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Biosorption of chromium by live and dead cells of *Bacillus nitratireducens* isolated from textile effluent

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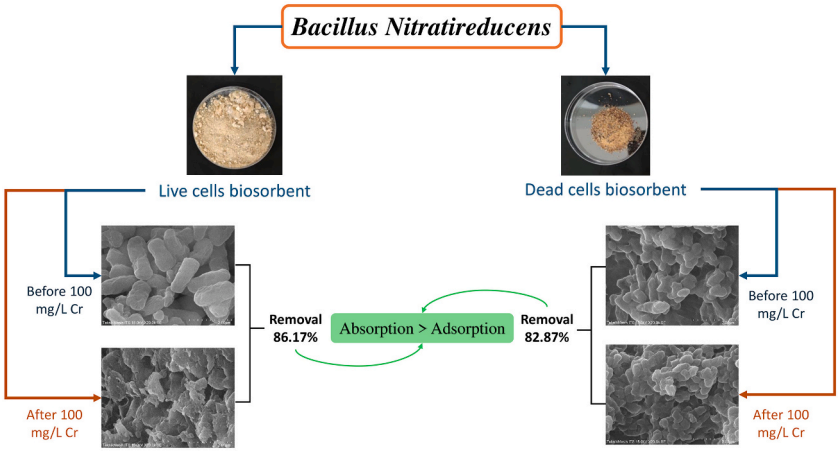
HIGHLIGHTS

- Live and dead cells of *Bacillus nitratireducens* performed biosorption of Cr.
- Live cells showed higher adsorption capacity.
- Active and passive mechanisms suggested to occur in live cells.
- Absorption > adsorption for both biosorbents.
- Maximum Cr removal >80% for both biosorbents.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacillus nitratireducens was isolated from textile effluent and showed high tolerance to chromium (Cr), reaching up to a 1000 mg/L MIC value. This research was aimed at utilizing biosorbents from live and dead cells of *B. nitratireducens* to remove Cr from an aqueous solution. A batch biosorption test was performed, and mechanisms analysis was approached by an adsorption-desorption test, SEM-EDS, and FTIR analysis. Cr removal by dead cells in 25, 50, and 100 mg/L of Cr were 58.99 ± 0.7%, 69.8 ± 0.2%, and 82.87 ± 0.11%, respectively, while that by live cells was 73.08 ± 1.9%, 80.27 ± 6.33%, and 86.17 ± 1.93%, respectively. Live cells showed significantly higher Cr removal and adsorption capacities as compared to dead cells. In all concentrations,

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Wastewater treatment

absorption contributed more than adsorption to the Cr removal by both live and dead cells. Absorption of Cr was subjected to occur due to passive mechanisms in dead cells while involving some active mechanisms in live cells. SEM-EDS confirmed the detection of Cr on the cell surface, while FTIR revealed the shifting of some peaks after the biosorption test, suggesting interactions between Cr and functional groups. Further TEM analysis is suggested to be conducted as a future approach to reveal the inner structure of cells and confirm the involvement of absorption mechanisms.

1. Introduction

Chromium (Cr) pollution generally comes from industrial activities such as the metallurgical industry to prevent corrosion, the chemical industry, dyes and paints, the heat-retaining materials industry as a material for making various types of steel, and the coal burning process (Anastopoulos et al., 2022; Ginting et al., 2023; Safitri et al., 2021). Cr pollution is reported in water bodies and also on land. Suteja et al. (2020) reported that Cr pollution reached 7 mg/L in the coastal area of Bali, Indonesia, and up to 24.6 mg/kg in the sediment. According to Bharagava and Mishra (2018), Cr pollution originated from tannery effluent in surface water and reached 5.7 mg/L. Mohanty et al. (2023) also reported Cr pollution in surface water in Sukinda Valley, India, with a value of 3.4 mg/L and in groundwater with a concentration of 0.6 mg/L, while the permissible limit is below 0.05 mg/L.

Trivalent chromium [Cr(III)] is classified as a dangerous contaminant for the environment and requires further treatment (Purwanti et al., 2017a; N.N. Ramli et al., 2023). Cr(III) levels in surface water and soil increased as many industries utilized Cr as a raw material (Ighalo et al., 2022b; Kurniawan et al., 2019; Purwanti et al., 2017b). Pushkar et al. (2021) mentioned that Cr(III) binds with nucleotide bases in DNA within the cell, resulting in genotoxic consequences. El-Shahawi et al. (2008) also reported the rapid kinetic change of Cr from trivalent to hexavalent, even when weak oxidants are present.

Efforts to reduce Cr levels are carried out by various methods, namely physical, chemical, and biological methods. Generally, Cr processing is carried out using physical and chemical methods due to the fact that it is fast and reliable (Choi et al., 2018; Faridah et al., 2018; Ighalo et al., 2023; Orooji et al., 2021). Even though it is fast, there are drawbacks to using this method, including the trace chemicals left as waste that can pollute the environment further (Ighalo et al., 2024; Oginawati et al., 2021; Purwanti et al., 2018; N. N. N.N.N.N. Ramli et al., 2023). Biological methods offer an alternative greener approach for treating Cr. Biological methods were proven to show good performance with less chemical residue that might pollute the environment (Ighalo et al., 2022a; Kurniawan et al., 2018; Titah et al., 2019; Wibowo et al., 2023).

