Supplementary Information

Yeast Biofilm Synapse: An Intra-kingdom Pathway to High-Density Current Output in Bioelectronic Devices

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The Supplementary Information provides a comprehensive overview of the methodologies employed in the study. It begins with the Experimental Section, detailing the reagents used, followed by the preparation process for the bioelectrodes. The section also includes a thorough description of vibrational spectroscopy techniques, specifically micro-FTIR, used to assess molecular interactions and structures. Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) methods are outlined, explaining how these techniques were employed to analyze surface topography and sample morphology. Electrochemical experiments, including protocols for cyclic voltammetry and open circuit potential (OCP) measurements, are also discussed. Furthermore, the section details the procedures for conducting Raman vibrational spectroscopy, particularly focusing on single-entity analysis to explore molecular vibrational modes. The section concludes with a list of references used throughout the Supporting Information and the main text.

S1. Experimental Section

S1.1 Reagents

Phosphate salts ($NaH_2PO_4.H_2O$, 137.99 g mol^{-1}, >98%; and $Na_2HPO_4.7H_2O$, 168.07 g mol^{-1}, >98%), sodium hydroxide (NaOH, 39,997 g mol^{-1}, 99%) and potassium permanganate ($KMnO_4$, 158,034 g mol^{-1}, 99%) were obtained from Synth®. Hydrochloric acid (HCl, 37%) and sulfuric acid (H_2SO_4, 98%) were obtained from Êxodo científica®. Anhydrous d-glucose ($C_6H_{12}O_6$, 180,156 g mol^{-1}, 99%) was obtained from Merck®. Agarose heteropolysaccharide was purchased from KASVI®. *Saccharomyces cerevisiae* was purchased from Fleischmann®. All aqueous solutions were prepared with deionized water (18 MΩ cm at 25 °C). Flexible carbon fiber electrode was manually manipulated, chemically treated and used as substrate material for the preparation of the bioelectrodes.

S1.2 Bioelectrodes Preparation

Flexible carbon fibers (FCFs) were chemically treated before bioelectrode preparation. The FCFs were submerged in a solution of 1 mol L^{-1} sulfuric acid containing 25 mmol L^{-1} of potassium permanganate and put in the ultrasonic bath for 3 hours. After that, they were washed once with concentrated hydrochloric acid and thoroughly washed with water until the solution had pH 7.[1]

The bioelectrodes prepared without agarose were obtained by incubating *Saccharomyces cerevisiae* in a 0.1 mol L^{-1} phosphate buffer solution (pH 7.2) with 1 mol L^{-1} of glucose, under N_2 atmosphere, at 40 °C. With the prepared solution, the treated FCF was immersed in the solution and yeast was added, resulting in the natural adsorption of *Saccharomyces cerevisiae* on the surface of the FCF, resulting in the FCF/Sacch bioelectrode.

For the preparation of the bioelectrode with agarose, 1.8 mg of agarose powder was dissolved in 5 mL of DI water upon stirring the solution, at 95 °C. The solution was slowly cooled down to ambient temperature (25 °C) before its use for the preparation of the bioelectrodes. 250 mg of *Saccharomyces cerevisiae* was suspended in the agarose solution and 100 µL of the suspension were drop-casted on the surface of each treated FCFs. After surface coverage, the treated FCFs were put in the vacuum desiccator overnight and later stored at 2 °C, resulting in the FCF/Sacch-agarose bioelectrode.

S1.3 Vibrational Spectroscopy (micro-FTIR)

micro-FTIR was performed using a Bruker Vertex 70V spectrometer with a Hyperion 3000 microscope attached (Bruker Gmbh, Ettlingen, Germany). Micro-FTIR spectra were obtained in the reflectance mode. The saccharomyces-modified gold surface samples were analyzed with a N_2-cooled mercury telluride and cadmium detector (MCT). The spectra were recorded in a matrix of points at several spots in the surface with an average of 4096 accumulations with a spectral resolution of 4 cm^{-1} and a spectral window of 4000–600 cm^{-1} using an IR objective lens with 36x magnification.

S1.4 Atomic Force Microscopy (AFM)

The AFM characterization was conducted using a Bruker Multimode 8 system paired with a NanoScope 6 controller. Intermittent contact mode was utilized, employing an aluminum-coated silicon probe with a spring constant of approximately 40 N/m and operating at a resonance frequency of around 320 kHz. To mitigate sample tilt and scanner motion artifacts, all images underwent first or second order flattening. Additionally, FFT filtering was applied as necessary to enhance signal-to-noise ratio. To capture finer details, slower scanning rates and increased line-sampling were implemented.

