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Determination of Chromium Oxidation State in Cultures of Dissimilatory Metal Reducing Bacteria by Electron Energy Loss Spectroscopy

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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. The valence state of Cr is important because it controls the geochemistry and toxicity of the element. Hexavalent Cr species are strong oxidants which act as carcinogens, mutagens, and teratogens in biological systems [1]. The high solubility, bioavailability, and toxicity of Cr(VI) make it a particular environmental concern. In contrast, Cr(III) species have low toxicity, in part, because their bioavailability is limited by their low solubility and their tendency to form strong complexes with organics and hydroxo complexes. 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 [2]. 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. Furthermore, the geochemical and microbiological processes of Cr oxidation/reduction drive both precipitation/dissolution and ion adsorption/desorption reactions in the environment. The study of these reactions has been hindered by the lack of an analytical technique that can determine the oxidation state of Cr at high spatial resolution. For instance, little is known about unstable redox intermediates with intermediate valence states between the valances of the stable end members of the oxidation/reduction process.

Electron energy loss spectroscopy (EELS) techniques have been developed to determine oxidation state of 3*d* and 4*d* transition metals at high spatial resolution [3-5]. However, only limited EELS studies of a few Cr oxidation states have been performed, and with the exception of our work [6], none have been applied to biological systems. We have previously reported the application of several EELS techniques in combination for the study of Cr(VI) reduction by the bacterium *Shewanella oneidensis* using conventional- and *in-situ* environmental cell (EC)- transmission electron microscopy (TEM) [6]. *Shewanella oneidensis (S. oneidensis)* is a gram-negative, facultative bacterium capable of respiring aerobically and anaerobically using a variety of compounds/metals as terminal electron acceptors including Cr(VI). In our previous work, a limited number of standards were examined. Here we report detailed EELS measurements of L_3/L_2 integrated-peak intensity ratios and L_3 peak positions for a greatly expanded range of chromium compounds, including additional oxidation states. Previously analyzed Cr standards are also reanalyzed, and we report less relative scatter in their data than in the original study [6], a consequence of repairs and upgrades to the energy loss spectrometer. Further, we report results of EELS studies of Cr reduction in anaerobic cultures of *S. oneidensis.*

References:

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[7] Work supported under ONR/NRL program element 0602236N.

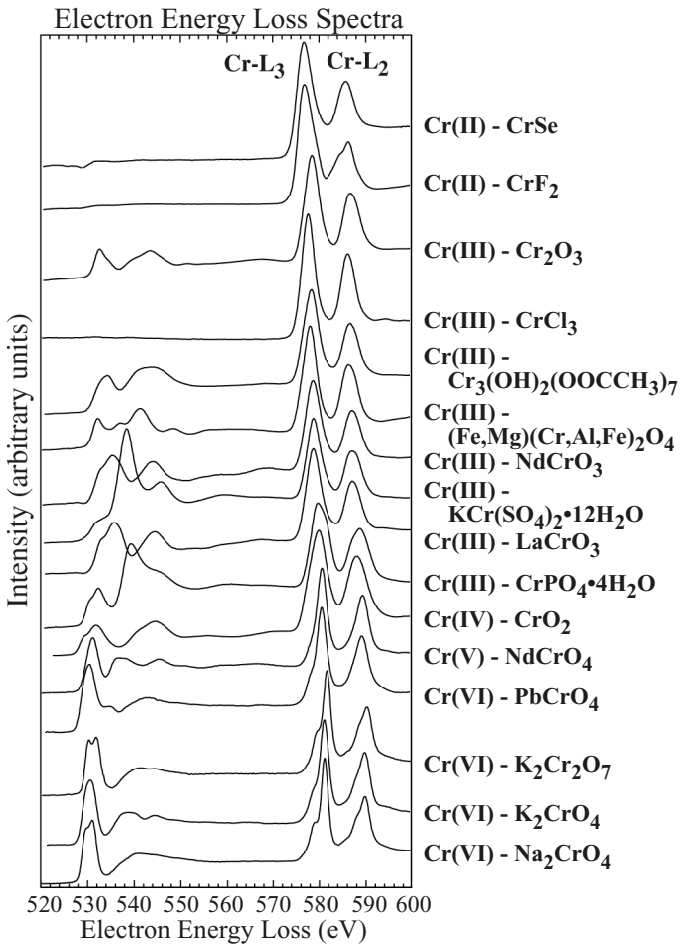

Figure 1. Comparison of core-loss EELS spectra of Cr oxidation state standards. Spectra were normalized to the intensity of the L_3 peak and offset from one another. They represent the sum of 25 individual spectra. The spectrometer was calibrated to the edge maxima of Ni-L_3 (NiO) = 855.00 ± 0.03 eV. Instrument details are given in Ref. 6.

