# Heterogeneity in microbial fuel cells stacking influences acidogenic metabolism towards bioelectricity generation, fatty acid synthesis and wastewater treatment

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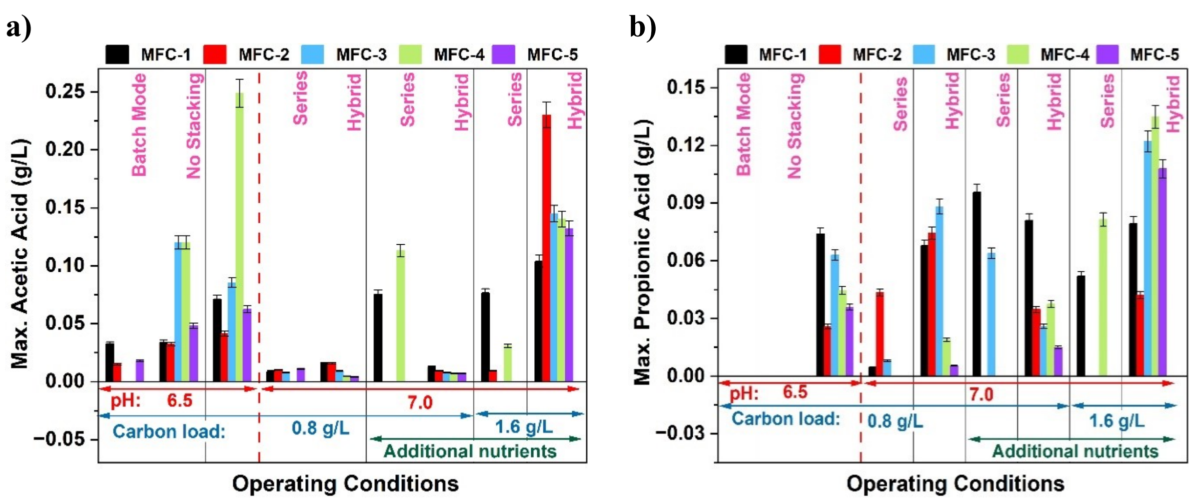
## Supporting information

## Results and discussion

### Metabolites Average productivities

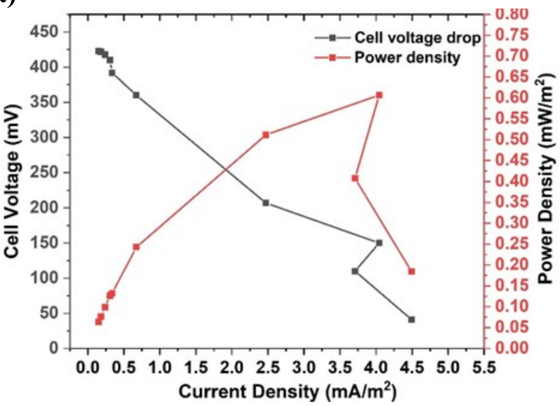
The average acid synthesis was observed as: B/S:0.8/6.5-0.021 g/L, C/S0.8/6.5-0.054 g/L, SR/S:0.8/6.5-0.038 g/L, SR/S:0.8/7-0.009 g/L, HY/S:0.8/7-0.009 g/L, SR/S:0.8/7/AD-0.094 g/L, HY/S:0.8/7/AD-0.008 g/L, SR/S:1.6/7/AD-0.027 g/L, HY/S:1.6/7/AD-0.123 g/L and B/S:0.8/6.5-0 g/L, C/S0.8/6.5-0 g/L, SR/S:0.8/6.5-0.038 g/L, SR/S:0.8/7-0.019 g/L, HY/S:0.8/7-0.043 g/L, SR/S:0.8/7/AD-0.079 g/L, HY/S:0.8/7/AD-0.037 g/L, SR/S:1.6/7/AD-0.045 g/L, HY/S:1.6/7/AD-0.096 g/L for acetic acid and propionic acid respectively (S Fig. 1).



