SUPPLEMENTARY INFORMATION

Electricity Generation Using a Microbial 3D Bio-anode Embedded Bio-photovoltaic Cell in a Microfluidic Chamber

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SECTION S1: Bioanode parts

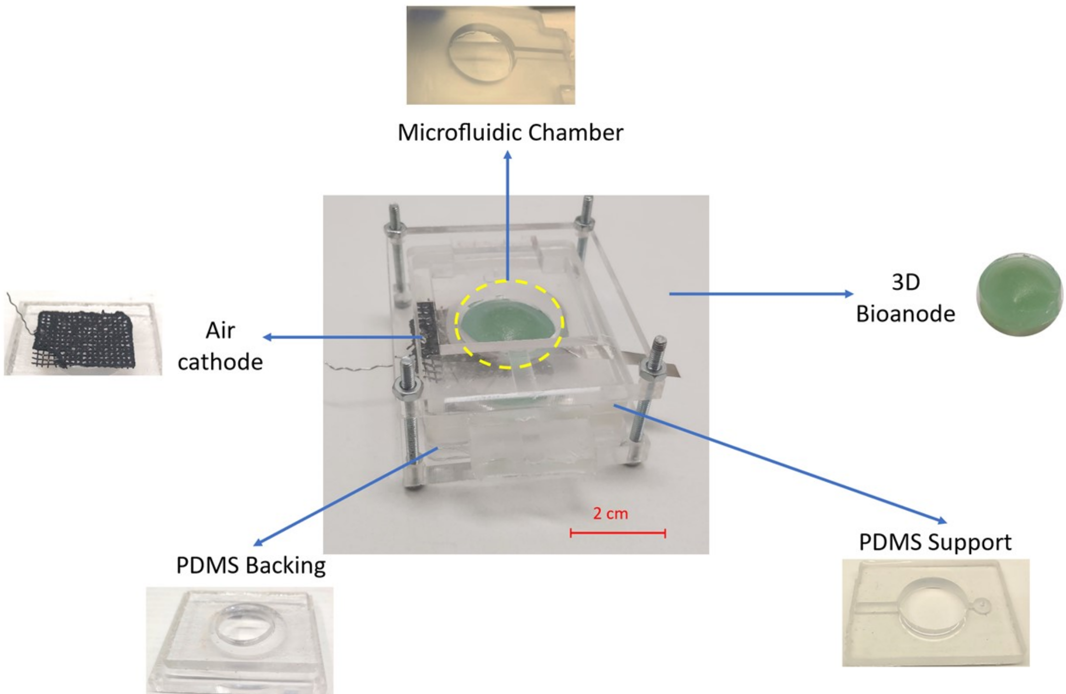

Figure S1: The main structure for the experimental setup of the 3D Bio-anode Embedded BPV Cell Unit

SECTION S2: OCV measurement stage

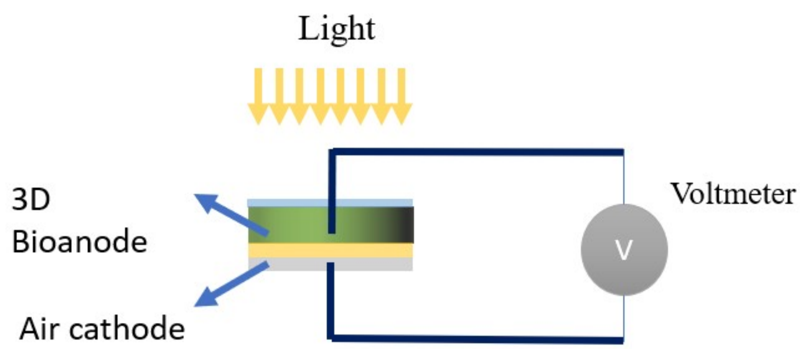

Figure S2: The block diagram of the BPV cell measurement system.

