Bioreduction and bioremoval of hexavalent chromium by genetically engineered strains (Escherichia coli MT2A and Escherichia coli MT3)

Şeyma Akkurt1, Merve Oğuz2, Aysel Alkan Uçkun1

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Abstract
The number of studies on the removal of hazardous metals from water using genetic engineering technologies is growing. A high rate of metal ion removal from the environment is ensured, particularly through the expression of cysteine and thiol-rich proteins such as metallothioneins in bacterial cells. In this study, we used recombinant strains created by cloning the human metallothioneins MT2A and MT3 into Escherichia coli Jm109 to assess the removal and reduction of hexavalent chromium (Cr(VI)) from aqueous solutions. MT2A was the most effective strain in both Cr(VI) removal (89% in 25 mg/L Cr(VI)) and Cr(VI) reduction (76% in 25 mg/L Cr(VI)). The amount of Cr adsorbed per dry cell by the MT2A strain was 22 mg/g. The biosorption of total Cr was consistent with the Langmuir isotherm model. Scanning electron microscope (SEM) images revealed that the morphological structures of Cr(VI)-treated cells were significantly damaged when compared to control cells. Scanning transmission electron microscope (STEM) images showed black spots in the cytoplasm of cells treated with Cr(VI). Shifts in the Fourier transform infrared spectroscopy analysis (FTIR) spectra of the cells treated with Cr(VI) showed that the groups interacting with Cr were hydroxyl, amine, amide I, amide II, phosphoryl and carbonyl. When all of the experimental data was combined, it was determined that both MT2A and MT3 were effective in removing Cr(VI) from aqueous solutions, but MT2A was more effective, indicating that MT2A may be employed as a biotechnological tool.

Graphical abstract

Extended author information available on the last page of the article
Keywords Cr(VI) bioreduction · Chromium removal · Genetical engineered bacteria · Bioremediation · Metalloproteins · Biological treatment

Introduction

Chromium (Cr) is a major contaminant in nature that is extremely harmful even at low concentrations (Kushwaha et al. 2018). Cr comes in two forms: trivalent (Cr(III)) and hexavalent (Cr(VI)). Because of its role in lipid and glucose metabolism, Cr(III) is advantageous to human health on a specific level (Pradhan et al. 2017). In contrast, Cr(VI) is a known mutagenic and carcinogenic component for both humans and all living organisms due to its strong oxidizing properties (Fernández et al. 2010). Cr(VI) is widely used in industrial applications due to its high resistance to corrosion and hardness (Lu et al. 2013). It is commonly used in many industries, such as electroplating, metallurgy, tanning of leather, wood defense, production of stainless steel, paint, pigment, and the production of pulp (Dhal et al. 2013; Jobby et al. 2018). Wastes containing Cr(VI), such as sludge, fly ash, and slag, are produced in these industries (Fernández et al. 2018). In humans, Cr(VI) causes a variety of diseases, including skin rashes, kidney and liver damage, internal bleeding, dental abnormalities, respiratory disorders, and lung cancer (Martone et al. 2013). Cr(VI) is 100 times more toxic and 1000 times more mutagenic than Cr(III) (Chang et al. 2019). Therefore, reduction of Cr(VI) to Cr(III) is an effective method to significantly reduce the toxic effects of Cr(VI) (Long et al. 2018; Pan et al. 2014). The United States Environmental Protection Agency (USEPA) recognizes Cr(VI) as one of the priority pollutants (USEPA 2014). It has been reported that the discharge limit of wastewater containing Cr(VI) made by the European Union (EU) to surface waters should be below 0.05 mg/L and that the total concentration of Cr should be reduced below 2 mg/L (Chang et al. 2019). The World Health Organization (WHO) has determined the maximum allowable total Cr content in surface water and drinking water to be 0.05 mg/L (WHO 2017). Various physical and chemical methods (oxidation–reduction process, flocculation, ion exchange, electrochemical methods, membrane filtering, and reverse osmosis) have been used to remove Cr(VI) from wastewater (Akkoyun et al. 2020; Geva et al. 2016). These methods, however, have a number of disadvantages, including high costs, secondary contamination, hazardous sludge production, and low treatment efficiency (Ahlulwalia and Goyal 2007). The bioremediation technique is a viable option for dealing with such issues (Anusha and Natarajan 2020). Bioremediation is a treatment method that uses microorganisms to eliminate toxins in an environmentally acceptable, clean, cost-effective, and efficient manner (Ayangbenro and Babalola 2017). Compared with plants and animals, microorganisms can develop effective resistance systems against the harmful effects of heavy metals (Yin et al. 2019). On the other hand, wild bacteria isolated from contaminated environments lack the ability to selectively bind metals and have limited ability to remove them (Ayangbenro and Babalola 2017). Genetic engineering applications allow for the selective removal of metals as well as an increase in the metal binding capacity of microorganisms (Yang et al. 2015). There are numerous strategies for increasing metal-binding capacity of microorganisms. The majority of these strategies rely on metal ion-binding polypeptides being added to the bacterial cell wall to bind metals to recombinant strains (Gupta and Singh 2017). To boost the metal binding capacity, proteins or peptides with a high affinity for metal ions, such as metallothioneins (MT) or poly-histidines, are cloned into the host cell (Ike et al. 2007). MTs are low-molecular-weight proteins with a high cysteine content found in all living species. Metal ions are bonded to sulfhydryl groups in MTs (Pinto et al. 2000). Many studies have found that genetically altered E. coli strains expressing the MT gene improve heavy metal removal (Cr, cadmium (Cd), zinc (Zn), copper (Cu), nickel (Ni), arsenic (As), and other heavy metals) (Deng and Jia 2011; Kim et al. 2005; Ma et al. 2011, 2019; Uçkun et al. 2021a, 2021b). The human metallothionein MT2A protein, which we use to remove Cr(VI) metal in our study, serves a number of purposes. Metal ions are chelated by MT2A proteins, which also remove large reactive oxygen (ROS) molecules and trigger antioxidant enzymes (Chen et al. 2014; Star ska et al. 2015). MT3 is another human metalloprotein that has been found to bind metals like Zn, Cu, Cd, and As (Toriumi et al. 2005; Uçkun et al. 2021a, 2021b). There is no research on the Cr removal of the MT2A and MT3 genes from water in the literature. The goal of this study was to use genetic engineering technologies to assure the removal and decrease of Cr(VI) from water. For this purpose, the human MT genes (MT2A and MT3) were cloned into host E. coli Jm109 and the ability of these cells binding to Cr was investigated. The Jm109 strain’s Cr(VI) removal and reduction activities were compared to those of other recombinant strains.
Materials and methods