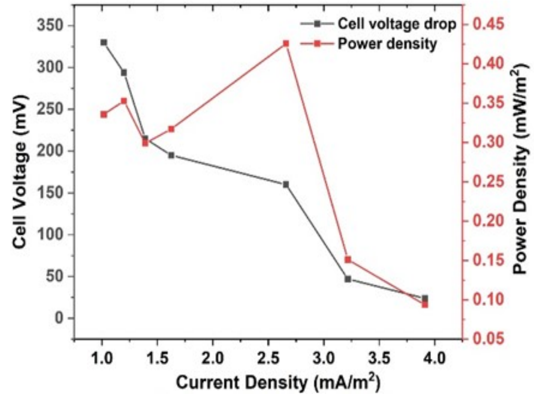

**S Figure 1:** Carboxylic acid synthesis trend (A) Maximum acetic acid synthesis; (B) Maximum propionic acid synthesis

**Cell design point of Individual fuel cells**

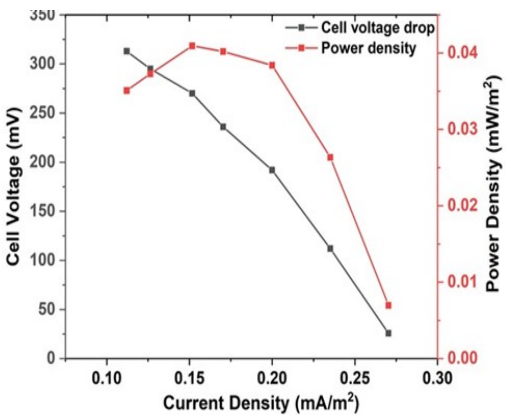
**a)**



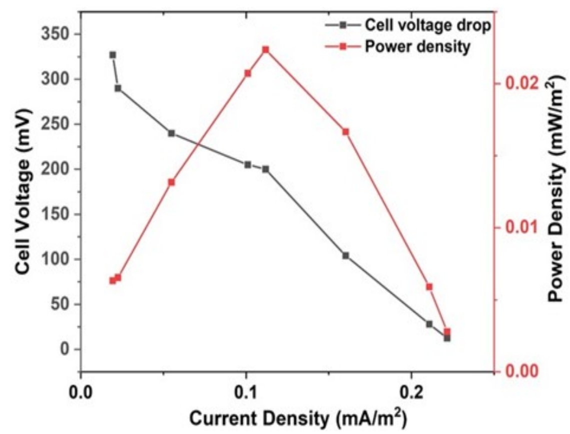

**b)**



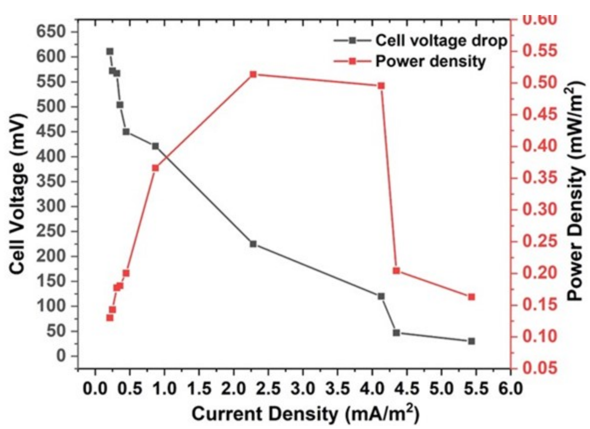

**c)**




**d)**




**e)**



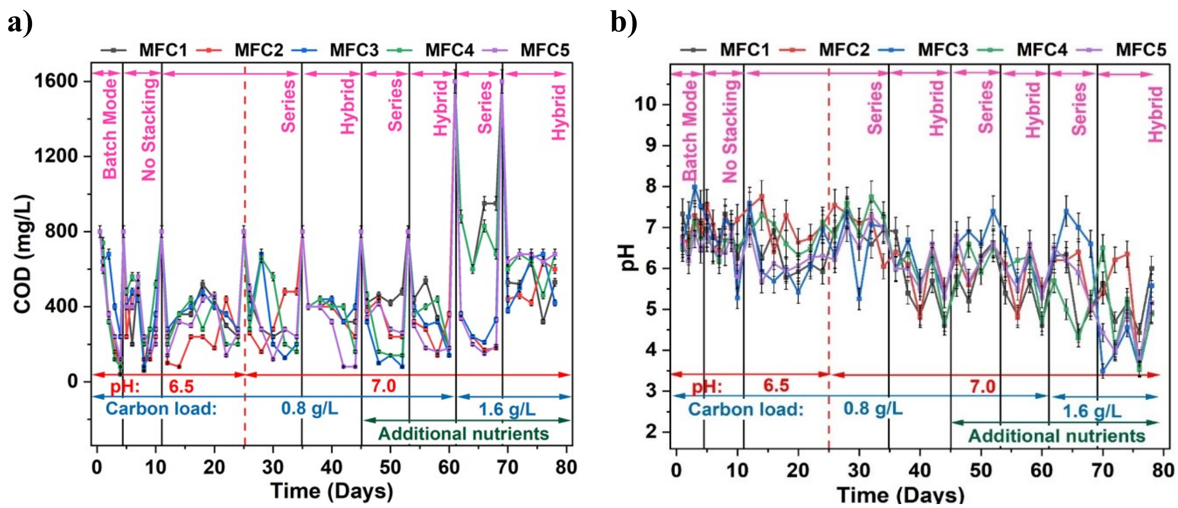

**S Figure 2:** Polarizations of individual cells a) MFC-1; b) MFC-2; c) MFC-3; d) MFC-4; e) MFC-5

**Substrate removal and treatment efficiency**

The treatment of domestic synthetic wastewater (as an anolyte) was monitored in terms of COD reduction. The COD removal was monitored by daily sampling during the analysing of all operating conditions. In the mode (B/S:0.8/6.5), gradual decrease in COD was observed daily with more reduction on the first day. The substrate reduction trends with time in each operating condition is illustrated in S. Figure 3a. At the end of 4 days 86% of COD reduction was observed on average. In this condition (B/S:0.8/6.5) MFC1 depicted highest treatment efficiency of 95% followed by MFC2 and MFC4 depicting equal reduction tendencies (90%). MFC3 and MFC5 depicted lower efficiency of 70% and 85% respectively. Whereas in continuous mode with flow rate of 1.2 ml/min (C/S:0.8/6.5) the average COD reduction was observed as 58.42% on an average. MFC2 (65%) exhibited maximum efficiency followed by MFC1 (61.25%), MFC3 (60%), MFC5 (59.17%) and MFC4 (46.67%). The reduced efficiency could be due to significant decrement in HRT from batch mode and continuous mode. To enhance the charge transfer affinity of biocatalyst by increasing the potential difference, the system was stacked in series configuration. In this condition (SR/S:0.8/6.5) MFC2 (72.86%) exhibited maximum efficiency, followed by MFC5 (63.21%), MFC4 (62.14%), MFC1 (56.07%) and MFC3 (54.29%). The average reduction in SR/S:0.8/6.5 was 61.72%. To prevent accumulation of charges in anolyte, the pH of the substrate was adjusted to 7 [34]. The COD reduction changed to 60.16%. In