

## Periodical charge/discharge can enhance the characteristic of electroactive biofilms

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Electroactive bacteria can use electrodes as terminal electron acceptors while oxidizing organic substrates. Electroactive biofilms (EABs) have been considered promising for several potential applications such as microbial fuel cells, microbial electrolysis cells or microbial biosensors. However, low current densities still limit their practicability and numerous questions remain on the parameters impacting their characteristics and performance. EABs are able to store electrons in the absence of external electron acceptors ("charge" process in open circuit). Once the microbial anode is polarized again, accumulated charges can be released and it produces an additional transient current ("discharge" process). This process has already been studied but solely for short-term and on already mature EABs. In particular, the effect of periodical "disconnections" on the characteristics of EABs has not been explored.

Here we applied periodical charge/discharge (i.e. OCV/– 0.1 V vs. Ag/AgCl) operations during the full growth of EABs on glassy carbon electrodes (i.e. starting from inoculation). We investigated the impact of the frequency of the signal on current generation, charge storage capacity, heme content, redox conduction and biofilm morphology.

When compared with a continuous polarization, the shortest half-periods of charge/discharge ( $\leq$  10 s) enhanced current production, increased the content of redox cofactors (and hemes) in the EABs, and improved the redox conduction. Oppositely, longer half-periods ( $\geq$  60 s) inhibited the growth and electroactivity of the EABs. Control EABs formed under continuous polarization were flat, while EABs formed under intermittent operations presented torus-shaped structures on their outer-layer. The results indicated that periodical charge/discharge can regulate the formation and electroactivity of EABs. In addition to the fundamental relevance, this may provide opportunities for future applications.