Biosorption is one of the biological processes that utilizes live and/or dead microorganisms to remove heavy metals (Kurniawan et al., 2022; Qian et al., 2020; Saha and Orvig, 2010). Various microorganisms, such as bacteria, yeast, algae, and fungi, can be used as biosorbents (Ayele and Godeto, 2021; Elahi et al., 2020; García et al., 2016; Wibowo et al., 2024). Several factors influence the Cr biosorption process: initial Cr concentration, pH, contact time, temperature, biosorbent characteristics, and the presence of other ions (Mangwandi et al., 2020; Pradhan et al., 2019).

Ramachandran et al. (2022) reported the performance of living cells of *Bacillus amyloliquefaciens,* reaching up to 79.90% Cr removal after 60 min of contact time. In another study, Elahi et al. (2022) mentioned that the live cells of *Bacillus cereus* b-525k removed up to 99% Cr after 8 days of treatment. The dead and live cells of *Bacillus sphaericus* were reported to biosorp Cr up to 44.5% and 22%, respectively (Velásquez and Dussan, 2009). To the best of our knowledge, the use of *Bacillus nitratireducens* for Cr biosorption is currently limited. A performance comparison between live and dead cells of this species in Cr biosorption is rarely even reported. To fill this gap, this research is aimed at comparing the

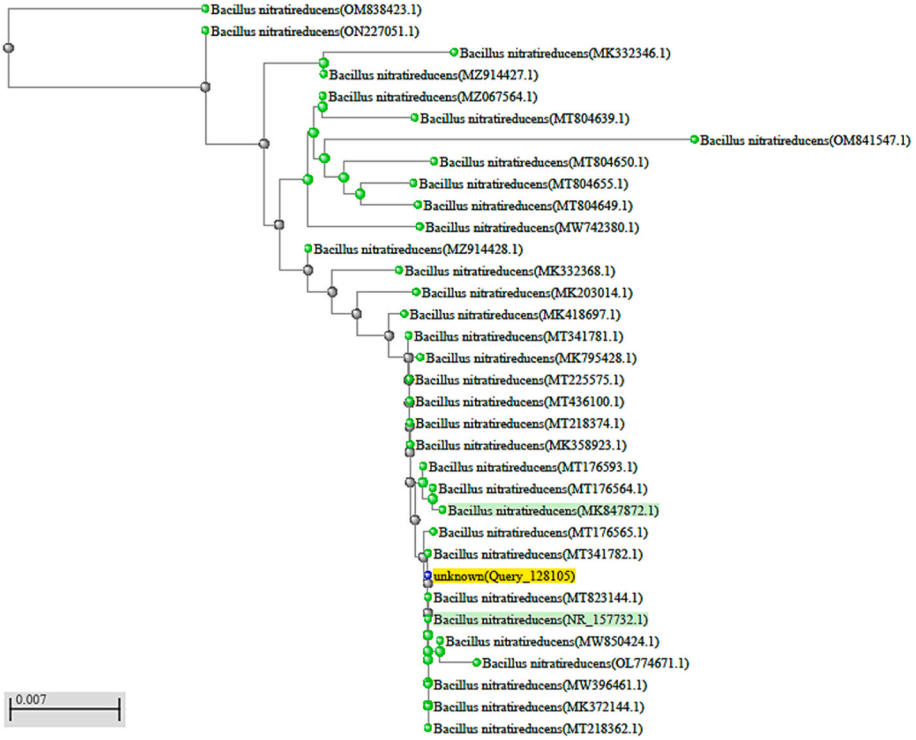


Fig. 1. Constructed phylogenetic tree for the isolated bacteria.

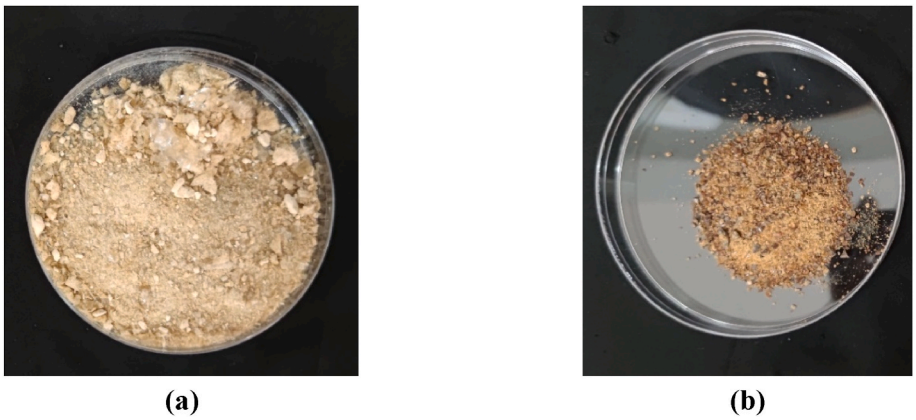


Fig. 2. Physical appearance of (a) live and (b) dead cells biosorbent of *Bacillus nitratireducens*.

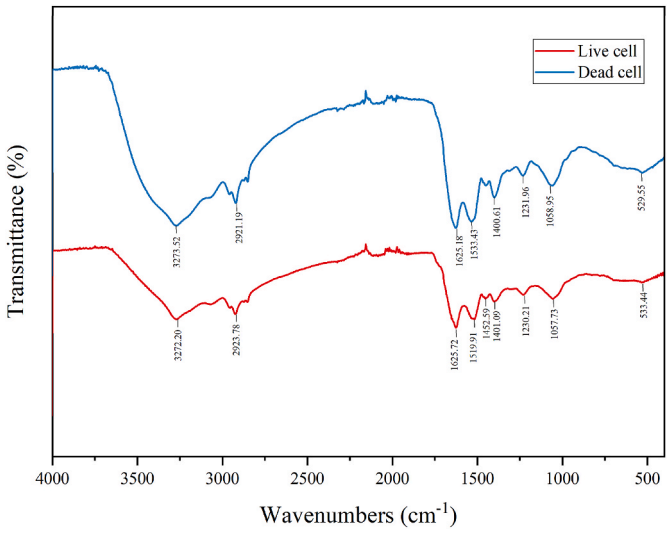


Fig. 3. FTIR of live and dead cells biosorbents.

