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ORIGINAL ARTICLE

Cr(VI) reduction by an extracellular polymeric substance (EPS) produced from a strain of *Pseudochrobactrum saccharolyticum*

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Abstract
A better understanding of the Cr(VI) reduction position and mechanisms by a Cr(VI)-reducing strain is important for the bioremediation of Cr pollution in the environment. In the present study, we were interested in figuring out the role of extracellular polymeric substances (EPS) as the main area for Cr(VI) reduction in the newly reported strain of *Pseudochrobactrum saccharolyticum* LY10. We investigated the subcellular distribution and reduction capability of each cellular component as the main area of Cr(VI) reduction by scanning electron microscopy and soft X-ray spectromicroscopy. The results suggested that most of Cr was presented in the supernatants as Cr(III) after reduction. In the cells, Cr was mostly distributed in the EPS and cell wall, while the EPS had the maximum Cr(VI) reduction rate (81.5%) as compared with the cell wall (30.1%). Soft X-ray spectromicroscopy analysis indicated that Cr accumulated more in the EPS. Therefore, the results suggested that the EPS were the main area for Cr(VI) reduction in the bacteria of *P. saccharolyticum* LY10.

Keywords EPS · *Pseudochrobactrum saccharolyticum* LY10 · Cr(VI) reduction · Subcellular distribution · Soft X-ray spectromicroscopy

Introduction

Chromium (Cr) might pose a severe threat to public health and the environment if discharged without adequate pretreatments (Zhong et al. 2014; Lai and Lo 2008; Beukes et al. 2017). Cr(VI) is one of the prevalent states found in the environment and is more toxic because of its mobility compared with the Cr(III). Therefore, the reduction of Cr(VI) has been widely studied and is of great concern to many researchers. Cr(VI) has also been identified as 1 of the 17 chemicals posing the greatest threat to humans by the United States Environmental Protection Agency (Lan et al. 2014). Compared with the traditional chemical reduction methods, microbial reduction of Cr(VI) has been considered as a promising process for its cost-effective and eco-friendly advantages (Cervantes et al. 2001; Chai et al. 2009; Morel et al. 2011; Ma et al. 2019; Su et al. 2019).

The microbial reduction of Cr(VI) has been reported for a variety of bacterial strains in aerobic or anaerobic conditions, such as *Paracoccus denitrificans* (Mazoch et al. 2004), *Bacillus subtilis* (Morokutti et al. 2005), *Vibrio fischeri* (Fulladosa et al. 2006), *Pseudomonas aeruginosa* (Kilic et al. 2010), and *Pseudomonas putida* (Dogan et al. 2011). A novel Cr(VI) reducing bacteria (*Pseudochrobactrum saccharolyticum* LY10) has also been isolated and characterized for its high Cr(VI) reducing ability, typical alkali tolerance, and halotolerance in our previous study (Long et al. 2013).

The direct enzymatic reaction or other indirect biotransformation to metabolites are the major processes for reduction of Cr(VI) (Lovley 1993; Lee et al. 2000; Valls et al. 2000). Basically, microbial reduction of Cr(VI) can

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be divided into two processes, intracellular and extracellular. Some microbes can directly use the related intracellular reductase to reduce Cr(VI), and some others use extracellular metabolites to reduce the highly toxic Cr(VI). Previous studies have shown that *P. putida* MK1 could use the soluble reductase ChrR to reduce Cr(VI) (Park et al. 2000), whereas *Bacillus megaterium* TKW3 uses a membrane-associated reductase to reduce Cr(VI) (Cheung and Gu 2005). Under anaerobic conditions, microorganisms can use extracellular metabolites with Cr(VI) reducibility to reduce Cr(VI) extracellularly. These studies found that sulfate-reducing bacteria can reduce the sulfate radical to the divalent negative sulfur. This reduction occurs by a series of enzymes, including sulfuric adenosyltransferase and adenylyl sulfate reductase (APS reductase), that generate the metabolite of H_2S as an effective Cr(VI) reducing agent (Tebo and Obraztsova 1998). In addition, some Fe(III) reduction microorganisms reduce Fe(III) to Fe(II) and use the resulting Fe(II) to reduce Cr(VI) (Wielinga et al. 2001). Therefore, there are different Cr(VI) reduction positions and mechanisms for different microorganisms.

Extracellular polymeric substances (EPS), mainly containing polysaccharides, proteins, extracellular DNA, and lipids, exhibit a dynamic double-layered structure with loosely bound EPS and tightly bound EPS (Flemming and Wingender 2010). EPS have an abundance of negatively charged functional groups that react with heavy metals as ligands for which the adsorption characteristics and kinetics have been reported previously (Wang et al. 2014a). The EPS produced by several bacterial species are recognized as bioflocculants, bioabsorbents, encapsulating materials, heavy metal-removing agents, drug delivery agents, ion exchange resins, a natural immunomodulator and antioxidant and antibiofilm agents (Kavita et al. 2013). In addition, previous studies have shown that the Cr(VI) sorption of loosely bound EPS and tightly bound EPS is followed by a charge transfer from the nitrogen and oxygen to the Cr(VI), and the transfer of charge can result in the reduction of Cr(VI) to Cr(III) (Wang et al. 2014b). However, whether the EPS are the main sites of Cr(VI) reduction have not been proven.

