Supporting Information

Strategic Regulation of Barrier Characteristics of Biofilm to Enhance the Extracellular Electrogenic Performance in MFC: An Electrochemical Dynamic Evaluation Study

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Preparation of Fe (III) modified electrode

All the chemicals were purchased from Sigma-Aldrich. The Fe_2TiO_5 composite was prepared by the thermal decomposition method as per the previous literature.[1,2] Briefly, The Fe_2TiO_5 composite was prepared by mixing the required quantity of $TICl_4$ and anhydrous $FeCl_3$ in isopropanol, which was then evaporated to dryness followed by heating at 120°C for 1h. The obtained dry powder was then subjected to annealing at 700°C for 2h in a muffle furnace.[3,4] Fe (III) modified electrode was prepared by electroless Ni-P coating method. For this, the mild steel (MS) substrate (AISI 304 grade) was mechanically polished using emery paper (60−1200 grade) followed degreased in 5% NaOH solution and 3% HCl (ASTM B 656). The cleaned MS was activated with required amount of $SnCl_2$ and $PdCl_2$ and immersed into the Ni−P bath [nickel sulfate (30 g/L), succinic acid (25 g/L), and sodium hypophosphite (25 g/L), Fe_2TiO_5 (2 g/L)] at pH - 4.5 and 80−85 °C with constant stirring for 2 h.[1,2] Previous studies in our lab invariably proved the biocompatibility of the developed Fe (III) modified electrode, which favours bacterial adhesion, growth and proliferation of microorganisms compared to electrode without Fe modification.[1]

Quantification of surface attached bacterial biofilm

Microtitre plate assay was used for the quantitative estimation of static biofilm. For this 96 well plate was inoculated with precultured LB media with an OD_{600} of 0.1 (350 µL/well) and incubated for required time period at 37 ˚C. After incubation, each well washed with 1X PBS (Phosphate buffer solution) twice to remove all planktonic cells, pellicle biofilm and other media components and dried. To thus 150 µL of 0.2% crystal violet

staining solution was added and incubated for 30 minutes at room temperature. After incubation rinse out the CV with distilled water and allowed to dry. Then 200 µL of 95% ethanol was added to each well to solubilize the crystal violet at 600 nm.[5]

Bacterial growth curve

The normal growth curve of *B. subtilis* was measured by turbidity assay at OD_{600} and shown in Figure S2. From the figure it is clear that bacteria exhibited an initial lag time of 2h, which is the time required for the adaptation in the new culture environment. It is the time where the enzymes, coenzymes and other metabolites are produced, which shift the initial electrode potential to 0.56 V. After 2h of lag phase, bacteria enters into the log phase, where the exponential growth of the bacteria takes place and OD_{600} raised in the range of 0.4 -0.5 within 10 h. After which the bacteria enters a stable phase in which number of bacteria in the solution was high. The cell proliferation is balanced with the cell death due to the accumulation of metabolites.

