact times; (b) with different adsorbent doses; (c) pH values, and (d) temperatures. e) Pseudo first analysis (PFO); (f) Pseudo second analysis (PSO); (g) nonlinear regression of Langmuir isotherm; (h) Freundlich isotherm model; (i) Temkin model; (j) D-R model, and k) intra particle diffusion method; (l) Elovich model. Error bars refer to SD (n = 3).

impede performance. Competition for binding sites and the buildup of chromium or its metabolites may further impede removal ability.

The bacteria showed resistance to Cr(VI) removal when the contact duration was beyond 60 min. *B. subtilis* can develop resistance to high concentrations of Cr(VI) via selective pressure from continuous exposure, genetic mutations that enhance tolerance, efficient stress response mechanisms, biofilm formation that provides protection, horizontal gene transfer of resistance traits, and metabolic adaptations that promote survival. These issues obstruct effective bioremediation efforts [49].

The study examined the effects of varying biomass concentrations (0.1 to 0.7 g). The investigation concentrated on the capacity of *B. subtilis* biomass to adsorb Cr(VI) ions [39]. The effects of different adsorbent doses were investigated while keeping other parameters constant. When the amount of adsorbent dose is increased from 0.3 to 0.5 g, there is a favorable response in terms of metal uptake. However, if the amount of biomass exceeds 0.5 g, the rate of absorption appears to decrease Fig. 4b. The peak degree of intake was noted at a dose of 0.5 g, which was determined to be the optimal dose. This could be attributed to the augmentation of binding sites provided by the biomass, hence improving the biosorption characteristics of the *B. subtilis* biomass. The biosorption level is highly influenced by the concentration of biosorbent. Higher concentrations of biosorbent typically result in greater levels of metal uptake, as observed in numerous studies.

The reduction in chromium removal efficiency with elevated dosages of *B. subtilis* is attributable to multiple causes. As the adsorbent dosage increases, binding sites may reach saturation, resulting in constrained chromium absorption. Elevated amounts may induce cell aggregation, hence diminishing the effective surface area for adsorption and escalating competition among cells for chromium ions. Moreover, accumulated chromium or metabolic byproducts may impede bacterial action, while alterations in local environmental circumstances, such as pH or nutrient concentrations, might further influence removal effectiveness.

3.6. Effect of pH

In the process of metal ion biosorption by bacteria, the selection of pH is an important determinant, because it impacts the contact between functional groups on the cell surface and the metal ions. This interaction is what makes the process possible. The ionization of functional groups on bacterial surfaces and the speciation of

