Targeting the Redox Activity of Biofilms

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Bacterial Biofilms

Biofilms are diverse aggregates of microorganisms that are enclosed in an extracellular polymeric substance which can adhere to various surfaces. Biofilm formation occurs via three main stages. Initial **adhesion** to a conditioning film via adhesins, **growth** of colonies which secrete an EPS matrix and finally **dispersion** of bacteria due to fluid shear, starvation or environmental pressures.

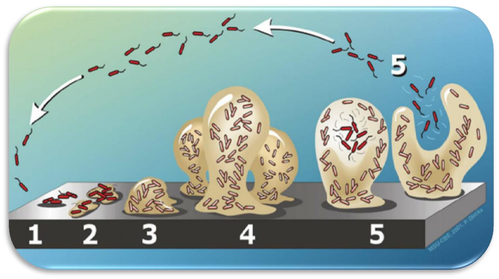


Figure 1. Stages of biofilm formation.[1]

Bacterial biofilms are associated with many diseases including cystic fibrosis. They are robust and more difficult to treat than planktonic bacteria:

- Slow penetration due to protective EPS matrix
- Resistant phenotypes due to local differentiation
- Antagonised antibiotic action due to altered microenvironments

Figure 2. Image showing the different regions in a biofilm which give rise to antibiotic resistance.[2]

Biosensors

Biosensors selectively recognise a biochemical response and transform this information into an electrical signal using a sensitive transducer. They can be used to detect electrons produced by bacteria during redox metabolism.

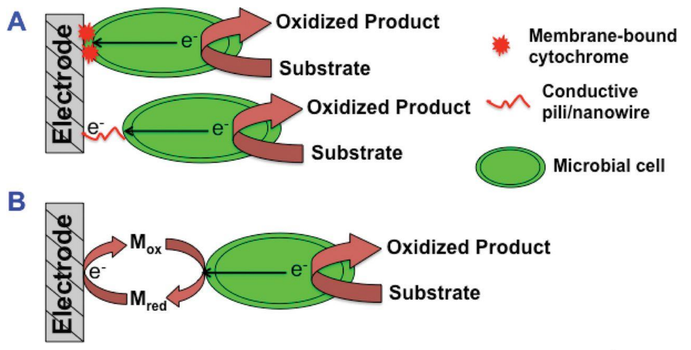


Figure 3. Direct (A) and mediated (B) electron transport.[3]

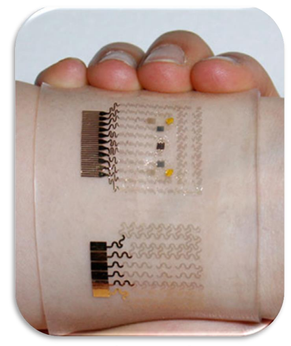


Figure 4. Glucose biosensor.[4]

If bacteria possess outer membrane redox proteins electrons can be transferred directly to the electrode via physical contact. (A) Alternatively, electrons can be transferred to the electrode surface via diffusion by utilising soluble redox active mediators. (B)

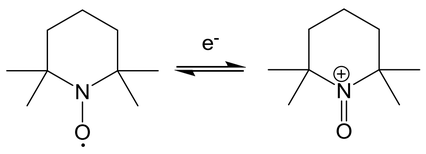
Incorporating Mediators into Graphite Paste Electrodes

Exogenous mediators could maximise the current generated by *Pseudomonas fluorescens* biofilms. To do this they must:

- Have the ability to penetrate cell membranes
- Have a potential which does not limit cell voltage
- Rapidly regenerate
- Have good solubility and stability in the electrolyte
- Not display cytotoxicity
- Not be consumed by bacteria as a nutrient
- Not affect metabolic processes

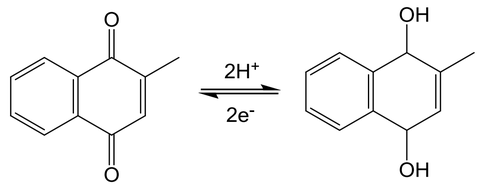
Electrodes were prepared by adding 200 µL of 20 mM mediator solution and 100 µL mineral oil to 0.15 g graphite before mixing to form a paste which was loaded in a platinum electrode. Electrochemical measurements were performed using a three-electrode cell in 10 mM PBS.

