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## Biosorption of hexavalent chromium in aqueous solution by *Bacillus cereus*

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### ABSTRACT

Hexavalent chromium is one of the toxic metals in water pollution. This study is aimed at analyzing the involvement of chromium reductase and biosorption potential in chromium-resistant species of *Bacillus cereus*. A total of 10 % (v/v) of *B. cereus* biomass was inoculated into a 90 mL chromium-contaminated solution with an initial concentration of 60 mg/L. Biomass digestion was carried out every day for a 5-day treatment period for chromium content analysis, while biomass characterization was carried out at the end of the treatment period, comparing the exposed vs. non-exposed bacteria. Results indicated that the highest chromium removal ( $16.12 \pm 0.63$  %) was obtained on day 3, while the maximum biosorption capacity was obtained on day 1, reaching  $0.461 \pm 0.02$  mg Cr/g dry cell of biomass. XRD showed the crystalline structure of the bacteria cell after being exposed to chromium, suggesting that interactions between polysaccharides and proteins in the membrane may occur during the treatment. In addition, FT-IR spectra also showed decreasing peaks and the involvement of hydroxyl, carboxyl, carbonyl, and nitroxide groups during the treatment. SEM-EDX results indicated that bacteria are experiencing cell structure alteration with more intense chromium spectra on the surface, while TEM images showed endospore formation by *B. cereus* under adverse environmental conditions.

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This study suggested that the removal of hexavalent chromium by *B. cereus* might be dominant via biosorption (translocated into cell biomass).

## 1. Introduction

Chromium is one of the most toxic heavy metal contaminants in the environment (Prasad et al., 2021). Most of the chromium pollution originated in the metallurgy, textile, tanning, and electroplating industries (Briffa et al., 2020; Nur-E-Alam et al., 2020; Sahinkaya et al., 2017). Effluent from these industries has led to chromium contamination in surface water, soil, and even groundwater (Mani Tripathi and Chaurasia, 2020; Purwanti et al., 2017b; Xie, 2024). High concentrations of chromium are categorized as carcinogenic, mutagenic, and teratogenic (Dasharathy et al., 2022; Léonard and Lauwerys, 1980). The toxicity of chromium may affect animals, plants, and even humans. Chromium can exist in two oxidation states: trivalent chromium [Cr(III)], and hexavalent chromium [Cr(VI)]. Its toxicity is magnified in the form of Cr(VI) compared to Cr(III) due to its higher reactivity, solubility, and mobility (Purwanti et al., 2017b; Sharma et al., 2022; Zulfiqar et al., 2023).

Some researches had highlighted the efficiency of chemical removal of hexavalent chromium from contaminated environments (Gomes et al., 2018; Oves et al., 2013a; Peng et al., 2019; Sahinkaya et al., 2017). Conventionally, the principle of Cr(VI) removal is to convert it into a less soluble, less reactive, and less mobile form [Cr(III)] and separate it from the contaminated environment using technologies like precipitation (Misganaw et al., 2024), adsorption (Juturu et al., 2024; Naous and Halfadji, 2024), membrane filtration (Peng and Guo, 2020), and electrocoagulation (Aguilar-Ascón et al., 2024). Despite its effectiveness, chemical treatment of chromium in a contaminated environment leaves chemical residues and potential harmful by-products formed from the processes, while physical processes have a relatively high cost (Kurniawan et al., 2022). To overcome this issue, biological treatment of Cr(VI) has become the main choice as it provides an environmentally friendly and sustainable process.

Bacteria are one of the treatment agents that can be used to remove Cr(VI) from various contaminated environments (Kamaruzzaman et al., 2019; Li et al., 2024). Many researchers have utilized bacteria to remove heavy metals, such as mixed heavy metals (Dodbiba et al., 2015; Oves et al., 2013b), Cr (Deepali, 2011; Halmi et al., 2017; Purwanti et al., 2017a; Qu et al., 2014; Sanjay et al., 2018; Shi et al., 2019), Al and Fe (Ismail et al., 2017; Titah et al., 2019), Mn and Pb (Hasan et al., 2016; Subari et al., 2018), and As (Titah et al., 2018), from contaminated environments, especially water, wastewater, and groundwater. In Cr(VI) removal, bacteria perform the same major pathway as chemical removal. The Cr(VI) will undergo some enzymatic reactions and be converted into Cr(III) (Joutey et al., 2015). Bacteria can even perform biosorption, an absorption by bacterial cells, as a complete mechanism to remove chromium from contaminated medium (Kurniawan et al., 2019; Oves et al., 2013a). The utilization of bacteria is considered to be one compact and effective treatment to remove Cr(VI) from contaminated medium.