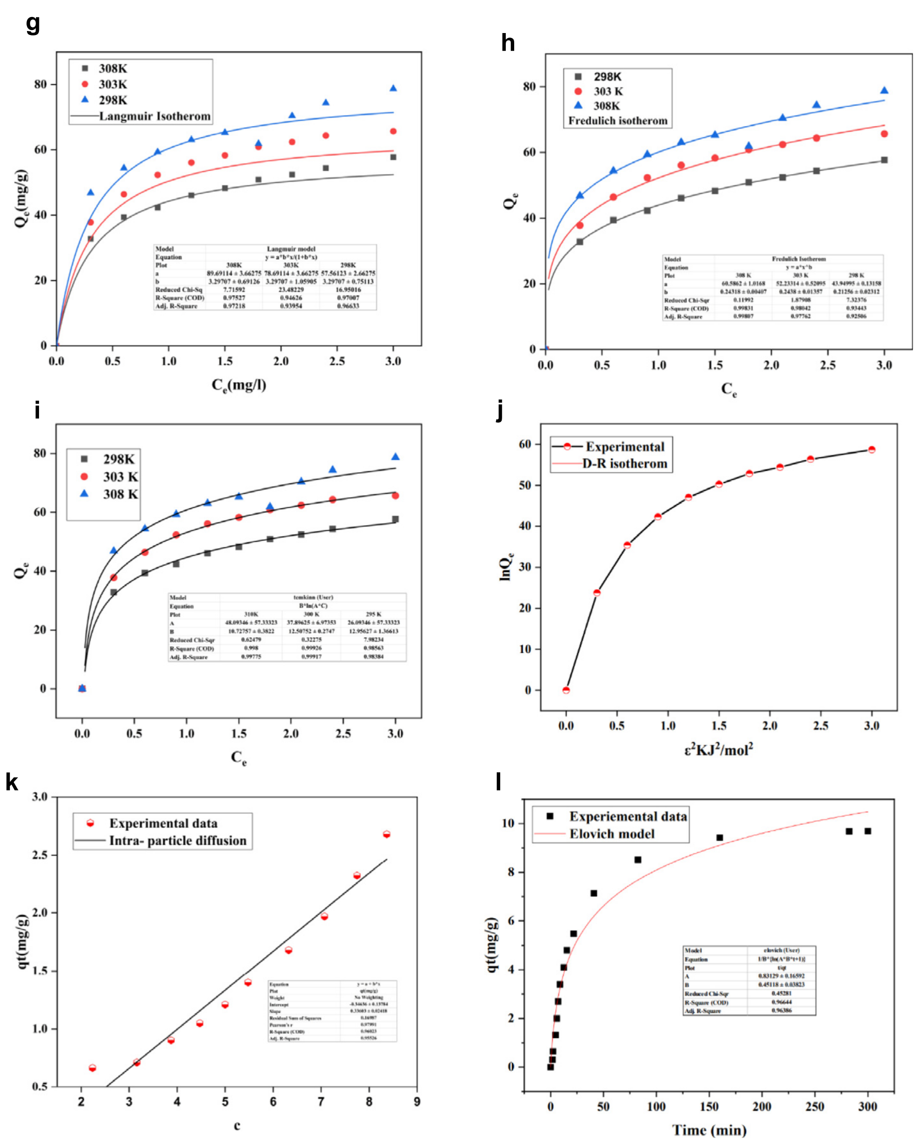

Fig. 4 (*continued*)

metal ions are both profoundly impacted by the pH, which is a critical determinant in the biosorption process [50]. In low pH environments, the biosorption efficiency is decreased because of the protonation of these groups and competition with H^+ ions, whereas the negative charge on bacterial surfaces is increased by deprotonation at elevated pH, which facilitates metal ion coupling. A significant factor that affects the solubility of metal ions is the pH rating. A low pH value increases the availability of unbound ions, whereas a high pH value may lead to metal precipitation. The optimal pH maximizes biosorption by equilibrating these variables. A pH range of 3 to 8 is particularly effective for the microbial reduction of Cr(VI). The research examined the removal of 91 ± 2.5 mg/L Cr(VI) utilizing *B. subtilis*, as illustrated in Fig. 4c The findings indicated that the peak absorption of Cr(VI) transpired at pH 6 following 48 h of incubation.

The proportion of reduction in Cr(VI) that is ideal by *B. subtilis* is achieved at pH 6, which is a result of numerous critical parameters that enhance the bacteria's bioremediation efficiency. *B. subtilis* demonstrates its highest metabolic activity at this pH, which facilitates the absorption of chromium ions. At pH 6, Cr(VI) is primarily present in a soluble form that bacteria can efficiently convert to Cr (III), a less poisonous and more readily digestible metal. The adsorption efficacy is enhanced by the surface charge of bacterial cells, which enables the binding of chromium ions at this pH. Additionally, a pH of 6 decreases the probability of chromium precipitate, thereby ensuring that a higher concentration of chromium is present in the solution for absorption. The removal of Cr(VI) with *B. subtilis* is made possible by the interplay of biological activity, advantageous speciation, and superior surface contacts at pH 6.

3.7. Effect of temperature

A significant correlation exists between the removal of Cr(VI) and temperature, as illustrated in Fig. 4d. Research findings suggest that a temperature range of 37 to 40°C is optimal for chromium biosorption and bioreduction by *B. subtilis*. Nonetheless, temperatures exceeding or falling below 40°C caused a slowdown in bacterial multiplication, which subsequently came about a reduction in Cr(VI) absorption. The observed results can likely be ascribed to structural damage to the bacterial membrane, the inactivation of certain enzyme functions, or the synthesis of intervening proteins.

