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Reduction of Chromium by Microbial Method: Transmission Electron Microscopic Study using Electron Energy Loss Spectroscopy *in-situ* Environmental Cells

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In the present study, we present the indirect Cr^{6+} reduction by *Shewanella oneidensis* using sulphur as energy source under aerobic and anaerobic conditions and the subsequent Cr^{3+} precipitation by *Shewanella oneidensis* under anaerobic conditions. Intermediate products such as sulfite and thiosulfite promote Cr^{6+} reduction. Both processes have been operated sequentially under continuous flow conditions to decontaminate 5mg/l^{-1} Cr^{6+} solutions.

The associated mechanism of Cr^{6+} reduction are technological and biological importance because the convert a toxic, mobile element into less toxic, immobile form. The results obtained through this investigation indicates possibility of capability to determine chemistry and valance state of reduction product associated with hydrated bacteria in more of less either than natural state *i.e.* (No. TEM specimen preparation) in an environmental cells.

Keywords: Spectroscopy, electron energy, Hexavalent chromium, microbial reduction, *in-situ* environmental cells.

Hexavalent chromium was classified as primary contaminant because of its mobility in soil and groundwater and its reported harmful effects on organisms including humans. Many industrial processes as ore processing, electroplating, leather-tanning process among others (Lawson, 1997; Thorat,2000) generate wastewaters containing Cr^{6+}. The reduction of toxic Cr^{6+} leads to the formation of stable and non-toxic Cr^{3+} this reduction is followed by precipitation or immobilization can be produced by chemical or biological action (Melhorn *et. al*., 1994; Rajwade and Parknikar 1997; Salunkhe *et.al*., 1998.). Industrial effluent containing chromium has toxicity as their dissolved Cr^{6+}. The Cr^{6+} salts can easily go into circulation system through lungs, in filtrate cells, combined with big molecules *in vitro* after being reduced to Cr^{3+} and eventually form the ultimate carcinogen (O'Brien *et.al*., 2003: Shakoon *et.al*., 2004).

Reduction of Cr^{6+} by bacterium, *Shewanella oneidensis* (previously classified *Shewanella putrefactions* strain MR-1), was studied by absorption spectrophotometry and in-situ, Environmental Cell (EC)-Transmission Electron Spectroscopy (TEM) coupled with Electron Energy Loss Spectroscopy (EELS). Shewanella oneidensis (*S. oneidensis*), a gram negative, facultative bacterium is capable of respiring aerobically and anaerobically using a variety of compounds, including O_2, Fe (III), Mn (IV), NO_2^-, NO_3^-, SO_2, SO_3^{2-}, thiosulphate(S_2O_3), triethanamine oxide, fumarate, U(VI), and Cr (VI) as terminal electron acceptor. *S. oneidensis*

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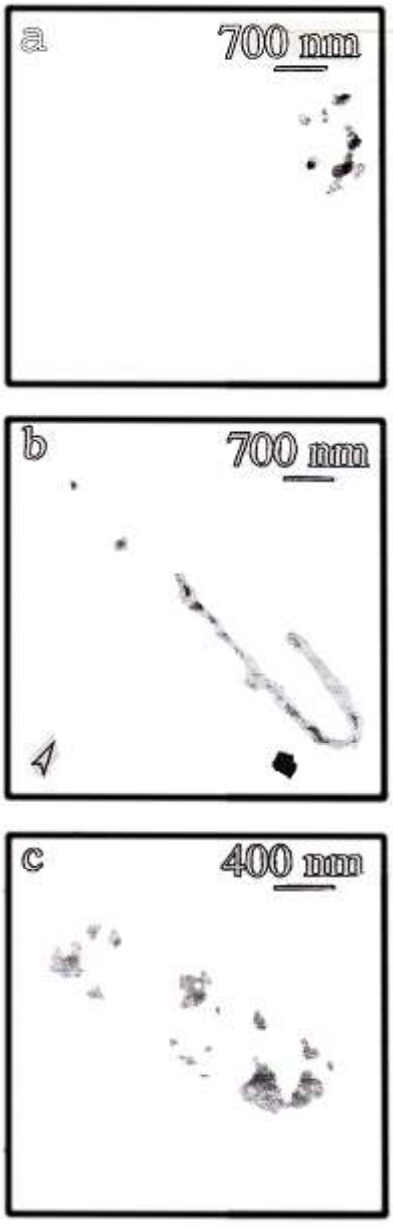
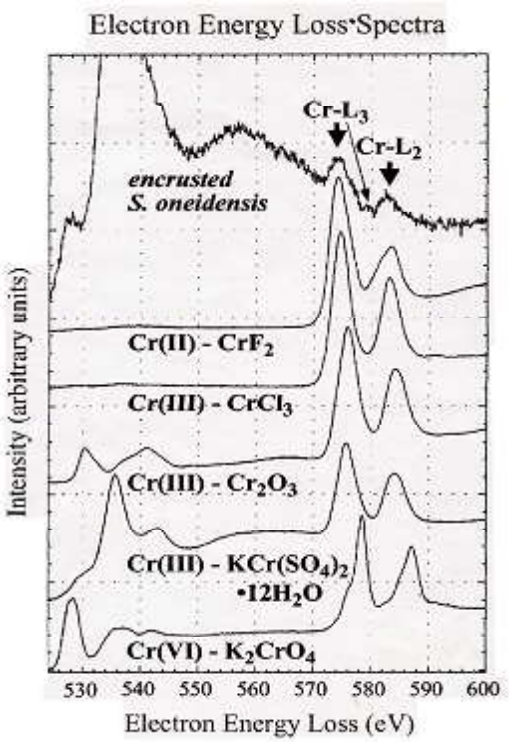
belongs to the gamma subclass of Proteobacteria and has been isolated from lascustrine and marine environment.

