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BIO-ELECTROCHEMICAL MANGANESE OXIDATION IN *Pseudomonas resinovorans* MOB-513

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The presence of high levels of manganese (II) in groundwater, a source of drinking water in many populations, causes organoleptic and health problems associated with its ingestion. Removal of Mn(II) from water is based on oxidation to insoluble states Mn(III/IV) and consequent filtration out of the water. While the addition of chemicals can achieve this, a more eco-friendly alternative involves a biological process where Manganese Oxidizing Bacteria (MOB) accelerate the rate of Mn mineralization. Therefore, the efficiency of biological filtration process depends on the presence of MOB that can form biofilms on the sand filters and oxidize Mn(II), like the environmental isolate *Pseudomonas resinovorans* MOB-513, which can perform both processes with high efficiency. A common limitation for biological oxidation processes arises when considering the dependence on acceptors, such as oxygen, leading to the application of an aeration step in these systems. In nature, there are microorganisms capable of transferring electrons to extracellular electron acceptor compounds (EETs). A special case of EET occurs when the acceptor is a polarized electrode. In these systems, the oxidation of donor compounds (organic matter, iron, urine, among others) can occur in the absence of traditional electron acceptors (oxygen, nitrate, sulfur). In this work, we studied bio-electrochemical Mn(II) oxidation in *P. resinovorans* MOB-513. For that, MOB-513 wild type and mutants obtained by transposon mutagenesis with altered Mn(II) oxidation capacities, were chosen. Each strain was inoculated in three-electrode reactors under potentiostatic mode, with graphite polarized at 0.2 V as working electrode (only electron acceptor), a graphite felt as the counter electrode, and a Ag/AgCl (3 M NaCl) reference electrode. Reactors were deoxygenated with a mixture of N₂:CO₂ (80:20) to maintain anaerobic conditions, and a minimal medium containing Mn(II) as the sole electron donor was used. Our results indicate that MOB-513 WT can oxidize Mn(II) anaerobically using an electrode as electron acceptor, thus producing an electric current. According to cyclic voltammetry analysis, MOB-513 presents at least three different redox processes somehow related to Mn(II) oxidation. Peak sizes and position of redox processes were observed to be different in mutants that do not oxidize Mn(II) and were, in consequence, unable to transfer electrons to the electrode. A superoxidant mutant, on the other hand, showed accelerated

electrochemical processes. These results provide new information about the bacterial Mn(II) oxidation process and may open the door to future applications of bioelectrochemical strategies for water cleaning and even Mn purification from groundwater.

Palabras clave: Manganese oxidation - Bioelectrochemistry - Biofilm - Pseudomonas