SECTION S3: Experimental Setup

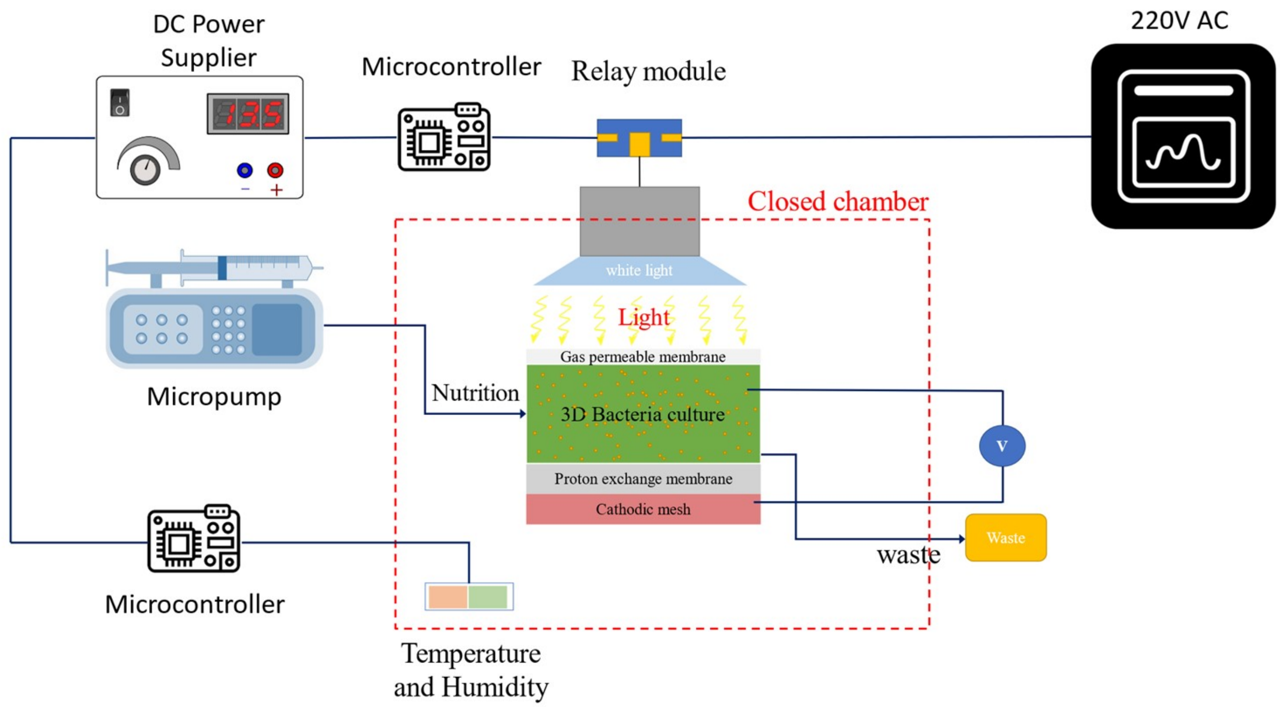

Figure S3: Electronic control and experimental setup configuration of the BPV cell.

SECTION S4: Internal resistance determination

In this section, the equivalent circuit model for different EIS results is presented. To create these models, the internal resistance value was determined using various passive circuit elements and electrochemical interactions. According to the results obtained, we found the internal resistance value to be 174.4±7.813 Ω. The effective surface area was determined as the sum of the surface areas of the ITO electrodes whose two surfaces touch the scaffold, that is, $2.\pi.(10mm)^2$. Thus, the total internal resistance related to surface area is 0.1096 $\Omega.m^2$.

A) ITO-ITO electrode configuration

In this section, the effects of similar and different electrodes were directly examined, but no major differences were obtained. When conductive materials are used as electrodes, there will not be a significant resistance effect, and thus, the scaffold pole can be obtained by the EIS method. In the first ITO-scaffold-ITO measurement made in this context, scaffold internal resistance of approximately 174 ohms was obtained.