The enzymatic reaction of Cr(VI) removal mostly involved the chromium reductase enzyme (Joutey et al., 2015). This enzyme plays an important role in utilizing free electrons from Cr(VI) and produces Cr(III) as the result of the process. Some studies provide information on the involvement of chromium reductase in the process of Cr(VI) removal (Duman et al., 2009; Joutey et al., 2015; Sanjay et al., 2018). Indigenous *YZ1* bacteria removed 86 % Cr(VI) from 8.57 mg/L initial concentration (Li et al., 2024). *Bacillus* sp. M6 reduced 45.9 % Cr(VI) to Cr(III) with 200 mg/L initial concentration after 72 h of exposure (Li et al., 2019). *Pseudochrobactrum saccharolyticum* W1 reduced 53.7 % Cr(VI) from 200 mg/L initial concentration after 60 h (Mengke-Li et al., 2019). In another research, *Comamonas acidovorans* removed 6 % of Cr(VI) from 100 mg/L initial concentration after 24 h of exposure. Even though there was much research exploring the removal and reduction of Cr(VI), the clear pathways of reduction and possible removal mechanisms of Cr(VI) are still scattered and lacking.

To fill this gap, this research aimed to (i) deeply analyze the performance of chromium-resistant bacteria of *Bacillus cereus* in the removal of Cr(VI) and (ii) to analyze the chromium biosorption potential from an aqueous solution. The presented results may shed light on the pathways of Cr(VI) reduction to Cr(III) and the possibility of removal mechanisms via oxidation-reduction by bacterial enzymatic reactions and biosorption by bacterial biomass.

## 2. Materials and method

### 2.1. Source of bacteria

One bacterial species of *Bacillus cereus* used in this study was isolated from the rhizosphere of *Scirpus grossus* in previous research conducted by Kamaruzzaman et al. (Kamaruzzaman et al., 2019). The isolated *B. cereus* was proven to have the ability to resist and reduce chromium. A fresh single culture of *B. cereus* was used in every step of this research.

### 2.2. Reagent and medium preparation

A total chromium stock solution was prepared by diluting 1 g of  $K_2Cr_2O_7$  (SYSTEM, Malaysia) in 1 L of distilled water and mixing completely until a homogenous solution was achieved. A certain concentration of spiked medium was prepared by diluting the 1000 mg/L of chromium stock solution with distilled water until the required concentration was achieved.

Bacterial inoculum was prepared by cultivating *B. cereus* overnight at 37 °C in trypticase soy broth under 150 rpm agitation (PROTECH S1–100D, Taiwan). Bacterial cells were harvested after reaching the lag phase using the 4000 rpm centrifuge for 15 minutes

(Eppendorf, Germany). Bacterial cell pellets were washed using a physiological solution (NaCl 8.5 %) to remove any residues. The initial inoculum of bacteria used in all stages of this research was  $OD_{600\text{ nm}} = 0.5 \text{ \AA}$ .

### 2.3. Experimental set up for biosorption process

Biosorption analysis was prepared using a chromium aqueous solution with an initial concentration of 60 mg/L. This concentration was selected based on a study carried out by Kamaruzzaman et al. (Kamaruzzaman et al., 2019), who found that this concentration was still tolerable by *B. cereus*. A 10 % (v/v) (10 mL) initial bacterial inoculum was inoculated into 90 mL of chromium-contaminated medium in a 250 mL Erlenmeyer reactor. All preparation, dilution, and mixing of solutions were conducted under aseptic conditions. There were 3 Erlenmeyer reactors representing treated reactors and 2 other reactors for control (for chromium solution without bacteria and for bacteria without chromium). All reactors were incubated for 5 days of exposure at 37 °C and 150 rpm in an incubator shaker (PROTECH S1–100D, Taiwan). On each sampling day, a total destruction approach was adopted, in which one reactor was taken daily and centrifuged at 4000 rpm for 15 minutes. The pH of the supernatant was also monitored daily by using a pH Meter (Hach Sensation+, USA). The supernatant was analyzed for chromium content, and the pellet was digested for chromium content analysis.

### 2.4. Analysis of total chromium

The total chromium was measured daily using an atomic absorption spectrophotometer (AAS) (Perkin Elmer, Analyst 800, Germany) in the solution and also inside the digested bacterial pellets. Pellet cells were dried in a 105 °C oven for 24 hours, then diluted with 10 mL of a 1:1:3 (HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub>:deionized water solution) for 24 hours at room temperature. The digested chromium concentration inside bacterial pellets was also measured using AAS.

### 2.5. Analysis of biomass under FTIR, XRD, SEM-EDX-Mapping and TEM

Bacterial pellets were dried and sent for Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analysis using the Nicolet 6700 FT-IR spectrometer (Thermo Scientific, U.S.) and the D8 Advance X-ray diffractometer (Bruker, U.S.), respectively. FTIR analysis was carried out to evaluate the shift in functional groups after Cr exposure.

