Bioreduction and Bioremoval of Hexavalent Chromium by Genetically Engineered Strains (*Escherichia Coli* MT2A and *Escherichia Coli* MT3)

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Research Article

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Abstract

Studies on the removal of toxic metals from water by genetic engineering applications are becoming more and more diverse. Especially by the expression of cysteine and thiol rich proteins such as metallothioneins in bacterial cells, a high rate of removal of metal ions from the environment is ensured. In this study, we evaluated the removal and reduction of hexavalent chromium Cr(VI) from aqueous solutions with the recombinant strains obtained by cloning the human metallothioneins MT2A and MT3 into *Escherichia coli* Jm109. *E. coli* MT2A was the most effective strain in both Cr(VI) removal (89% in 25 mg/L Cr(VI) application) and Cr(VI) reduction (76% in 25 mg/L Cr(VI) application). Cr(VI) adsorbed per dry cell (at 25 mg/L) were 17 mg/g, 22 mg/g and 19 mg/g for Jm109, MT2A and MT3, respectively. Scanning electron microscope (SEM) images showed that the morphological structures of Cr(VI) treated cells were significantly damaged compared to control cells. Scanning transmission electron microscope (STEM) images showed black spots in the cytoplasm of cells treated with Cr(VI). From the shifts in the Fourier transform infrared spectroscopy analysis (FTIR) spectra of the cells treated with Cr(VI) compared to the control cells, it was determined that the groups interacting with Cr were amino, hydroxyl, methyl and sulfhydryl. When all these experimental data were evaluated together, it was concluded that all three species were effective in removing Cr(VI) from aqueous solutions and MT2A strain could be used as the most effective biotechnological tool.

Introduction

Chromium (Cr) is one of the important pollutants in nature that has high toxicity even at low doses (Kushwaha et al. 2018). Cr is usually found in two forms, trivalent Cr(III) and hexavalent Cr(VI). Cr(III) is beneficial to human health at specific level due to its participation in lipid and glucose metabolism (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 widespread 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 production of pulp (Dhal et al. 2013; Jobby et al. 2018). In these industries, wastes containing Cr(VI) such as sludge, fly ash and slag are produced (Fernández et al. 2018). Cr(VI) causes many diseases in humans, such as skin rash, kidney and liver damage, internal bleeding, dental abnormalities, respiratory disorders, and lung cancer (Martone et al. 2013). Cr(VI) is 100 times toxic and 1000 times more mutagenic compared to Cr(III) (Chang et al. 2019). Reducing Cr(VI) to Cr(III) in Cr(VI) removal is therefore an effective method for significantly reducing the toxic effect of Cr(VI) (Long et al. 2018; Pan et al. 2014). Cr(VI) is recognized as one of the priority pollutants by the United States Environmental Protection Agency (USEPA) (USEPA 2014). It has been reported that the discharge limit of wastewater containing Cr(VI) made by 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 chromium content in surface water and drinking water to be 0.05 mg/L (WHO 2017).

Various physical and chemical methods to remove Cr(VI) from wastewater (oxidation-reduction process, flocculation, ion exchange, electrochemical methods, membrane filtration and reverse osmosis) are used (Akkoyun et al. 2020; Geva et al. 2016). However these methods have some significant disadvantages, such as high costs, secondary pollution formation, toxic sludge formation, and low treatment efficiency (Ahluwalia and Goyal 2007). To overcome such problems, the bioremediation process is an important alternative (Anusha and Natarajan 2020). Bioremediation is an environmentally friendly, clean, inexpensive and efficient treatment process in which contaminants are removed using microorganisms (Ayangbenro and Babalola 2017). However, wild bacterial strains isolated from contaminated environments do not have the ability to selectively bind metals and have a low ability to remove them (Ayangbenro and Babalola 2017).

On the other hand, today genetic engineering applications enable both selectively removing the metals and increasing the metal binding capacity of microorganisms (Yang et al. 2015). There are many strategies to increase the metal binding