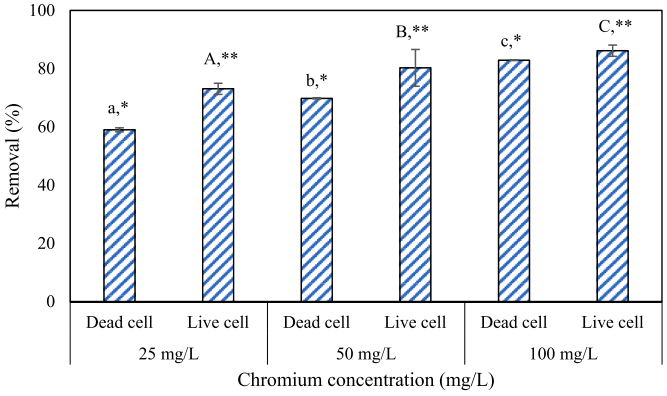


Fig. 4. Removal of Cr during biosorption tests by live and dead cells of *Bacillus nitratireducens. The data* is presented as mean ± standard deviation. Letters a, b, and c indicate significant differences in Cr removal by dead cells among different concentrations. Letters A, B, and C indicate significant differences in Cr removal by live cells among different concentrations. The asterisks (* and **) indicate significant differences in Cr removal between live and dead cells in the same concentration.

Table 1
Functional groups of biosorbent.

No	Wavenumbers (cm^{-1})		Functional groups	Compound class	Appearance
	Live cell	Dead cell			
1	3272.20	3273.52	O–H stretching	Carboxylic Acid	Strong, Broad
2	2923.78	2921.19	C–H stretching	Alkene	Medium
3	1625.72	1625.18	C═C stretching	Conjugated Alkene	Medium
4	1519.91	1533.43	N–O stretching	Nitro Compound	Strong
5	1452.59	–	C–H bending	Alkane	Medium
6	1401.09	1400.61	S═O stretching	Sulfonyl Chloride	Strong
7	1230.21	1231.96	C–N stretching	Amine	Medium
8	1057.73	1058.95	C–O stretching	Primary Alcohol	Strong
9	533.44	529.55	C–I stretching	Halo Compound	Strong

performance of live and dead cells of *B. nitratireducens* in Cr biosorption, focusing on the biosorbent's physicochemical characteristics changing after treatment to reveal possible mechanisms. The presented result is expected to contribute to the knowledge of the biosorption of metals as well as to provide an alternative option for Cr-containing wastewater

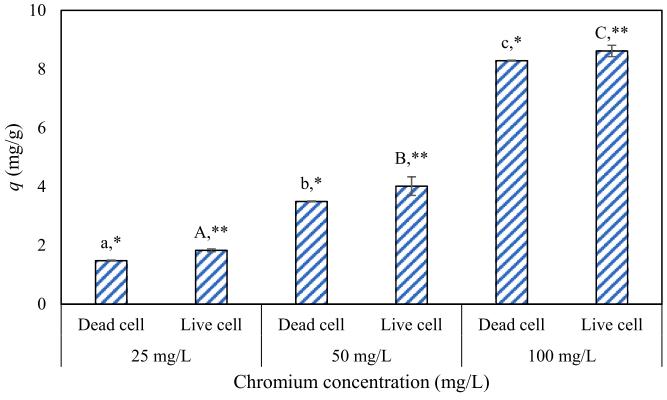


Fig. 5. Cr adsorption capacity during biosorption tests by live and dead cells of *Bacillus nitratireducens*. The data is presented as mean ± standard deviation. Letters a, b, and c indicate significant differences in the adsorption capacity of dead cells among different concentrations. Letters A, B, and C indicate significant differences in the adsorption capacity of live cells among different concentrations. The asterisks (* and **) indicate significant differences in adsorption capacity between live and dead cells in the same concentration.

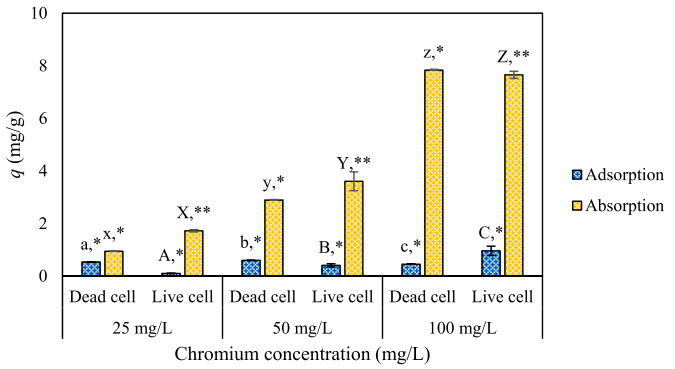


Fig. 6. Adsorption and absorption during biosorption tests by live and dead cells of *Bacillus nitratireducens. The data* is presented as mean ± standard deviation. Letters a, b, and c indicate significant differences in the adsorption by dead cells among different concentrations. Letters A, B, and C indicate significant differences in adsorption by live cells among different concentrations. Letters z, y, and z indicate significant differences in the absorption by dead cells among different concentrations. Letters X, Y, and Z indicate significant differences in absorption by live cells among different concentrations. The asterisks (* and **) indicate significant differences in adsorption or absorption between live and dead cells in the same concentration.

treatment. This research also contributes to Sustainable Development Goals (SDGs) 12 and 14 to create responsible consumption and production and maintain the quality of life below water.