SR/S:0.8/7 MFC5 depicted 64.50% efficiency, followed by MFC1 (63%), MFC3 (61.30%), MFC2 (58.50%). MFC4 depicted the least substrate reduction of 53.50%. Under the same conditions (HY/S:0.8/7) the COD reduction was limited to 56.40% in hybrid stacking. The treatment efficiencies were in the following order: MFC5 (68%), MFC2 (56%), MFC4 (55%), MFC1 (53%) and MFC3 (50%). Under same operating conditions, the hybrid stacking is observed to yield lower treatment efficiencies. The higher potential difference in series stacking results in higher charge transfer affinity and might be the reason for better treatment efficiencies. Inclusion of additional nutrients increased COD reduction 63% in series stacking. MFC3 (80%) depicted the highest reduction followed by MFC4 (71.87%). MFC2 and MFC5 exhibited 59.37% efficiency, whereas MFC1 depicted 44.37%. The treatment efficiency in HY/S:0.8/7/AD followed the order: MFC5 (74.37%), MFC2 (65.62%), MFC3 (63.75%), MFC4 (56.87%) and MFC1 (53.12%) with an average of 62.75% reduction. Addition of nutrients improved treatment efficiency by 3% and 6% in series and hybrid staking respectively. In doubled carbon load (SR/S:1.6/7/AD), improved the charge transfer efficacies were observed with average efficiency of 70.82%. MFC5 depicted highest of 86.25%, followed by MFC2 (85.17%), MFC3 (82.19%), MFC4 (53.28%) and MFC1 (47.19%). In hybrid stacking under the same operating conditions (HY/S:1.6/7/AD), the average efficiency depicted was 64.70%. MFC1 (68.25%) depicted maximum reduction, followed by MFC2 (68%), MFC3 (67%), MFC4 (61.75%) and MFC5 (58.50%). Doubling the carbon load along with additional nutrients yielding highest treatment efficiency in both stacking configurations. Presence of additional nutrients and optimum carbon load increased the COD reduction up to 10.66%/ This could be due to enhanced metabolic activity of microorganisms. The maximum VTR (Volumetric treatment rate) was depicted in both series and hybrid stacking was with high carbon load in presence of additional nutrients as 21.78 kg COD/$m^3$ day & 19.90 kg COD/$m^3$ day respectively.