S1.5 Scanning Electron Microscope (SEM)

Scanning electron microscopy experiments (SEM, LEO – 440) of the FCF-modified electrodes were operated at 15 keV. After metallization of the FCF and FCF-modified electrodes the samples under vacuum with a thin gold film, the samples were fixed on a cylindrical stainless-steel base with a circular area of approximately 1 cm^2 with a conductive carbon tape for fixation of the samples on the support. The FCF and FCF-modified samples were then placed under vacuum for image acquisition (< $2x10^{-10}$ mbar).

S1.6 Electrochemistry Experiments

The experiments were conducted in a conventional jacket glass electrochemical cell, containing a homemade Ag/AgCl/KCl_{sat} reference electrode, a Pt wire counter electrode and FCF bioelectrodes as working electrode. The experiments were performed at 40 ^oC and under N_2 atmosphere, using a 0.1 mol L^{-1} phosphate buffer solution (pH 7.2) as the supporting electrolyte, containing 1 mol L^{-1} of glucose. A GE® Multitemp IV thermostatic circulator (±0.1

^{o}C) was used for temperature control during the experiment. Before all measurements, the solution was degassed with N_2 gas for 15 minutes.

All the electrochemical measurements were conducted on an Autolab PGSTAT 204 (Metrohm, Swiss) potentiostat with software Nova 2.1.5. and the measured currents were normalized by the geometric area of the bioelectrodes, resulting in current density (*j*). **Figure S1** and **Figure S2** show the obtained cyclic voltammograms for the FCF/Sacch and FCF/Sacch-Agarose respectively. **Figure S3** shows the evolution of the OCP values for the bioelectrodes, respectively.

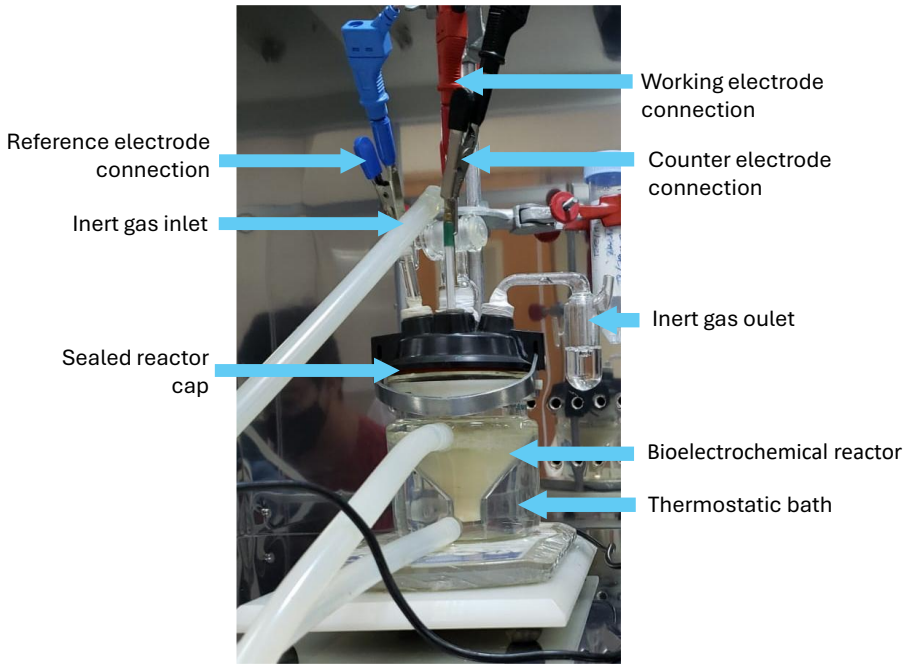

Figure S1. Photo of the bioreactor used as electrochemical cell.

