Supporting Information for

Combining hyperspectral imaging and electrochemical sensing for detection of *Pseudomonas aeruginosa* through pyocyanin production

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Contents

Reference

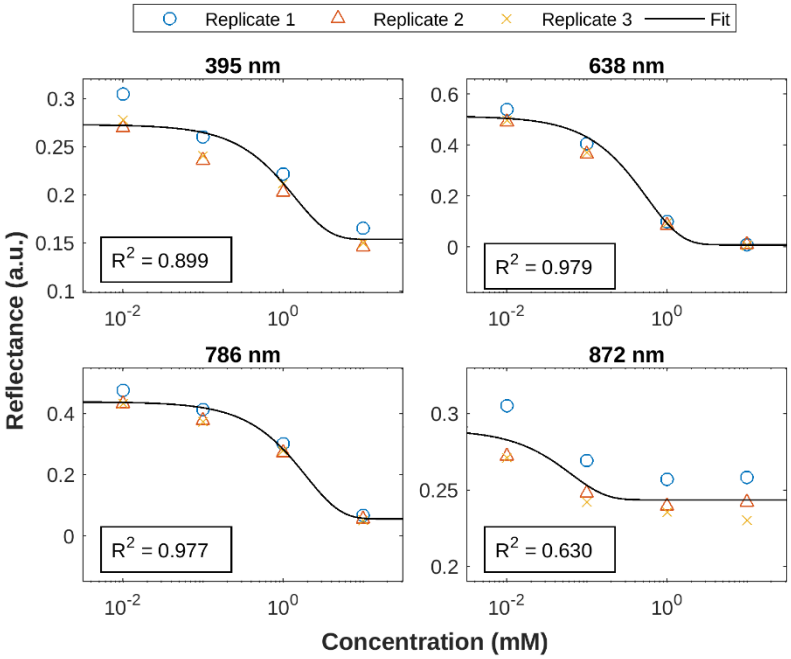

Fig. S1 Exponential curves fit to additional characteristic wavelength bands. Along with Fig. 4c, these wavelengths show the highest difference between each concentration. The wavelength of 638 nm, which had the R^2 value closest to 1, was used for pyocyanin estimations.

Table S1 Estimated mM of pyocyanin quantity using SWV linear correlation. (n=3 for each condition)

	t = 0	*t* = 24 h	*t* = 48 h
LESB58 OD_{600} 0.5	(0.49)	2.03	1.96
LESB58 OD_{600} 1.0	(0.96)	2.2	1.61
PA14 OD_{600} 0.5	0.16	(0.53)	0.46
PA14 OD_{600} 1.0	(0.49)	(0.52)	0.36

Peak detection was run in the [-0.35;-0.15] V region and current amplitudes for each replicate reported. Pyocyanin estimations were calculated for each replicate then averaged. The following equation fits the SWV dose response data with an adjusted R^2 = 0.99892:

SWV peak amplitude = 0.152017 + 2.75069* pyocyanin concentration

Peaks detected for pyocyanin were only recorded if their amplitude was higher than LB in that region and values in parentheses correspond to peak 2, not exactly overlapping the pyocyanin standard potential.