In this study, treatment efficiencies achieved were higher as compared to similar contemporary studies, considering lower HRTs employed. For HRT of more than 24 hours, Nikhil et al., and Das et Al., reported 80% reduction in batch mode, whereas Kuchi et al., reported 82% reduction in continuous mode. It is clearly observed from the results that series stacking exhibited better efficiency in all operating conditions as compared to hybrid stacking. Series stacking exhibited higher voltages in each operating condition. Higher voltage attests enhanced microbial activity, which, in turn, depicts efficient electron flow. Also, higher redox potential means ease in microbial electron transfer and less internal resistances. All these factors are evident by improved treatment efficiencies of series stacking. It is also observed that each cell exhibited dynamic performance which reinforces the inherent dynamic nature of biocatalysts as mixed culture is being used. The enrichment of microorganisms takes place individually in each cell making each microbial environment distinctive from one another.

As redox reactions and metabolic activity are largely governed by pH, with neutral pH evidently depicting higher microbial activity and hence better results. Deviation of optimal pH range (7 to 7.5) of exoelectrogens and fermentative bacteria can significantly hamper the overall performance of an MFC. To prevent such predicament pH control strategy of adjusting the buffer to neutral pH of 7.0 was employed throughout the operation of the MFCs in stacked configuration, after initial stages. The pH trends remained in neutral with gradual decrease with time due to formation of carboxylic acids (S. Figure 3b). The hybrid stacking observed lower pH throughout in each operating condition due to bioproduction of higher concentration of carboxylic acids as compared to series stacking, as discussed in previous section.

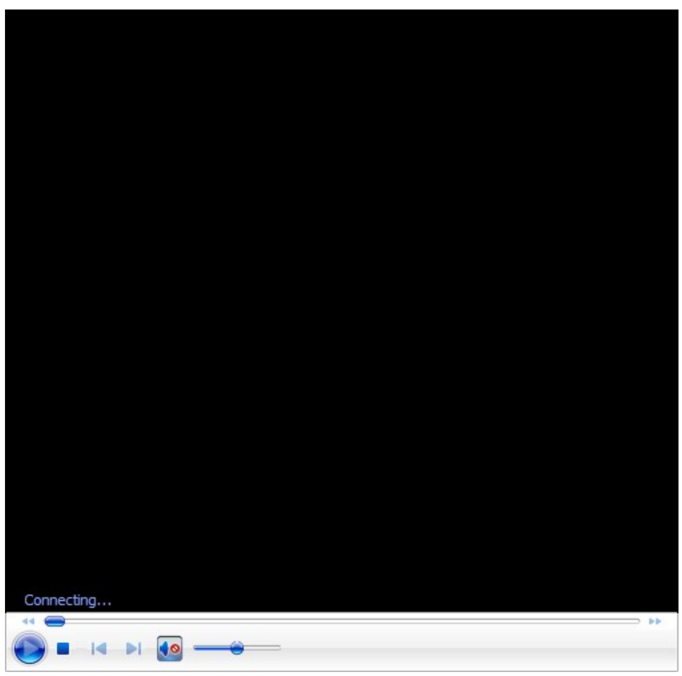



**S. Figure 3:** a) Substrate consumption and b) pH profile with time in all MFCs across the different operating conditions.