The scanning electron microscopy (SEM) and the energy dispersive X-ray diffraction (EDX) analyses were conducted using a Field Emission Scanning Electron Microscope (FE-SEM) (Zeiss Model Supra VP, Germany). The purpose of this analysis was to analyze the existence of chromium on the surface of bacterial cells. Examination of bacterial cells was conducted after fixation and vacuum freeze drying. Fifty mL of bacterial culture was centrifuged at 4000 rpm for 10 minutes. The pellet of bacterial cells was fixed for 24 hours in 4 % glutaraldehyde solution, followed by a graded series of ethanol dehydration (30, 50, 70, 80, 90, and 100 %, 10 minutes for each concentration). The fixed pellet was dried using an automated critical point dryer (Leica EM CPD300, Singapore) under a CO<sub>2</sub> atmosphere for 30 minutes. A fixed pellet was mounted on a stainless-steel stab, and a thin layer of platinum was placed on the surface as a cover under vacuum conditions. The analytical condition parameters during analysis were backscattered electron mode, 15,000 × magnification, and 15 kV beam voltage.

The transmission electron microscopy (TEM) analysis was conducted using a transmission electron microscope (TEM) (Thermo Fisher Model Talos 120 C, U.S.). The purpose of this analysis was to give an intra-cellular structure visualization of Cr(VI) exposure to bacterial cells (Winey et al., 2014). Bacterial cells were chemically fixed using glutaraldehyde for 12–24 hours at 4 °C, followed by osmium tetroxide for 2 hours at 4 °C. Fixed cells were then dehydrated by a graded series of ethanol, i.e., 30, 50, 70, 80, 90, and 100 % (10 minutes for each concentration). Samples were then infiltrated with several ratios of the ethanol-resin mixture (1:1 for 1 hour, 1:3 for 2 hours, 0:1 overnight, and 0:1 for 2 hours). Samples were then embedded into a beam of plastic, filled with resin, and then polymerized in a 60 °C oven for around 24–48 hours. Samples were ultrathin sectioned with a size of 60–90 nm and stained using uranyl acetate replacement for 15 minutes and lead citrate for 10 minutes, followed by rinses with water.

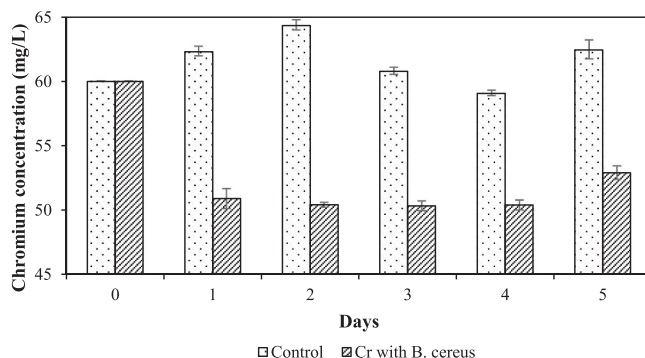


Fig. 1. Remaining Cr in solution during treatment by *B. cereus*.

## 2.6. Statistical analysis

All statistical analysis was conducted using Minitab 16. Tukey's HSD was used to rank the data and further analyze the significant differences between the obtained results (Kurniawan and Imron, 2019a, 2019b). All statistical evaluations and conclusions were evaluated at a 95 % confidence interval under  $\alpha = 0.05$ . All sampling and analysis were conducted in triplicate, and the data was presented as the mean  $\pm$  SD.

## 3. Results and Discussion

### 3.1. Elimination and recovery of Cr

The result showed that the chromium removal fluctuated throughout the 5-days incubation period. The remaining chromium inside reactors is depicted in Fig. 1. The lowest chromium remaining in solution was obtained after 3 days of incubation, in accordance with the highest removal of chromium. After 3 days of incubation, the remaining chromium in the solution showed a value of  $50.33 \pm 0.38$  mg/L, while the removal percentage reached  $16.12 \pm 0.63$  %. After day 3, the remaining chromium in the solution was increased, reaching the final concentration of  $52.90 \pm 0.52$  mg/L at the end of the test period. This result showed that 3 days of incubation was the optimum treatment time for chromium removal by *B. cereus*. The increase in chromium concentration from day-4 to day-5 was expected to occur due to bacteria forming endospores (based on the result of TEM analysis). Endospores are formed due to a lack of nutrients and extreme environmental conditions (Hao et al., 2010; Li et al., 2014). When endospore formation occurs, the bacteria cell is under stress and undergoes cell lysis (Decker and Ramamurthi, 2017), resulting in an unstable condition of the cell that can initiate the release of sorbed heavy metal back to the living medium (Imron et al., 2019a). During the endospore phase, bacteria might produce enzymes to exploit alternative resources (Liu et al., 2004).