The soft X-ray spectromicroscopy has been proved to be a powerful technology for the spectral imaging and chemical speciation analysis of metals. It combined the advantages of scanning transmission X-ray microscopy with high spatial resolution and the near-edge absorption fine structure spectroscopy with high-energy resolution (Smith 2001; Chao et al. 2005). Compared with transmission electron microscopy and other technologies, the soft X-ray spectromicroscopy analysis causes relatively little damage to the samples and can be used in nano-scale research on a variety of forms such as solid and liquid (Jacobsen et al. 2000). For environment pollutants, the soft X-ray spectromicroscopy can also be used to study the chemical distribution of organic compounds and inorganic metals by K-edge and L-edge, respectively.

Overall, we hypothesized that the EPS were the main sites for Cr(VI) reduction in the newly reported strain of *P. saccharolyticum* LY10. To prove this, the Cr(VI) subcellular distribution and the reduction capability of various components of *P. saccharolyticum* LY10 cells were investigated in the present study. Furthermore, scanning electron microscopy (SEM), and specially, the soft X-ray spectromicroscopy method were applied to observe the images of cells or single cell under Cr(VI) stress to confirm the main area of Cr(VI) reduction in the cell.

Methodology

Bacteria and media

The novel Cr(VI)-reducing strain of *P. saccharolyticum* LY10, isolated in our previous study (Long et al. 2013) and characterized for its high Cr(VI)-reducing ability, alkali tolerance and halotolerance, was selected for the present study. The common Luria–Bertani (LB) medium with tryptone (10 g), yeast extract (5 g), NaCl (5 g), and distilled water (1 L) in pH 8.0 was used during the incubation of bacteria (McLean and Beveridge 2001; Chen et al. 2012). Cr(VI) was added into the LB medium of K_2CrO_4 (200 mM) after the filtration by 0.22 μm membrane.

Subcellular components separation

Pseudochrobactrum saccharolyticum LY10 was inoculated in liquid LB medium containing 110 mg L^{-1} Cr(VI) and 220 mg L^{-1} Cr(VI), and then was cultured at 28 °C, 160 rpm for 96 h. The broth was centrifuged for 30 min at 6000*g* and then, the supernatant and cells was separated. The concentration of total Cr and Cr(VI) in the supernatant was measured. The obtained cells were further separated for the subcellular components (Chen 2011). The cells were first resuspended in 10 mL 0.9% NaCl solution and centrifuged at 10,000*g*, 4 °C for 30 min. The remaining supernatant was the extracellular polymeric substances (EPS). The broth was further resuspended with 10 mL 25 mM Tris–HCl buffer (pH 7.0), and the cells were broken by ultrasonic (200 w, 30:30 s, 20 times). The solution was centrifuged (2500*g*, 4 °C, 20 min) to remove the unbroken cells, which were redissolved in 10 mL 25 mM Tris–HCl buffer and broken completely by ultrasonic. The mixed broken solution was centrifuged (30,000*g*, 4 °C, 30 min) to obtain the precipitate (cell wall). While the remaining supernatant was centrifuged at 110,000*g*, 4 °C for 2 h, the precipitate was the cell membrane, and the rest of the supernatant was soluble fraction of cells.

The pellet obtained after centrifugation was digested with concentrated nitric acid at 200 °C for 2 h and diluted with 5% hydrochloric acid at constant volume. The soluble components were treated with concentrated nitric acid (5%) overnight. A Thermo Element MKII-M6 atomic absorption spectrophotometer was used to measure the total Cr content in different components. Colorimetric method was used to determine the concentration of Cr(VI) at 540 nm using the DPC method with a detection limit of 0.2 μg L^{-1}.

Cr(VI) reduction by different cell components

The *P. saccharolyticum* LY10 was incubated in LB liquid medium without Cr(VI) and cultured at 28 rpm for 24 h. The EPS, cell wall, cell membrane, and soluble fraction were obtained by the centrifugation method. Following purification, the ability of different cell components to reduce Cr(VI) was tested. Generally, 10 mL of phosphate buffer (0.1 M, pH 7.0) was added separately into the cell components, and a Cr(VI) stock solution was then added to a final Cr(VI) concentration of 5.2 mg L^{-1} in the system. The Cr(VI) was sampled in each system under 28 °C, 160 rpm for 16 h to measure the remaining Cr(VI) concentration and further analyze the Cr(VI) reduction capability of different cellular components.

