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# New method for characterizing electron mediators in microbial systems using a thin-layer twin-working electrode cell

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## Abstract

Microbial biofilms are significant ecosystems where the existence of redox gradients drive electron transfer often via soluble electron mediators. This study describes the use of two interfacing working electrodes (WEs) to simulate redox gradients within close proximity (250  $\mu\text{m}$ ) for the detection and quantification of electron mediators. By using a common counter and reference electrode, the potentials of the two WEs were independently controlled to maintain a suitable “voltage window”, which enabled simultaneous oxidation and reduction of electron mediators as evidenced by the concurrent anodic and cathodic currents, respectively. To validate the method, the electrochemical properties of different mediators (hexacyanoferrate, HCF, riboflavin, RF) were characterized by stepwise shifting the “voltage window” (ranging between 25 and 200 mV) within a range of potentials after steady equilibrium current of both WEs was established. The resulting differences in electrical currents between the two WEs were

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