Cultivation of strains

E. coli Jm109 was used as the control strain in our study. Cells were grown at 37 °C with 150 rpm shaking (MS, Major Science orbital shaker) in Luria Bertani (LB) medium containing beef extract (10 g/L), peptone extract (10 g/L), and NaCl (3 g/L). Unlike the control Jm109 cell, recombinant cells (MT2A and MT3) were planted in LB medium containing ampicillin (Merc).

Preparation of Cr(VI) solution

Chemicals for the preparation of stock metal solutions were acquired from Merc. The Cr(VI) stock solution was made by dissolving potassium dichromate (K₂Cr₂O₇) metal salt in distilled water. The Cr(VI) solution was then autoclave sterilized and kept at 4 °C.

Gene synthesis, cloning and bacterial transformation

The human MT (MT2A and MT3) gene sequences were provided by Twist Bioscience. The following accession numbers can be used to access the MT2A and MT3 GenBank databases: NM_005953.5 for MT2A and NM_005954.4 for MT3. The MT2A and MT3 gene fragments and the pET21 plasmid were double digested with EcoRI and NotI restriction enzymes (Thermo Scientific). These genes were then ligated to the pET21 plasmid individually. Host Jm109 strain (ATCC 53323) was made competent according to the chemical CaCl₂ protocol (Merc). Recombinant pET21 plasmids (Sigma-Aldrich) were transformed into competent Jm109 cells. Then, recombinant (MT2A and MT3) strains were plated on LB agar plates supplemented with ampicillin (50 µg/mL). X-gal (for blue/white cell screening) and IPTG and incubated at 37 °C overnight. The QIAGEN Qiaprep Spin Miniprep Kit (Cat no: 27104) was used to isolate the plasmid, and PCR was used to confirm the existence of the positive transformant. To validate additional genes, sequencing libraries of isolated plasmids were prepared and sequenced using Miseq platform (Table 1).

Table 1 Sequencing libraries for the isolated plasmids

| Name | Tube ID | Insert Length | Construct Length | Insert Sequence | Construct Sequence | Yield (ng) | NGS Yield | Expression Vector |
|------|---------|---------------|-----------------|----------------|-------------------|------------|------------|-------------------|
| MT3  | tSHPw0903B | 300           | 5646            | ATGGACCCCT     | ATGGACCCCT       | 612        | PASS       | PASS              |
| MT2A | tSHPw0903B | 300           | 5646            | ATGGATCCCA     | ATGGATCCCA       | 523        | PASS       | PASS              |

Determination of minimum inhibition concentration

The resistance capacities of Jm109 and its recombinant strains (MT2A and MT3) to Cr(VI) were determined by the minimum inhibition concentration (MIC). MIC experiments were performed with a few minor changes in the method applied by Anusha and Natarajan (2020). Three independent control groups (just LB, LB + metal solution and LB + cell culture) were used in the analysis. Cells were shaken in LB medium containing varying concentrations of Cr(VI) (0–1000 mg/L) for 96 h at 37 °C to determine the Cr(VI) MIC concentration. Culture samples were taken from the medium at regular time intervals (every 8 h) and measured at 600 nm in the microplate reader (Thermo Flash 2000).