3.8. Biosorption kinetics

For the purpose of gaining a deeper comprehension of the response mechanism along with the intensity of solute absorption in biosorption approach, kinetic analysis is fundamental, offering valuable information about the time frame of the chromium interaction at the exterior of the boundary layer [51]. This investigation focused on the interaction phases that regulate Cr(VI) ion assimilation by *B. subtilis*. The PFO model as depicted in **[Equation 4]** proposes that a single adsorptive species occupies a single reactive spot of the adsorbing material, whereas the PSO model **[Equation 5]**, indicates that a single adsorptive entity occupies multiple reactive sites. Experimental data were used to calibrate both the PFO and PSO models. These analyses are indispensable for the development of bulk biosorption systems, as they enable the estimation of the biosorption rate. Lagergren's PFO and PSO were employed for assessing the findings Fig. 4e,f, thereby clarifying the biosorption rate kinetics.

The fact that the PSO model has a higher correlation coefficient (R^2) than other models demonstrates that it is suitable for predicting the kinetics of Cr(VI) adsorption onto bacterial biomass. The adsorption mechanism for Cr(VI) is comprised of multiple phases, including: i) Film diffusion, in which Cr(VI) ions are moved to the exterior of the biosorbent; ii) Intra-particle diffusion, in which Cr (VI) ions migrate farther into the inner pores of the biosorbent; and adsorption, in which ions bond to the interior pores of the biosorbent respectively. In general, the kinetic analysis reveals that the PSO model describes the process in the most correct manner. This highlights the significance of intra-particle diffusion in the process of Cr(VI) adsorption onto bacteria biomass biosorbent. With this newfound knowledge, the adsorption process can be optimized for use in water treatment applications that are more commonly encountered.

Lagergren linear PFO expression is as:

$$log(q_e - q_t) = logq_e - \frac{K_1 t}{2.303} \quad (4)$$

where q_e (mg/g) is the optimum uptake of contaminant ions and q_t (mg/g) signifies uptake at t (min). The PFO rate parameter for biosorption is represented by K_1 (min^{-1}).

The PSO R^2 values are relatively satisfactory, as indicated by data. In the current context, the PSO seems more fit to accurately represent the kinetic biosorption of Cr(VI) within the framework of biosorption kinetics. Due to its reliance on chemisorption, biosorption is classified as a second-order process, the below non-linear representation corresponds to the PSO.

$$t/qt = 1/(K_2 q_e 2) + t/q_e \quad (5)$$

As can be seen from this data, the outcomes of R^2 of pseudo-second-order are relatively high. In this particular setting, PSO is the one that is most likely to correctly represent the kinetic behavior of Cr (VI) biosorption within the kinetics context.

3.9. Biosorption isotherm models

To facilitate the isotherm experiment, the content of Cr(VI) in synthetic wastewater ranged from 10 mg/L to 100 mg/L. The assessment consisted of incorporating Cr(VI) into flasks that were 100 mL in capacity and contained a solution of Cr(VI). The contents underwent mechanical shaking at 120 rpm for a specified amount of time at a range of temperatures. In the subsequent step, the sample filtration was done using Whatman filter paper, with 0.45 μm pore size of. A non-linear fitting was performed on the data obtained from the experiment in order to determine the various isotherms, as will be laid out in the following paragraphs.

The Langmuir isotherm is stated as follows **[Equation 6]:**

$$q_e = \frac{q_{mK_l} C_e}{1 + K_l C_e} \quad (6)$$

where q_m is the monolayer utilization effectiveness of the material, which is measured in mg/g. *Ce* is the residual level of contaminant in the solution, which is measured in mg/L. K_l is the Langmuir adsorption constant, which is measured in mg/L and corresponds to the free energy of sorption. q_e is the highest possible adsorption material degree on the sorbent, which is measured in mg/g. Illustrations of the graphs of the non-linear Langmuir isotherm are shown in Fig. 4c.

With regard to the sorption surface, the Freundlich model postulates that there is an intrinsic diversity [52]. The Freundlich isotherm serves as a well fit for adsorption that is capable of addressing multilayer uptake on diverse heterogeneous interfaces. In **[Equation 7]** the isotherm is stated in its current form.

