MICROBIAL REDUCTION OF CHROMIUM(VI): IN-SITU ENVIRONMENTAL CELL TRANSMISSION ELECTRON MICROSCOPY STUDY USING ELECTRON ENERGY LOSS SPECTROSCOPY

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The geochemistry and toxicity of chromium is controlled by its valence state. Chromium is a redox active 3*d* transition metal with a wide range (-2 to +6) of possible oxidation states; however only Cr(III) and Cr(VI) are stable. Hexavalent Cr(VI) can be readily reduced to the trivalent state by Fe^{2+}, S^{2-}, organic compounds, wetland plants, and several species of microorganisms.[1] The associated mechanisms of Cr(VI) reduction are technologically and biologically important because they convert a toxic, mobile element into a less toxic, immobile form.

Reduction of Cr(VI) by the bacterium, *Shewanella oneidensis (*previously classified *Shewanella putrefaciens* strain MR-1), was studied by absorption spectrophotometry and *in-situ*, environmental cell (EC) - transmission electron microscopy (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-}, thiosulfate ($S_2O_3^{2-}$), trimethyamine oxide, fumarate, U(VI), and Cr(VI) as terminal electron acceptors. *S. oneidensis* belongs to the γ-subclass of *Proteobacteria* and has been isolated from lascustrine and marine environments.[2] In this work, *S. oneidensis* was grown in a synthetic medium containing 100 µM of Cr(VI). The microbial reduction of Cr(VI) in these cultures were measured spectrophotometry using colorimetric techniques. Microanalysis of individual bacteria and their associated reduction products was performed by EC-TEM.

A JEOL JEM-3010 transmission electron microscope equipped with a 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 typical 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(VI): those that exhibit low image contrast (Fig. 1a) and heavily precipitate-encrusted cells exhibiting high image contrast (Figs. 1b & 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 (a) the chemical shift and (b) intensity ratios of Cr-$L_{2,3}$ adsorption peaks. The precipitate encrusted bacteria are shown to contain a reduced form of Cr in oxidation state +3 or lower (Fig. 2 & Table I). Furthermore, evidence is found for Cr(VI) fixation or complexation by encrusted bacteria or their encapsulating extracellular polymeric excretions. Strong direct evidence indicating that the bacteria are the active sites of Cr(VI) reduction is provided by the EELS spectra. Microanalysis suggests that *S. oneidensis* can sequester Cr(VI) from its environment by both complexation and reduction into insoluble forms. These results demonstrate the capability to determine chemistry and valence state of reduction products associated with hydrated bacteria, in more or less their natural state (i.e. no TEM specimen preparation), in an environmental cell.

References:

1.) S. Fendorf *et al*., *Internat. Geol. Rev.* 42 (2000) 691-701
2.) K. Venkateswaran *et al.*, *Int. J. Syst. Bacteriol.* 49 (1999) 705-724
3.) Work supported under NRL 0602233N and ONR & ASEE Postdoctorial Fellowship programs.