SEM analysis

Pseudochrobactrum saccharolyticum LY10 was inoculated in liquid LB medium that contained 55 mg L^{-1} Cr(VI), 110 mg L^{-1} Cr(VI), or 220 mg L^{-1} Cr(VI). Cultures were maintained at 28 °C, 160 rpm for 96 h, and then the cells were collected after centrifugation (6000 rpm, 4 °C, 10 min). The cells were placed in 2.5% glutaraldehyde solution overnight at 4 °C. The samples were rinsed with 0.1 M, pH 7.0 phosphate buffer three times for 15 min. The samples were fixed for 1–2 h with 1% osmium tetroxide solution and rinsed with 0.1 M, pH 7.0 phosphate buffer three times for 15 min each. The samples were dehydrated for 15 min with different concentrations of ethanol solution: 50%, 70%, 80%, 90%, and 95%, followed by dehydration with 100% ethanol twice for 20 min. The samples were treated with a mixture of ethanol and isoamyl acetate (V/V = 1/1) for 30 min, then pure isoamyl acetate for 1–2 h. Then, the samples were dried at the critical point and plated. The processed samples were analyzed using an XL30ESEM environmental scanning electron microscope (Netherlands).

Analysis of soft X-ray spectromicroscopy

Sample preparation

Pseudochrobactrum saccharolyticum LY10 was inoculated in liquid LB medium that contained 220 mg L^{-1} Cr(VI) with sampling after 24 h and 48 h. The cultures were centrifuged at 5000 rpm, 4 °C for 1 min, and the cells were washed twice with sterile water to remove any Cr interference from the culture medium. The clean bacterial cells were resuspended in sterile water. The cells were dripped to a silicon nitride window, allowed to air dry, and analyzed by the soft X-ray spectroscopy micro-beam line.

Sample measurement and data analysis

The sample detection by soft X-ray spectromicroscopy was completed at beam line 08U of the Shanghai Synchrotron Radiation Facility (SSRF). Data ware analyzed using dual-energy ratio-contrast image method. For each specimen, two absorption images were scanned separately with energies E1 and E2, of which E1 was focused on the absorption edge of the chosen element and E2 was focused below the edge (Smith 2001; Chao et al. 2005). Because the change in photon energies was only a few percent or less, the absorption of X-rays by all the other elements in the specimen was negligible, and consequently any observed difference in the transmitted X-ray flux between the two images obtained at the two energies could be attributed to the presence of the element being studied (Smith 2001; Chao et al. 2005). The total electron yield (TEY) method was applied to detect the Cr(VI) reference sample of $K_2Cr_2O_7$ and the Cr(III) reference sample of $CrPO_4$. The absorption edge (E1) of Cr(VI) and Cr(III) was confirmed to be 579.8 eV and 576.8 eV, respectively. The E2 which was below the edge focused at 573.8 eV. The three energies were used to scan the sample for image. The scanning range was determined according to the sample of which the scanning step was 0.03 μm and every pixel acquisition time was 20 ms. The distribution map of Cr(VI) was obtained by digital division of two absorption-contrast images at energies of 579.8 eV (at the peak) and 573.8 eV (pre-peak). Similarly, the Cr(III) maps was obtained by the digital ratios of 576.8 eV and 573.8 eV (Smith 2001; Chao et al. 2005). Microsoft Excel and SPSS software version 13.0 were used for the data processing and analysis. In addition, the probability value of 0.05 was used to determine significance in the analysis of variance.

Results

Subcellular distribution of Cr in cells

The Cr content was determined for each component as shown in Fig. 1a. It was found that the respective Cr contents were 102.3 and 208.8 mg L^{-1} in supernatants of the 110 and 220 mg L^{-1} cultures, which accounted for more than 92% of the total amount of Cr, while the immobilized, cellular Cr was only 5.1–7.1% of the total amount. However, most of

the Cr was in the form of Cr(III) in the supernatants, which accounted for 76.9 and 54.0% of the total Cr in supernatants for the two treatments, respectively. To further study the distribution of Cr in different subcellular components, the gradient centrifugation method was applied to divide the whole cells into four components including the EPS, cell wall, cell membrane, and intracellular soluble fraction. Figure 1b showed that the Cr contents were mostly distributed in the EPS and cell wall, accounting for more than 90% of the total Cr in cells. It was noted that the content of Cr was more in the EPS (47.1%) compared with the cell wall (43.3%) when the initial concentration of Cr(VI) was increased to 220 mg L^{-1}. There was only 6.4 and 7.5% of the intracellular soluble fraction at 110 and 220 mg L^{-1} of Cr(VI) concentration, respectively, whereas the Cr distribution was lowest in the cell membrane (1.5 and 2.1%, respectively).