2. Materials and methods

2.1. Isolation and identification of bacteria

The bacteria used in this research were isolated from the sediment in a textile effluent canal. Isolation was carried out by adding 10 g of sediment to the nutrient broth medium containing 1000 mg/L of Cr(III). The mixture was then shaken for 24 h at a speed of 160 rpm at room temperature (26 ± 1 °C). Serial dilution (using physiological saline) was then conducted to isolate the growing colonies until 10^6 dilution (Imron et al., 2019). A total of 1 mL from the last dilution was cultured in nutrient agar medium (Marck, Germany). The appeared colony was selected to be analyzed further for identification using PCR (Bio-rad T100 Thermal-cycler, Singapore). The constructed phylogenetic tree (Fig. 1) showed that the isolated bacteria was closely related to the species of *Bacillus nitratireducens;* afterward, the isolated bacteria was used and mentioned as *B. nitratireducens*.

Table 2
SEM results.

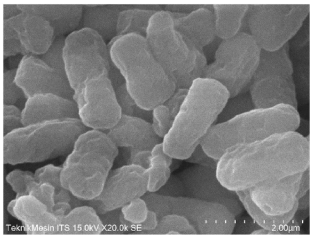
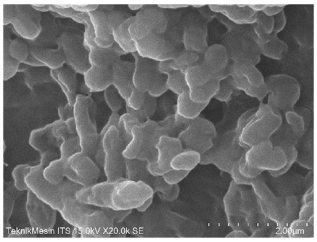
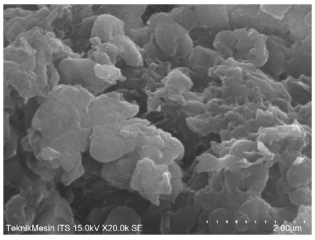
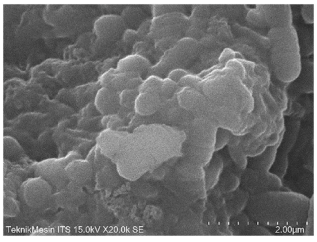
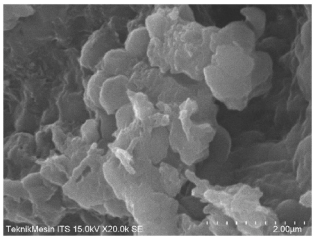
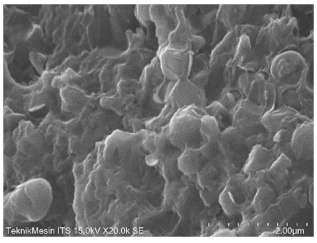
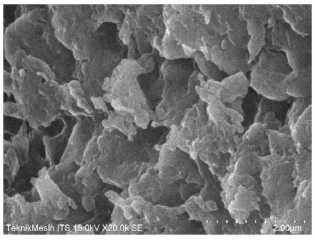
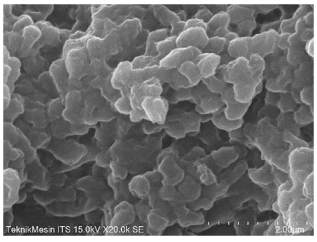
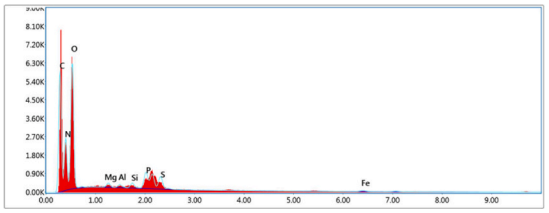
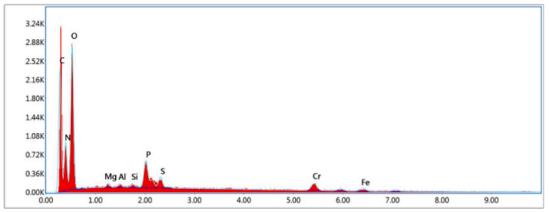
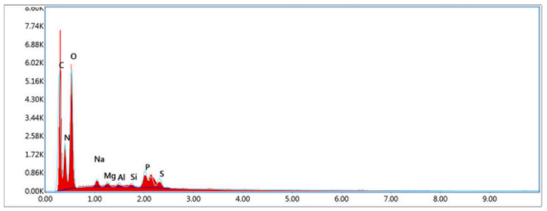
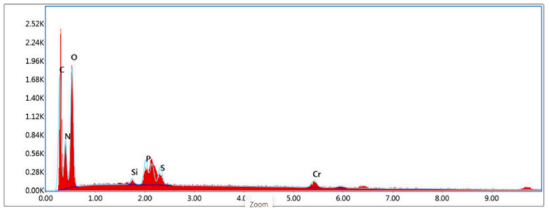
Cr concentration (mg/L)	Live cell	Dead cell
0		
25		
50		
100		

Table 3
EDS results.