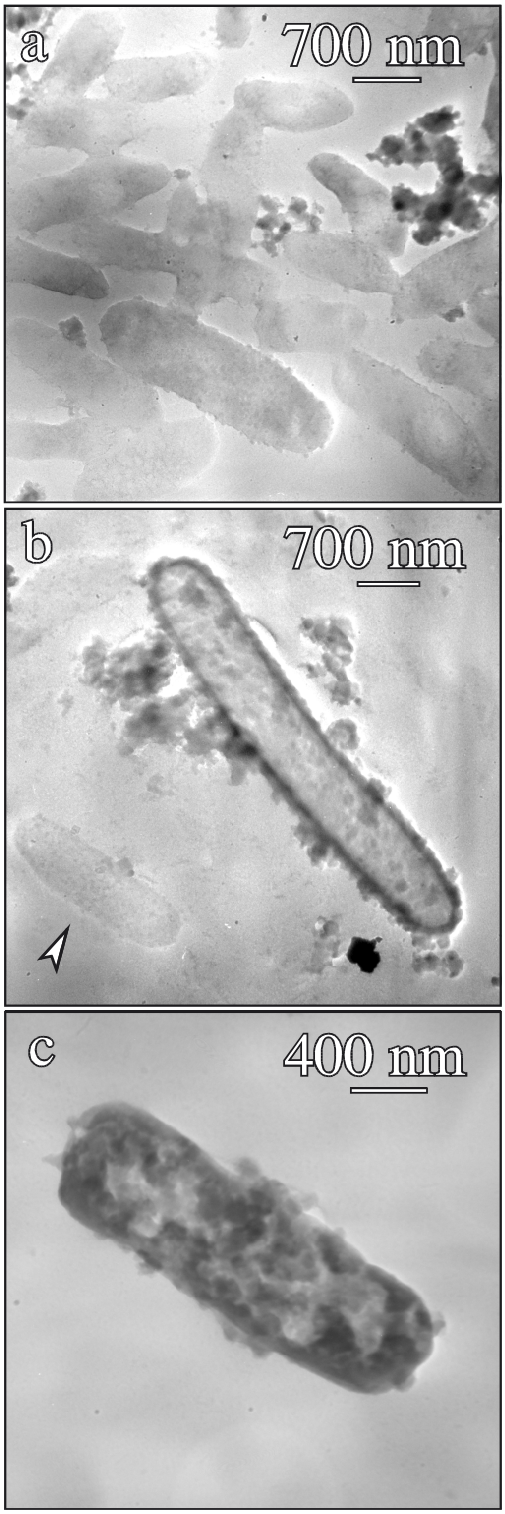

Figure 1. *Shewanella oneidensis,* in a hydrated state, imaged in the EC at 100 Torr. (a) bacteria exhibiting low contrast in bright-field and (b, c) bacteria encrusted/impregnated with electron dense particulates. The arrow in panel (b) points to a low contrast bacterium in the same field of view as a bacterium with electron dense particulates, illustrating the dramatic contrast difference.

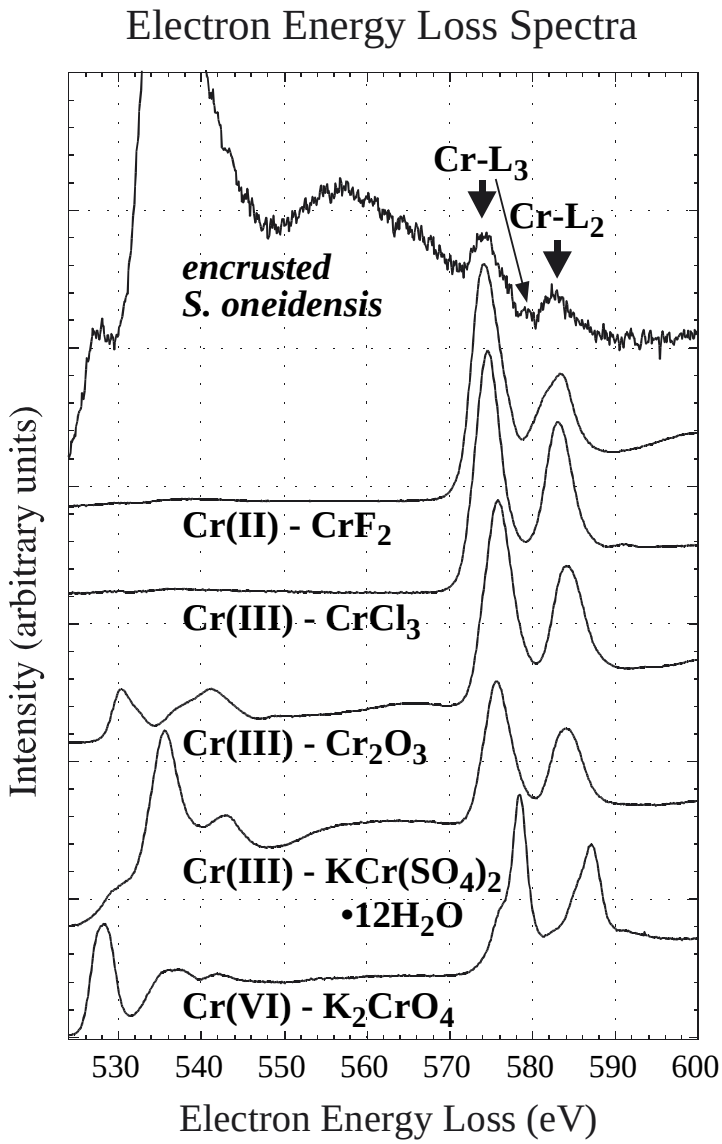

Figure 2. A comparison of the core-loss EELS spectra of *Shewanella oneidensis* in the EC at 100 Torr and Cr standards of known oxidation state. The spectra were offset from one another after being normalized to the intensity of the L_3 peak.

Table I
Cr-L_3 ($2p_{3/2}$) and Cr-L_2 ($2p_{1/2}$) Adsorption-Edges

Specimen	Formal Valence	Cr-L_3 (eV)	Cr-L_2 (eV)	L_3/L_2 ratio
S. oneidensis		574.4 ± 1.5 579.3 ± 1.5	583.0 ± 1.5	2.75 ± 0.25
CrF_2	II	574.2 ± 0.1	583.5 ± 0.1	2.99 ± 0.07
$CrCl_3$	III	574.9 ± 0.1	583.4 ± 0.1	1.74 ± 0.02
Cr_2O_3	III	575.9 ± 0.1	584.3 ± 0.1	1.81 ± 0.01
$KCr(SO_4)_2$	III	576.0 ± 0.1	584.5 ± 0.1	1.77 ± 0.02
K_2CrO_4	VI	578.6 ± 0.1	587.2 ± 0.1	1.42 ± 0.01