Bacteria capable of performing biosorption were also assessed, and the results of this analysis are presented in Fig. 2. It can clearly be seen that the biosorption capacity ( $q$ ) of *B. cereus* cells was increased from day 0 to day 1, reaching up to  $0.461 \pm 0.02$  mg Cr/g dry cell weight. On days 2–4, a decrease in biosorption capacity occurred. The decrease in biosorption capacity was suspected to be related to the unstable condition of bacterial cells inside the reactor due to the occurrence of endospores (Li et al., 2014). On day 5, the result of biosorption capacity increased again, up to  $0.131 \pm 0.00$  mg Cr/g dry cell weight. Bioaccumulation can occur with the help of living cells and dead cells. Some researchers demonstrate the capability of dead cells in performing passive biosorption of metals and show even greater capability when compared to living cells (Dadrasnia et al., 2015; Diep et al., 2018; Li et al., 2018). The result of biosorption capacity on day 5 confirmed that the biosorption of chromium was not only performed by living *B. cereus* cells but also occurred due to the passive biosorption by dead *B. cereus* cells. A comparison with previous studies is summarized in Table 1. Based on data presented, Cr removal performance by *B. cereus* in this study was considered to be low. This result might be obtained due to the use of pure Cr(VI) solution without any additional carbon and nutrient source in the medium. Further optimization can be carried out to enhance the removal capability of *B. cereus*, as discussed further in Section 4.

### 3.2. XRD analysis

The state changes in *B. cereus* membrane molecules were validated using x-ray diffraction (XRD) analysis. Fig. S1(a) represents the *B. cereus* membrane surface without being exposed to Cr showed a broad XRD peak, which indicates the amorphous state of the bacterium membrane. In contrast with Fig. S1(a), Fig. S1(b) represents the *B. cereus* membrane surface after being exposed to Cr for one day. Fig. S1(b) showed sharp and intense peaks, indicating the presence of a crystalline structure molecule, in this case, Cr, on the *B. cereus* membrane surface. A comparison between these two figures indicates that there are changes in *B. cereus* membrane state before and after being exposed to Cr. The crystalline peak profile may suggest that there is an interaction between Cr, polysaccharides, and protein molecules on the bacterial membrane surface (Biswas and Paul, 2020). However, this interaction is not an assurance that

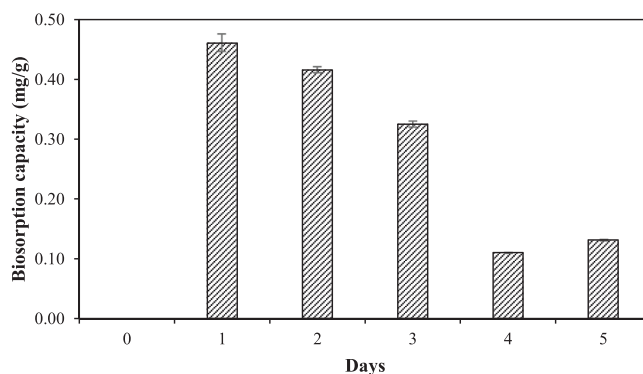


Fig. 2. Biosorption of Cr by *B. cereus*.

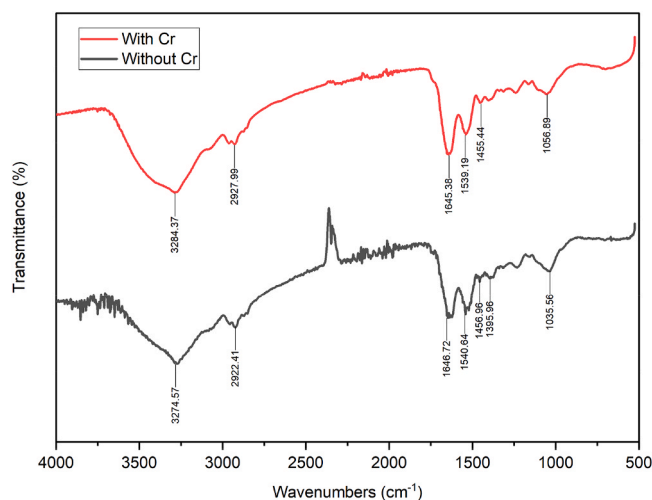
**Table 1**  
Total Cr removal by various bacterial species.