Biosorbent	Cr (mg/L)	Spectra
Live cell	0	
	100	
Dead cell	0	
	100	

2.2. Preparation of live and dead cells biosorbent

B. nitratireducens was inoculated in 2 nutrient broth culture media (Merck, Germany) at pH 7.0 ± 0.2 using an incubation shaker (KS 130 Basic IKA, Germany) at room temperature (26 ± 1 °C) with a rotation speed of 160 rpm. After incubation for 24 h, bacteria were harvested by centrifugation (OHAUS FC5706 Frontier, USA) at 5000 rpm for 10 min. The dead cell biosorbent was prepared by oven-drying the obtained pellet at 105 °C for 24 h, and the live cell biosorbent was obtained by freeze-drying (Christ Alpha 1–2 LDplus, Ukraine) the obtained pellet for 24 h (Velásquez and Dussan, 2009).

2.3. Preparation of Cr stock solution

All chemicals used were analytical grade, and pure H_2O (OneMed, Indonesia) was used as a diluent. A Cr stock solution of 1000 mg/L was prepared by dissolving 0.5125 mg of $CrCl_3$ (Merck, Germany) in 1000 mL of distilled water (Purwanti et al., 2017b). The Cr working solution was prepared by diluting the stock solution according to the desired concentration, namely 25, 50, and 100 mg/L.

2.4. Experimental set up of biosorption

The biosorption process by live and dead cell biosorbents of *B. nitratireducens* was carried out at room temperature (26 ± 1 °C) by mixing 1 g of each biosorbent into a 250 mL Erlenmeyer (Pyrex, Germany) containing 100 mL of Cr solution with concentrations of 25 mg/L, 50 mg/L, and 100 mg/L. The mixture was shaken at a speed of 160 rpm for 24 h, then centrifuged at a speed of 5000 rpm for 15 min. The Cr concentration in the supernatant was determined by an AAS (Atomic Absorption Spectrophotometer) (PerkinElmer AAnalyst 800, USA) with three replications. Biosorption removal efficiency (%) for Cr and biosorption capacity were calculated by using Equations (1) and (2).

$$\text{Biosorption removal efficiency } (\%) = \frac{C_0 - C_{eq}}{C_0} \times 100 \quad (1)$$

$$\text{Biosorption capacity } (\text{mg}/\text{g}) = \frac{C_0 - C_{eq \times V}}{m} \quad (2)$$

where, C_0 = initial concentration (mg/L), C_{eq} = final concentration (mg/L), m = mass of biosorbent (g), v = volume of solution (L).

2.5. Recovery of Cr

The recovery analysis was carried out by using the Cr desorption method. The harvested pellets after the Cr biosorption test were desorbed using eluents. Distilled water, 1.0 mol NH_4NO_3, and 0.1 mol EDTA-Na_2 as eluents were used in this stage (Li et al., 2018a). Eluents are sorted by desorption, namely: (1) H_2O desorbs the weakest bonds such as physical traps that occur on the cell surface; (2) ion exchange with K^+, Ca^{2+}, Na^+, and Mg^{2+} in the cell wall by NH_4NO_3; and (3) EDTA-Na_2 desorbs complex bonds with functional groups. The remaining metal was considered sorbed and cannot be desorbed via EDTA. Desorption experiments were carried out in a batch reactor loaded with cell biosorbent in a 100 mL Erlenmeyer flask containing 50 mL of each aforementioned eluent. After 2 h of reaction in a shaker at 28 °C, the

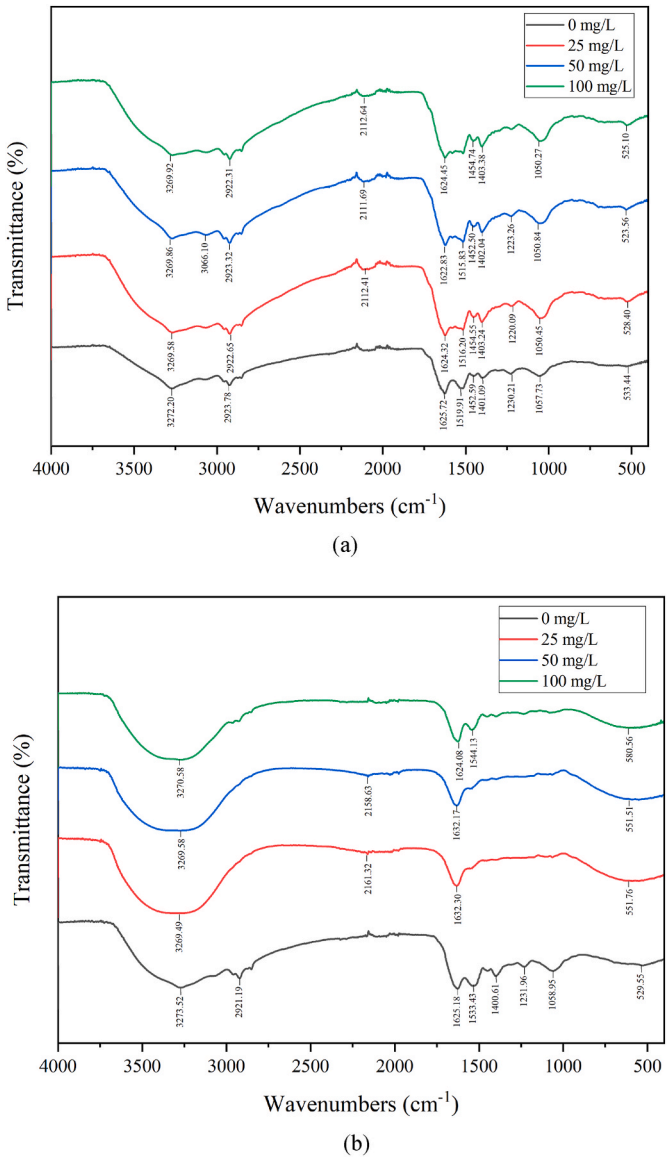


Fig. 7. FTIR graph of (a) live and (b) dead cells after exposed to different Cr concentrations.

supernatant was analyzed with AAS in triplicate sampling.