2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO)



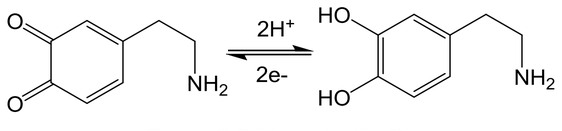
$E_{1/2}$ = 0.59 V vs. Ag/AgCl

2-Methyl-1,4-naphthoquinone (menadione)



$E_{1/2}$ = -0.24 V vs. Ag/AgCl

Dopamine hydrochloride



$E_{1/2}$ = 0.2 V vs. Ag/AgCl

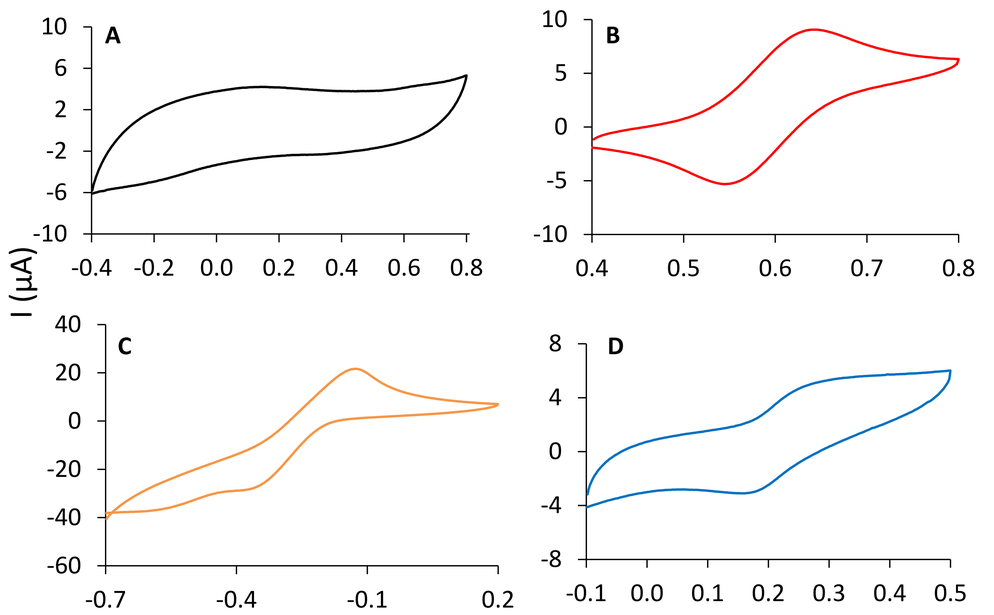


Figure 5. Cyclic Voltammogram of graphite paste electrode (A) doped with 200 µL of 20 mM TEMPO (B), menadione (C), and dopamine (D). CV performed in 10 mM PBS, using an Ag/ AgCl reference electrode and Pt counter electrode.

The Effect of MnO_2 on the Electrochemical Activity of Biofilms

After background measurements, the mediator doped electrodes were immersed in a lysogeny broth culture of *Pseudomonas fluorescens* to promote biofilm formation.

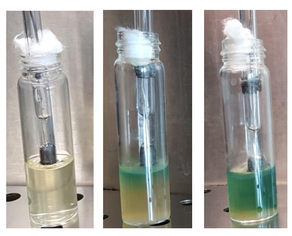


Figure 6. Photograph showing graphite paste electrode immersed in Pseudomonas fluorescens culture at 0 (left) 48 (middle) and 72 (right) hours.