Quantitative analysis of total Cr in the medium

In the quantitative analysis of Cr, the method of Shamim et al. (2014) was modified and used. The strains were incubated in LB broth containing Cr(VI) (0.05–25 mg/L) for 24 h at 37 °C and 150 rpm. Samples were taken after the incubation was completed to determine the amount of Cr remaining in the medium. The samples were centrifuged for 20 min at 4 °C at 5000 rpm, and the supernatant was filtered through a syringe filter. The remaining pellet was kept at 4 °C for the next analysis. Total Cr accumulations in the medium were measured by inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer, USA & Nexion 350X).

Cr accumulation on the cell surface

In order to determine the accumulation of metal on the cell surface, 1 mL of 50 mM EDTA (Merck) solution was applied to the pellet separated in the previous step. The samples were centrifuged and the supernatant filtered with a syringe filter and transferred to another tube for the ICP-MS analysis. The total amount of Cr in the filtrates was analyzed by ICP-MS. The formula given below was used to determine the amount of metal removed by the strains (Anusha and Natarajan 2020).

Removal of Cr (%) : \((\frac{(C_i - C_f)}{C_i}) \times 100\)
Cr: Remaining concentration of Cr after bioaccumulation (mg/L), Ci: Initial concentration of Cr added into the medium (mg/L).

**Diphenyl carbazide method**

The 1.5 diphenyl carbazide method (DPC) was used to determine the reduction of Cr(VI) to Cr(III) (Thacker et al. 2006). Supernatants of the samples prepared for Cr removal analysis in bioremoval experiments were stored at +4 °C. These samples were also used to detect Cr(VI) reduction in this experiment. Sulfuric acid (6 M 330 µL) and DPC (400 µL) were added to a 10 mL falcon tube containing 200 µL of sample. The final volume of the reaction solution was made up to 10 mL with distilled water. For this reaction to occur, the samples were kept at room temperature for 5–10 min. A standard curve was drawn using known chromate concentrations (1–10 mg/L). The Cr(VI) amount of the samples was calculated according to this curve. The absorbance values of the samples were measured at a wavelength of 540 nm by a spectrophotometric method using a microplate reader.

The percent decrease in Cr(VI) was determined in accordance with the formula below.

\[ \% \text{Cr(VI) reduction} = \left( \frac{C_i - C_f}{C_i} \right) \times 100 \]

Ci: The initial Cr(VI) concentration mg/L, Cf: The final Cr(VI) concentration mg/L.

**Biosorption experiments**

To obtain the amount of adsorbed Cr per dry cell, the method of Srinath et al. (2002) was used. Because the most successful strain in bioremoval and bioreduction experiments was proven to be MT2A, the quantity of Cr adsorbed per dry weight was computed just for this strain. In a 500 mL flask of LB containing 100 g/mL ampicillin, the MT2A strain was inoculated and incubated for 24 h at 150 rpm at 37 °C. After incubation, the cells in the late exponential phase were centrifuged at 6000 rpm for 30 min at 4 °C. The pellet that settled at the bottom was washed with distilled water. 0.1 M HCl was applied to the pellet for 1 h and dried in an oven at 80 °C for 24 h. The optimum pH was 2.0 and the temperature was 25 °C. 0.1 g of prepared dry biomass was weighed and added to 100 mL of solution containing 25 mg/L Cr. This mixture was shaken at 150 rpm for 2 h at 25 °C and then centrifuged at 6000 rpm for 30 min. The supernatants were taken and passed through 0.45 µm filters and the amount of Cr was determined by ICP-MS. The dry biomass sorption capacity was calculated in accordance with the formula below (Bazzazzadeh et al. 2020).

\[ q_e = \frac{(C_i - C_f)V}{M} \]

q_e: the amount of Cr adsorbed at equilibrium (mg Cr/g dry biomass), C_i: the initial Cr concentration (mg/L), C_f: the final Cr concentration (mg/L), V: the solution volume (L), M: the dry biomass weight (g).

**Adsorption isotherms**

Langmuir and Freundlich equilibrium isotherm models, which are commonly used in water and wastewater treatment, were employed to evaluate the total Cr adsorption capacity of the MT2A strain.