2.6. Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM -EDX) analysis

Harvested pellets were tested by SEM-EDX (Hitachi Flexsem, Japan) after being fixed and freeze-dried. The pellet was then fixed for 24 h in a 4% glutaraldehyde solution in refrigerator temperature conditions, then dehydrated with a graded series of ethanol (30, 50, 70, 80, 90, and 100%) with respective contact times of 10 min each (Othman et al., 2022; Purwanti et al., 2019; Titah et al., 2019). The sample was then dried in a desiccator for 48 h before being read. The SEM-EDX was performed for non-exposed cells (control 0 mg/L Cr) and exposed cells.

2.7. Fourier transformed infrared (FTIR) analysis

FTIR (Thermo Scientific Nicolet iS10, USA) was performed on live and dead cell samples to compare the functional groups of the biosorbents. In addition, FTIR was also performed after the biosorption test on the pellet for each given concentration of Cr exposure (0, 25, 50, and 100 mg/L) to compare the changes in functional groups between live and dead cells under different Cr exposures.

2.8. Statistical analysis

Each stage of this research was carried out in triplicate. Each piece of data was tested using the Kolmogrov-Smirnov normality test and Levene's homogeneity test. A one-way ANOVA was performed to analyze the significant differences between tested factors, followed by a Duncan post-hoc test for significant factors (Kurniawan and Imron, 2019a, 2019b).

3. Results and discussion

3.1. Characteristics of biosorbent

The identification of biosorbent characteristics was carried out based on physical and chemical characterization. The characteristics of the biosorbent can influence the Cr biosorption mechanism that occurs during exposure.

3.1.1. Physical characteristics of live and dead cells of Bacillus nitratireducens

The live and dead cells of *B. nitratireducens* are depicted in Fig. 2. Based on the color appearance, live cells showed a lighter color as compared to dead cells. The changing color of biosorption was affected by the thermal treatment, which can disrupt the cell wall's structure, causing an alteration in physical appearance (Cebrián et al., 2017).

3.1.2. FTIR results of biosorbent

The results of the FTIR test for biosorbents before biosorption can be seen in Fig. 3, and the detected functional groups are tabulated in Table 1. Based on Fig. 3, there were no differences between live and dead cell biosorbent functional groups. It can also be seen that there were OH and CH group bonds at 3273.52 cm^{-1} and 2921.19 cm^{-1}. Naiu et al. (2020) stated that the O–H bond in alcohol appeared at a higher wave number than an acid, between 3230 and 3550 cm^{-1}. The absorption occurs at a higher wavelength when the alcohol lacks hydrogen bonds, such as in its gaseous form. The absorption of the C–H bond was slightly below 3000 cm^{-1}, and the absorption in the 1000 and 1100 cm^{-1} regions comes from the C–O bond.

Based on Table 1, there were no big differences in terms of functional groups between live and dead cells of the bacteria. The OH functional groups were detected in the range of 3230–3550 cm^{-1} and C–H in the range of 2900–3000 cm^{-1}. The only difference that appeared was the presence of C–H bending correlated to alkane in the live cell but not in the dead cell. Based on these results, alcohol and amine groups may serve as ionic bonding sites for adsorption mechanisms (Kurniawan et al., 2021). The obtained results were in accordance with Huang et al. (2013) which mentioned that live and dead cells of *Bacillus cereus* RC-1 were having similar functional groups. The slight differences were observed on the reducing of O–H stretching and increasing peak of –COOH stretching after autoclaving. Mohapatra et al. (2019) also mentioned that live and dead cells of *Bacillus xiamenensis* PbRPSD202 showed similar functional groups with slightly different peak intensity.

3.2. Performance of biosorbents

3.2.1. Cr removal efficiency

Fig. 4 shows the Cr removal efficiency during the biosorption test. Based on Fig. 4, it can be seen that the increase in Cr concentration up to 100 mg/L gave a significant increment to the removal. Cr removal by dead cells in 25, 50, and 100 mg/L of Cr were 58.99 ± 0.7%, 69.8 ± 0.2%, and 82.87 ± 0.11%, respectively. In the live cells, Cr removals were 73.08 ± 1.9%, 80.27 ± 6.33%, and 86.17 ± 1.93% for 25, 50, and 100 mg/L of Cr concentration, respectively. Comparing the live and

Table 4
FTIR results of live and dead cells after Cr exposure.

No	Wavenumbers (cm^{-1})				Functional groups	Compound class	Appearance
	Cr concentration (mg/L)						
	0	25	50	100			
Live cell							
1	3272.20	3269.58	3269.86	3269.92	O–H stretching	Carboxylic acid	Strong, broad
2	–	–	3066.10	–	N–H stretching	Amine salt	Strong, broad
3	2923.78	2922.65	2923.32	2922.31	C–H stretching	Alkene	Medium
4	–	2112.41	2111.69	2112.64	C ≡ C stretching	Alkyne	Weak
5	1625.72	1624.32	1622.83	1624.45	C═C stretching	Conjugated alkene	Medium
6	1519.91	1516.20	1515.83	–	N–O stretching	Nitro compound	Strong
7	1452.59	1454.55	1452.50	1454.74	C–H bending	Alkane	Medium
8	1401.09	1403.24	1402.04	1403.38	S═O stretching	Sulfonyl chloride	Strong
9	1230.21	1220.09	1223.26	–	C–N stretching	Amine	Medium
10	1057.73	1050.45	1050.84	1050.27	C–O stretching	Primary alcohol	Strong
11	533.44	528.40	523.56	525.10	C–I stretching	Halo compound	Strong
Dead cell							
1	3273.52	3269.49	3269.58	3270.58	O–H stretching	Carboxylic acid	Strong, broad
2	2921.19	–	–	–	C–H stretching	Alkene	Medium
3	–	2161.32	2158.63	2111.67	C ≡ C stretching	Alkyne	Weak
4	1625.18	1632.3	1632.17	1624.08	C═C stretching	Conjugated alkene	Medium
5	1533.43	–	–	1544.13	N–O stretching	Nitro compound	Strong
6	1400.61	–	–	–	S═O stretching	Sulfonyl chloride	Strong
7	1231.96	–	–	–	C–N stretching	Amine	Medium
8	1058.95	–	–	–	C–O stretching	Primary alcohol	Strong
9	529.55	551.76	551.51	580.56	C–I stretching	Halo compound	Strong