Figures

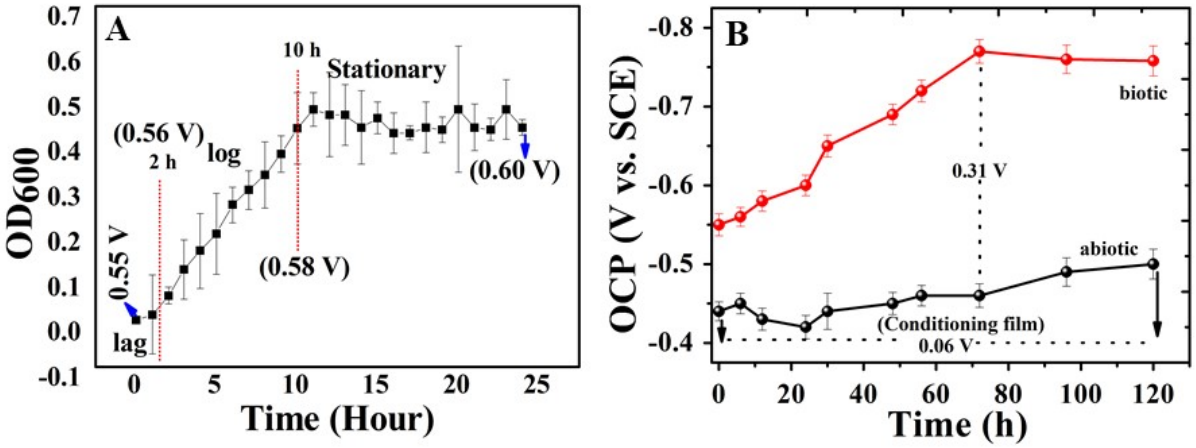

Figure S1: (A) Growth curve of *B. subtilis* showing different phases of bacterial growth (lag, log and stationary phase) and (B) OCP of developed electrode immersed in LB medium under abiotic and biotic condition. The variation in electrode potential with respect to bacterial growth phase is illustrated in A.

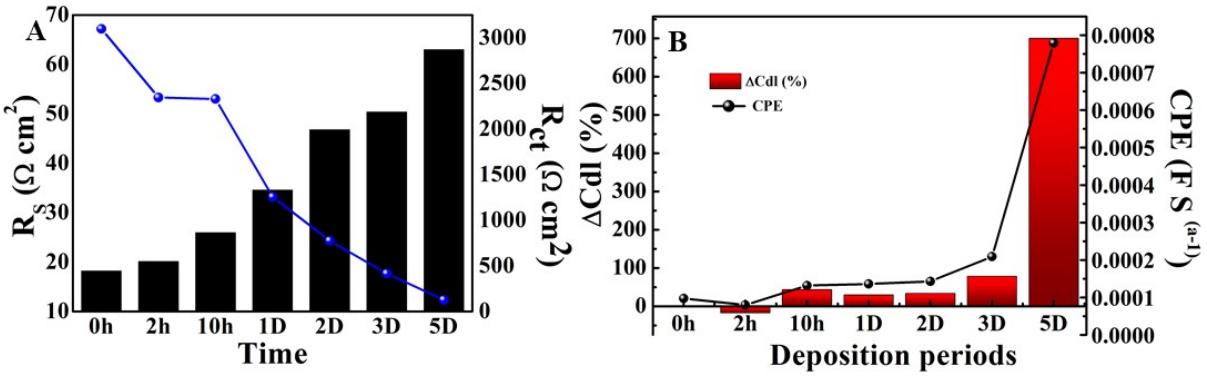

Figure S2: (A) Contradictive response of solution resistance (Rs) and charge transfer resistance (Rct) and (B) comparison of capacitance of the coated MS with different phases of bacterial growth.