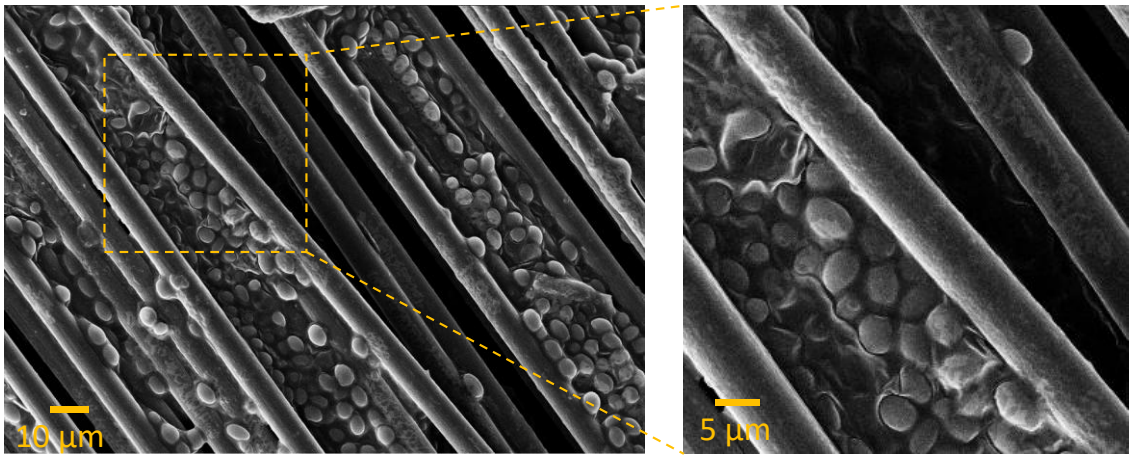

Figure S2. (a) SEM image of the yeast entrapped on to the flexible carbon fibers and (b) a zoomed image highlighting the biofilm between the carbon fibers.

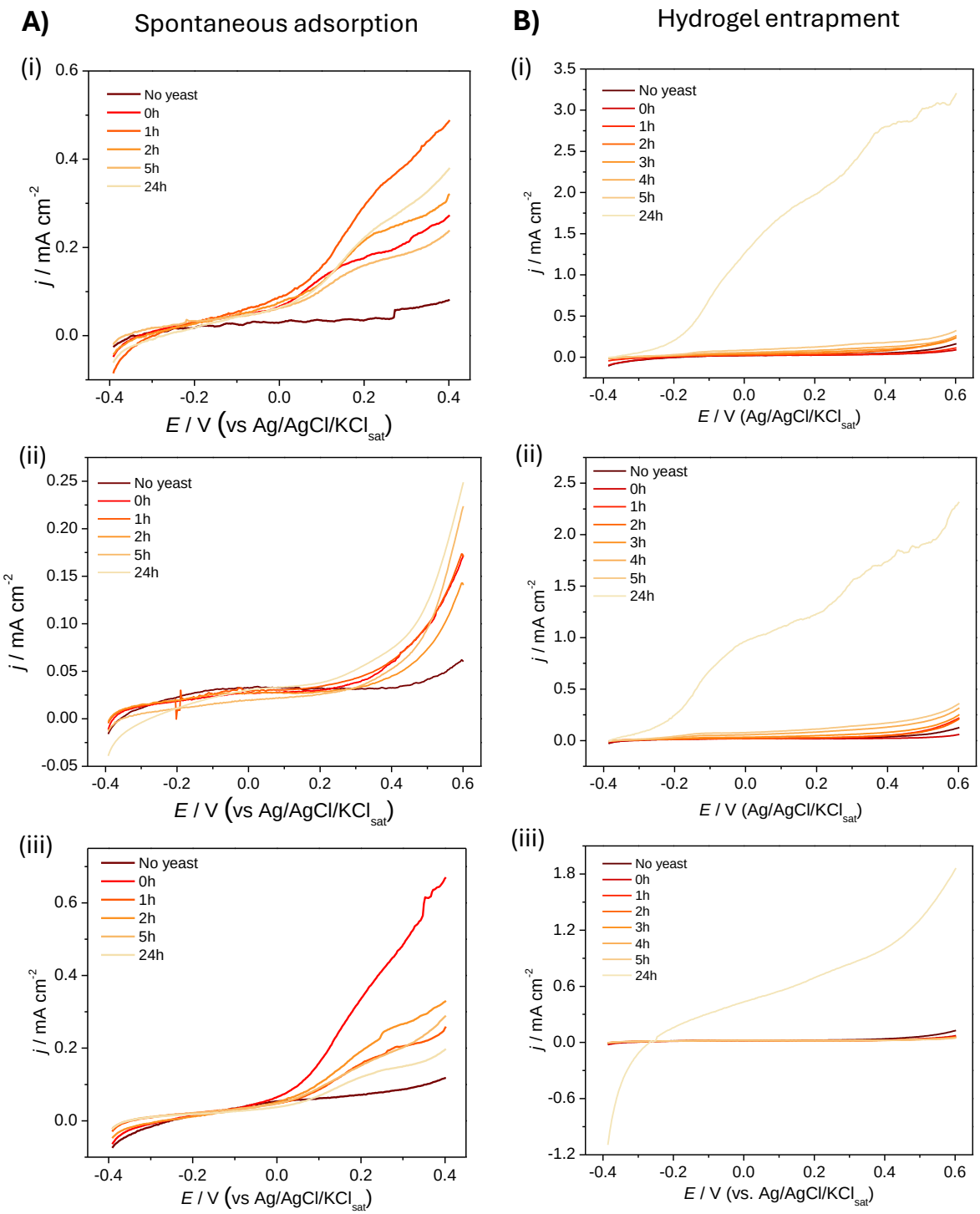

Figure S3. Replicates (i, ii, and iii) of the linear voltammograms (5 mV s^{-1}) for spontaneous adsorption method and (b) hydrogel entrapment method, using phosphate buffer (pH 7.2) as supporting electrolyte.