**Langmuir isotherm**

In the Langmuir isotherm, the molecules adsorbed on the surface are adsorbed in a single layer. Adsorption takes place only in the determined region, and there should be no interaction between the molecules attached to the surface (Vijayaraghavan et al. 2006). The Langmuir isotherm is expressed by the following equation (Maleki et al. 2015).

\[ \frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{C_e}{q_m} \]

C_e: The amount of absorbed Cr at equilibrium (mg/g), q_m: The maximum Cr uptake of the cell (mg/g), K_L: Adsorption constant of Langmuir, C_e: Cr concentration in solution at equilibrium (mg/L).

According to this equation, 1/C_e values versus 1/q_e graph is drawn and q_m is calculated from the slope of the line, and the K_L constant is calculated from the shift value. In order to determine the conformity of the adsorption process to the Langmuir isotherm model, the dimensionless separation constant R_L is calculated. The R_L value is calculated with the equation given below. If R_L > 1, it is called unfavorable, R_L = 1 is called linear, and R_L = 0 is irreversible. If the R_L value is between 0 and 1, it is suitable for the Langmuir isotherm (Al-Ghouti and Da’ana 2020).

\[ R_L = 1 + K_L C_0 \]

R_L: The dimensionless separation constant, K_L: Adsorption constant of Langmuir, C_0: The initial substance concentration (mg/L).

**Freundlich isotherm**

In the Freundlich model, unlike the Langmuir isotherm, it is not limited to the formation of a single layer, but multilayer adsorption is possible. The Freundlich isotherm model describes the heterogeneity of the surface as well as the exponential distribution of active sites and the energies of active sites. In general, systems with ideal adsorption are those that fit the Langmuir model. As a result of the homogeneity of the surface of the adsorbent and the interactions

\[ C_f = \frac{1}{K_C q_m} + \frac{C_f}{q_m} \]

K_C: Adsorption constant of Freundlich

\[ q_e = k_F C_e^{1/n} \]

k_F, n: Freundlich constants, C_e: Cr concentration in solution (mg/L), q_e: the amount of adsorbed Cr at equilibrium (mg Cr/g dry biomass).
between the adsorbed molecules, deviations from the Langmuir equation occur. Non-ideal systems may conform to some empirical isotherms, such as the Freundlich isotherm (Al-Ghouti and Da’ana 2020).

\[
\log q_e = \log K_F + \frac{1}{n} \log C_e
\]

\( q_e \): The amount of substance that remains in solution at equilibrium (mg/L), \( q_e \): The amount of adsorbed Cr in equilibrium (mg/g), \( K_F \): A constant that gives the adsorption capacity (L/g), \( n \): A constant that gives the adsorption density. The Freundlich isotherm constants are calculated by plotting the \( \log q_e \) versus the \( \log C_e \) isotherm. \( 1/n \) represents the intensity of adsorption or surface heterogeneity, which indicates the heterogeneity of adsorbate sites. When \( 1/n \) is higher than zero (\( 1/n > 0 \)), the adsorption process is favorable; when \( 1/n > 1 \) the adsorption is unfavorable; and when \( 1/n = 1 \), adsorption not reversible (Ayawei et al. 2017).

**SEM, EDX and STEM analyses**

SEM analysis was conducted to view morphological changes on the surface of Cr(VI) treated cells. STEM (ZEISS, Germany) was used to determine the metal distribution inside bacteria. MT2A cells were grown in a LB medium treated and untreated Cr(VI) (25 mg/L) at 150 rpm at 37 °C for 24 h. The cells were then centrifuged at 6000 rpm for 20 min at 4 °C, the liquid part was discarded and washed three times with the pellet phosphate buffer (PBS, pH 7.2). Then the pellet was fixed to 2.5% of glutaraldehyde for 4–6 h at 4 °C. The cells were washed with PBS and treated with 1% osmium tetraoxide for 1–2 h. After fixation, the cells were washed again with PBS and dehydrated by increasing concentrations of ethanol (15, 30, 60, 90 and 100% v/v). The fixed samples were critically dried thoroughly and gold-platinum (QUORUM) coated (Bharagava and Mishra 2018). SEM–EDX analysis of MT2A cells was performed using a ZEISS & EVO (LS 10, Germany) with an EDX microprobe attached. While the morphological images of the cells were taken with the SEM device, elemental analyzes were made from the EDX probe. By making minor changes in the SEM analysis protocol for STEM analysis, samples of MT2A cells that were Cr(VI) treated and non Cr(VI) treated were prepared.

**FTIR analysis**

FTIR (Perkin Elmer 400 FT-IR/FT-FIR spectrometer) was performed to detect Cr(VI) binding functional groups on the MT2A cell surface. Cr(VI) (25 mg/L) and Cr(VI) untreated MT2A cells were incubated for 24 h. The samples were then centrifuged at 5000 rpm for 20 min at 4 °C. The supernatant was discarded and the pellet was lyophilized after washing twice with PBS (Karthik et al. 2017). Samples were analyzed on the FTIR Spectrometer between 400 and 4000 cm⁻¹ wavelength.