The Freundlich model:

$$q_e = K_f C_e 1/n \quad (7)$$

In this context, K_f represents an equilibrium indicative of uptake capability, and *1/n* denotes an observational parameter. This delineates the degree of removal, which varies according to the dissimilarity of the substance. The figures of K_f and *1/n* were determined by nonlinear regression analysis. Fig. 4h illustrates the graphs of the non-linear Freundlich isotherm. The figures of *1/n* ranging from '0′ to '1′ demonstrated that the Cr(VI) uptake onto *B. subtilis* was effective within examined circumstances and the equations are summed up in Table 3.

The model's alignment with the observational data was studied by linear regression examination, as shown in Table 4. R^2 values achieved using Langmuir isotherm were the greatest at all temperatures. The high finding indicates that Langmuir isotherm is the most appropriate for Cr(VI) uptake, signifying monolayer uptake on inhomogeneous surfaces. Comparable findings were noted when polypyrole composite adsorbents were utilized for the extraction of Cr(VI). The maximum extraction capacities, q_{max}, were 57.96, 78.69, and 89.6 mg/g at temperatures of 293 K, 303 K, and 308 K, respectively. The computed values of equilibrium state and separation state constant indicated that RL varied from 0.13 to 0.35, signifying the advantageous nature of the adsorption phenomenon. The value of (1/n) is inferior to one. The accumulation technique is favored, the surface is inhomogeneous, and interactions among the contaminant ions are reduced. Cr(VI) uptake happens via multi-molecular and multi-anchorage uptake phenomenon. It was hypothesized that Cr(VI) biosorption happened through a chemisorption mechanism, as the E value was above 8 kJ/mol. The Temkin isotherm model aims to investigate the relationship between the adsorption of Cr(VI) onto biomass and variations in temperature. This model indicates that with rising temperature during the adsorption process, greater heat absorption occurs, signifying that ion exchange and chemisorption are efficient methods for the removal of Cr(VI). This understanding of temperature-dependent adsorption behavior is crucial for comprehending the thermodynamics of Cr(VI) adsorption and can offer significant insights for enhancing nitrate removal systems utilizing biomass as the adsorbent [53].

The Temkin isotherm incorporates a component that explicitly considers adsorbent-adsorbate interactions Fig. 4i [54]. The derivation, as indicated by the equation, is defined by a uniform distribution of binding energies and is articulated as shown in **[Equation 8]**.

$$q_e = \frac{rt}{bt} \ln(a_t c_e) \tag{8}$$

The Dubinin-Radushkevich isothermal approach plays a vital part in differentiating the physical and chemical characteristics of adsorption through EDR (mean adsorption energy) findings, which assess the uptake energy. The EDR results recorded are 114.38 kJ/mol at 308 K, as illustrated in Fig. 4j. The EDR values exceed 8 kJ/mol, indicating that chemisorption has occurred throughout the adsorption phase [55].

In conclusion, the findings of our research indicate that the Langmuir and Freundlich isotherms are in close agreement with the experimental data that we have collected. This indicates that ion exchange and complexation mechanisms are key factors that influence the way that Cr(VI) binds to B. subtilis biomass. These findings offer useful insights into the adsorption behavior of Cr (VI) and have the potential to inform techniques for the efficient removal of Cr(VI) by utilizing *B. subtilis* biomass as an adsorbent.

This research contrasts the adsorption capacities of several adsorbents from previous studies with those employed in this investigation, as outlined in Table 5. This comparison demonstrates that bacterial biomass possesses considerable promise as an adsorbent in wastewater treatment, evidenced by its exceptional performance.

3.10. Thermodynamic studies

In an attempt to acquire a comprehensive comprehension of the adsorption of Cr(VI) ions atop the exterior membrane of *B. subtilis*, researchers employ a variety of parameters, such as enthalpy (ΔH °), free energy (ΔG°), and entropy (ΔS°) [53]. This comprehensive understanding is achieved through the utilization of biological procedures. These evaluations offer significant insight about the energy and efficiency of the surface binding. They also offer important insights into the mechanisms that drive and the natural tendency of Cr(VI) ion adsorption onto the bio adsorbent. The values were computed utilizing the subsequent **[Equation 9]**:

$$\Delta G = -RTlnKc \tag{9}$$

T stands for temperature in Kelvin (K), *R* for the universal gas constant, as well as $K_{d'}$ for the distribution coefficient. 8.314 J/K mol is the value of the universal gas constant *R*. **[Equation 10]** can be used to find out what $K_{d'}$ is.

$$K_d = \frac{q_e}{c_e} \tag{10}$$

q_e and c_e are Cr(VI) optimum capacity to absorb and an equilibrium adsorbate levels respectively. Use the **[Equation 11]** to ascertain the changes in enthalpy ($\varDelta H°$) and entropy ($\varDelta S°$) over biosorption.

$$lnkd = \frac{\Delta s}{R} - \frac{\Delta H}{RT} \tag{11}$$

Table 3
The equations of kinetic and adsorption isotherms.