Table S1: Model parameters

Parameter	**Value**	**% Error**	**Units**
R1	174	4.48	ohm
R2	3.22E+05	6.615	ohm
Yo1	3.20E-06	0.11	S*s^a
a1	7.96E-01	1.63	
C1	1.35E-09	8.37	F
R3	210	3.5	ohm

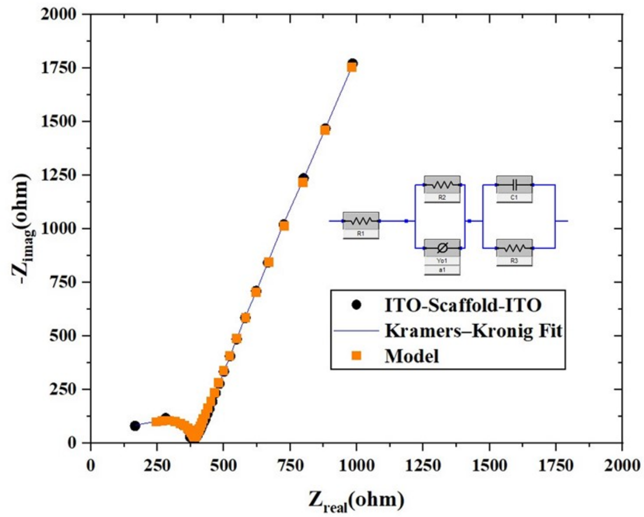

Figure S4: Measured impedance values, Kramers-Kronig fit, and equivalent circuit model for scaffold with ITO-ITO electrodes.

B) Pt/Air cathode electrode configuration in open circuit EIS measurement

The utilization of Pt/Air cathode electrodes induced a noteworthy reduction in internal resistance, primarily attributed to the insertion of platinum into the scaffold. Paradoxically, this augmentation led to an increase in double-layer capacitance, consequently impeding charge transfer efficiency. Notwithstanding that the paramount source of resistance emanates from the internal resistance factor, it is worth noting that the configuration of the air cathode electrode also poses challenges to effective charge transfer. Although parameters like charge transfer resistance and double-layer capacitance wield influence in the realm of internal resistance measurement through Electrochemical Impedance Spectroscopy (EIS), an equivalent circuit model provides an avenue to discern an averaged internal resistance value. The resultant value for internal resistance for the scaffold with Pt/Air cathode electrodes is 67.7 ohm, but the insertion of platinum into the scaffold reduced the exact value. Furthermore, employing similar electrodes on both surfaces (ITO-ITO) effectively nullifies the electrode-induced resistance effect. However, it does modify charge transfer dynamics, mainly attributed to the formation of a double-layer capacitance between the electrolyte (scaffold) electrodes. Given the precise fitting of the equivalent circuit model to the measurements, we can confidently ascertain the internal resistance at 174.4 ohms. When scaled according to surface area, this equals π.(10mm)^2 = 314.2 x 10^{-6} m^2, which is needed for maximum power density calculation.

Table S2: Model parameters

Parameter	Value	± Error	Units
R1	67.7	1.488	ohm
C1	6.97E-09	3.49E-10	F
R2	92.5	3.576	ohm
R3	1.26E+06	2.77E+05	ohm
Yo1	1.30E-05	4.08E-07	S*s^a
a1	7.89E-01	7.25E-03	
R4	46.24	12.89	ohm
Yo2	1.08E-05	6.73E-06	S*s^a
a2	7.89E-01	1.67E-01	

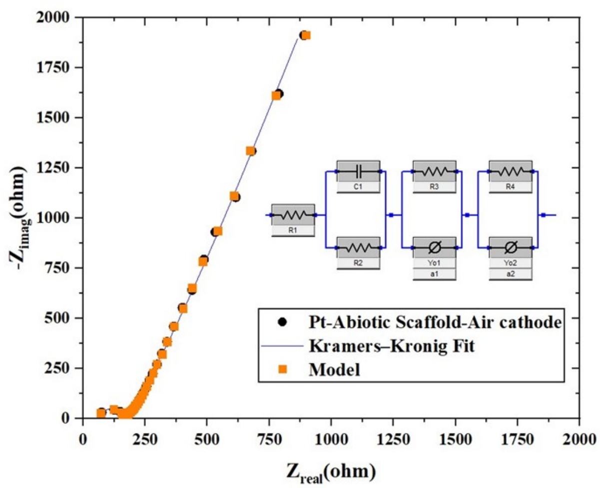

Figure S5: Measured impedance values in open circuit mode, Kramers-Kronig fit, and equivalent circuit model for scaffold with Pt/Air cathode electrodes.

C) Pt/Air cathode electrode configuration in closed circuit EIS measurement

Here, we tested only a 560 kΩ resistor to develop an equivalent circuit model, which has almost similar internal resistance to **SECTION S4, B**.