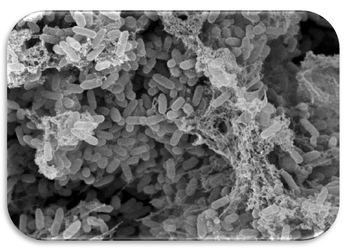


Figure 7. SEM image showing formation of a Pseudomonas fluorescens biofilm after 48 hours of drop casting culture on a screen printed carbon electrode.[5]

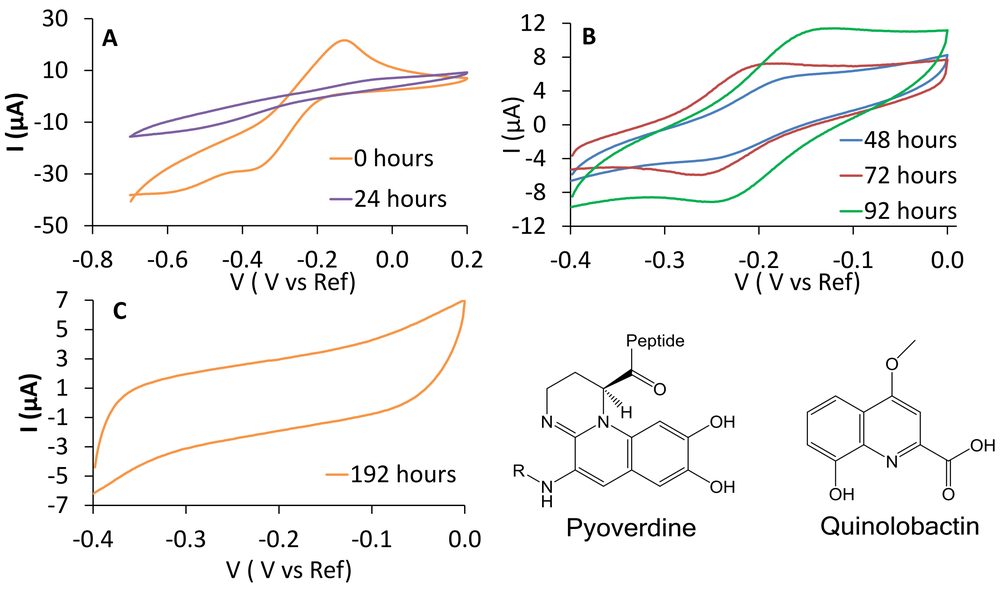


Figure 8. Cyclic voltammograms of menadione doped electrode which highlight redox activity observed during biofilm formation . Siderophore structures shown bottom right.

$$C_s = \frac{1}{2mv(V_f - V_I)} \int_{V_i}^{V_f} I(V)dV$$

Electrode	Specific capacitance / mF g^{-1}			
	48 h	72 h	96 h	168 h
Undoped	19.01	24.91	32.52	35.33
TEMPO	9.05	5.74	8.44	5.37
Menadione	23.37	33.32	48.65	28.81
Dopamine	34.85	40.24	44.21	31.04

Table 1. Specific capacitance calculated for the four graphite paste electrodes during biofilm maturation.

Conclusion

- Prepared and characterised redox mediator doped graphite paste electrodes.
- Immobilised *Pseudomonas fluorescens* biofilms on electrode surfaces.
- Probed the electrochemical activity of biofilms and confirmed the formation of redox active species during biofilm maturation using CV, DPV and SWV.
- Menadione and dopamine facilitated electron transport, showing increased response signals but cytotoxicity of TEMPO reduced current generated.

References

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2. J. Chambles, Appl. Environ. Microbiol. **2006** , 72, 2005-13
3. K. Hasan, Bio. Soc. Tran. **2012**, 40 , (6), 1330-1335
4. H. Lee *et al. Nature Nanotechnology, **2016**, 11, 566–572*
5. E. Knowles, R. Kataky, MChem project, **2017**

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