Model	Equation	Representation plot
Kinetic Pseudo-first-order (PFO)	$log(q_e - q_t) = logq_e - \frac{K_1 t}{2.303}$	$log(q_e - q_t$ *vs t*
Pseudo-second-order (PSO)	$t/q_t = 1/(K_2 q_e 2) + t/q_e$	*t/qt* v t
Inter-particle diffusion (ID)		$q_t = K_P t_2^1 + C$
Isotherm Langmuir	$q_e = \frac{q_{mK_l} C_e}{1+K_l C_e}$	Q_e *vs Ce*
Freundlich	$q_e = K_f C_e 1/n$	$q_e \upsilon s c_e$
Temkin	$q_e = \frac{RT}{bt}$ ln (A_T C_e)	$q_e \upsilon s c_e$
Dubinin – Radushkevich	$q_e = lnq_e - k\,\varepsilon^2$	ln q_e *vs* ε^2

Table 4
Studying how temperature affects Cr(VI) adsorption isotherms on *B. subtilis*.

Adsorption equilibrium model	Isotherm parameters	Temperature 298 K	303 K	308 K
Langmuir	q_{max} (mg/g)	57.56	78.69	89.69
	K_L	2.69	1.79	0.93
	R^2	0.99	0.99	0.97
Freundlich	q_{max}(mg/g)	43.94	52.23	60.58
	1/n	0.2	0.21	0.23
	R^2	0.92	0.97	0.99
Temkin Model	K_t	58.61	23.56	15.60
	R^2	0.999	0.997	0.996

Table 5
Comparative analysis of different bio adsorbents for Cr(VI) elimination.

Microorganisms	Concentrations of Cr(VI)	Mechanism of Cr(VI) removal	Reduction (%)	Reference
Halomona sp.	40 mg/L	Reduction	93.3	[56]
Sporosarcina saromensis W5	200 mg/L	Reduction	100	[57]
Aeromonas hydrophila ATCC 7966	25 mg/L	Reduction	70	[58]
Cellulosimicrobium funkei AR8	250 mg/L	Reduction	78.18	[59]
Bacillus sp. *CRB-B1*	100 mg/L	Reduction	100	[60]
Bacillus cereus XMCr-6	100 mg/L	Reduction	100	[61]
Spirulina platensis	100 mg/L	Biosorption	61.97	[62]
Acinetobacter sp.	100 mg/L	Biosorption	67	[63]
Brevibacillus laterosporus	100 mg/L	Biosorption	92	[64]
Bacillus subtilis	2–10 mg/l	Biosorption	95	Current Study

Table 6
Evaluation of thermodynamic parameters for *B. subtilis* for Cr(VI) biosorption.

Temperature K	$-\Delta G$ kJmol^{-1}	$-\Delta H$ kJmol^{-1}	ΔS kJmol^{-1} k^{-1}
298	36.77	97.56	176.76
303	42.79		
308	47.89		

Table 6 provides a concise summary of the thermodynamic properties that are associated with biosorption. These features encompass the variation in enthalpy ($\Delta H°$), the change in entropy ($\Delta S°$), and the change in free energy ($\Delta G°$). The fact that the free energy change was negative is evident that the process was carried out without any external intervention. A decrease in enthalpy signifies that bioaccumulation is an exothermic phenomenon, culminating in the emission of heat to the surrounding environment. Research has shown that increasing the temperature has a negative impact on the ability of biosorption to absorb substances [65]. The increase in entropy reinforces the successful implementation of the procedure, signifying an enhancement in systemic turmoil.