**Bioelectricity harness**

**a)** **b)**

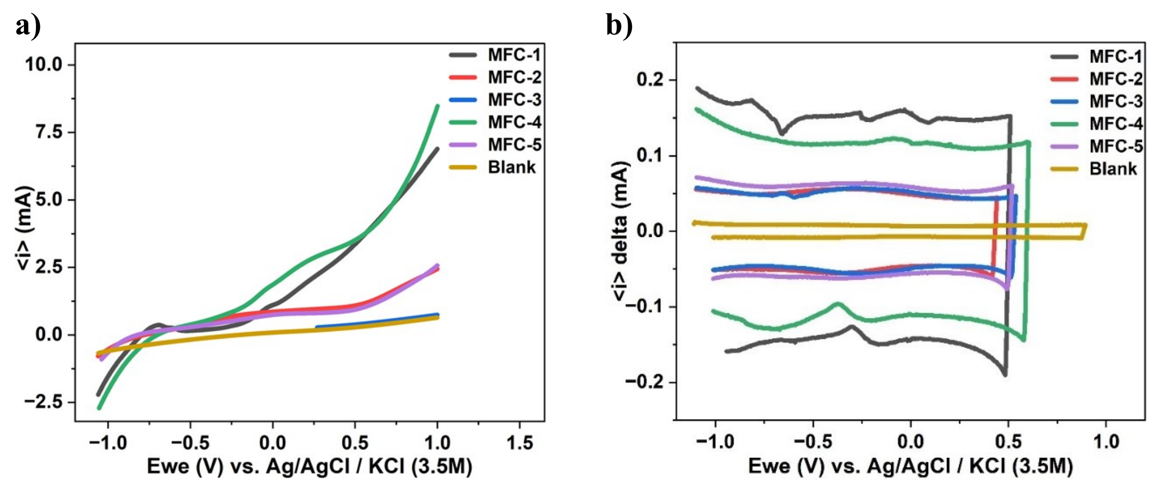
**c)**




**S. Figure 4:** Photographic representation of a) MFCs stack module; b) Feed tank opted gravity inflow with optimal flow rate. C) Videographic representation of powering LED bulb. *(Note: Turnoff the* ***design mode*** *application in* ***developer*** *ribbon bar before playing the video)*