Cr(VI) reduction by different subcellular fractions

The four subcellular fractions were freshly obtained by the gradient centrifugation method and incubated for Cr(VI) reduction in medium containing 5.2 mg L^{-1} Cr(VI) for 16 h. The ability of different subcellular fractions to reduce Cr(VI) was compared and presented in Fig. 2. The results indicated that the four fractions had obviously different abilities to reduce Cr(VI). Interestingly, the EPS had the maximum Cr(VI) reduction rate (81.5%) within 16 h, while the cell wall had the lowest capacity of Cr(VI) reduction with only 30.1% of Cr(VI) reduced within the same amount of time. The reduction rate of Cr(VI) was 69.2 and 49.3% for intracellular soluble fraction and cell membrane, respectively.

SEM observation of cells under Cr(VI) stress

SEM was used to observe the micro-appearance of *P. saccharolyticum* LY10 under different Cr(VI) stressors. In the

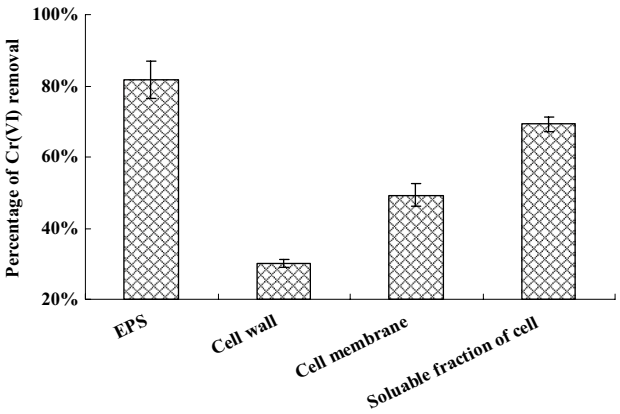

Fig. 2 The Cr(VI)-reducing abilities of different subcellular fractions of *P. saccharolyticum* LY10 [experiments were conducted with initial 5.2 mg L^{-1} Cr(VI)]

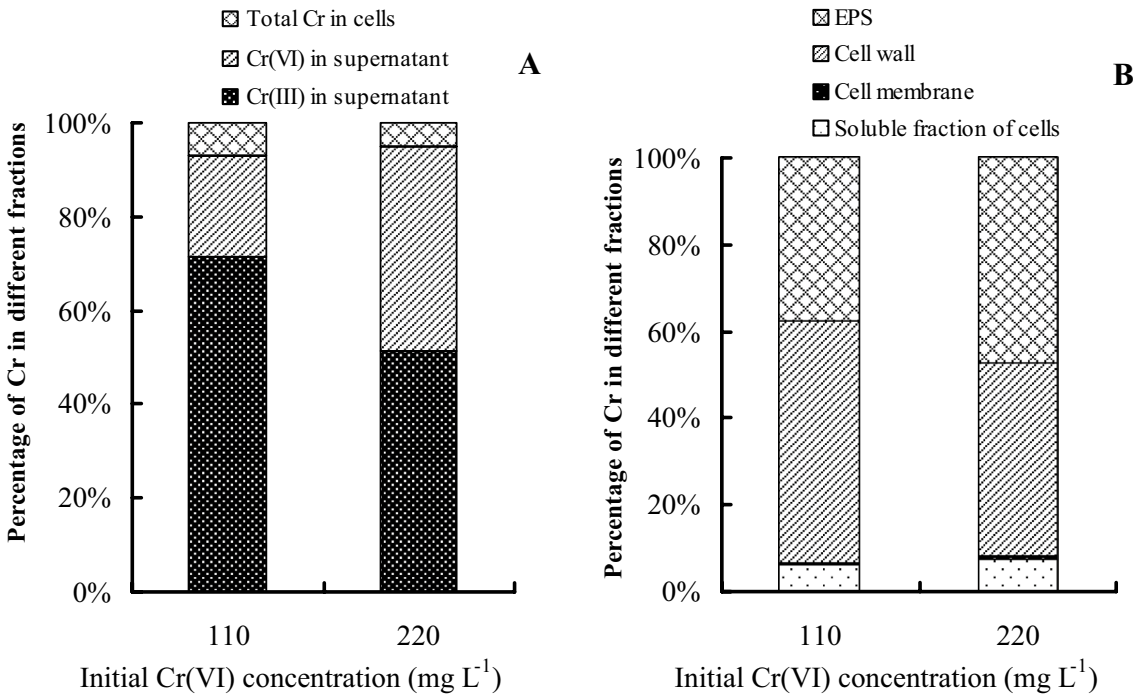

Fig. 1 Distribution profiles of Cr in different fractions of *P. saccharolyticum* LY10 culture (**a** distribution in supernatant; **b** the subcellular distribution profile of Cr in cells)

absence of Cr(VI), SEM results revealed that the cells were rod shaped with regular shapes, and they were dispersed throughout the system with no clear connections by extracellular materials (Fig. 3a). At the 55 mg L^{-1} Cr(VI) concentration, EPS was clearly outside of each cell (Fig. 3b). With the increase of Cr concentration, the shape of the cells changed (Fig. 3c). When the *P. saccharolyticum* LY10 was exposed to 220 mg L^{-1} of Cr(VI) for 96 h, a large number of EPS emerged surrounding the cells, and some cells were wrapped up by EPS.