3.11. Exploration of adsorption mechanisms and regeneration strategies

In order to eliminate heavy metals from wastewater, biosorption technologies make use of the metal-binding capabilities of a wide variety of biological substances [66]. These applications leverage metabolically mediated and physico-chemical absorption mechanisms from the biological materials. The procedure involves a complicated interaction of components, which makes it difficult to develop a strategy that is applicable to all situations. For the purpose of metal binding, a number of different mechanisms have been postulated. These mechanisms include coordination, chemisorption by ion exchange, complexation, chelation, and physical adsorption. Despite this, the precise mechanism is still not fully understood, as different biosorbents and species may choose entirely different courses of action. When it comes to acquiring metals, bacteria mainly rely on ion exchange and coordination. However, fungus and algae may rely on surface adsorption or complexation. Certain species might make use of active transport routes in order to integrate metals into their metabolic processes. The potential mechanism is illustrated in Fig. 5.

To evaluate the bacteria's potential for reuse, we implemented five consecutive adsorption–desorption cycles with identical samples (Fig. 6). The microorganisms were separated by centrifugation after the adsorption procedure was completed. Subsequently, the adsorbed ions were eliminated by eluting the isolated bacteria with a diverse array of chemical solutions. Specifically, 25 ml of chemical solutions was administered to samples weighing 0.1 g containing 0.05 M HNO_3 and deionized water. After being handled, the specimens were then placed in cylindrical containers put on a shaker and subjected to a shaking rate of 3000 rpm for a duration of 10 min. The medium's metal ion concentrations were investigated. Two approaches were taken to regenerate the bacteria: the first method involved the exclusive use of distilled water, while the second method involved the use of a 2% sodium hydroxide mixture. The main goal of this renewal operation was to get rid of the positive hydrogen ions as soon as possible, up to the pH hit 5. The reinvigorated bacteria were extensively rinsed with copious quantities of Ultra-pure water to neutralize the pH. Afterward, the bacteria were desiccated at 60°C to facilitate their reutilization in innovative adsorption processes.

As a result of the mechanical stress, chemical alterations, and surface modifications that occur during the regeneration process, repeated adsorption–desorption cycles have the potential to eventually compromise the structural integrity and function of the biosorbent. It is possible that the adsorption capacity of the biosorbent will decrease as a consequence of the active binding sites gradually deteriorating or becoming saturated during the experiment. Additional factors that may have an effect on the efficacy of the biosorbent include exposure to harsh desorption agents or frequent washing, both of which have the potential to alter the structure of the cell wall and the functional groups that are associated with biosorption. Alternative regeneration methods should be studied. Using alkaline solutions (e.g., NaOH), salts (e.g., NaCl, KCl), heating the biosorbent to moderate temperatures (e.g., 100–300°C), enzymes or other biochemical agents that can degrade Cr complexes or restore functional groups on the biomass surface, electrochemical processes, or a combination of treatments may offer a superior regeneration method [67]. The assessment of the cost-effectiveness of the regeneration methods of *B. subtilis* and comparison to other adsorbents is to be done in the future.

The use of *B. subtilis* as a biosorbent for wastewater treatment raises important environmental and biosafety considerations. As a GRAS microorganism, *B. subtilis* has been widely utilized in industrial applications due to its safety profile. However, the large-scale application of microbial biosorbents requires careful assessment of potential risks, including the release of microorganisms into the environment. The introduction of *B. subtilis* into aquatic ecosystems can lead to biofilm formation. This may reduce water flow in natural or man-made systems, provide a niche for opportunistic pathogens, and disrupt the balance of natural microbial communities [68]. Moreover, the production of secondary metabolites by *B. subtilis*, such as antibiotics and enzymes, can inhibit or outcompete indigenous microorganisms, further disrupting the existing microbial equilibrium [69]. In this study, the strain used was not genetically modified, and no antibiotic resistance was detected, reducing concerns about horizontal gene transfer or ecological disruption. However, these risks are relevant and need to be addressed from the beginning. To mitigate environmental risks, closed-loop systems and proper disposal of biosorbents post-treatment are recommended. Future research should focus on evaluating the long-term environmental impact of *B. subtilis*-based biosorbents, including their interaction with native microbial communities and

