## Manganite reduction by Shewanella putrefaciens MR-4

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## ABSTRACT

Previous studies have documented dissimilatory growth of bacteria on solid Mn<sup>4+</sup> oxide, but Mn<sup>3+</sup> oxides have not been previously studied; here we have demonstrated for the first time the bacterial reduction of manganite. Strain MR-4 of Shewanella putrefaciens was able to grow on and rapidly reduce insoluble needle-shaped crystals of synthetic manganite (MnOOH), converting them to soluble  $Mn^{2+}$  in the process. The rate of  $Mn^{3+}$  reduction was optimal at pH of 7.0 and 26 °C consistent with an enzymatic reaction. In addition the rates of reduction were in proportion to the amount of manganite added, but nearly independent of the cell concentration present (e.g., cell number had only a small effect on the rate of Mn<sup>3+</sup> reduction at early stages of growth) suggesting that surface properties were dictating the rates of metal reduction. This thesis was supported by major differences in reduction rates when Mn oxides of different surface areas were studied. Removal of the carbon source (formate or lactate) or addition of metabolic inhibitors reduced the rate of metal reduction. No  $Mn^{3+}$  reduction was observed when the samples were oxygenated, nor when the cells were separated from the Mn<sup>3+</sup> oxide by a dialysis membrane. Environmental scanning electron microscopy (ESEM) images showed close contact of the cells with the needle-shaped crystals during early stages of reduction. In later stages, the closely associated cells were coated with a layer of extracellular polymeric material that obscured the cells when viewed by ESEM. When manganite crystals were dried and gold coated, and viewed by standard scanning electron microscopy (SEM), abundant bacteria could be seen on the surface of the metal oxide in a thin biofilm-like layer. The layer of extracellular polymer is a new finding, and neither the composition, function, nor importance in the manganese reduction process have been elucidated.