Distribution of Cr in the single cell by soft X-ray spectromicroscopy analysis

The soft X-ray spectromicroscopy analysis was used to study the distribution of different forms of Cr in a single cell of *P. saccharolyticum* LY10. The results indicated that an obvious accumulation of EPS could be observed surrounding the single cell with exposure to 220 mg L^{-1} Cr(VI), even when the cell has been washed with sterilized water twice prior to analysis (Fig. 4). This result confirmed the conclusion that large amounts of EPS appeared and closely connected with the cells under the toxic Cr(VI) stress, which was previously observed by SEM.

The distribution of Cr(VI) or Cr(III) in a single cell, determined by the soft X-ray spectromicroscopy analysis, was presented in the Fig. 5. The figures indicated that the distribution of both Cr(VI) and Cr(III) was greater in the EPS surrounding the cell, while there was rarely any Cr(VI) and Cr(III) inside the cells. After 24 h incubation, the surface density of Cr(VI) in the single cell ranged from 1.54×10^{-6} to 4.84×10^{-6} g/cm^2, while the surface density of Cr(III) was 1.09×10^{-6} to 2.78×10^{-6} g/cm^2. However, the surface density of Cr(VI) decreased to 1.29×10^{-6} to 2.91×10^{-6} g/cm^2 after exposure for 48 h, while the surface density of Cr(III) increased to 1.76×10^{-6} to 5.22×10^{-6} g/cm^2. The calculated surface density suggested that the Cr(VI) in the EPS was gradually reduced to Cr(III) with incubation time (Fig. 5, 48 h).