Species	Initial Cr concentration (mg/L)	Removal (%)	Adsorption capacity (mg/g)	Exposure time (hour)	Proposed removal mechanisms	Reference
<i>Bacillus cereus</i>	60	16.12	0.46	72	Oxidation-reduction, biosorption	This study
<i>Bacillus cereus</i> strain PY3	100	99	-	24	Oxidation-reduction, biosorption	(Wani et al., 2024)
<i>Bacillus endophyticus</i>	100	50	-	120	Oxidation-reduction, extracellular precipitation	(Panneerselvam et al., 2013)
<i>Bacillus pumilis</i> CrK08	200	51	-	24	Oxidation-reduction	(Rehman and Faisal, 2015)
<i>Comamonas acidovorans</i>	100	6	-	24	Biosorption	(Rudakiya, 2013)
<i>Pseudomonas stutzeri</i>	1900	-	27.47	120	Biosorption	(Yaashikaa et al., 2019)
<i>Trichoderma koningiopsis</i> LBM 253	200	23	-	96	Biosorption, extracellular and intracellular reduction	(Tatarin et al., 2024)

the reduction of Cr occurs on the membrane surface. The Cr interaction and intake mechanism into the cell can be validated using FTIR analysis, where changes in the bacteria membrane functional group can suggest a binding interaction between Cr and bacteria membrane biomolecules (Biswas and Paul, 2020). On the other hand, biotransformation of the Cr(VI) ion to Cr(III) on the bacteria membrane surface showed an amorphous XRD pattern during chromium exposure. Analysis of sludge bacteria showed that although biotransformation of Cr(VI) occurs on the membrane, the XRD peak profile showed no significant difference between pre- and post-treatment. This suggests that the transformed Cr(VI) was bound to the bacteria's membrane biomolecules and no longer exists in its crystal form (Jin et al., 2017). A similar finding was mentioned for the bacterium strain *Bacillus* sp. (CSB-4), where the reduced Cr (VI) showed no significant difference in the XRD pattern (Dhal et al., 2010). In summary, the XRD peak pattern in this study showed that there is a possibility for Cr in the system to interact with the *B. cereus* membrane. However, binding between Cr and membrane biomolecules is yet to be determined, as more validation analyses are required.

### 3.3. FTIR analysis

FTIR was conducted to analyze and identify the functional groups of bacteria involved in the biosorption process. The result of the FTIR analysis was presented in Fig. 3 and Table 2. The result showed that the functional groups on the surface of bacterial cells with or without Cr exposure contained hydroxyl, carboxyl, nitroxides, and carbonyl groups. Similar to Nguema et al. (Nguema et al., 2014), carboxyl, amide, and hydroxyl groups are functional groups in *B. cereus* that play a role in the Cr biosorption process. According to Fig. 3, Cr exerts an effect on the change and loss of functional groups on the bacterial cell surface. The disappearance of the peak and the displacement of the peak indicate a biosorption process between the functional groups and Cr (Shroff and Vaidya, 2012). Based on Fig. 3, peak 1035.56 (C-O stretching as a carbonyl group) shifted to peak 1056.89 after exposure to Cr. In addition, peak 1540.64 (N-O stretching as nitro compounds) on the cell surface also shifted to 1539.19, indicating that this functional group also plays a role in the