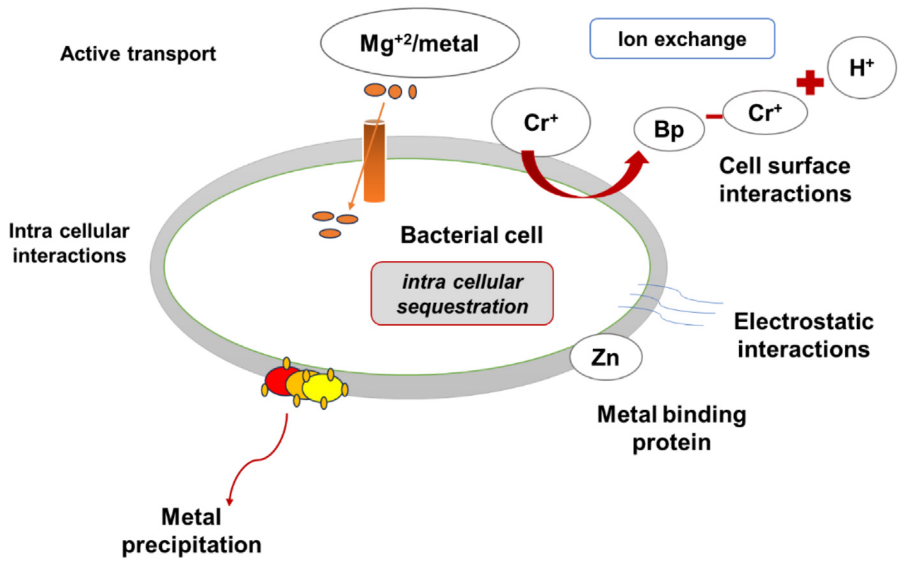

Fig. 5. Possible mechanism of biosorption.

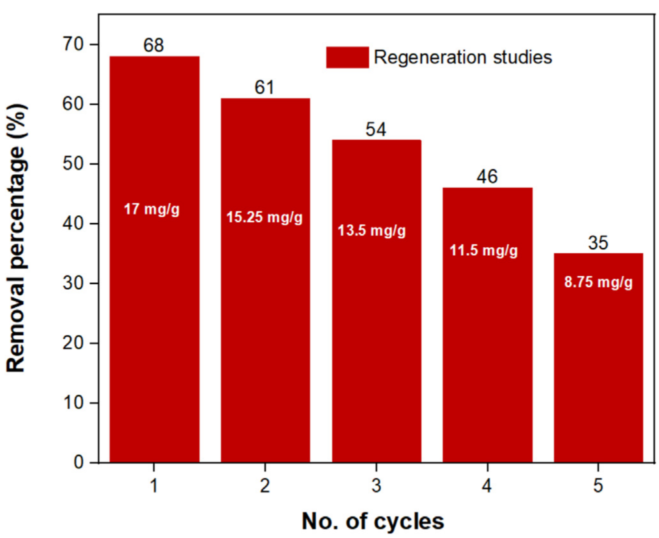

Fig. 6. Regeneration mechanisms: A comprehensive study.

biodegradability. Regulatory frameworks and monitoring protocols will also be essential to ensure the safe and sustainable implementation of microbial biosorption technologies.

4. Conclusions

This study has demonstrated the potential of *B. subtilis* as an efficient biosorbent for the removal of Cr(VI) from synthetic wastewater. The efficiency of biosorption was high, 95%. The optimal conditions were pH 6, a contact time of 60 min, 0.5 g/L of adsorbent dose, and a temperature of 40°C. The efficiency was comparable to many conventional remediation methods, which are often costly and environmentally burdensome. This study offers a framework to develop a sustainable and cost-effective solution for mitigating heavy metal contamination in wastewaters. However, the technique must be studied using real wastewater samples, where the variation of the results will be larger. *B subtilis* performance will vary in different environmental conditions, and thus, several different real samples should be studied. Moreover, conditions that are industrially relevant such as temperature should be studied further. Possibly heat-stabilized or immobilized forms of *B. subtilis* could be used and need to be studied.