dead cells, significant differences were obtained, with live cells showing higher Cr removal efficiency in all concentrations. The obtained results were in accordance with Hlihor et al. (2017) which stated that the increment of initial Cr concentration gave significant increases in the Cr removal by both live and dead cells of *Arthrobacter viscosus*. Comparing the live and dead cells, Hlihor et al. (2017) mentioned that live cells performed slightly better than dead cells due to the involvement of complex metabolic mechanisms for Cr removal (43.5% vs 74.8%).

Since the increase in Cr concentration still gave significant improvement to the Cr removal by biosorbents, this suggested that the biosorption was still not at the equilibrium stage. The equilibrium stage can be indicated by the stagnant removal of Cr by increasing the concentration (Michalak et al., 2013). The biosorbents performed well by showing a Cr removal value of more than 50%. Physical adsorption was observed at the beginning of biosorption, followed by chemical adsorption mechanisms (Raji et al., 2023). In this research, Cr ions in the solutions possibly interacted well with alcohol and amine groups to form ion-polar interactions (Bandara et al., 2020). To confirm the adsorption capability of biosorbents, an adsorption capacity analysis was presented in Section 3.2.2, in which the different mechanisms involved in biosorption will be further detailed in Section 3.2.3.

3.2.2. Adsorption capacity

Based on Fig. 5, the same finding was obtained with the Cr removal efficiency. There was a significant increase in adsorption capacity obtained by the increase in concentration in both live and dead cells. There was also a significant difference when comparing live and dead cells, with live cells showing a higher capacity for all Cr concentrations. Cr adsorption capacity by dead cells in 25, 50, and 100 mg/L of Cr was 1.47 ± 0.01 mg/g, 3.49 ± 0.01 mg/g, and 8.29 ± 0.01 mg/g, respectively. For the live cells, the adsorption capacities were 1.82 ± 0.05 mg/g, 4.01 ± 0.31 mg/g, and 8.62 ± 0.19 mg/g for 25, 50, and 100 mg/L of Cr concentration, respectively.

Referring to previous research, *Bacillus sphaericus* also showed an adsorption capacity of Cr(VI), with values ranging from 1.6 to 7.82 mg/g (Velásquez and Dussan, 2009). The obtained findings were in accordance with Mohapatra et al. (2019) which stated that live cells of *B. xiamenensis* PbRPSD202 gave higher Pb adsorption capacity 216.75 mg/g as compared to the dead cells (207.4 mg/g). However, Huang et al. (2013) reported different findings that *Bacillus cereus* RC-1 showed Cd adsorption capacities of 24.01 and 31.95 mg/g in live and dead cells, respectively, while Dadrasnia et al. (2015) also mentioned that the dead cells of *Bacillus salmalaya* Strain 139SI showed higher Cr adsorption capacity (40 mg/g) than the live cells (30 mg/g). Adsorption capacities are highly related to the amount of metal removed from the solution and the amount of biosorbent used during the treatment (Raji et al., 2023). The differences in adsorption capacity with previous research were subjected due to the different mechanisms involved during the biosorption by both live and dead cells. The higher adsorption capacity in live cells was subjected to the capability of live cells in performing active adsorption which add-up to the only passive adsorption that occurred in the dead cells (Mohapatra et al., 2019). The higher the adsorption capacity, the less biosorbent is used to adsorb certain amounts of metal, which is more beneficial.

3.2.3. Biosorption mechanism by live and dead cells of *B. nitratireducens*

Biosorption of Cr by live and dead cells of *B. nitratireducens* occurred via adsorption and absorption mechanisms. Adsorption occurs when metals adhere to the surface membrane of the cells, while absorption occurs when metals enter the cells. Based on Fig. 6, the higher the concentration of Cr, the higher both the adsorption and absorption of Cr into the cells (both live and dead cells). Comparing dead and live cells at concentrations of 25 and 50 mg/L, there was a significant difference in absorption capacity (higher for live cells) but not for adsorption. At a concentration of 100 mg/L, there was a significantly lower absorption capacity by live cells as compared to the dead cells.

In dead cells, passive biosorption occurs via adsorption and physical interaction mechanisms in which Cr can stick to the surface membrane cavities, filling pores and also having weak interaction bonds (Huang et al., 2013), while absorption can occur due to the disruption of the cell membrane after thermal treatment, creating some hollow structures inside cells (Thompson et al., 2020). In live cells, adsorption and physical interaction mechanisms also occur, accompanied by active biosorption, in which metal ions are accumulated inside cells via some enzymatic reactions (Chojnacka, 2010). Results for 25 and 50 mg/L of Cr indicated that live cells performed better biosorption of Cr, which was facilitated by not only passive but also active mechanisms. However, at 100 mg/L of Cr, absorption was significantly higher in dead cells, suggesting that a 100 mg/L concentration affected some enzymatic reactions in the live cells, thus reducing its absorption efficiency (Imron

et al., 2021; Titah et al., 2018).