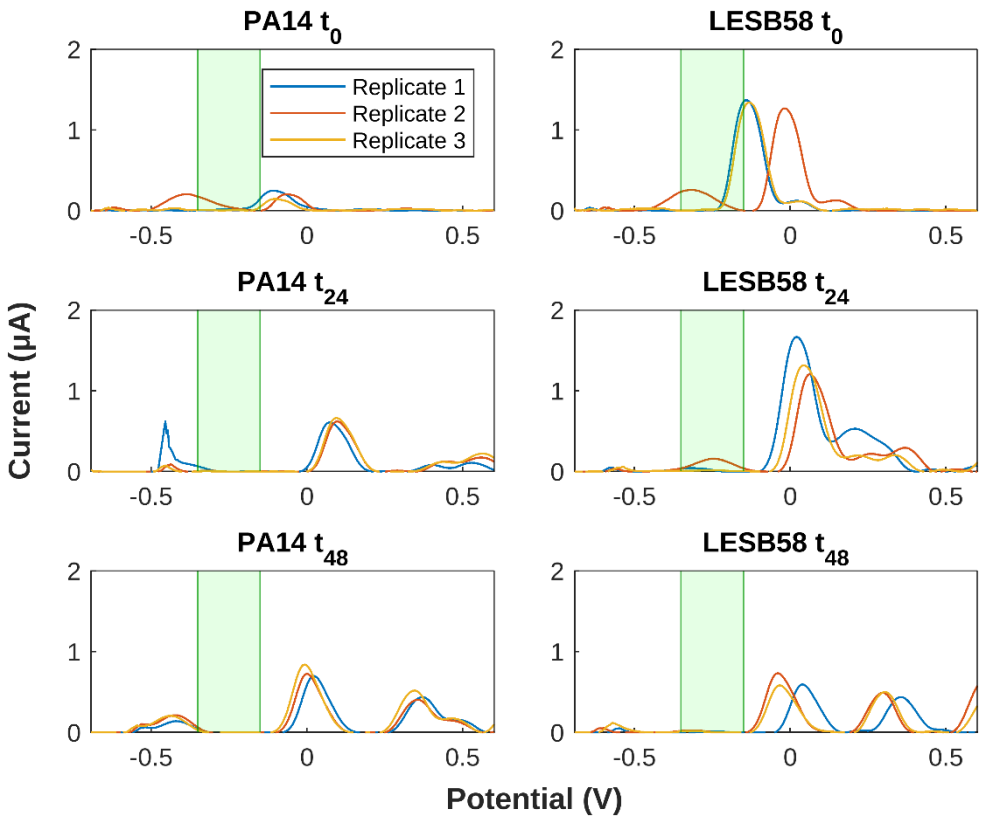

Fig. S2 Square Wave Voltammograms of PA14 (left) and LESB58 (right) at initial concentrations of OD_{600} = 0.5 grown in the custom-built test cell at 0, 24 and 48 hours. The pyocyanin detection area is represented in green. Replicates show slight shifts in response over time and pyocyanin production is not the main metabolic end product as peaks outside of the detection zone display higher current amplitudes.

Square Wave Voltammograms show variable sample behaviour regarding pyocyanin production when grown in the sample plate. PA14 replicates show the likely presence of 5-MCA around -0.1 V upon inoculation, and of 1-hydroxyphenazine towards -0.45 V after 24 and 48 hours, indicating another enzyme is more active than the one leading to pyocyanin production. A series of unidentified peaks between 0.2 and 0.6 V might result from another quorum sensing pathway[1]. For LESB58 samples, replicates behave differently from one another. Initially, replicate 2 exhibits lower pyocyanin production with a majority of other phenazine precursors and replicates 1 and 2 seem to contain pyocyanin when comparing to the standard behaviour at the same time point. After 24 hours, replicates 2 and 3 have produced small quantities of pyocyanin at -0.25 V, which is not yet the case for replicate 1. After 48 hours, pyocyanin production has not varied, except for replicate 1 where a small quantity can be observed. However, compounds outside of pyocyanin detection range have increased in amplitude, suggesting that other metabolic pathways were favoured over pyocyanin production.

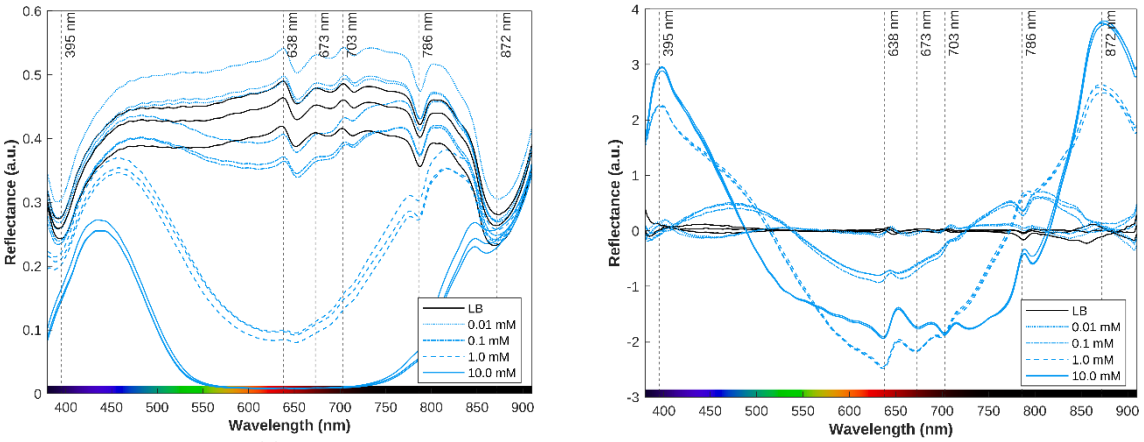

Fig. S3 (a) Reflectance spectra of pyocyanin in LB at four concentrations. Each line represents one of three replicates. (b) The same spectra normalised using SNV and baseline subtraction. The normalisation process corrects for constant offsets in the individual spectra that result from slight variations in the imaging conditions and are not indicative spectral properties.