Table S3: Model parameters

Parameter	Value	± Error	Units
R1	9.28E+01	4.25E+00	ohm
C1	3.71E-09	4.29E-10	F
R2	8.16E+01	4.11E+00	ohm
R3	6.34E+01	7.53E+01	ohm
Yo1	7.00E-06	1.29E-05	S*s^a
a1	8.14E-01	2.11E-01	
R4	1.10E+03	1.38E+03	ohm
Yo2	3.71E-06	7.28E-06	S*s^a
a2	9.64E-01	2.87E-01	

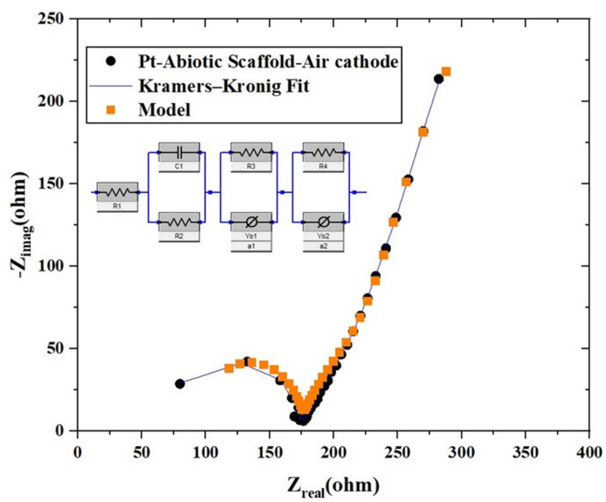

Figure S6: Measured impedance values during closed-circuit mode with 2MΩ resistor, Kramers-Kronig fit, and equivalent circuit model for scaffold with Pt/Air cathode electrodes.

D) Pt/Air cathode electrode configuration with biotic scaffold during open circuit EIS measurement

Based on the EIS measurement taken on the first day, it was observed that the presence of bacteria did not significantly affect the internal resistance. However, there was a rapid decrease in the double-layer capacitance, possibly due to the interaction between the bacteria and the electrode surface.

Table S4: Model parameters

Parameter	Value	± Error	Units
R1	103.7	5.232	ohm
C1	2.67E-09	2.78E-10	F
R2	112.1	5.032	ohm
R3	143.5	36.6	ohm
Yo1	3.87E-06	7.21E-07	S*s^a
a1	8.28E-01	2.85E-01	
R4	2.17E+03	1.41E+02	ohm
Yo2	7.56E-06	4.02E-06	S*s^a
a2	8.41E-01	2.75E-01	

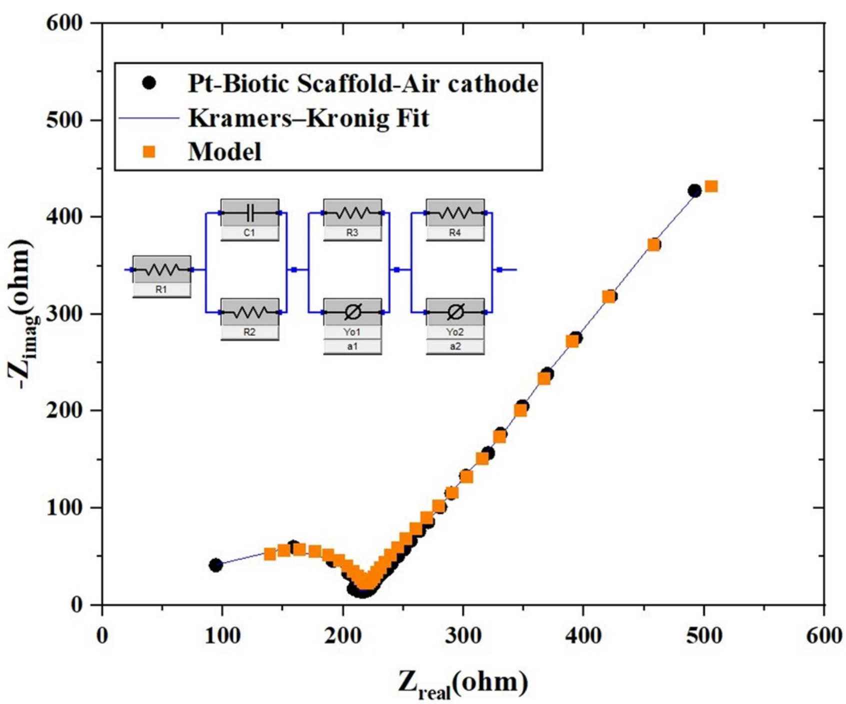

Figure S7: Impedance measurement of the biotic scaffold during the open-circuit mode, Kramers-Kronig fit, and equivalent circuit model for biotic scaffold with Pt/Air cathode electrodes.