3.2.4. SEM-EDS analysis

To enhance the discussion on the mechanisms involved during biosorption, SEM-EDS was used to analyze the cell structures and components detected on the cell surface. Table 2 showed that the live cells of *B. nitratireducens* showed a basil shape with intact structure and a smooth surface, while the dead cells showed an irregular shape with interconnected wrinkly structure and a coarse surface. This may be an indication that pores and cavities created by heat (during the inactivation of the cells) can be a good surface for Cr to adhere to (Hossan et al., 2020; Huang et al., 2013; Li et al., 2018b). After being exposed to Cr, a significant alteration of shape occurred in the live cells of *B. nitratireducens.* Live cell biosorbents lost their intact shape and showed a wrinklier structure. Interestingly, the dead cells showed that they maintained their shape after being exposed to Cr, even at a concentration of 100 mg/L. Comparing the exposed cells at 100 mg/L Cr, the structure of live cells seems to be more wrecked than that of dead cells. This may be a reason for the significantly lower absorption capacity of living cells in 100 mg/L of Cr, in which the bacterial structure altered significantly.

Referring to the EDS results (Table 3), some elements were detected on the surface of the cells. Cr was confirmed to be detected on the surface of the live and dead cells of bacteria, supporting the involvement of the adsorption mechanism during the biosorption test. For the live cells, Mg, Al, Si, and Fe were detected on both control (0 mg/L Cr) and exposed sample (100 mg/L Cr). Interestingly, Na, Mg, Al, and Si were detected on the control sample of dead cells, while only Si was presented on the exposed sample. This result suggested that the adsorption mechanism for dead cells involved the interactions of Cr with these cations (Na, Mg, Al) on the surface of the biosorbents. To further enhance the discussion and confirm the involvement of absorption mechanisms, TEM analysis is suggested to be conducted to reveal the inner structure of the biosorbents.

3.2.5. FTIR analysis

FTIR results after the biosorption test (Fig. 7 and Table 4) revealed the changing of some functional groups and indicated the absorption of inorganic ions, as shown by a peak at 2200–2000 cm^{-1} for biosorbents exposed to 25 mg/L, 50 mg/L, and 100 mg/L of Cr. There was also a strong notion that the dead cell biosorbent absorbs Cr ions due to the disappearance of most of the functional groups. With each increase in concentration, the transmittance of dead cell bacterial cell walls consisting of C–H, O–H, and C–I weakens. Huang et al. (2013) stated that the absorption of metal ions in cells caused the weakening of cell wall transmittance. It also showed the presence of functional groups that experience a shift in wave number, which indicates the involvement of these groups in forming bonds with Cr during biosorption (Mohapatra et al., 2019). The most striking shift was the loss of peak in the functional group markers C–H, –NO, O–H, O, and C–O.

3.3. Environmental implications and future research directions

The environmental implications of Cr removal research using biosorbents are significant. The development of biosorbent research for the elimination of Cr can greatly contribute to the protection of aquatic environments and human health. Live and dead cells of bacteria are categorized as green sorbents, which can be an alternative option to commercially available sorbents that also leave chemical residue after usage (Pattnaik et al., 2020). The presented result can be implemented in the current wastewater treatment system as a tertiary treatment with adsorption or a fixed bed column to reduce Cr concentration before final disposal to the water bodies (Boeris et al., 2018).

The presented results unravel the Cr removal by live and dead cells of *B. nitratireducens,* especially for the surface adsorption mechanisms. Recovery analysis revealed a glimpse of absorption mechanisms into cells, while conducting further profound absorption analysis is suggested to clarify the still-blurry area of this research. Transmission electron microscopy (TEM) analysis is suggested to be conducted to reveal the intracellular absorption mechanisms by live and dead cells of *B. nitratireducens.* In addition, this research can be extended to the analysis of the use of live and dead cell biosorbents to remove other pollutants. Future research directions can address the long-term sustainability of using biosorbents to remove Cr, with a focus on the recovery of sorbed metals, the kinetics of biomass growth, the regeneration of biosorbents after treatment disposal, and the optimization of operational conditions during biosorption analysis.

4. Conclusion

Bacillus nitratireducens, which has a high tolerance to Cr, was used to eliminate Cr from aqueous solutions. Batch biosorption, adsorption-desorption, SEM-EDS, and FTIR analyses revealed that live cells exhibited higher Cr adsorption and absorption capacities. Absorption is confirmed to be a major mechanism contributing to Cr removal as compared to adsorption by both live and dead cells. The presence of Cr on the cell surface was verified by SEM-EDS analysis. Additionally, FTIR analysis revealed shifts in peaks following the biosorption test, indicating interactions between Cr and functional groups. Additional transmission electron microscopy (TEM) investigation is recommended to gain a deeper comprehension of the cellular structure and absorption processes.

CRediT authorship contribution statement

Muhammad Fauzul Imron: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Wahyu Setiawan:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Trisnadi Widyaleksono Catur Putranto:** Supervision, Funding acquisition. **Siti Rozaimah Sheikh Abdullah:** Supervision, Funding acquisition. **Setyo Budi Kurniawan:** Writing – review & editing, Writing – original draft, Methodology, Data curation.

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.

Data availability

Data will be made available on request.

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