**Oxidative tendencies**

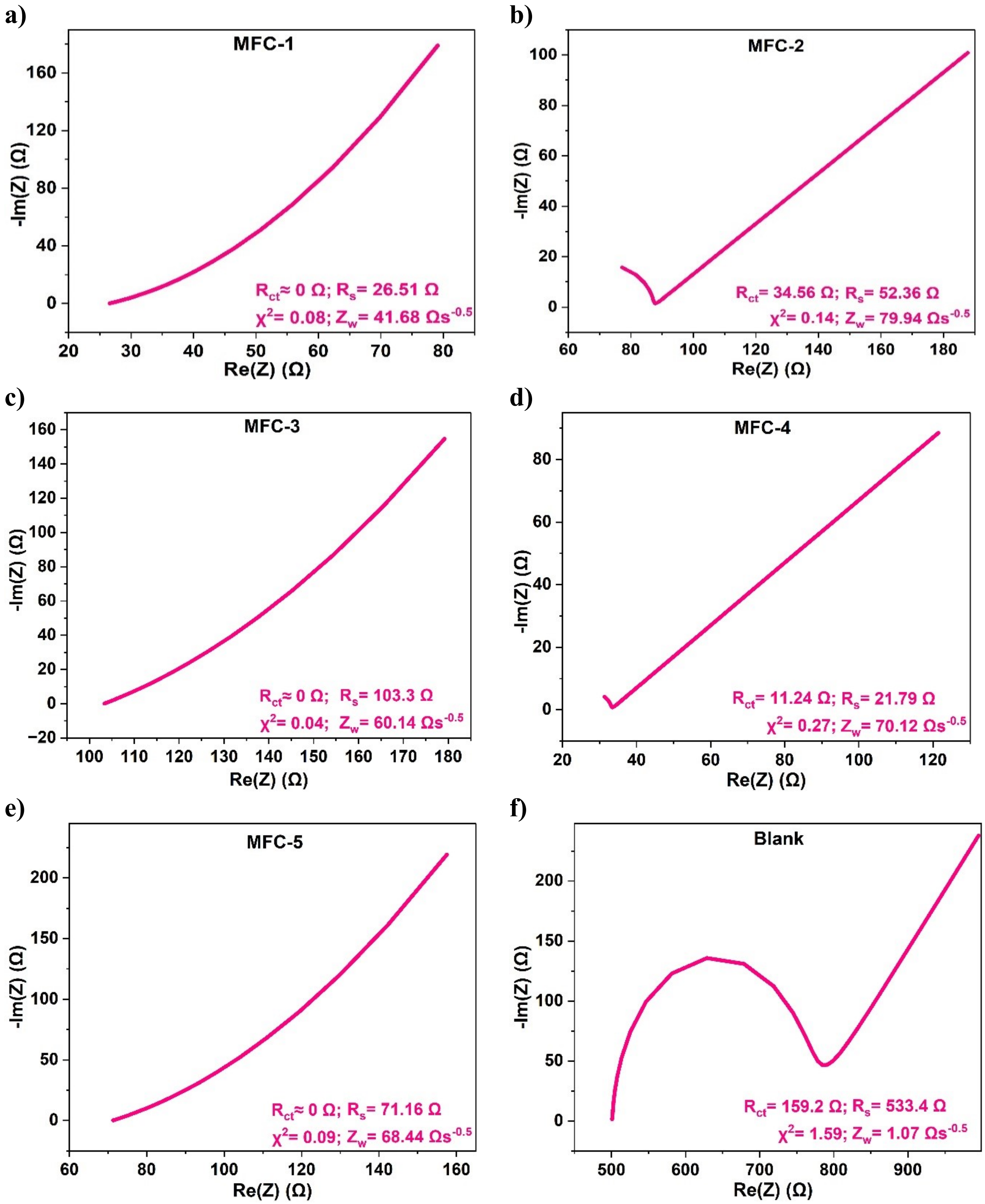
Oxidative tendencies of the biocatalyst were assessed using linear sweep voltammetry. The range for LSV was kept -1 V to 1 V with a scan rate of 10 mV. The blank/non-turnover LSV (Linear sweep voltammetry) depicts very less peak oxidation current of less than 1 mA. After inoculation significantly higher oxidative currents were observed. This shows a higher tendency of biocatalyst to oxidize the substrate and hence, higher charge transfer tendency between biocatalyst and anode, facilitating power generation. MFC4 depicted maximum oxidative currents 8.8 mA followed by MFC1 with 7.6 mA. MFC2 and MFC5 depicted peak oxidation current of 2.5 mA. MFC3 depicted 0.74 mA. Similar trend was observed in cyclic voltammograms. This shows higher metabolic activity and oxidative tendencies in MFC1 and MFC4. The lowest voltage value at which the oxidation product is produced by the biocatalyst at the anode in the given conditions is referred to as onset potential. Onset potential provides insight to the relative activity of the electrode and electrolyte. It is observed in all MFCs that the onset potential is occurring near to -0.3 V, which shows that the cells are depicting higher oxidative tendencies. The half-wave potential is a characteristic of electroactive species which is defined as half of the potential required to reach limiting current density obtained from LSV curve [29]. The half wave potential of all MFCs is occurring at 0.35 V. As half wave potential is an indicator of ORR performance, it could be concluded that higher ORR performance is observed after inoculation. The pulse wave form was imposed on the system to record delta current generated. Differential pulse voltammetry (DPV) was recorded with a starting potential of -1 V and limit potential of 0.8 V followed by reverse scan rate of -1 V. Pulse height of 2.5 mV, width of 100 mV was set along with 5 mV as step height and 500 ms step time. Before inoculation, the delta current recorded was -0.01 mA and 0.01 mA, which rose up to -0.15 mA and 0.2 mA in MFC1. The delta current recorded in other MFCs was as follows: MFC4 (-0.1 mA and 0.16 mA), MFC5 (-0.06 mA and 0.06 mA), MFC3 (-0.05 mA and 0.05 mA) and MFC2 (-0.05 mA and 0.05 mA). The peaks in the DPV curve correspond to different redox couples undergoing reactions. The intensity of peaks is directly proportional to the concentration of the biologically active species present.