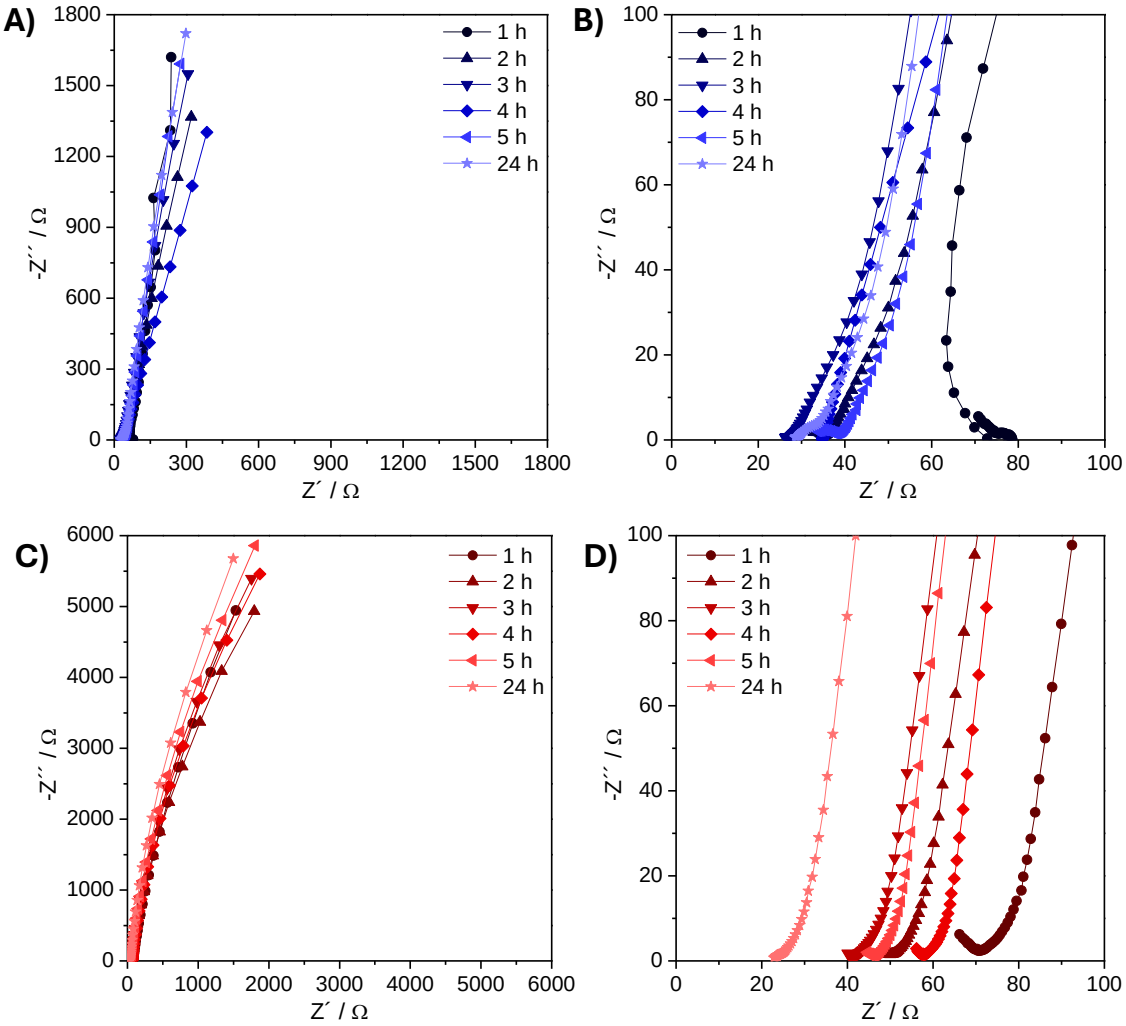

Figure S4. Nyquist plots obtained with FCF electrodes modified with Saccharomyces cerevisiae immobilized by (A, B) spontaneous adsorption and (C, D) hydrogel entrapment at different incubation times. B and D are zoomed views of A and C, respectively.