The use of advanced characterization techniques, such as Fourier Transform Infrared Spectroscopy Field Emission Scanning Electron Microscopy and X-ray Diffraction, elucidated the biosorption mechanisms at the molecular level, demonstrating the structural and morphological changes in *B. subtilis* due to Cr (VI) adsorption. In the future, X-ray Photoelectron Spectroscopy (XPS) and Nuclear Magnetic Resonance (NMR) studies will offer more mechanistic understanding. Photoelectron Spectroscopy identifies the elemental composition, oxidation states, and chemical bonding environment of surface species. It demonstrates the reduction of toxic Cr (VI) to less toxic Cr(III) and its subsequent adsorption or precipitation on biosorbents. NMR provides information about the local chemical environment and dynamic behavior of atoms in the adsorbent. The reuse potential of the biosorbent was shown to be good when evaluated through five consecutive adsorption–desorption cycles. This improves the sustainability and economy of the treatment. The thermodynamic studies provided further evidence of the spontaneity and feasibility of the biosorption process, reinforcing the practical applicability of this method.

The biosorption process followed the PSO kinetic model more closely than the PFO model, indicating chemisorption as the dominant mechanism. The thermodynamic analysis revealed favorable enthalpy and entropy changes, supporting the biosorption process's endothermic and spontaneous nature. Furthermore, the presence of co-existing ions, such as sodium sulfate or metals, did not significantly interfere with Cr(VI) uptake, underlining the robustness of *B. subtilis* in complex wastewater environments. These findings collectively establish the efficacy and resilience of *B. subtilis* in heavy metal remediation. However, studies using many real wastewater are needed.

The adsorption efficiency achieved surpasses many previously reported biosorbents. For instance, several studies on agricultural by-products or other microbial biosorbents have shown lower adsorption efficiencies and less favorable kinetics for Cr(VI) removal. Moreover, the reuse potential of *B. subtilis* demonstrated here exceeds the operational cycles reported for many alternative materials, highlighting its practical advantage. Additionally, the favorable adsorption performance under relatively mild conditions (pH 6 and ambient temperature) offers an edge over other biosorbents requiring more stringent operational parameters.

In conclusion, this study underscores the promise of *B. subtilis* as a robust, efficient, and sustainable bioremediation agent for Cr (VI) removal. The combination of high adsorption efficiency, reusability, and minimal interference from co-existing ions positions *B. subtilis* as a competitive alternative to synthetic adsorbents and traditional remediation techniques. Future work should focus on scaling up this approach for industrial applications and exploring its efficacy against other toxic metals in real-world wastewater systems, further establishing *B. subtilis* as a cornerstone in the field of bioremediation.

5. Recommendations for further studies

To properly analyze heavy metal biosorption from water settings, precise future suggestions are needed. Column-based experimental equipment is recommended for optimization experiments. The upcoming research will also examine *B. subtilis* implementation issues, commercialization, and how to apply effective remediation procedures on a broad scale and garner universal support. Future research should examine the efficacy of other bacterial strains or mixed microbial consortia to improve bioremediation and Cr(VI) removal in various situations. *B. subtilis* biomass could also be combined with chemical methods such as chemical precipitation or electrocoagulation to achieving synergistic benefits.

The initial chemical precipitation reduces Cr(VI) concentration, decreasing the load on bacterial biomass. Subsequently, the biomass adsorbs residual Cr(VI), achieving lower effluent concentrations than either method alone [70]. Electrocoagulation reduces Cr(VI) levels and produces flocs that can entrap bacterial biomass, enhancing overall Cr(VI) removal efficiency. The biomass further adsorbs Cr(VI), leading to improved purification [71]. Future research should examine *B. subtilis*' environmental impacts and scalability in real-world settings through extensive ecological studies and field experiments. The bacteria's long-term ecological effects, effluent performance, and survivability should also be studied. Future research should focus on prototype research to determine the feasibility and effectualness of *B. subtilis* bioremediation in wastewater treatment environments. *B. subtilis* is non-pathogenic and harmless; however, frequent use in open circumstances can pose biosafety and biosecurity risks. Horizontal gene transfer by *B. subtilis* is a big concern. This communication could spread antibiotic resistance or other problems. Biofilms can colonize surfaces and damage agricultural machinery and water systems. These issues require rigorous risk assessments, environmental monitoring, and containment measures to ensure future field uses.

CRediT authorship contribution statement

Khawla E. Alsamhary: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Conceptualization.

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Declaration of competing interest

The author contends that there certainly are no competing motives to disclose.

Supplementary material

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Data availability

Data will be made available on request.

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