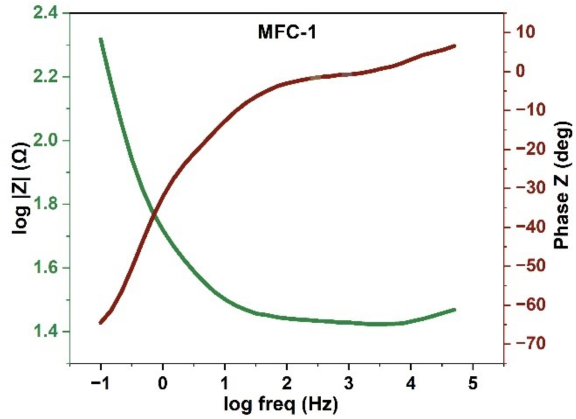

**S Figure 5:** a) Individual cell LSV; b) Individual cell DPV (reverse scan rate= -1 V)

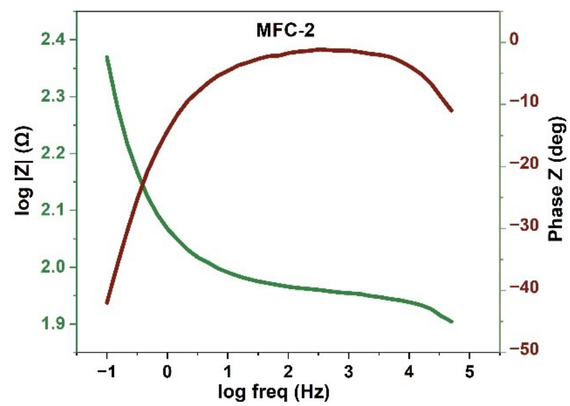
**Cumulative impedances and wave frequencies**

g)    h)