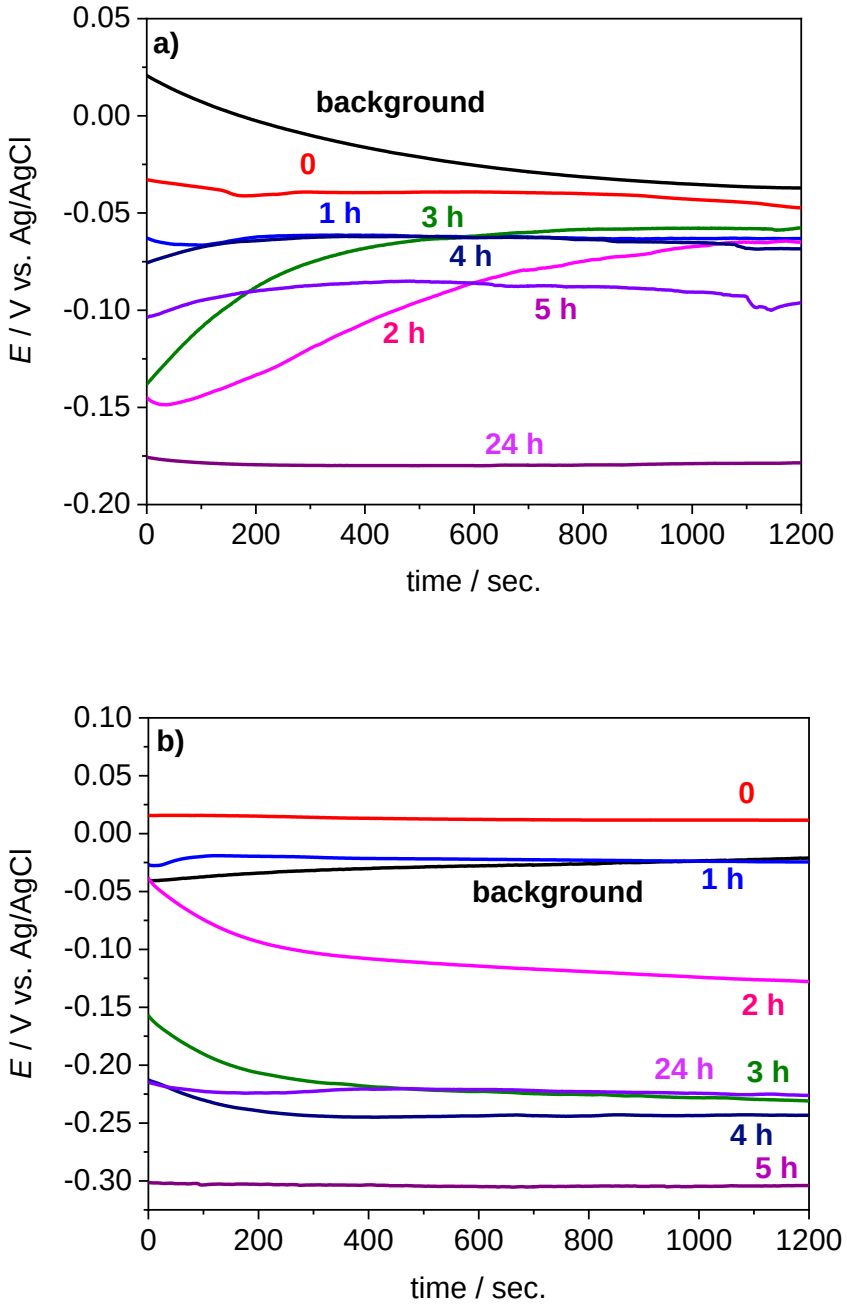

Figure S5. Chronopotentiometry of the bioelectrodes registered through 1200 seconds for the yeast activity time of 0 hours (red), 1 hour (blue), 2 hours (pink), 3 hours (green), 4 hours (dark blue), 5 hours (lilac) and 24 hours (purple). **a)** OCP for FCF/*Saccharomices* bioelectrode. **b)** OCP for FCF/Sacch-agarose bioelectrode.

S1.7 Raman Vibrational Spectroscopy

Raman spectra of the bioelectrodes were obtained on LabRam HR Evolution equipment, equipped with a 1600 x 200 CCD detector (symphony; liquid N_2 cooled), an 1800 L mm^{-1} grating, a HeNe laser with an excitation wavelength of 635 nm (red laser), filter of 10% and a 50x objective. The spectrometer was calibrated by determining the wavenumber position of Raman line of a silicon substrate at 520.7 cm^{-1} before data acquisition. **Figure S6** shows the Raman spectra for FCF, FCF/Sacch and *S. cerevisae*. [2,3]