capacity of microorganisms. Most of these strategies are based on the binding of metals to recombinant strains by providing metal ion binding polypeptides to the bacterial cell wall (Gupta and Singh 2017). Proteins or peptides such as metallothioneins (MT) or poly-histidins, which have high affinity for metal ions, are cloned to the host cell to increase the metal binding capacity (Ike et al. 2007). MTs are proteins with low molecular weight and high cysteine content found in all living organisms. Metal ions are bonded to sulfhydryl groups in MTs (Pinto et al. 2003). Because of the high thiol content of MTs, they help detoxify heavy metal group I-II, protect cells and organs from the harmful effects of metals, and are considered free radical scavengers (Hussain et al. 1996). There are four distinct isoforms of mammalian MTs (MT1, MT2A, MT3, MT4) (Miles et al. 2000). In many studies on this subject have shown that genetically engineered *E. coli* strains expressing the MT gene increase the removal of various heavy metals (Cr, zinc (Zn), copper (Cu), nickel (Ni), arsenic (As), etc.) (Deng and Jia 2011; Kim et al. 2005; Ma et al. 2011, 2019; Uçkun et al. 2021). Human metallothionein MT2A protein, which we use for the removal of Cr(VI) metal in our study, has several functions. MT2A proteins participate in cell processes and chelate the metal ions, clear large reactive oxygen (ROS) molecules and stimulate antioxidant enzymes (Chen et al. 2014; Starska et al. 2015). Another human metalloprotein that is known to bind metals such as Zn, Cu, cadmium (Cd) (Toriumi et al. 2005), As (Uçkun et al. 2021) is MT3. In the literature, there is no study related to the Cr removal of MT2A and MT3 genes from water. The aim of this study was to ensure the removal and reduction of Cr(VI) from water using genetic engineering technology. 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. Cr(VI) removal and reduction activities of Jm109 strain and other recombinant strains were compared.

Materials And Methods

Cultivation Of Strains

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

Preparation Of Cr(Vi) Solution

Chemicals used in the preparation of solutions for stock metals were obtained from Merc. In this study, Cr(VI) stock solution was prepared by dissolving potassium dichromate ($K_2Cr_2O_7$) metal salt in distilled water. Then, the Cr(VI) solution was sterilized in autoclaves and stored at +4 °C.

Gene Synthesis, Cloning And Bacterial Transformation

The human MTs (MT2A and MT3) gene sequences were provided from Twist Bioscience. MT2A and MT3 gene fragments and the pET21 plasmid were double digested with EcoRI and NotI restriction enzymes (Thermo Scientific). Then these genes were individually ligated to the pET21 plasmid. Host Jm109 strain (ATCC 53323) was made competent according to the chemical $CaCl_2$ protocol (Merc). Recombinant pET21 plasmids (Sigma-Aldrich) were transformed into competent Jm109 cells. Then, recombinant (MT2A and MT3) strains were seeded 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 plasmid was isolated using the QIAGEN Qiaprep Spin Miniprep Kit (Cat no: 27104), and the presence of the positive transformant was confirmed using PCR. 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	tSHPs0903B	300	5646	ATGGACCCT	ATGGACCCT	612	PASS	PASS	pET-21
MT2	tSHPs0903B	300	5646	ATGGATCCC	ATGGATCCC	523	PASS	PASS	pET-21

Minimal Inhibitory Concentration (Mic) Of Cells Against Cr(Vi)

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

Residue of Cr in the medium and bioreduction of Cr(VI)

In the bioaccumulation experiments, the method of Shamim et al. (2014) was modified and used. The strains were incubated at 37°C and 150 rpm for 24 hours in LB broth containing Cr(VI) (0.05-25 mg/L). Samples were taken after the incubation was completed to determine the amount of Cr remaining in the medium. The samples were centrifuged and the supernatant was filtered with a syringe filter. Total Cr accumulations in the samples were measured by inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer, USA & Nexion 350X). The Cr(VI) concentration of the supernatant was analyzed at 540 nm by the 1,5-diphenylcarbazide (DPC) method (Kathiravan et al. 2011).

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

% Cr(VI) reduction: $((C_i - C_f)/C_i) * 100$

C_i: initial Cr(VI) concentration mg/L

C_f: final Cr(VI) concentration mg/L

Bioremoval Of Cr Analysis

In order to determine the accumulation of metal on the cell surface, 1 ml of 50 mM EDTA (Merck) solution was applied to the cells precipitated after centrifugation in the previous step. The samples were centrifuged and the supernatant was taken into another tube for ICP MS analysis. The total amount of Cr in the filtrates was analyzed in the ICP-MS device. To determine the amount of metal removed by the strains, the formula given below was used (Anusha and Natarajan 2020).

