Production of Extracellular Polymeric Substances by the Facultative Iron-Reducing Bacterium *Shewanella oneidensis* Strain MR-1: Implications for Metal Reduction and Sequestration under Aerobic Conditions

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The dissimilatory iron-reducing bacterium *Shewanella oneidensis* strain MR-1 formed cell aggregates (flocs) when cultivated in a chemically defined medium with dissolved oxygen concentrations ranging from 10 to 100% of air saturation. These flocs, with diameters between ten to several hundred microns, were composed of hundreds of cells bound in a matrix of extracellular polymeric substances (EPS). The EPS contained polysaccharides and proteins, including c-type cytochromes, and exhibited an abundant cation sorption capacity. Colloidal gold particles (1.4 nm), which were coated with positively charged polyamines, served as contrasting agents for transmission electron microscopy, and clearly revealed the homogeneous distribution of charged groups throughout the EPS matrix. When ferrous iron solutions were added to anaerobic suspensions of flocs, iron precipitates formed throughout the EPS matrix, as visualized by electron microscopy. Identification of these precipitates is pending low temperature Mossbauer spectroscopy. However, the greenish color of pelleted flocs and associated precipitates coupled with electron microscopic evidence suggest the formation of a poorly crystalline green rust similar to that which forms on surfaces of MR-1 grown under anaerobic conditions.

Aerobic suspensions of flocculated MR-1 reduced Co(III)–EDTA and Tc(VIII). Although the rates of reduction were significantly slower than observed from anaerobic suspensions, reduction of Tc(VIII) resulted in the formation of black Tc(IV) precipitates. In contrast, no reduction was detected in aerobic suspensions of cells that were prevented from flocculating by omitting Ca^{2+}, a divalent cation that is involved in assembly of the EPS matrix, or in aerobic cultures that were deflocculated by the addition of EDTA. These results are consistent with our hypothesis that cell aggregates establish an oxygen gradient that decreases with depth. Hence, cells located toward the center of the floc are oxygen-limited and reduce metals under suboxic or anaerobic conditions that are not represented by the aerobic bulk aqueous phase. These results present new evidence that iron (metal) reduction can occur in biofilm communities present within aerobic media, and that the fate of the reduction products (cations) is controlled by charged groups within the EPS.