**Fig. 3.** FT-IR analysis of *B. cereus* in control (without Cr) and exposed in Cr.

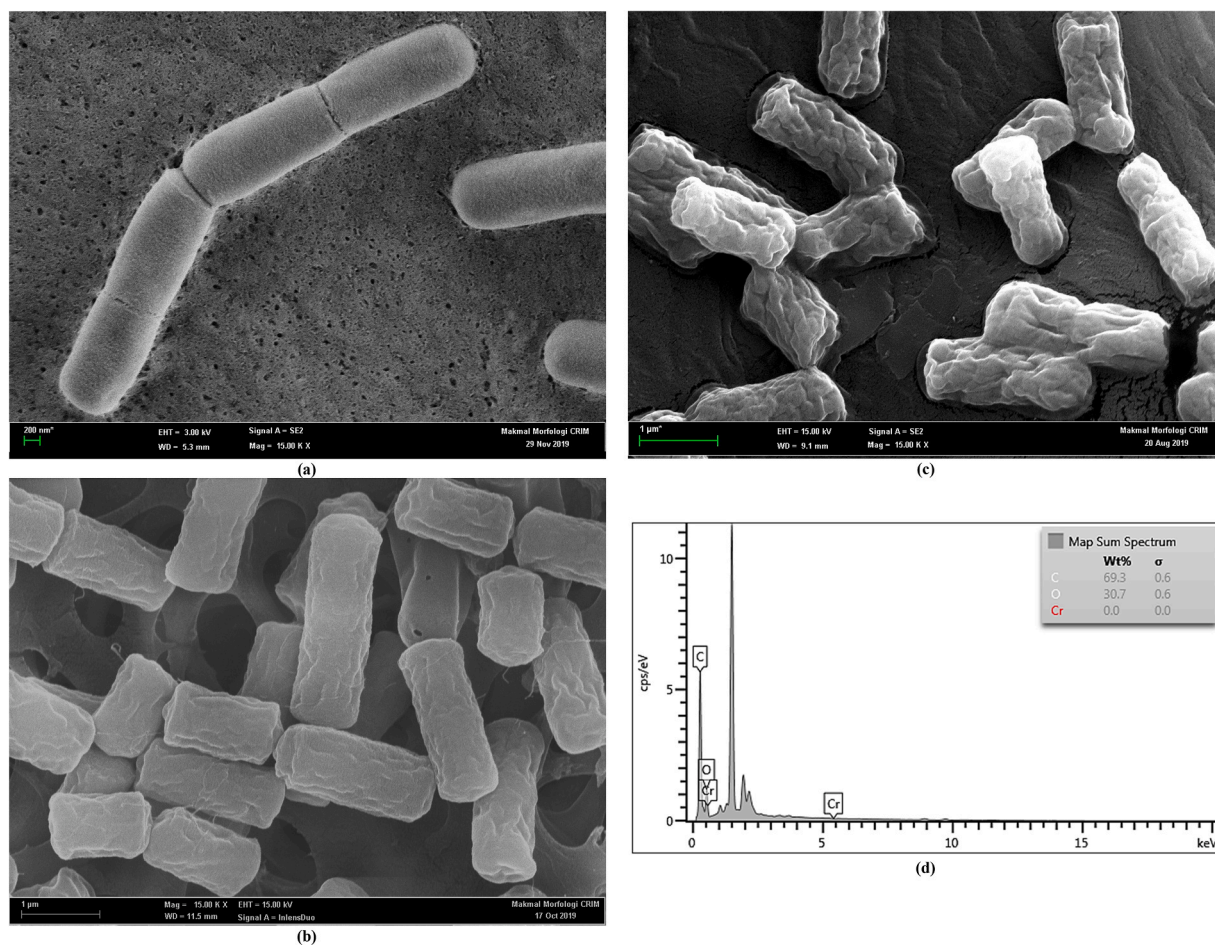
**Table 2**  
Functional groups of *B. cereus* in control (without Cr) and exposed in Cr.

Wavenumbers		Appearance	Functional groups	Compounds
Before	After			
3274.57	3284.37	strong, broad	O-H stretching	Carboxylic acid
2922.41	2927.99	medium	C-H stretching	Alkane
1646.72	1645.38	strong	C=C stretching	Alkene
1540.64	1539.19	strong	N-O stretching	Nitro compound
1456.96	1455.44	medium	C-H bending	Alkane (methyl group)
1395.96	-	medium	O-H bending	Carboxylic acid
1035.56	1056.89	strong	S=O stretching	Sulfoxide

metal biosorption process. Similar to Yokoyama et al. (Yokoyama et al., 2013) and Yokoyama et al. (Yokoyama et al., 2012), the reaction between nitro compounds and Cr can form chromium crystals, and it is also evident from the XRD results that there are crystal molecules on the surface of bacteria. Nguema et al. (Nguema et al., 2014) also reported that the shift peak also occurs at the amino groups because of interactions between the nitrogen of the amino group and the Cr(VI) ions. In conclusion, based on previous studies, the functional groups involved in the Cr biosorption process were hydroxyl, carboxyl, carbonyl, and nitroxide groups (Nguema et al., 2014), suggesting that this research obtained the same result.

### 3.4. Microscopic examination of biomass

The surface morphology of *B. cereus* was performed by FESEM. It was done for *B. cereus* in nutrient broth, control, and Cr medium to



**Fig. 4.** SEM micrographs at magnification of 15,000  $\times$  for *B. cereus* (a) in nutrient broth, (b) in control (without Cr) and (c) in Cr medium and EDX micrographs at magnification of 15,000  $\times$  for *B. cereus* (d) in control (without Cr) and (e) in Cr medium; and mapping for Cr (f) in control (without Cr) and (g) in Cr medium.