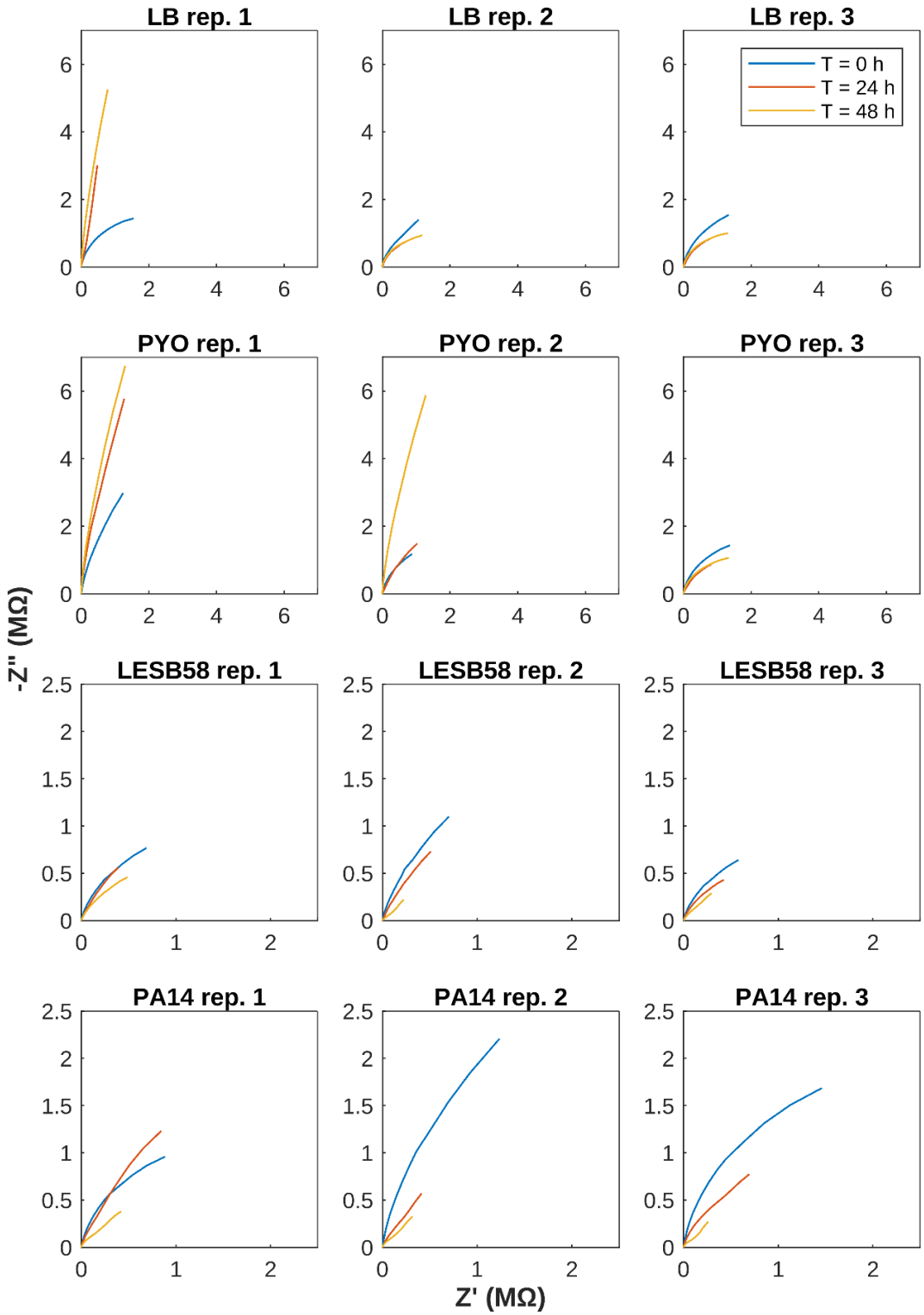

Fig S4. Nyquist plots corresponding to data acquired from the test support with the top row corresponding to the medium control, the second row to the pyocyanin standard, the third row to LESB58 inoculated at OD_{600} = 0.5 at t=0 and the bottom row to PA14 inoculated at the same initial concentration.

Reference

1 J. Oziat, M. Gougis, G. G. Malliaras and P. Mailley, Electroanal, 2017, 29, 1332–1340.