SECTION S5: Maximum power transfer theorem

This section aims to determine how much maximum power can be provided from an electricity generating system. It is explained how the BPV system we developed can be analyzed using the maximum power transfer theory.

The maximum power transfer theorem, which is valid for both alternating direct current circuits, states that the load can draw maximum power from the power supply when the load resistance is equal to the source's internal resistance [S1].

In a circuit that includes a source and a load, as shown in **Figure S8**, we can model it using the Thevenin method and the Thevenin equivalent circuit model exhibits a resemblance akin to that of a voltage source.

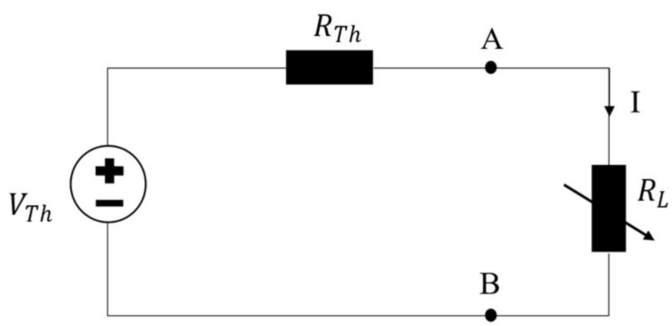

Figure S8: Power supplier and a variable resistor circuit diagram.

The total dissipated power (P_T) across the load resistor (R_L) is calculated as:

$$P_T = I^2 R_L \tag{S1}$$

where $I = \dfrac{V_{Th}}{R_{Th} + R_L}$ (A) is the current flowing across the circuit. We can then calculate the power depending on R_L as:

$$P_T = V_{Th}^2 \left[\frac{R_L}{(R_{Th} + R_L)^2} \right] \tag{S2}$$

To calculate the maximum or minimum values for total power, we take the derivative of the equation (S2) respect to R_L:

$$\frac{dP_L}{dR_L} = 0 = \frac{d}{dR_L}\left[V_{Th}^2 \frac{R_L}{(R_{Th} + R_L)^2}\right]$$

(S3)

This leads to $R_{Th} = R_L$, which implies that the maximum power is transferred when the internal resistance of DC power is equal to the load resistance, or the impedance of AC source is equal to the load impedance.

For calculating the maximum power, we use $R_{Th} = R_L$ in equation (S3):

$$P_T = V_{Th}^2 \frac{R_L}{(R_{Th} + R_L)^2} = \frac{{V_{Th}}^2}{4R_L}$$

(S4)

Here, the power source is BPV, and the internal resistance of abiotic and biotic scaffold will be the internal resistance of the developed device. Then, we can obtain the peak power as:

$$P_{max} = \frac{{V_{OC}}^2}{4R_{int}}$$

(S5)

where P_{max} (W) is the maximum power transferred from source to load, V_{OC} (V) is the open-circuit voltage of the source, and R_{int} is the internal resistance of the source with total effective electrode area.

The maximum power density is calculated by using the maximum power, but the internal resistance of the source should be given with the total effective electrode area. Thus, the internal resistance becomes equal to 0.1096 Ω.m^2. According to the given parameters, we can calculate the maximum power density as:

$$D_{max} = \frac{P_{max}\,(W)}{Total\ effective\ electrode\ area\ (m^2)}$$

(S6)