**Statistical analysis**

Statistical analysis was performed using the computer software package, SPSS 22. Differences between groups were tested by a one-way analysis of variance (ANOVA). The significance of differences between the strains were determined by Student’s t test. Differences at \( p \leq 0.05 \) were considered statistically significant.

**Results**

**Cr bioremoval and Cr(VI) bioreduction capacity of the strains**

The MIC values for Jm109, MT2A, and MT3 cells were 50 mg/L, 140 mg/L, and 75 mg/L, respectively. Figure 1 depicts the total Cr removal rates of bacterial cells from the medium. The Cr removal rates of *E. coli* Jm109 from media containing Cr at 0.5, 10, 25, and 50 mg/L concentrations were 80%, 70%, 68%, and 35%, respectively. Figure 1 shows the significance of statistical difference between strains (\( p < 0.05 \)).

![Fig. 1 Total Cr removal rates of the strains from the medium. a,b,c Showed the significance of statistical difference between strains (\( p < 0.05 \))]
At a Cr concentration of 0.5 mg/L, only MT2A differed statistically from the other strains, whereas at higher Cr concentrations, all three species showed statistically significant differences (p < 0.05). The removal rates decreased as the Cr concentration increased. The Cr(VI) reduction rates in both MT2A and MT3 strains were statistically significantly different from those of *E. coli* Jm109 at all Cr concentrations. There was a significant difference between MT2A and MT3 strains only at 25 mg/L and 50 mg/L Cr concentrations. As in total Cr removal, the reduction rate decreased as the Cr concentration increased in Cr(VI) reduction. The amount of Cr adsorbed per dry cell from the medium containing the highest Cr concentration (25 mg/L) was 22 mg/g for the MT2A strain.

The growth tolerance of the MT2A cell against different Cr(VI) concentrations (0–400 mg/L) of the MT2A strain is shown in Fig. 3. Cells grew at low Cr(VI) concentrations, whereas they were inhibited at increasing Cr(VI) concentrations. When a Cr concentration of 100 mg/L or above was applied, the growth was significantly reduced. The first inhibition occurred between 12 and 18 h at concentrations of 100, 200, and 400 mg/L Cr(VI). On the 24th h, a Cr(VI) concentration of 75 mg/L was added to this concentration range.

**Equilibrium isotherm models**

Adsorption isotherms are used to mathematically determine the maximum capacities, surface properties, and biosorbent coefficients of the biosorbent. Equilibrium biosorption isotherms provide a better understanding of the mechanisms of the biosorption process. Langmuir and Freundlich isotherm curves of MT2A cells plotted according to total Cr concentrations in the range of 50–250 mg/L at constant temperature were shown in Fig. 4 and isotherm parameters were shown in Table 2. The correlation value of MT2A in the Langmuir model (0.9981) has a higher R² value compared to the Freundlich model (0.8549). The R_L values calculated for the initial Cr concentrations (50–250 mg/L) were found to be between 0.11 and 0.25. The R_L values between 0 and 1 verify that the total Cr biosorption of MT2A biomass is in accordance with the Langmuir isotherm model. The *q_m* (maximum biosorption capacity) of total Cr estimated by the Langmuir model was 133.33 mg/g.

**SEM and STEM images of the cells**

Since MT2A is the most effective strain in Cr removal, SEM and STEM images of this strain were taken. The appearance of MT2A cells unexposed to Cr was uniform, without any deformation, and bacillus shaped (Fig. 5a).
After exposure to Cr, it was observed that the shape of the cells elongated, overlapped, and agglomerated, and the surface of some was significantly dispersed (Fig. 5b). Cr accumulation in the cell wall of Cr(VI) treated cells was confirmed by Cr peaks in the EDX spectra. While no Cr peak was observed in the EDX of MT2A control cells (Fig. 5c), a Cr peak was observed in the Cr-treated cells (Fig. 5d). STEM images of 25 mg/L Cr(VI) treated cells had obvious black spots that could represent intracellular Cr precipitation (Fig. 6b). These black spots were
absent in the control cells that were not treated with Cr(VI) (Fig. 6a).