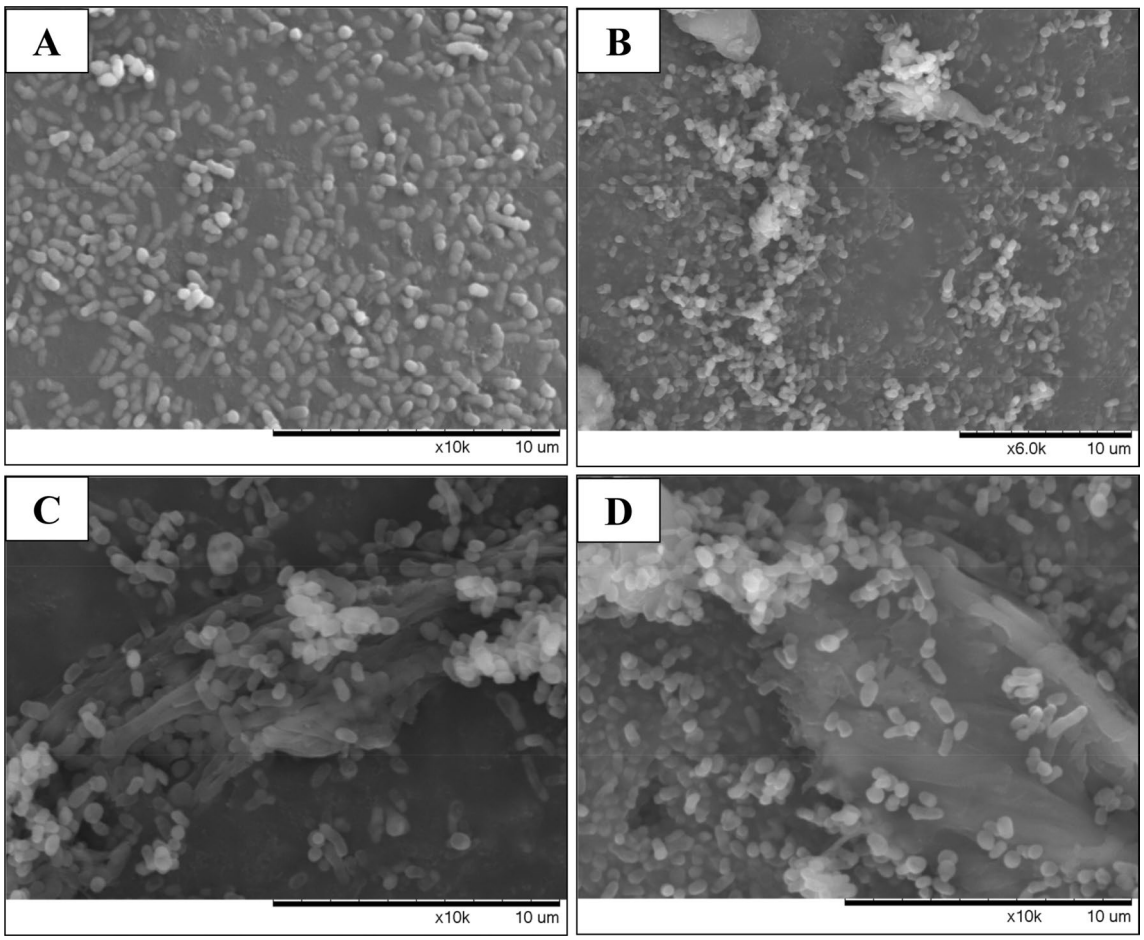

Fig. 3 SEM images of *P. saccharolyticum* LY10 cells [**a** cells were not treated with Cr(VI); **b** cells were incubated with 55 mg L^{-1} Cr(VI); **c** 110 mg L^{-1} Cr(VI); **d** 220 mg L^{-1} Cr(VI)]

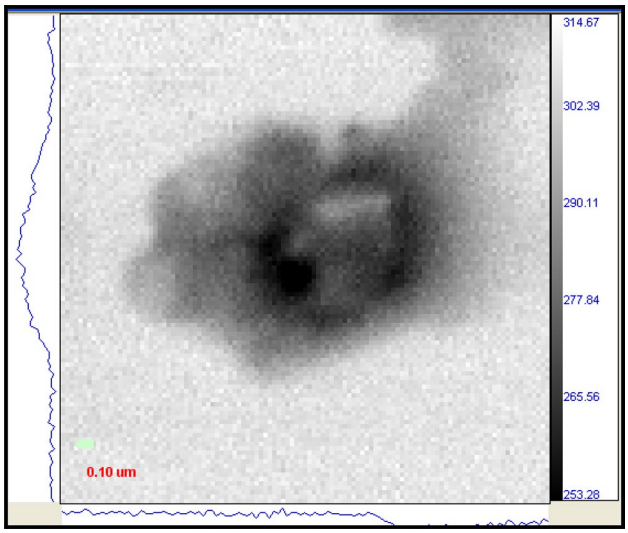

Fig. 4 Morphology of a single *P. saccharolyticum* LY10 cell by soft X-ray spectromicroscopy analysis (3.3 µm × 3.3 µm)

Discussion

After 96 h of incubation, the Cr(VI) concentration decreased from 110 to 220 mg L^{-1} to 23.6 and 96.1 mg L^{-1}, respectively, which confirmed the high efficiencies of Cr(VI) removal by *P. saccharolyticum* LY10 (Long et al. 2013). The Cr(VI) reduction by the microorganism could be divided into intracellular and extracellular reduction (Pal and Paul 2008). The Cr(VI) compounds had a tetrahedral structure, a similar molecular structure with SO_4^{2-}. Thus, Cr(VI) could transfer into the cells using the sulfate channels on the cell membrane (Ramirez-Diaz et al. 2008). On the contrary, the Cr(III) compounds had a octahedral structure and were unable to pass the cell membrane (Peterson et al. 1997). Therefore, the extracellularly reduced Cr(III) could not enter the cell membrane, and it was also difficult to discharge the Cr(III) reduced from Cr(VI) intracellularly through the cell membrane. Figure 1 showed that a large percentage of total Cr (more than 92%) was present in the supernatants, most of which was in the form of Cr(III), and only 5.1–7.1% was