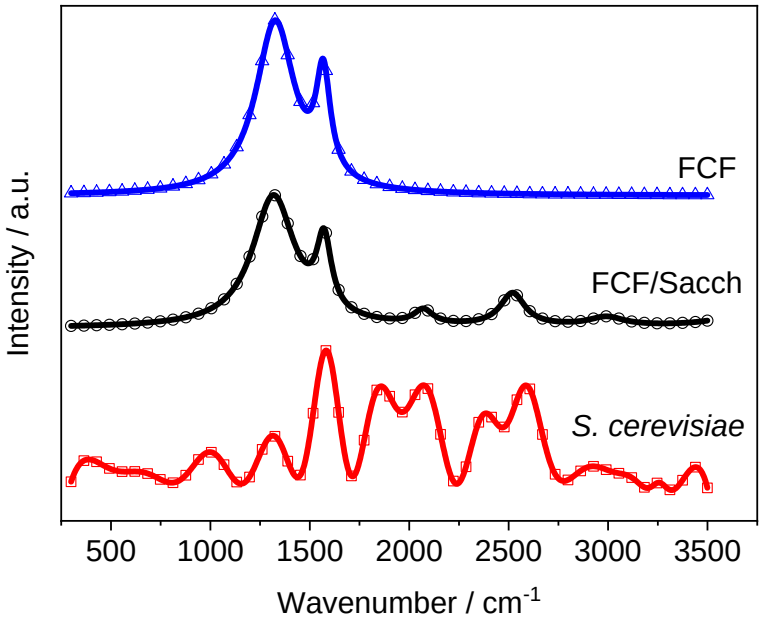

Figure S6. Raman spectra of the treated FCF (blue), FCF/Sacch bioelectrode (Black) and a *Saccharomyces cerevisiae* single cell on the surface of the bioelectrode (red).

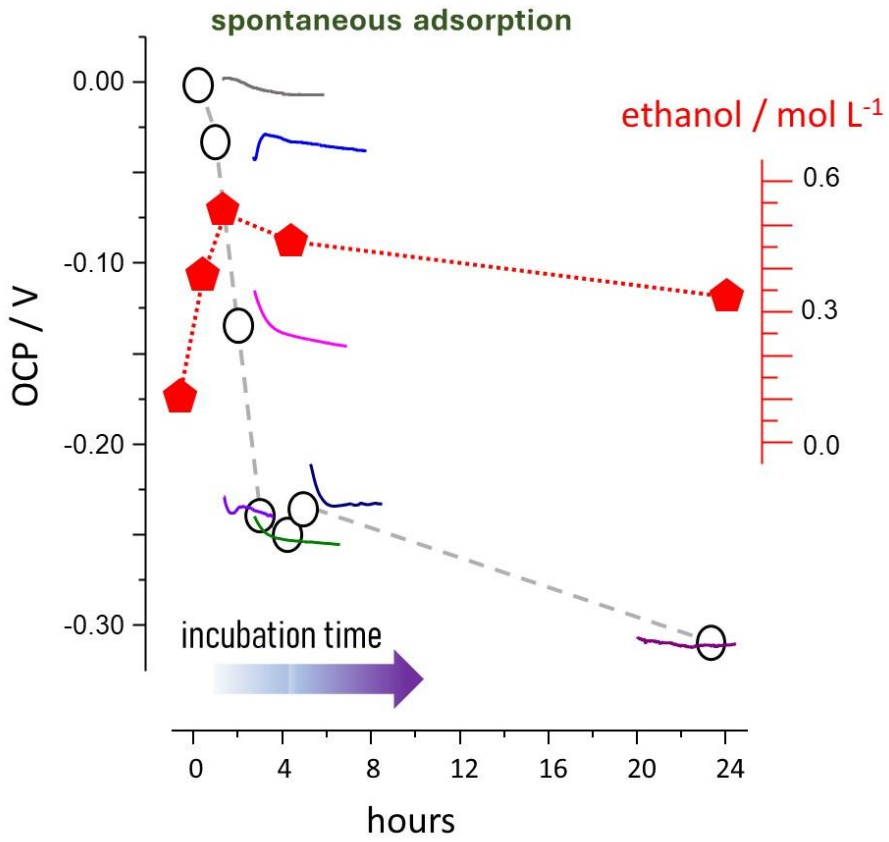

Figure S7. Polarization curves correlated with the OCP (open circuit potential) and the amount of metabolite (ethanol) produced during the formation of the EPS.