**FTIR spectra of the cells**

FTIR analysis was performed only for the MT2A strain. Figure 7 shows the FTIR spectra of MT2A bacterial cells treated with 25 mg/L Cr(VI) and untreated with Cr(VI) (control). In the FTIR spectra, it was seen that the location and intensity of the important peaks changed after 25 mg/L Cr treatment compared to the control. The peaks belonging to the oligosaccharide, protein, and lipid groups, which are the bacterial structural components, were clearly seen. After treatment with Cr(VI), the peaks found at 1233 cm⁻¹, 1379 cm⁻¹, 1514 cm⁻¹ and 1632 cm⁻¹ changed to 1225 cm⁻¹, 1387 cm⁻¹, 1531 cm⁻¹ and 1626 cm⁻¹. It is the hydrogen bond stretching vibration of –OH groups around 3300–3600 cm⁻¹ (Ma et al. 2021). The small shoulder peak seen in the range of 3000–3300 cm⁻¹ was the hydrogen peak of the N–H bond in the protein structure. Generally, the peaks seen in the range of approximately 2850 cm⁻¹ and 2950 cm⁻¹ are aliphatic C–H stress peaks (Ma et al. 2021). These peaks are due to protein and carbohydrate structures. The amide I peak at 1620 cm⁻¹ and the amide II peak at 1530 cm⁻¹ are amide (peptide) bond C=O stretching vibrations caused by bacterial proteins (Zhang et al. 2019). The peak at approximately 1220 cm⁻¹ was observed for the P=O bond, which originates from the groups in the bacterial cell wall structure. Especially with carbonyl (C=O) and P=O groups and their interaction with Cr ions and the formation of coordination structures, peak positions have changed. The reason for this change is the formation of the coordination structure, the ring stability, and the vibration of the bonds due to the coordination bond stability. Cr ions generally form

![Fig. 6 STEM images of bacterial cells a untreated Cr(VI) cells b 25 mg/L Cr(VI) treated cells](image)

![Fig. 7 FTIR spectra of control (untreated Cr(VI)) and 25 mg/L Cr(VI) treated MT2A cell](image)
coordination with protein and polysaccharides in bacterial cell structures, and some of these structures interact with free –OH groups in polysaccharides. The free –OH groups of the polysaccharide structures usually give a distinct and severe H-bond stretch band at 3300–3600 cm\(^{-1}\). However, as a result of interaction with Cr ions, the intensity of this band peak decreased compared to the control. This change is due to Cr ion coordination. Another change observed under the influence of Cr ion was the broadening of the peak observed around 518 cm\(^{-1}\). In addition, it has been stated in the literature that not only Cr(III) binding of hydroxyl and carboxyl groups in the cell wall is involved, but bacteria may also play a role in Cr(VI) reduction. Therefore, it can be said that due to this change in the FTIR spectrum, some group peaks in the control’s FTIR spectrum decreased by entering into redox reactions and the peak intensities of some groups increased. For this reason, the intensity of the peak at around 518 cm\(^{-1}\) increased. It can be seen from all these peaks that Cr coordinations have been formed with the protein and polysaccharide groups in the bacterial cell structure. In addition, P=O groups and Cr coordinations in phospholipid structures were observed. Also, it appears that Cr(III) is reduced to Cr(IV). Due to these changes, bacterial cell surface functional groups can coordinate with Cr ions and be used to reduce heavy metal toxicity in the environment.

**Discussion**

Genetically engineered microorganisms have a higher metal binding capability than wild ones (Yang et al. 2015). As the Cr concentrations we applied increased, the Cr removal and Cr(VI) reduction percentages of the strains decreased, and the difference between the strains became significantly wider. This may be because, under high metal stress, removal and reduction are inhibited due to the stressed metabolic pathway and the biological activities of the cells, even though the strain may develop (Zhang et al. 2012; Pan et al. 2014). Parallel to our findings, Tan et al. (2020) reported that the Bacillus sp. CRB-B1 strain isolated from sewage treatment plant sludge removed 86.15% and 43.1% of Cr(VI) under 200 mg/L and 300 mg/L Cr(VI) concentrations, respectively, and they suggested that this strain was able to efficiently remove Cr when its concentration was less than 300 mg/L, but the reducing capacity was compromised at higher Cr concentrations. Similarly, Huang et al. (2021) determined that the Sporosarcina saroimensis W5 strain, which is a novel facultative anaerobic bacterium, achieved complete removal at 50 mg/L Cr(VI) concentration in the aerobic system, and removal efficiencies gradually decreased with increasing Cr(VI) concentration. In the literature, there are many studies (Zeng et al. 2019; Li et al. 2019; Sathishkumar et al. 2017) on the removal of Cr(VI) from water by wild strains. Bharagava and Mishra (2018) discovered that a Cellulosimicrobium sp. strain isolated from leather industry wastewater reduced Cr(VI) by 99% and 97% at 50 mg/L and 100 mg/L concentrations in 24 and 96 h, respectively. However, at 200 mg/L and 300 mg/L Cr(VI) concentrations, only 84% and 62% reductions were observed after 96 h, respectively. From this, it is clear that, as in our study, the reduction ratio decreases as the Cr(VI) concentration increases. Also, the reduction rate of Cr(VI) increases as the contact time increases. In our study, there was a statistically significant difference between the recombinant species and the wild species in terms of Cr removal and reduction. Among the recombinant strains, the dominant strain was MT2A in both Cr removal and reduction. When we look at the studies on Cr(VI) removal with recombinant strains, as a result of the cloning of the ChrA, ChrB, and ChrAB genes associated with Cr(VI) resistance of Serratia sp. to E. coli BL21, the ChrA and ChrAB genes show high resistance against Cr(VI) and can confer Cr(VI) resistance ability to the strains. However, it has been observed that the protein ChrB does not have such a capacity (He et al. 2018). Pei et al. (2020) isolated the most effective Cr(VI) removal genes from waters containing 200–600 μM Cr(VI) as E-mcr and E-gsr (at least 50% Cr(VI) removal efficiency) and cloned them into E. coli. Wang et al. (2021) expressed a Cr(III) adsorption protein (MerP) on the cell surface of E. coli and then combined it with a magnetic pellet system to facilitate Cr(III) adsorption. They determined that the obtained E. coli M-BL21 strain absorbed 2.38 mmol/g Cr(III). These studies support our findings that more Cr is removed from aqueous solutions by recombinant strains than by wild strains. However, the Cr removal efficiency varies according to the bacterial strain used, the characteristics of the cloned gene, the Cr application time, the initial Cr concentration, and the environmental conditions (pH, temperature, etc.) of the medium (Fernández et al. 2018).