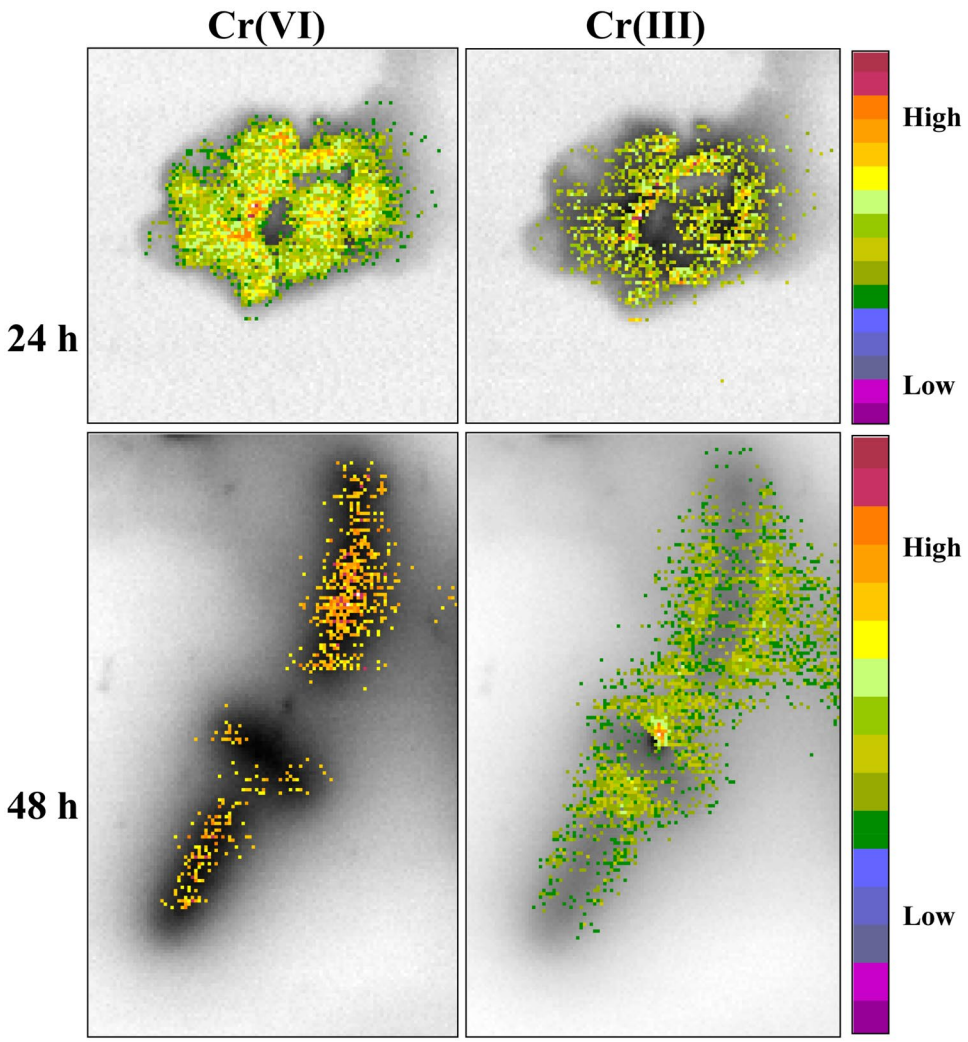

Fig. 5 The distribution of different Cr species in a single cell of *P. saccharolyticum* LY10 by soft X-ray spectromicroscopy analysis (24 h: 3.3 µm × 3.3 µm; 48 h: 3.3 µm × 5.4 µm)

immobilized by the cells, suggesting that Cr(VI) reduction occurred mainly extracellularly rather than intracellularly.

Previous studies on the subcellular distribution of heavy metals in the cell have already suggested that the intracellular soluble fractions were the main distribution area (Kong et al. 1992; Keim et al. 2001). However, the present study revealed that Cr contents were mostly distributed in the EPS and cell wall compared with other parts in the cell, as shown in Fig. 1b. Considering the Cr(VI) reduction capacity of different fractions, following the order: EPS > soluble fraction of cell > cell membrane > cell wall (Fig. 2), the cell wall had limited capacity of Cr(VI) reduction. Therefore, the removal of Cr(VI) by the strain *P. saccharolyticum* LY10 was mainly achieved extracellularly, and the EPS was the main area for the reduction process.

EPS produced by microorganisms are of particular relevance to the bioremediation process because of their involvement in flocculation and binding of metal ions from solutions (Salehizadeh and Shojaosadati 2003; Kachlany et al. 2001). Because EPS have many functional groups such as hydroxyl, amino, carboxyl, and phosphoric acid groups, the EPS can not only provide numerous binding sites for heavy metal ions but also effectively prevent the entry of toxic heavy metals into the microbial cells, and reduce the damage from toxic heavy metals to the cells (Valdman et al. 2001; Ueshima et al. 2008; Comte et al. 2006; Priester et al. 2006). Previous studies revealed that EPS of a biofilm has a much greater Pb^{2+} and Cd^{2+} fixation capacity than the microbial cells in the water environment (Kang et al. 2005). It was also found that more Cr was removed when large amounts of EPS were produced, suggesting that the bacterial EPS did contribute to the Cr(VI) removal (Jia et al. 2014). The removal of heavy metals by the bacterial EPS would not only increase the sensitivity of bacteria to heavy metals but also significantly reduce the cell immobilization and absorption ability of metal ions (Ueshima et al. 2008; Liu et al. 2002; Fang et al. 2011). Yang et al. (2007) studied the Cr(VI) resistance mechanism of *Enterobacter cloacae* using an atomic force microscope. Their results showed that the cell size of *E. cloacae* changed with the increase of Cr(VI) concentration, and the cell length increased from 2.3 ± 0.6 μm without Cr(VI) stress to 3.2 ± 0.7 μm under 400 mg L^{-1} Cr(VI). Specially, the EPS might be highly produced to hinder the entry of Cr(VI) into the cells, thereby protecting the cells.