% of Remove of Cr: $((C_i - C_s)/C_i) * 100$

Cr in supernatant (C_s): Remaining concentration of Cr after bioaccumulation (mg/L)

Initial Cr concentration (C_i): Initial concentration of Cr added into the medium (mg/L)

Sem, Stem And Edx 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 hours. The cells were then centrifuged at 6000 rpm for 20 minutes 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 hours at 4°C. Cells were washed with PBS and treated with 1% osmium tetroxide for 1–2 hours. 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. The images of the samples were taken using SEM and Energy Dispersive X-Ray (EDX) (ZEISS & EVO LS 10, Germany) devices. 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 cells 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 hours. The samples were then centrifuged at 5000 rpm for 20 minutes 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 cm⁻¹ and 4000 cm⁻¹ wavelength.

Results

Cr Bioremoval And Cr(Vi) Bioreduction Capacity Of The Strains

MIC values of Jm109, MT2A and MT3 cells were found to be 50 ppm, 140 ppm and 75 ppm, respectively. Cr removal rates of bacterial cells from the medium are shown in Fig. 1. Cr removal rates of Jm109 from media containing Cr at 0.05, 0.1, 0.3, 0.5, 10, and 25 mg/L concentrations are 90%, 90%, 85%, 80%, 70% and 68%, respectively. These rates were 97%, 95%, 94%, 92%, 90%, and 89% for the MT2A strain, while 95%, 91%, 89%, 83%, 80%, and 76% for the MT3 strain. While the removal rates were close to each other in the first three Cr concentrations (0.5 mg/L, 0.1 mg/L, and 0.3 mg/L) in each strain, the removal rates decreased as the concentration increased. Cr(VI) reduction rates from media containing the same Cr concentrations are shown in Fig. 2. As with total Cr removal, Cr(VI) reduction was close to each other and very high at low Cr(VI) concentrations (in the range of 92–99%), while at high Cr concentrations it was low (range of 50%-82%). The amounts of Cr adsorbed per dry cell from the medium containing the highest Cr concentration (25 mg/L) were 17 mg/g, 22 mg/g, and 19 mg/g for Jm109, MT2A and MT3, respectively (Table 2).

Table 2
Amount of total Cr (25 mg/L) remaining in the medium and cell in aqueous solution

Strains	Amount of Cr in the medium (mg/L)	Amount of Cr in the cell (mg/L)	Amount of adsorbed Cr per dry cell (mg/g cell)	Percentage of Cr removal of cells (%)
Jm109	17	8	17	68
MT2A	22	3	22	88
MT3	19	6	19	76

The growth tolerance of the MT2A cell against different Cr(VI) (0-400 mg/L) concentrations of the MT2A strain is shown in Fig. 3. Cells grew at low Cr(VI) concentrations, whereas it was inhibited at increasing Cr(VI) concentrations. When a Cr concentration of 100 mg/L and above was applied, growth was significantly reduced. The first inhibition occurred at 100,

200, 400 mg/L Cr(VI) concentrations between 12 and 18 hours. At the 24th hour, 75 mg/L Cr(VI) concentration was added to this concentration range.

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. 4a). 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. 4b). Cr accumulation in the cell wall of Cr(VI) treated cells is confirmed by Cr peaks in the EDX spectra. While no Cr peak was observed in the EDX of MT2A control cells, a Cr peak was observed in Cr treated cells. STEM images of 25 mg/L Cr(VI) treated cells had obvious black spots that could represent intracellular Cr precipitation (Fig. 5). These black spots were absent in control cells that were not treated with Cr(VI).

Ftir Spectra Of The Cells

FTIR analysis was performed only for the MT2A strain. Figure 6 shows the FTIR spectra of MT2A bacterial cells treated with 100 mg/L Cr(VI) and untreated with Cr(VI) (control). Increases were observed in the FTIR spectra of the Cr(VI) treated cells compared to the control. These increases are due to the activity of functional groups on the cell surface and suggest that Cr(VI) binds to the cell membrane. In the Cr(VI) treated cells, peak 3267 cm^{-1} indicates strong OH peak. The presence of OH peak in the Cr(VI) treated cell may have been caused by low concentration of Cr(III) hydroxide precipitation. A similar result to ours was observed by Wu et al. (2019) in the formation of an OH peak due to chromium oxide deposition after Cr(VI) application to *Bacillus* sp. CR7. In this study, the spectrum of 2923.4 cm^{-1} in control shifted to three separate spectra (2962.8 , 2917.7 , 2840.4 cm^{-1}) in the bacterial cells exposed to Cr(VI). These shifts mean that Cr binds to the methyl group of the cells. The shift of the 1514.4 cm^{-1} band in the control cell to the 1531.2 cm^{-1} band in the Cr(VI) applied cell, may be due to the interaction between nitrogen groups (N-O) and Cr(VI) on the cell surface. The 1379.1 cm^{-1} peak in the control cell shifted to 1387.1 cm^{-1} in the Cr(VI) applied cell. This shift indicates that sulfate groups (S-O) are effective in the absorption of Cr(VI).