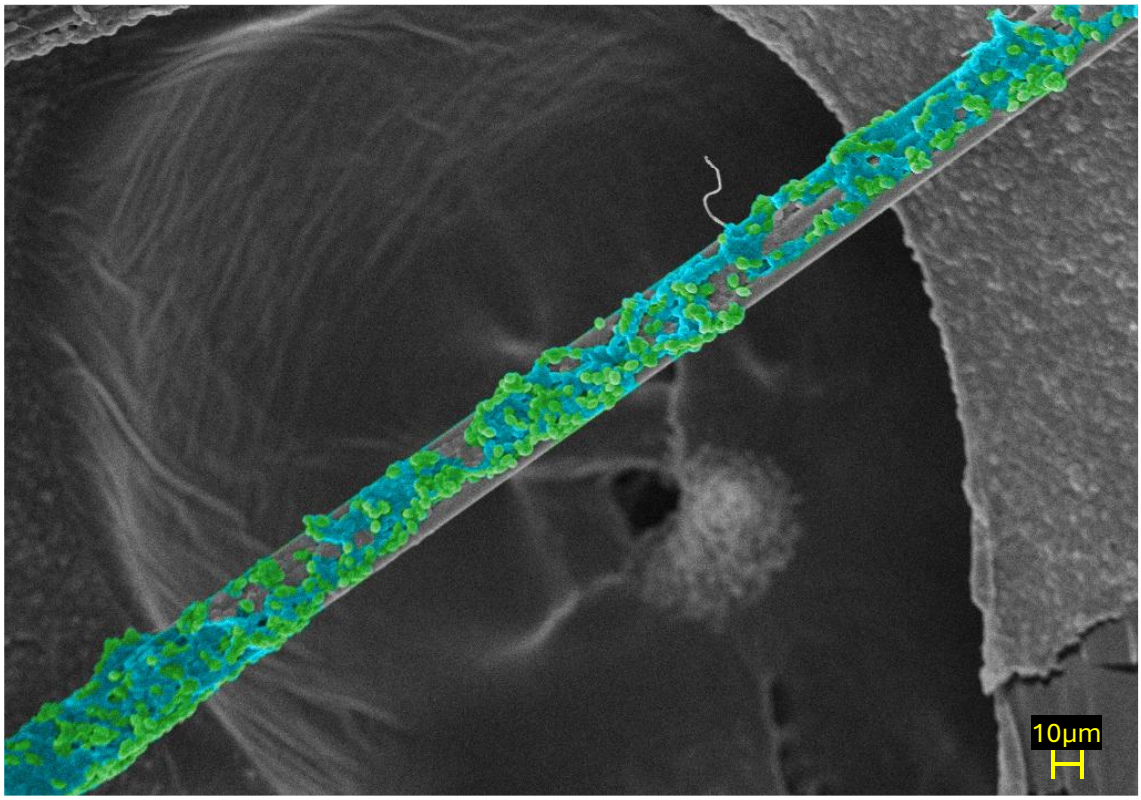

Figure S8. Single carbon fiber showing *Saccharomyces cerevisiae* colonization, used in the single-entity Raman spectroscopy experiment.

Table S1. Performance of *S. cerevisiae*-based bioanodes.

#	Electrode Material	Entrapment method	Redox mediator	Electrolyte	Fuel	Current/Current Density	Ref.
1	Glassy carbon/ Carbon nanotubes	Nafion	none	Phosphate buffer, pH 7.00	Not reported	22.8 μA cm^{-2} (by CV at 20 mV s^{-1})	4
2	Glassy carbon/ Vulcan carbon/ graphene oxide ink	None	Ferrocene	50 mM phosphate buffer, pH 7.0	10 mM glucose	332.7 μA cm^{-2} (by CA at 0.20 V)	5
3	Screen-printed carbon/ oxidized multi-walled carbon nanotubes	Nafion	None	Phosphate buffer pH 7.0	Not reported	42.5 μA cm^{-2} (CV at 100 mV s^{-1} and 0.65 V)	6
4	Platinum microelectrode	None	Menadione and Ferricyanide	PBS solution, pH 6.0	2 g L^{-1} glucose	47.5 ± 1.9 nA (by LSV at 10 mV s^{-1} and 500 mV vs Ag/AgCl)	7
5	Platinum microelectrode	None	Potassium Hexacyanoferrate and 2,3,5,6-TMPD	50 mM phosphate buffer, pH 7.0	7.5 mM glucose	350 ± 15 nA (LSV at 10 mV s^{-1} and 425 mV vs Ag/AgCl)	8
6	Bow-shaped carbon fiber	None	None	-	10 % glucose and 0.25% yeast extract	4.363 μA cm^{-2} (calculated by Ohm's law)	9
7	Flexible carbon fiber	None	None	0.1M phosphate buffer, pH 7.2	1 M glucose	0.023 mA cm^{-2} (by CV 5 mV s^{-1} and -0.08 V vs Ag/AgCl)	10
8	Pyrolysed photoresist film	None	Osmium bypiridine	50 mM phosphate buffer, pH 7.0	Not reported	55 nA (by CV at 5 mV s^{-1})	11
9	Flexible carbon fiber	Agarose hydrogel	None	0.1 M phosphate buffer, pH 7.2	1 M glucose	2.62 ± 0.50 mA cm^{-2} (by LSV at 5 mV s^{-1} and 0.50 V)	This work

