Manganite reduction by *Shewanella putrefaciens* MR-4

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**ABSTRACT**

Previous studies have documented dissimilatory growth of bacteria on solid Mn$^{4+}$ oxide, but Mn$^{3+}$ 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$^{3+}$ 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$^{3+}$ 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.

**INTRODUCTION**

Bacteria capable of dissimilatory growth on oxidized manganese were first described by Myers and Nealson (1988a) and Lovley and Phillips (1988). In the former report, the bacteria, identified as *S. putrefaciens*, were shown to grow on solid Mn$^{4+}$ oxides, using lactate as the carbon source. Mn$^{4+}$ reduction required cell contact and was inhibited by oxygen as well as several metabolic inhibitors. Subsequent studies showed that both Fe$^{2+}$ produced from ferrous iron reduction or H$_2$S, produced by thiosulfate reduction could also reduce Mn$^{4+}$ oxide, leading to an indirect reduction of MnO$_2$, by chemical intermediates (Myers and Nealson 1988b). Thus, the ability of *S. putrefaciens* to reduce other compounds like Fe$^{2+}$, thiosulfate, or elemental sulfur (Moser and Nealson 1996) results in products that cause rapid Mn$^{4+}$ reduction and obscure the measurement of direct (enzymatic) manganese reduction (Nealson and Saffarini 1994).

Several mineral forms of insoluble Mn$^{4+}$ oxides of the general formula MnO$_2$, were shown previously to be reduced at quite different rates (Burdige et al. 1992), such that amorphous oxides with high surface area are the most reactive, while highly crystalline, low surface area oxides like pyrolusite are almost totally inert to bacterial reduction. For Mn$^{4+}$ oxides, surface area is a very important parameter, although almost certainly not the only factor controlling reduction rate. In studies of soluble Mn$^{4+}$ pyrophosphate, Kostka et al. (1995), reported the stability of some Mn$^{4+}$ containing ligands and the ability of *S. putrefaciens* to reduce the bound Mn$^{4+}$ to soluble Mn$^{2+}$.

Mn$^{4+}$ oxides can be readily reduced by any of a variety of organic compounds that were also capable of reducing Mn$^{4+}$ oxides (Bricker 1965; Stone and Morgan 1984a, 1984b; Stumm and Giovanoli 1965; Xyla et al. 1992). In