SECTION S6: Scaffold optical properties

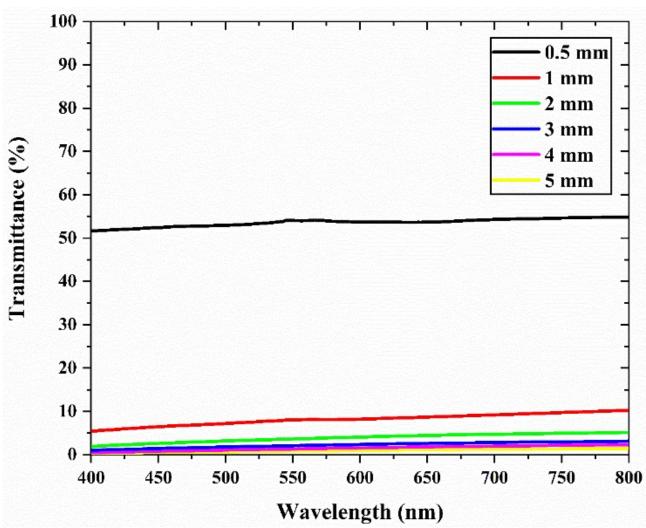

Figure S9: Light transmission of 3D bioanode structure. Depending on the increase in scaffold thickness, the light transmittance decreases sharply.

SECTION S7: Power conversion efficiency

A) Light properties

We used a light source that had the total light power density D_{light};

$$D_{light} \sim 16.7 \frac{W}{m^2} \quad \text{(S7)}$$

B) Power conversion efficiency

Here, we have enough information to calculate the power conversion efficiency by dividing output power (bioanode maximum power output) to input power (light power).

$$\mu_{CE}\% = \frac{P_{bioanode}}{P_{light}} x100 = \frac{0.0534\ Wm^{-2}}{16.7\ Wm^{-2}} x100 = 0.32\%$$

(S8)

where $P_{bioanode}$ is the output power of the bioanode, P_{light} is the total light power on the scaffold, and the μ_{CE} is the conversion efficiency of the bioanode.

C) Polarization curves for power density determination

The HPPC test data contains relevant polarization curves that show voltage and current density profiles during different pulses. The power density at different operating points can be calculated by utilizing polarization curves. The formula for power density is expressed as Power Density =

Voltage×Current Density. It helps to understand the dynamic performance of the electrochemical device and to identify the optimal operating conditions based on power density analysis.

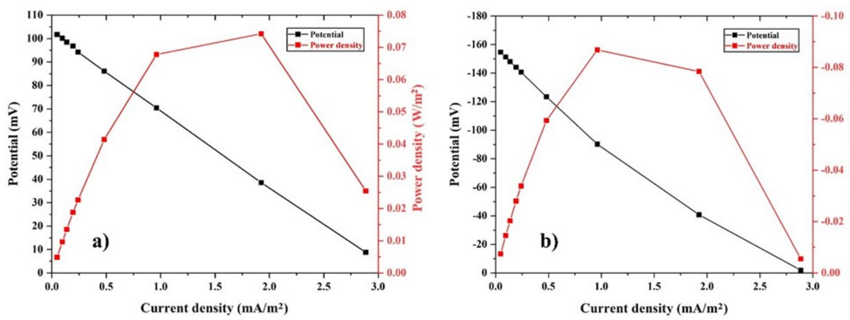

Figure S10: Polarization curves of **a**) biotic scaffold under white light and **b**) biotic scaffold during dark hours.

SECTION S8: Cyclic Voltammetry Analysis

Cyclic voltammograms were obtained in biotic structures using Pt/air cathode electrodes at different scan rates between ±1V. Since there were bacteria in both environments, the difference in voltammograms is because of light. As seen in Section S8, Figure S11, the light effect provided a further increase in the current density in the negative direction for each scan rate. This result can be associated with the negative values obtained under light in open circuit voltage measurements.