MATERIAL AND METHODS

Cultures were carried out in a reactor vessel containing 12gm of powered sulfur and 0.61 of iron free 9K medium (Silverman and Lundgren, 1959), inoculated at 10% with the pH was maintained with the automatic condition of 1.25 adjusted to 2.0, 4.0 or 6.0. pH was maintained at 30°C and stirred at 400rpm. When free bacterial population reached 5 x 10^8 cells ml^{-1}, 100ml. of the culture were filtered through blue ribbon filter paper and then through a 0.45u filter; then 20ml of solution of $K_2Cr_2O_7$ containing 10mg.l^{-1} Cr^{6+} at pH 2.0 added cells and sulfur particles of size less than 3um) during 20 min to allow Cr^{6+} reduction. Afterwards, the solution was forced through the membrane using vacuum pump. Membranes were washed with sulfuric acid (pH 2.0) and fixed with 10% glutaraldehyde in phosphate buffer (pH 7.0 to allow microscopic / EDTA examination.

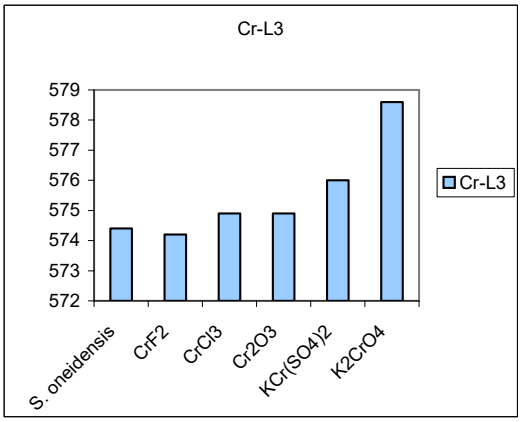
In this work, *S. oneidensis* was grown in a synthetic medium containing 100 M of Cr^{6+}. The microbial reductions of Cr^{6+} in these cultures were measured spectrophotometrically using colorimetric techniques. Microanalysis of individuals bacteria and their associated reduction products was performed by EC-TEM.

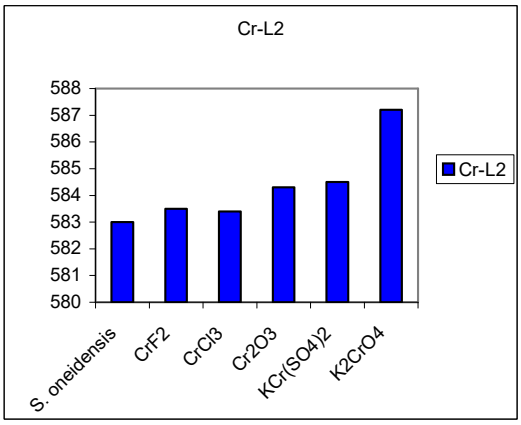


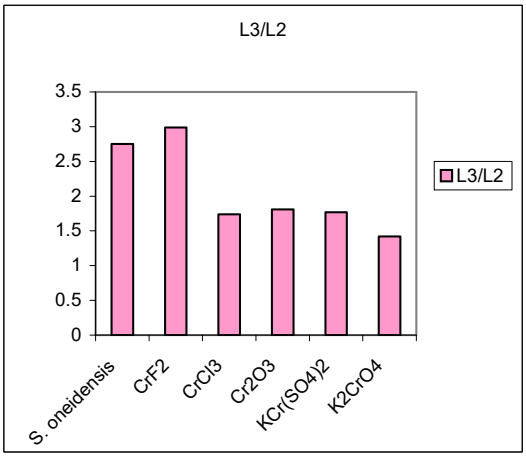


RESULTS AND DISCUSSION

A JEOL JEM-3010 transmission electron microscope equipped with JEOL EC system was used in this study. Confinement of the pressurized environment within the EC is achieved with electron transparent windows. Bacterial cultures were examined by EC-TEM at 100 Torr, under a circulation of air saturated with water vapor. *S. oneidensis* exhibit rod shaped morphology typically of this polarly flagellated and non-spore forming species (Fig. 1). The EC-TEM micrographs demonstrate that bacterial membranes are intact and do not show evidence of rupture by partial decompression. The cells remain plump/ hydrated while the extracellular polymeric substances retain moisture and appear as a continuous capsule surrounding the cells. Bright field images show two distinct populations of *S. oneidensis* in incubated cultures containing Cr^{6+} those that exhibit low image constract (Fig.1a) and heavily precipitate encrusted cells exhibiting high image constrast (Fig. 1b and 1c). Several EELS techniques were applied to determine the oxidation state of the Cr associated with the encrusted cells examined in the EC. Oxidation state was determined by measuring the chemical shift and intensity ratio of Cr- L2, 3 adsorption peaks. The precipitate encrusted bacteria are shown to contain a reduced form of Cr in oxidation state +3 of lower (Fig.2 and Table 1). Furthermore, evidence is found for Cr^{6+} fixation or complexation by encrusted bacteria of their encapsulating extracellular polymeric excretions. Strong direct evidence indicating that the bacteria are the active sites of Cr^{6+} reduction is provided by EELS spectra. Microanalysis suggests that *S. oneidensis* can sequester Cr^{6+} from its environment by both complexation and reduction into insoluble forms. These results demonstrate the capability to determine chemistry and valence state reduction products associated with hydrated bacteria, in more or less their natural state (*i. e.* no TEM specimen preparation), in an environmental cells.







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