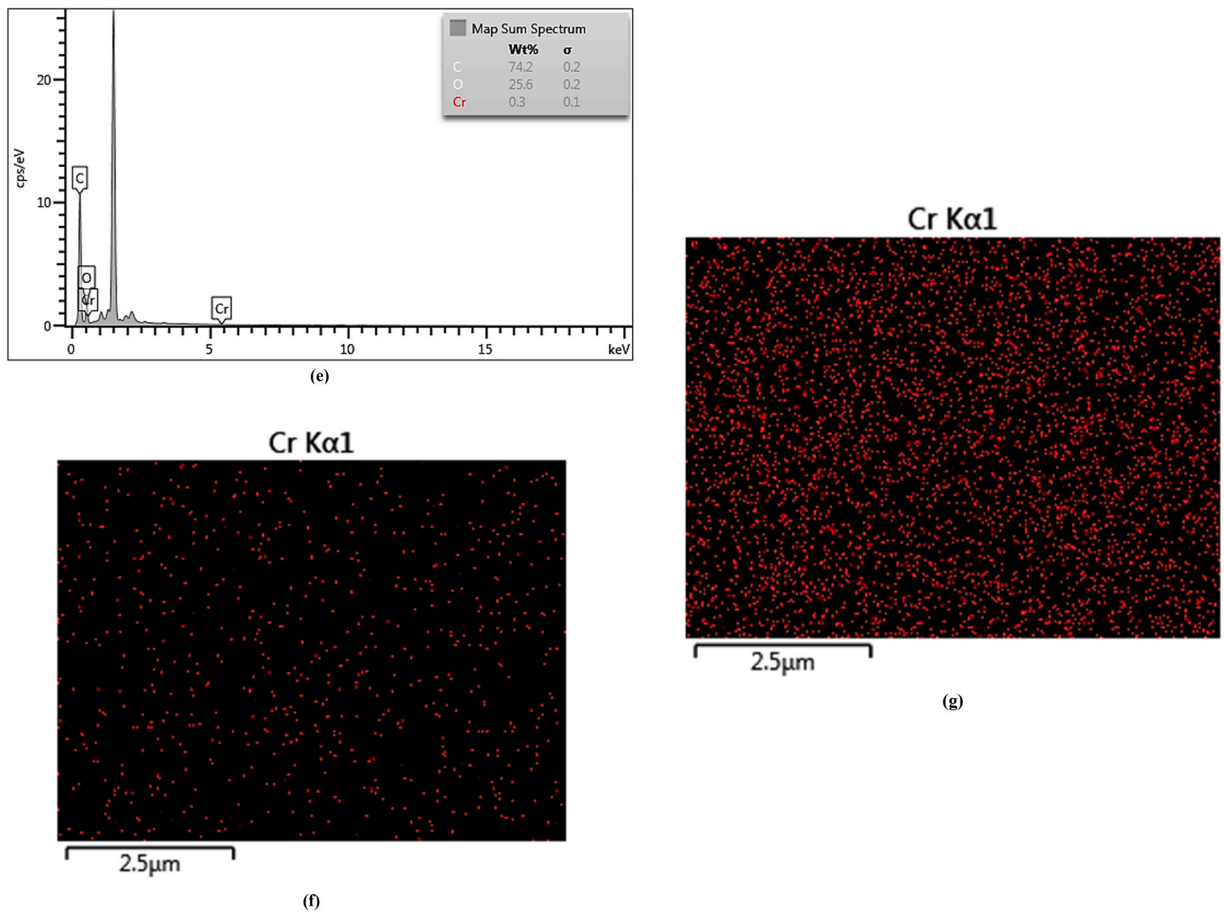
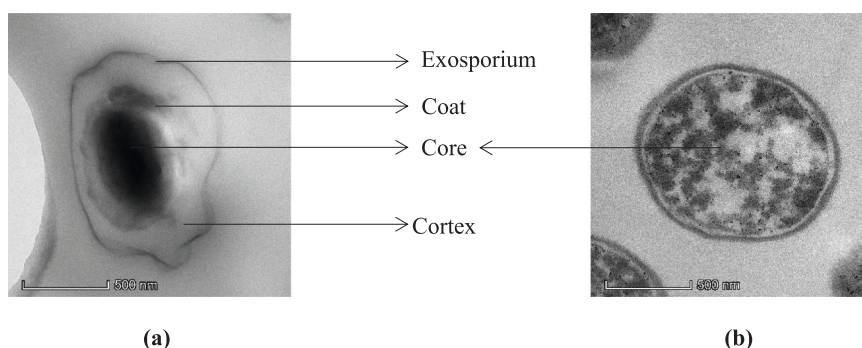


Fig. 4. (continued).

observe the Cr effects on the surface of cells. SEM micrographs of *B. cereus* at 15,000  $\times$  magnification in nutrient broth (Fig. 4a), in control (Fig. 4b), and in Cr medium (Fig. 4c) The *B. cereus* was rod-shaped and was much smoother in nutrient medium when compared with cells in control and in Cr medium. The absence of nutrients in control and the presence of Cr affects the morphology of *B. cereus*, where the cells are shrunk and have a wrinkled surface. These effects are more severe for *B. cereus* exposed to Cr in the medium. This pattern was similar to other findings showing changes in membrane cell surface after exposure to Cr(VI) (Hossan et al., 2020; Xu et al., 2014). The wrinkle on the *B. cereus* surface was probably due to the chemical interaction between metal ions and biomolecules on the membrane outer layer. Another possibility is due to the changes in the bacteria's envelope constituent during its growth period in the presence of heavy metals (Hemambika and Kannan, 2012).