The decrease in growth tolerance of the MT2A cell as the Cr(VI) concentration increases indicates that Cr(VI) has a toxic effect on the cells at high doses. Already, as the applied concentration increased, the MT2A strain’s Cr(VI) bioremoval and bioreducing capacities decreased. Similar to our findings, the cells were inhibited due to the increase in Cr(VI) toxicity on Ochrobactrum sp. (He et al. 2009), B. anthracis (Xu et al. 2015), and Microbacterium paraoxydans (Mishra et al. 2021) strains due to the increase in Cr(VI) concentration.

When the R\(^2\) values of the adsorption isotherm models were examined, it was determined that the biosorption of total Cr by MT2A was consistent with the Langmuir isotherm model. This means that the total Cr adsorption takes place on the monolayer and homogeneous biosorbent surface. Similar to our findings, Yin et al. (2016) reported that Hg\(^{2+}\) biosorption with E. coli Top10/pBATE2 strain is in
accordance with the Langmuir isotherm model, which is a physicochemical process. There are studies in the literature showing that the Cr adsorption of *Aeromonas caviae* (Loukidou et al. 2004), *Laminaria japonica* (Wang et al. 2008), *Pseudomonas sp* (Ziagova et al. 2007) are in accordance with the Langmuir isotherm model.

In the SEM images, MT2A cells exposed to Cr were overlapping, elongated, and dispersed. Cr adsorbed on the bacterial surface causes changes in the shape of the bacterium. Another possible reason is that when bacteria are exposed to toxic metals, the bacterial cells may clump or stick together, protecting themselves as a defense mechanism (Karthik et al. 2017; Varnca et al. 2021). Naik et al. (2012) suggest that the change in the cell shape due to heavy metal exposure is an adopted mechanism to resist the toxicity of heavy metals. Stress-induced morphological changes may have an important role in cell survival and metabolic activity and, thus, in the absorption of Cr from wastewater (Garg et al. 2013). Changes in the morphology of cells exposed to Cr(VI) in SEM images and the Cr peak in the EDX spectra may indicate accumulation of Cr(III) on the cell surface or interior as a protective strategy for cells against Cr(VI) toxicity. Based on this information, we think that the Cr form accumulated in the cells in our study may be Cr(III). Similar to our findings, Das et al. (2021) suggested that the Cr particles adsorbed or precipitated on the outer surface of the cells from the EDX peaks obtained from the bacterial cells to which Cr(VI) was applied may be the reduced form of Cr(III). On the other hand, Zhu et al. (2008) reported that the EDX peaks of the bacterial cells to which they applied Cr(VI) may be due to Cr(OH)₃ binding to the cell surface. Karthik et al. (2017) reported that Cr(VI) treated cells showed coarse and cluster-like morphology that increased with increasing Cr(VI) concentrations, and they suggested that cluster formations of the cells might have protected bacterial cells from exposure to chemical stress induced by Cr. Garg et al. (2013) made a similar observation, noting differences in SEM morphology between unexposed (control) and exposed *P. putida* cells to 500 mg/L Cr(VI). In another study, after treatment with 100 mg/L Cr(VI), *Rhodobacter sphaeroides* SC01 bacterial cells were observed to become irregular and wrinkled, and cell debris appeared (Su et al. 2021). The black dots in the cell that we found in STEM images could be Cr(III) accumulating in the cell. Similar to our comment, Pei et al. (2020) suggested that the black dot clusters seen inside the cells in the TEM images of recombinant *E. coli* with a cloned E-mcr gene to which they applied Cr(VI) could be Cr(III). It has been reported by different researchers (Karthik et al. 2017; Liu et al. 2019; Sturm et al. 2018; Su et al. 2021; Zeng et al. 2019) that these black dots observed inside the cell as a result of the application of Cr(VI) to various bacterial cells may be Cr(III). There is no study of SEM or STEM images of Cr-treated recombinant strains in the literature, but we can discuss the morphological structures of the same recombinant strains that we exposed to Cd and As in our previous studies. We observed that the morphological structures of the cells were disrupted and many had broken ends in the SEM images of the *E. coli* MT3 strain that we treated with 25 mg/L Cd, the presence of Cd in the EDX peaks, and the clusters reflecting the accumulation of Cd in the cells in the STEM images (Uçkun et al. 2021a). From the SEM images of *E. coli* MT2, which we treated with 1 mg/L, we observed that the cells were adhered to each other, slightly elongated, and had a slightly spongy appearance compared to the control (Uçkun et al. 2021b).