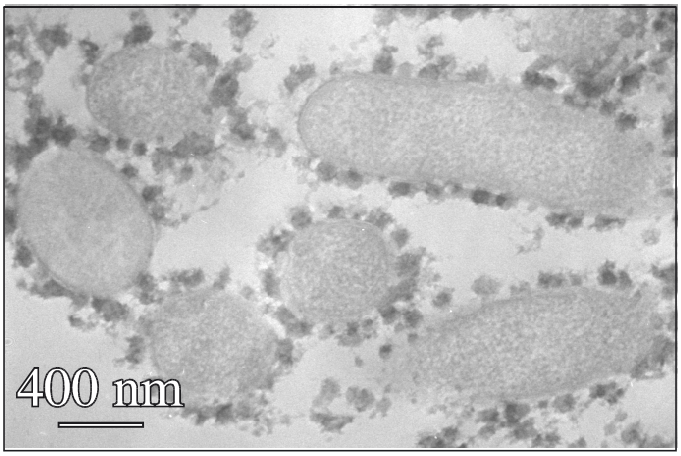

Figure 2. Encrusted *S. oneidensis* in cross-section.

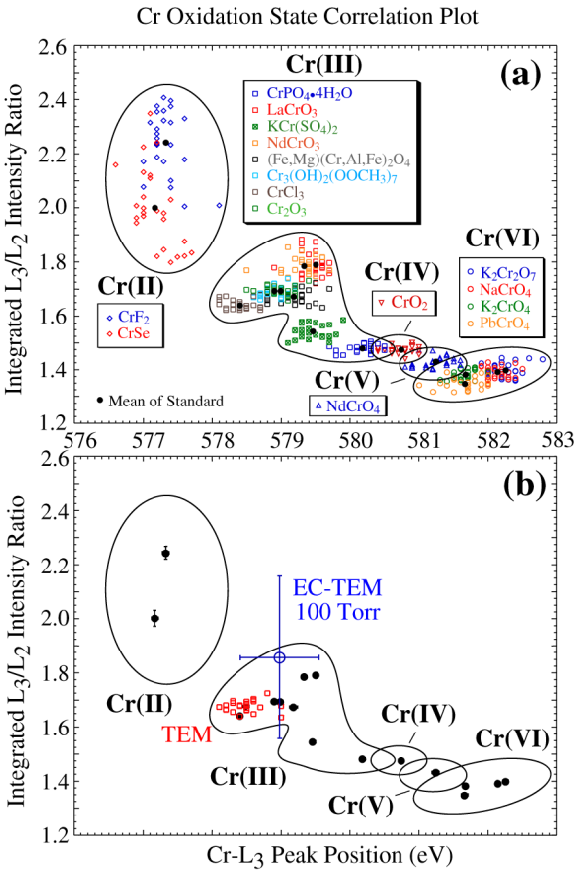

Figure 3. Correlation between L_3/L_2 integrated-peak intensity ratios and L_3 peak positions for Cr oxidation-state standards (a). For analysis details see Ref. 6. Solid data points represent the mean of the data for a particular standard. Different Cr oxidation states fall within well-separated regions in the correlation plot. Within a given oxidation state, spectra for the individual standards fall within separate groupings reflecting possible differences in atom coordination, spin-orbit interactions, crystal field splitting, atomic coulomb repulsion, and exchange effects between the compounds. These factors also influence $L_{2,3}$ fine structure and must be considered to properly interpret absorption edges. The correlation plot represents a map of the possible range in fine structure (including influences from factors other than valence state) that a particular Cr oxidation state can display. The mean oxidation state of an unknown can be determined by plotting its L_3 peak position and L_3/L_2 integrated-peak intensity ratio in a correlation plot of oxidation state standards as shown for Cr precipitates encrusting *S. oneidensis* in (b).