In addition, an increase in the length of *B. cereus* was observed after Cr exposure. Cells without Cr exposure had an average length of 1.951  $\mu\text{m}$ , while cells exposed to Cr had an average length of 2.0187  $\mu\text{m}$ . The same results were also observed when *Sinorhizobium* sp.

Fig. 5. TEM images at scale of 500 nm for *B. cereus* (a) in control and (b) in Cr medium.

SAR1 was exposed to Cr (Jobby et al., 2019). EDX and mapping provided confirmation regarding the elemental attributes of biomass. This analysis confirmed the Cr signals in their respective spectra of biomass exposed to Cr medium (0.3 wt%), while the spectrum of biomass in control did not show such signals (0.0 wt%) (Fig. 4e and Fig. 4d, respectively). Moreover, mapping of Cr on the surface of biomass, represented as red dots, supported the findings that the presence of Cr was more intense in biomass with Cr medium (Fig. 4g) as compared with the control (Fig. 4f).

The TEM analysis of the cross section of *B. cereus* was carried out to monitor any changes in the cells before and after 5 days of Cr introduction. Fig. 5a and Fig. 5b demonstrated the inner cells in control (minimal media without Cr) and in minimal media in the presence of Cr. Both images show the endospore of *B. cereus* formed in response to adverse environmental conditions such as a lack of nutrients for *B. cereus* in control and the presence of toxic Cr for *B. cereus* in Cr medium. Fig. 5a shows the endospore of *B. cereus* in control with a clear endospore structure labeled as exosporium, coat, core, and cortex (Ali et al., 2017; Lv et al., 2019). In contrast with Fig. 5b, *B. cereus* endospore exposed to Cr medium does not contain exosporium and cortex, with the core of the endospore exposed to Cr medium burst and expand. At this stage, the exposed core indicated an initial stage of vegetative division. During the initial cell vegetative division, the endospore, exosporium, and coat will diffuse, exposing the core to the environment. This latter will be followed by the binary diffusion of the core and the formation of a new cell (Abel-Santos, 2015). This study suggests Cr from the environment was translocated into the *B. cereus* cell. At certain Cr concentrations, *B. cereus* cells showed intolerance towards Cr toxicity, and cell lysis started to occur. During this process, *B. cereus* endospores start to form. After 5 days of exposure, endospores start to germinate and are ready to undergo vegetative division or binary diffusion.

#### 4. Practical applications and future research prospects

The use of bacterial biomass as an adsorbent has been carried out by many researchers. Some surface modification can also be conducted to enhance its adsorption performance (Pradhan et al., 2017). In terms of practical application, the production of dried *B. cereus* biomass can be conducted to obtain ready-to-be-used adsorbent for Cr-contaminated water treatment using a column reactor (Igwegbe et al., 2021). Future research can also focus on the regeneration of dried biomass after saturation/breakthrough point is achieved.

Discussing the improvement of overall Cr removal, further optimization of Cr biosorption tests can be conducted, focusing on adjustment of environmental conditions such as pH (Kurniawan et al., 2018), carbon source (Imron et al., 2019b), nutrient source (Kurniawan et al., 2022), and temperature (Dampang et al., 2021). Since biosorption was involved, the ratio of initial biomass concentration optimization can also be conducted to increase the removal performance (Dhal et al., 2018).

#### 5. Conclusions

*Bacillus cereus* showed good potential for treating hexavalent chromium in an aqueous solution. Maximum removal of  $16.12 \pm 0.63\%$  was obtained on day 3, while maximum absorption capacity of  $0.461 \pm 0.02$  mg Cr/g dry cell of biomass was obtained on day 1 of the treatment. Results showed the involvement of hydroxyl, carboxyl, carbonyl, and nitroxide groups during the treatment, as well as the presence of crystalline structure molecules of Cr on the bacterial membrane surface, suggesting the occurrence of oxidation-reduction mechanisms. Biosorption of chromium was evidenced by SEM-EDX and TEM imaging, with physical and internal structure alteration indicated by the formation of endospores during the treatment. Further research can be conducted to optimize the environmental conditions to obtain higher Cr removal, focusing on the carbon source, nitrogen source, and temperature.

#### CRedit authorship contribution statement

**Azimah Ismail:** Resources. **Hajjar Hartini Wan Jusoh:** Resources. **Setyo Budi Kurniawan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Data curation. **Israa Abdulwahab Al-Baldawi:** Methodology. **Siti Rozaimah Sheikh Abdullah:** Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Mohd Aidil Kamaruzzaman:** Methodology. **Nur Izzati Ismail:** Writing – original draft, Investigation, Formal analysis, Data curation. **Muhammad Fauzul Imron:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Ahmad Razi Othman:** Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Hassimi Abu Hasan:** Supervision, Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eti.2025.104015](https://doi.org/10.1016/j.eti.2025.104015).

### Data availability

Data will be made available on request.

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