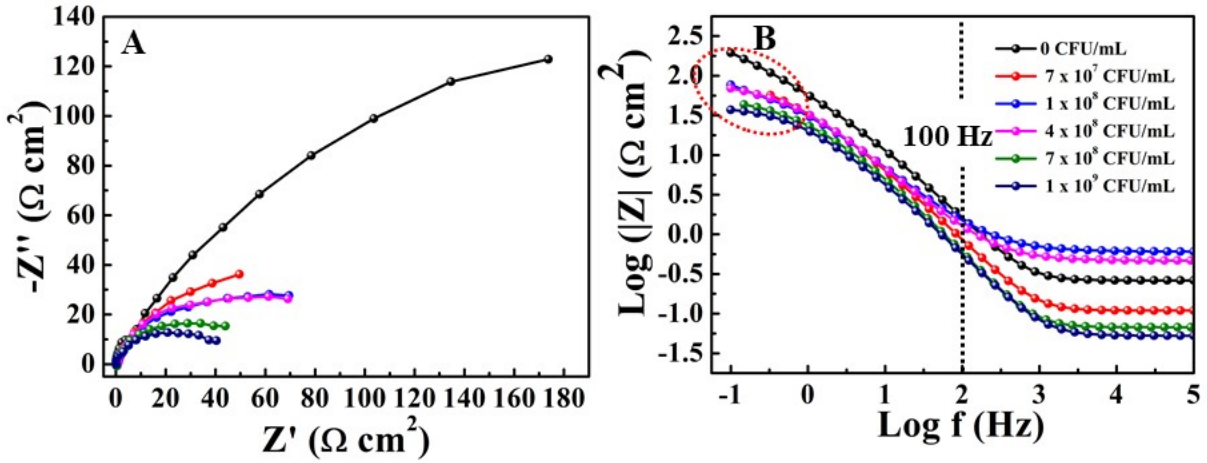

Figure S3: (A) Nyquist plots and (B) frequency-impedance spectra of the MS immersed in LB containing different concentration of *B. subtilis* cell suspension and pellicle biofilm.

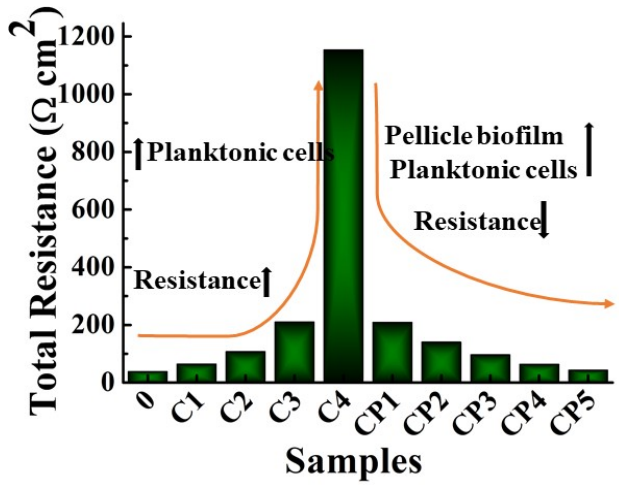

Figure S4: Comparison of total impedance value with increase in concentration of planktonic cells and pellicle biofilm.

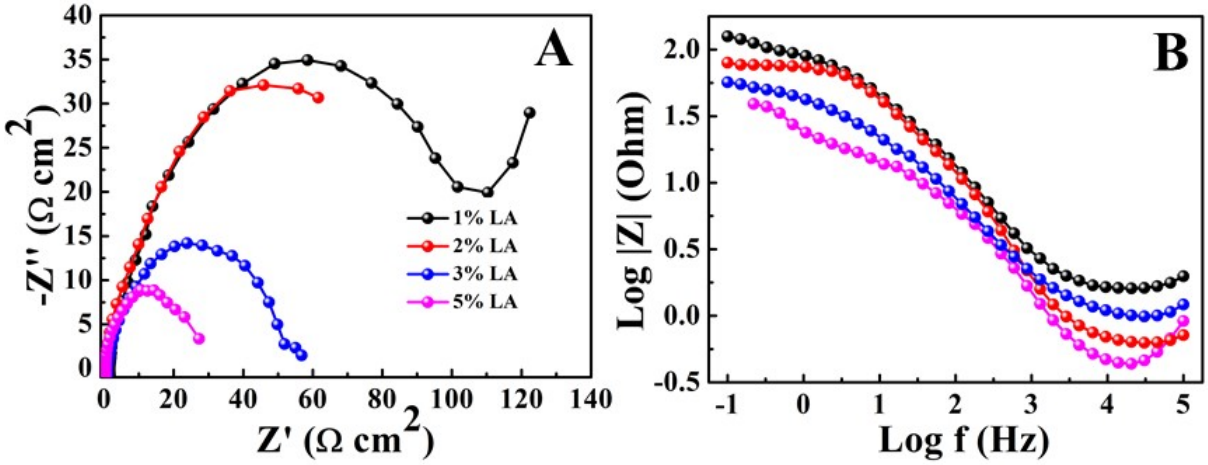

Figure S5: Nyquist plots and (B) frequency-impedance spectra of the MS immersed in LB medium inoculated with *B. subtilis* and different concentration of biofilm disruptor (L-Arginine (LA).