CA: chronoamperometry; CV: cyclic voltammetry; LSV: linear sweep voltammetry; MFC: microbial fuel cell.; OCP: open-circuit potential; PBS: phosphate buffer saline; TMPD: N,N,N´,N´´-tetramethylphenylenediamine.

S2. References

(1) Pereira, A. R.; de Souza, J. C. P.; Iost, R. M.; Sales, F. C. P. F.; Crespilho, F. N. Application of Carbon Fibers to Flexible Enzyme Electrodes. *J. Electroanal. Chem.* **2016**, *780*, 396–406. https://doi.org/10.1016/j.jelechem.2016.01.004.

(2) Movasaghi, Z.; Rehman, S.; Rehman, I. U. Raman Spectroscopy of Biological Tissues. *Appl. Spectrosc. Rev.* **2007**, *42* (5), 493–541. https://doi.org/10.1080/05704920701551530.

(3) de Siqueira e Oliveira, F. S.; Giana, H. E.; Silveira, L. Discrimination of Selected Species of Pathogenic Bacteria Using Near-Infrared Raman Spectroscopy and Principal Components Analysis. *J. Biomed. Opt.* **2012**, *17* (10), 107004. https://doi.org/10.1117/1.JBO.17.10.107004.

(4) Restrepo, I. A.; Naranjo, L. B.; Hoyos-Arbeláez, J.; Vázquez, M. V.; Granados, S. G.; Palacio, J. Electrochemical Determination of Saccharomyces Cerevisiae Sp Using Glassy Carbon Electrodes Modified with Oxidized Multi-Walled Carbon Nanotubes Dispersed in Water – Nafion®. *Curr. Res. Food Sci.* **2022**, *5*, 351–359.

(5) García-Villagomez, A. D.; Galindo-de-la-Rosa, J.; Dector, A.; Alvarez, A.; Rodríguez-Morales, J. A.; Olivares-Ramírez, J. M.; Arjona, N.; Chavez-Ramírez, A. U.; Vallejo-Becerra, V. Immobilization of Saccharomyces Cerevisiae for Application in Paper-Based Microfluidic Fuel Cell. *Int. J. Hydrogen Energy* **2023**, *48*, 5301–5312.

(6) Acevedo-Restrepo, I.; Blandón-Naranjo, L.; Vázquez, M. V.; Restrepo-Sánchez, N. Evaluation of the Electrochemical Response of Saccharomyces Cerevisiae Using Screen-Printed Carbon Electrodes (SPCE) Modified with Oxidized Multi-Walled Carbon Nanotubes Dispersed in Water – Nafion®. *Curr. Res. Food Sci.* **2023**, *6*, 100401.

(7) Zhao, J.; Yang, Z.; Gong, Q.; Lu, Y.; Yang, Z. Electrochemical Insights into the Glucose Metabolism Pathways Within Saccharomyces Cerevisiae. *Anal. Lett.* **2005**, *38*, 89–98.

(8) Rawson, F. J.; Downard, A. J.; Baronian, K. H. Electrochemical Detection of Intracellular and Cell Membrane Redox Systems in Saccharomyces Cerevisiae. *Sci. Rep.* **2014**, *4*, 5216.

(9) Shrivastava, A.; Pal, M.; Sharma, R. K. Simultaneous Production of Bioethanol and Bioelectricity in a Membrane-Less Single-Chambered Yeast Fuel Cell by Saccharomyces Cerevisiae and Pichia Fermentans. *Arab. J. Sci. Eng.* **2022**, *47*, 6763–6771.

(10) Sedenho, G. C.; Modenez, I.; Mendes, G. R.; Crespilho, F. N. The Role of Extracellular Polymeric Substance Matrix on Saccharomyces Cerevisiae Bioelectricity. *Electrochim. Acta* **2021**, *393*, 139080. https://doi.org/10.1016/j.electacta.2021.139080.

(11) Rawson, F. J.; Gross, A. J.; Garrett, D. J.; Downard, A. J.; Baronian, K. H. R. Mediated Electrochemical Detection of Electron Transfer from the Outer Surface of the Cell Wall of Saccharomyces Cerevisiae. *Electrochem. commun.* **2012**, *15*, 85–87.