Discussion

The metal binding capacity of genetically modified microorganisms increases even more than wild strains (Yang et al. 2015). In our study, the strain that removed the most Cr at each concentration and most effective in reducing Cr(VI) was MT2A. In the literature, there are many studies on the removal of Cr(VI) from water. In a study conducted by Zeng et al. (2019), it was found that *Oceanobacillus oncorhynchi* W4 a newly isolated Cr(VI) reducing bacterium, removed 74.2% of Cr(VI) from aqueous solution in 72 h. Bharagava and Mishra (2018) observed that *Cellulosimicrobium* sp. strain isolated from common effluent treatment plant of tannery industries reduced Cr(VI) by 99.33% in 24 h at concentrations of 50, 100, 200 and 300 mg/L Cr(VI). It was determined that *Bacillus* sp. M6 had a 45.9% removal at 200 mg/L Cr(VI) concentration, but the Cr(VI) concentration in the control did not change significantly (Li et al. 2019). It has been reported that *Pseudomonas stutzeri* L1 and *Acinetobacter baumannii* L2 strains reduce Cr(VI) by 40–97% and 84–99%, respectively, at concentrations of 100–1000 mg/L (Sathishkumar et al. 2017). 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, 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) determined the most effective genes in Cr(VI) removal from waters containing 200–600 μM Cr(VI) as E-mcr and E-gsr (at least 50% Cr(VI) removal efficiency) that they cloned in to *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).

As the Cr(VI) concentration of the MT2A cell increases, the decrease in its growth tolerance indicates that Cr(VI) has a toxic effect on the cells at high doses. Already, the Cr(VI) bioremoval and bioreducing capacity of the MT2A strain decreased as the applied concentration increased. 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.

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 strategy for cells to protect themselves 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 caused to protect bacterial cells from exposure to chemical stress induced by Cr. A similar observation was reported by Garg et al. (2013) who noted differences in SEM morphology of 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 we detected in STEM images may reflect the accumulated Cr(III) 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 cloned E-mcr gene to which they applied Cr(VI) could be Cr(III). Similarly, 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).

FTIR analysis results showed that –OH, methyl, sulfate groups of MT2A cells were effective in Cr(VI) binding. In FTIR spectra, functional groups such as amine, hydroxyl and carboxyl in the cell wall ensure the absorption of heavy metals (Pradhan et al. 2019). Cells can withstand harsh conditions by modifying their functional groups in the cell wall while adsorbed 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). When FTIR analysis results of *Cellulosimicrobium funkei* AR8 cell applied Cr(VI) were examined, it was found that alkanes, amide and amine functional groups were effective in Cr(VI) absorption (Karthik et al. 2017). A similar result is in the study conducted by Bharagava and Mishra (2018), it was determined that there are different functional groups such as alkene, carbonyl, nitro in the results of the FTIR analysis of the Cr applied cell.

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 (Tiquia-Arashiro 2018). In this study, Cr(VI) removal with genetically modified species was higher than that of 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 with 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/enzymes have been used in the literature for Cr(VI) removal from water, but studies are continuing to discover the most effective biomaterial. When compared with the studies in the literature, it was concluded that the MT genes are the most effective gene that has been tried so far in Cr(VI) removal. With this study, we have added new information to these studies. In the light of this information, it can be decided which gene, protein or enzyme will be the most effective to be attached to a biosorbent in Cr(VI) removal applications from water.

Declarations

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

Availability of data and material Not applicable

Code availability Not applicable

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Figures

Figure 1

Cr removal rates of bacterial cells from the medium

Figure 2

Cr(VI) reduction rates from media

Figure 3

Growth curve of MT2A strain exposed to different Cr(VI) concentration

Figure 4

SEM images and EDX spectrums of bacterial cells a SEM image of untreated Cr(VI) cells b SEM image of 25 mg/L Cr(VI) treated cells c EDX spectrum of untreated Cr(VI) cells d EDX spectrum of 25 mg/L Cr(VI) treated cells

Figure 5

STEM images of bacterial cells a untreated Cr(VI) cells b 25 mg/L Cr(VI)treated cells

Figure 6

FTIR spectra of control (untreated Cr(VI)) and 25 mg/L Cr(VI) treated MT2A cell

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