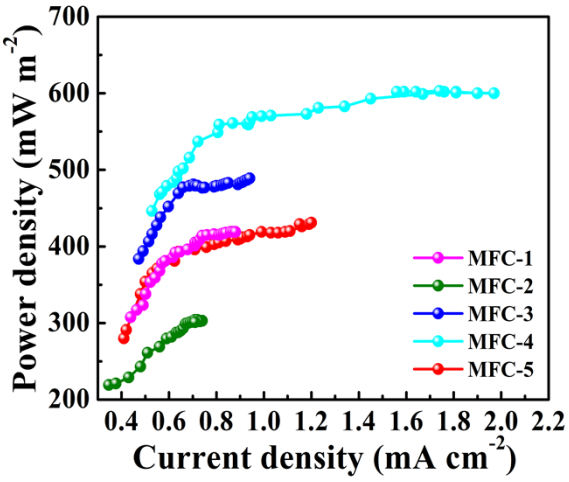

Figure S6: The power generation profile of MFCs inoculated with different phases of *B. subtilis*.

Tables

Table S1: Nyquist parameters of MS immersed in LB medium inoculated with different concentration of *B. subtilis* cell suspension in complexed with the pellicle biofilm

Conc.	Rs (Ω cm^2)	Rd (Ω cm^2)	Rct (Ω cm^2)	Q_1 (F. s $^{(a-1)}$)	Q_3 (F. s $^{(a-1)}$)
0	1.73×10^{0}	7.56×10^{-2}	4.13×10^{-2}	4.83×10^{-3}	0.85×10^{-3}
7×10^7	1.06×10^{0}	1.23×10^{-2}	8.43×10^{-2}	6.30×10^{-3}	1.60×10^{-3}
1×10^8	0.60×10^{0}	9.03×10^{-1}	4.98×10^{-1}	4.40×10^{-2}	6.79×10^{-3}
4×10^8	0.46×10^{0}	6.47×10^{-1}	3.11×10^{-1}	5.09×10^{-3}	4.52×10^{-2}
7×10^8	0.06×10^{0}	4.87×10^{-1}	1.46×10^{-1}	2.68×10^{-3}	4.64×10^{-3}
1×10^9	0.05×10^{0}	4.26×10^{-1}	3.88×10^{1}	2.41×10^{-3}	6.95×10^{-3}

Table S2: Nyquist parameters of MS immersed in LB medium inoculated with *B. subtilis* and different concentration of biofilm disruptor L-Arginine (LA).

Conc. of LA	Rs (Ω cm^2)	Rct (Ω cm^2)	Rt (Ω cm^2)	Qdl (F. s $^{(a-1)}$)
1%	1.61×10^{0}	7.91×10^{-1}	8.97×10^{-1}	0.63×10^{-3}
2%	0.90×10^{0}	5.00×10^{-1}	5.32×10^{-1}	0.75×10^{-3}
3%	0.64×10^{0}	3.48×10^{-1}	4.48×10^{-1}	1.75×10^{-3}
5%	0.43×10^{0}	4.95×10^{0}	4.03×10^{-1}	5.41×10^{-3}

Table S3: Effect of L-Arginine (LA) in bacterial deposition by *B. cereus* on the electrode surface

LA (%)	Total carbohydrates (µg/cm^2)	Total proteins (µg/cm^2)
1	0.856 ± 0.06	0.279 ± 0.05
2	0.537 ± 0.12	0.255 ± 0.05
3	0.284 ± 0.13	0.092 ± 0.03
5	0.048 ± 0.03	0.025 ± 0.05

References

1 P. Chandraserkharan Meenu, B. R. Sreelekshmy, R. Basheer, S. M. Sadasivan, R. M. Vijayakumari Ramakrishnan, and S. M. A. Shibli, *ACS Appl. Bio Mater*, 2018, **1**, 1124-1133.

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3 Shibli, S. M. A., and J. N. Sebeelamol, *Int. J. Hydrog. Energy*, 2013, **5**, 2271-2282.

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5 G. A. O'Toole, (2011). *JoVE*, 2011, **47**, e2437.