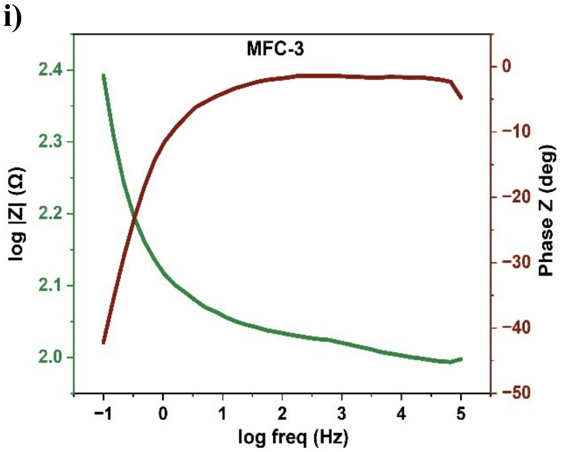


a)
MFC-1
$R_{ct}$≈ 0 Ω; $R_s$= 26.51 Ω
$\chi^2$= 0.08; $Z_w$= 41.68 Ωs$^{-0.5}$
b)
MFC-2
$R_{ct}$= 34.56 Ω; $R_s$= 52.36 Ω
$\chi^2$= 0.14; $Z_w$= 79.94 Ωs$^{-0.5}$
c)
MFC-3
$R_{ct}$≈ 0 Ω; $R_s$= 103.3 Ω
$\chi^2$= 0.04; $Z_w$= 60.14 Ωs$^{-0.5}$
d)
MFC-4
$R_{ct}$= 11.24 Ω; $R_s$= 21.79 Ω
$\chi^2$= 0.27; $Z_w$= 70.12 Ωs$^{-0.5}$
e)
MFC-5
$R_{ct}$≈ 0 Ω; $R_s$= 71.16 Ω
$\chi^2$= 0.09; $Z_w$= 68.44 Ωs$^{-0.5}$
f)
Blank
$R_{ct}$= 159.2 Ω; $R_s$= 533.4 Ω
$\chi^2$= 1.59; $Z_w$= 1.07 Ωs$^{-0.5}$
-Im(Z) (Ω)
Re(Z) (Ω)

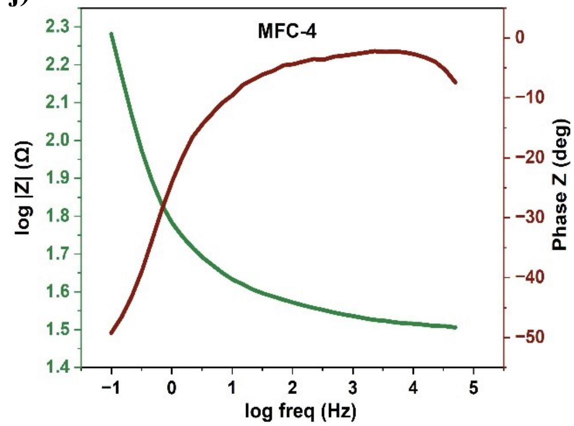


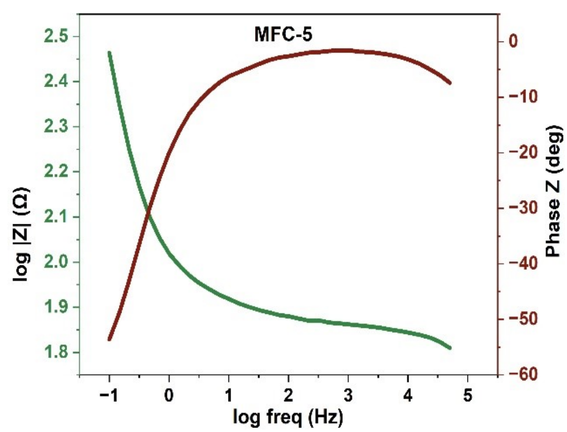

i)




j)




k)




**S Figure 6:** Z Fit curve of individual cells a) MFC-1; b) MFC-2; c) MFC-3; d) MFC-4; e) MFC-5; f) Blank; and Bode curve of individual cells against log of frequency, g) MFC1; h) MFC2; i) MFC3; j) MFC4; k) MFC5.

**Statistical significance of the outcomes**

**Table 1:** Analysis of variance between and within group (A) COD; (B) Acetic acid; (C) Propionic acid

| | **Source of Variation** | **SS** | **$d_f$** | **MS** | **F** | **P-value** | **F Crit** |
|---|---|---|---|---|---|---|---|
| (A) | Between Groups | 29230.04 | 4 | 7307.51 | 0.591962 | 0.024456 | 2.431965 |
| | Within Groups | 1851685 | 150 | 12344.57 | | | |
| | | | | | | | |
| | Total | 1880915 | 154 | | | | |
| (B) | Between Groups | 0.000806 | 4 | 0.000202 | 0.455055 | 0.002522 | 2.578739 |
| | Within Groups | 0.01993 | 45 | 0.000443 | | | |
| | | | | | | | |
| | Total | 0.020736 | 49 | | | | |
| (C) | Between Groups | 0.000987 | 3 | 0.000329 | 0.916064 | 0.000749 | 3.008787 |
| | Within Groups | 0.008621 | 24 | 0.000359 | | | |
| | | | | | | | |
| | Total | 0.009608 | 27 | | | | |