FTIR analysis results showed that hydroxyl, amine, amide I, amide II, phosphoryl, and carbonyl groups of MT2A cells were effective in Cr(VI) binding. Pradhan et al. (2019) suggested that functional groups such as amine, hydroxyl, and carboxyl in the cell wall ensure the absorption of heavy metals. Cells can withstand harsh conditions by modifying their functional groups in the cell wall while absorbing heavy metals (Zakaria et al. 2007). Karthik et al. (2017) determined that the functional groups differed according to the Cr(VI) concentration in the FTIR results of the cells to which they applied different concentrations of Cr(VI). A similar result to ours was observed by Wu et al. (2019) in the formation of a hydroxyl peak due to chromium oxide deposition after Cr(VI) application to *Bacillus* sp. CR7. When the FTIR analysis results of the *Cellulosimicrobium funkei* AR8 cell were examined, it was discovered that alkanes, amides, and amine functional groups were effective in Cr(VI) absorption (Karthik et al. 2017). A similar result was obtained in the study conducted by Bharagava and Mishra (2018), in which it was determined that there were different functional groups such as alkene, carbonyl, and nitro in the results of the FTIR analysis of the Cr applied cell. Maurya et al. (2022) found that the amide I group, hydroxyl, carboxyl, and carboxyl functional groups were effective in binding Cr(VI) to the bacterial cell surface in *B. vallismortis* cells exposed to Cr(VI). Su et al. (2021) discovered that amine, hydroxyl, carboxyl, phosphoric, and sulfate functional groups were efficient in the physical binding of Cr to the cell wall. These studies have similar results to our FTIR results and support our study.

**Conclusion**

Genetic engineering applications enable us to design microorganisms with desirable properties to enable overexpression of metal chelating proteins and peptides and the ability to increase metal accumulation for enhanced bioremediation (Tituia-Arashiro 2018). In this study, Cr(VI) removal with genetically modified species was higher than that with the
wild type. In biosorption, metal accumulation takes place on the cell surface. Metal accumulation on the cell surface occurs in both living and dead cells. The genetically modified bacteria used in this study can accumulate Cr ions inside the cells, in addition to surface adsorption, due to the MT genes we cloned. To our best knowledge, the efficiency of human MT genes in Cr(VI) removal from aqueous solutions and reduction to Cr(III) was determined for the first time in this study. In addition, it was determined that MT2A removed more Cr(VI) than the MT3 gene. Thus, it has been demonstrated that both genes can be used as a tool in the removal or reduction of Cr(VI) from water, but MT2A is more effective. Many genes, proteins, and enzymes have been used in the literature to remove Cr(VI) from water, but research is ongoing to find the most effective biomaterial. When compared with the studies in the literature, it was concluded that the MT genes are the most effective genes that have been tried so far in Cr(VI) removal. With this study, we have added new information to these studies. Based on this knowledge, it is possible to determine which gene, protein, or enzyme will be most effective when attached to a biosorbent in Cr(VI) removal applications from water.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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Authors and Affiliations

Şeyma Akkurt1 · Merve Oğuz2 · Aysel Alkan Uçkun1

1 Aysel Alkan Uçkun
auckun@adiyaman.edu.tr

2 Department of Environmental Engineering, Faculty of Engineering, Erciyes University, Kayseri, Turkey

Present Address: Department of Environmental Engineering, Faculty of Engineering, Adıyaman University, Altınşehir Neighborhood, Ataturk Boulevard, No. 1, Central Campus, 02040 Central, Adıyaman, Turkey