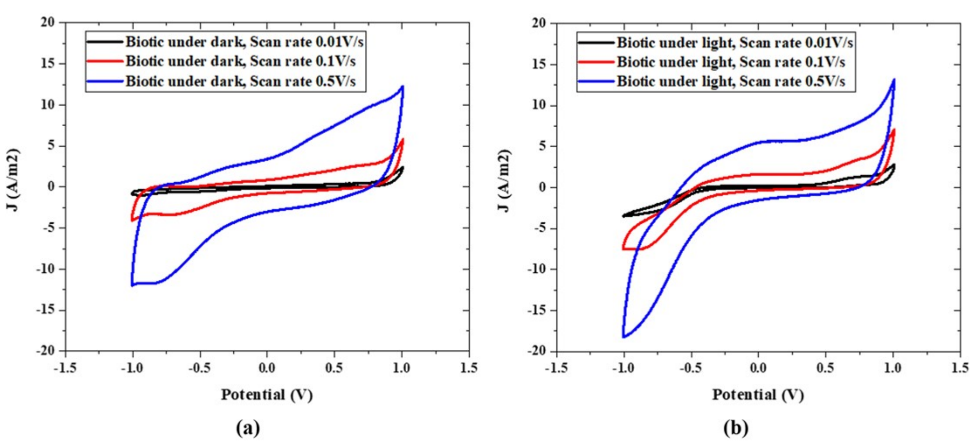

Figure S11: CV scans of Pt-air cathode under dark or light-adapted conditions. **(a)** CV scans of Pt-air cathode under dark conditions. **(b)** CV scans of Pt-air cathode attached electrodes on biofilm under light irradiation. All experiments were conducted in BG11 medium (pH 8.0) at 25°C.

SECTION S9: Statistical Analysis

Statistical analysis was applied to peak powers for both abiotic and biotic data under light and dark conditions. Here, we used abiotic peak powers as control data and applied the student's t-test.

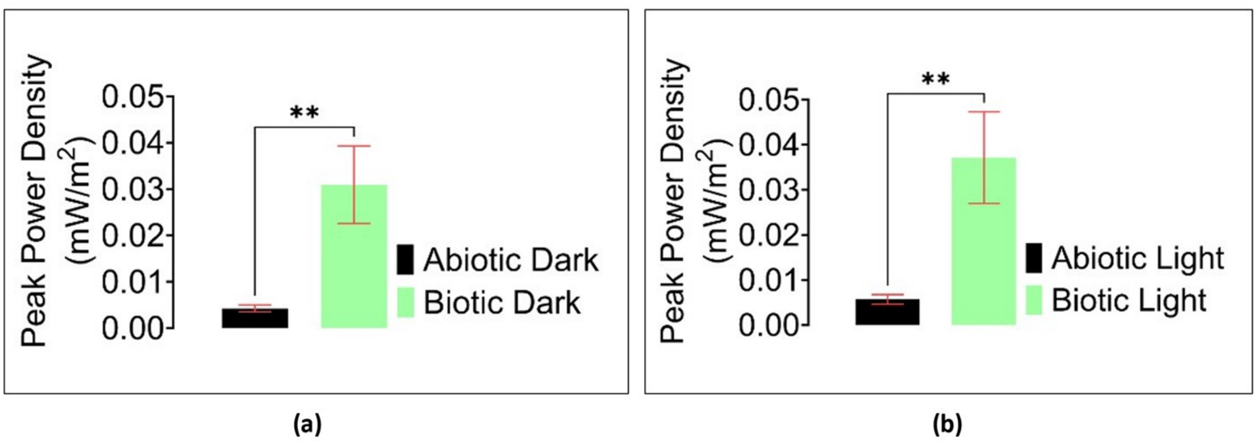

Figure S12: Statistical results of peak powers. (**a**) Comparison between abiotic and biotic peak powers under dark, and (**b**) comparison between abiotic and biotic peak powers under light conditions.

The required statistical analysis is provided for Abiotic and Biotic peak power results under dark conditions. A student's t-test was conducted to compare the abiotic power density (control) under dark conditions (mean: 0.00423 mW/m², standard deviation: 0.002128299) with the biotic power density under dark conditions (mean: 0.03094 mW/m², standard deviation: 0.023623713).

Similarly, the student's t-test was utilized for Abiotic and Biotic peak power results under light conditions. Then, it was employed to find the statistical outputs using abiotic power density (control) under dark conditions (mean: 0.00571 mW/m^2, standard deviation: 0.003001375) and biotic power density under dark conditions (mean: 0.03713 mW/m^2, standard deviation: 0.028748628). According to the Figure S12, the results show that the significant differences occur between abiotic and biotic 3D scaffolds and also light and dark conditions.

REFERENCES

S1. Nahvi, M. and J.A. Edminister, Maximum Power Transfer Theorem, in Schaum's Outline of Electric Circuits. 2018, McGraw-Hill Education: New York.