The SEM results confirmed that there were no obvious EPS appearing between the cells without Cr(VI) exposure (Fig. 3a). However, with increasing Cr(VI) concentration, the EPS increased and tightly gathered near the cells, which might served as a protection mechanism for the cells of *P. saccharolyticum* LY10 (Fig. 3b–d). This was also confirmed by TEM-EDS analysis, which revealed that at high concentration, Cr(VI) exposure over time resulted in Cr accumulation in the extracellular of *P. saccharolyticum* LY10 in our previous studies (Long et al. 2013). Further, when the strain *P. saccharolyticum* LY10 was exposed to 220 mg L^{-1} Cr(VI) for 96 h, black particles emerged surrounding the cells, and some cells were wrapped up by these extracellular particles. The black particles were further analyzed by energy dispersive X-ray spectrum (EDX), and the results showed that a large amount of Cr (15.1%) was contained in the black particles, whereas the Cr content in the cells was less than 10% (Long et al. 2013). These findings were consistent with results that EPS was the main area of Cr(VI) reduction in *P. saccharolyticum* LY10 which was obtained from the subcellular distribution of Cr in cells and the comparison of Cr(VI) reduction by different subcellular fractions.

The differences in Cr(VI) and Cr(III) distribution revealed that the majority of Cr(VI) was trapped outside the cells in EPS and was gradually reduced to Cr(III). Due to the octahedral structure of Cr(III), it could not pass through the cell membrane (Peterson et al. 1997), and, as a result, accumulated in the EPS and the cell wall (Fig. 5). This finding, again, suggested that the EPS secreted by *P. saccharolyticum* LY10 could most effectively trap Cr(VI) outside the cells and reduced it to Cr(III). Therefore, EPS was the main area of Cr(VI) reduction in the *P. saccharolyticum* LY10. Due to the high concentration (220 mg L^{-1}) of Cr(VI), EPS could not trap and reduce all of the Cr(VI) outside the cells. Consequently, a small amount of Cr(VI) with tetrahedral structure got into the cells using the sulfate channels on the cell membrane (Ramirez-Diaz et al. 2008). Subsequently, little Cr(VI) existed after 48 h exposure as shown in Fig. 5.

Microbial reduction of Cr(VI) has been identified as having two major enzymatic mechanisms (Cheung and Gu 2007). Aerobic reduction was thought to be a detoxification mechanism where cells used a soluble enzyme to reduce Cr(VI) to Cr(III) internally or externally to the plasma membrane, while reduction might also proceed through the use of CrO_4^{2-} as a terminal electron acceptor during anaerobic respiration (McLean and Beveridge 2001). Currently, many soluble enzymes have been found in different microorganisms with the ability to reduce chromate (Park et al. 2000; Cheung and Gu 2005, 2007). Cr(VI) served as a non-essential element to microorganisms (Ramirez-Diaz et al. 2008) and had a high cytotoxicity (Coetzee et al. 2018). To protect the cells from toxic damage due to the high concentration of Cr(VI) strain *P. saccharolyticum* LY10 secreted more EPS to wrap the cells. In the EPS Cr(VI) was reduced to Cr(III), and intercepted by adsorption. This combined mechanism could help the cells to survive under high Cr(VI) stress. With the increase in Cr(VI) concentrations, the protection mechanism of *P. saccharolyticum* LY10 became more apparent. The extracellular soluble reductase was the key enzyme found in the EPS and was potentially involved in Cr(VI) reduction (Viti et al. 2014). Compared with the intracellular reduction, extracellular reduction by EPS could efficiently decrease

the toxicity of Cr(VI) for key subcellular components and, provide better protection for the cells. Overall, the results suggested that EPS was the main Cr(VI) reduction site of *P. saccharolyticum* LY10, and an absorbed soluble reductase apparently catalyzed reduction.

Conclusion

The main area of Cr(VI) reduction in the strain of *P. saccharolyticum* LY10 was investigated in the present study. The results indicated that most of Cr was presented in the supernatants in the form of Cr(III) after reduction. In the cell, Cr was mostly distributed in the EPS and cell wall, accounting for more than 90% of the total Cr. However, EPS had the maximum Cr(VI) reduction rate (81.5%) compared with the intracellular fraction, cell wall and cell membrane, suggesting that the main pathway of Cr(VI) removal was extracellular reduction by the EPS rather than intracellular immobilization. The analysis of SEM and TEM confirmed that EPS appeared around the external walls with the Cr(VI) stress applied, and a large amount of Cr was contained in the EPS. Based on the soft X-ray spectromicroscopy analysis, the distribution of the Cr(VI) or Cr(III) in a single cell was much greater in the EPS surrounding the cell, while there was rarely chromium inside the cell. Therefore, we concluded that EPS were of great importance in the Cr(VI) resistance and reduction in the bacteria of *P. saccharolyticum* LY10 and were the main area for Cr(VI) reduction.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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