

Microbial Fuel Cells: A Bioelectrochemical Respiration Assay Using *S. oneidensis* Cytochrome Complex mtrCAB

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In this study, microbial fuel cells (MFCs) of our own design were inoculated with organisms of the species *Shewanella oneidensis*. *S. oneidensis* is a gram-negative, flagellum-motile bacterium known for its metabolic adaptability. This bacterium, which forms biofilms in both natural and laboratory conditions, is a facultative anaerobe and an exoelectrogen, meaning it can use substances other than oxygen as a terminal electron acceptor, such as heavy metals or in this case an anode in a galvanic system. The electrochemical communication between a *Shewanella* biofilm and an electrode is the key mechanism by which electrical energy is generated in an MFC. We were able to successfully demonstrate that our system is capable of producing bioelectricity in an external circuit. This occurs via the coupling of the respiratory chain of *S. oneidensis* MR-1 to a galvanic system, consisting of a biotic, anaerobic anode chamber and an abiotic, aerobic cathode chamber. We tested our assay on several factors to prove its sensitivity, achieving very high statistical significance. To this end, we conducted several experiments, such as a partial aerobic reactor start which allowed us to visualize the process of oxygen scavenging in the recorded data. While the maximum voltage remained unchanged, energy production was delayed by approximately 48 hours. Furthermore, we investigated the combined effects of anode potential and sodium and potassium ion concentrations. Demonstrating the sensitivity of our system to various influencing factors is essential for the application of MFCs as a microbiological assay. To expand the potential application range of the MFC assay to all bacteria, we established a genetic toolkit using the extracellular electron transport (EET) Cytochrome Complex mtrCAB.

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