# SI

## Yeast Bio-battery

**Frank N. Crespilho[1,2,4] ✉, Ricardo Brito-Pereira[2,3], Rita Policia[2,3], Nelson Pereira[2,3], Graziela C. Sedenho[1], Carlos M. Costa[2,3], Senentxu Lanceros-Méndez[2,4,5] ✉**

**S1. Reagents and materials**

Phosphate salts ($NaH_2PO4.H_2O$, 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 anhydrous d-glucose (99%) was acquired from Merck®. Iota-carrageenan was obtained from Alfa Aesar®. Carbon ink was obtained from BareConductive®. All aqueous solutions were prepared using deionized water (18 MΩ cm at 25 °C).

**S2. Biobattery materials fabrication**

**Carbon electrodes:**

The carbon-interdigitated electrodes on PET (with 24 $mm^2$) were prepared via screen printing. The carbon ink was homogeneously screen printed (DSTAR, model DX-305D) over the PET substrates, with a polyester mesh with 120 wires. After the spreading step, the samples were placed inside an oven (JP Selecta, Model 2000208) for 20 min at a temperature of 80°C, for complete removal of the solvent.

**Iota-carrageenan hydrogel preparation:**

Iota-carrageenan hydrogel films were obtained by dissolving 0.3 grams of carrageenan powder in 10 ml of water with magnetic agitation for 3 h at 150 rpm and then allowing them to stand in petri dishes for 48 hours to the complete evaporation of the solvent. Iota-carrageenan hydrogel films were prepared by dissolving 0.3 grams of carrageenan powder in 10 ml of water with magnetic stirring at 150 rpm for 3 hours. After complete dissolution of the polymer, the solution was placed in petri dishes for 48 hours until complete solvent evaporation.The bioanodes were prepared through a process involving the cultivation and incubation of Saccharomyces cerevisiae. Baker's yeast was suspended on a ratio of 0.05 g/mL in a sterile phosphate buffer solution with a concentration of 0.10 mol $L^{-1}$ at a controlled pH of 7.2, supplemented with a concentration of 1.00 mol $L^{-1}$ glucose to promote yeast growth and activity. The suspension was prepared to ensure uniformity and sterility. The Saccharomyces cerevisiae was then allowed to incubate under these conditions during 72h, fostering optimal growth and metabolic activity, thereby preparing the bioanodes for subsequent electrochemical studies. The carrageenan hydrogel was then immersed in

the solution containing yeast to adsorb the Saccharomyces cerevisiae EPS, and the hydrogel with EPS was then placed over the electrode surface, forming the bioanode.

The biocathode was constituted by a hydrogel of carrageenan previously soaked in a saturated solution of potassium hexacyanoferrate for 10 minutes. The gel was removed from the solution and positioned on the surface of interdigitated electrode. Subsequently, the gel was hydrated with 50 microliters of sodium phosphate buffer solution.

**Experimental characterization:**

Scanning electron microscopy (SEM) images were acquired at the Central de Análises Químicas Instrumentais of São Carlos Institute of Chemistry (CAQI/IQSC/USP) using a Zeiss LEO 440 microscope located in Cambridge, England, equipped with an Oxford (model 7060) detector operating at 15 kV and 2.82 A, with a probe current of 200 pA. Subsequently, the electrodes were extracted from the incubation solution and gently washed with deionized water. Prior to analysis, the samples were coated with a 6 nm thick layer of gold using a Coating System Bal-Tec MED 020 from Bal-Tec, Liechtenstein, and stored in a desiccator until measurement. The metallization process parameters were as follows: chamber pressure of $2.00 \times 10^{-2}$ mbar, current of 60 mA, and deposition rate of 0.60 nm $s^{-1}$. The optical microscopy images were obtained using two microscopes. The first one is a Leica S APO Stereo zoom metallographic microscope. For high-resolution images, a Leica DM500 with a 100x objective was used. Electrodes with and without Saccharomyces cerevisiae were compared for analysis.

**S3. Half-cell experiments**

The experiments were conducted in a conventional jacket glass electrochemical cell, equipped with an $Ag/AgCl/KCl_{sat}$ reference electrode, a Pt wire counter electrode, and carbon as working electrodes. Experimental conditions included 0.1 mol $L^{-1}$ phosphate buffer solution (pH 7.2) containing 1 mol $L^{-1}$ glucose as the supporting electrolyte. Electrochemical measurements were performed using an Autolab PGSTAT 204 (Metrohm, Swiss) potentiostat with Nova 2.1.5 software. The measured currents were normalized by the geometric area of the bioelectrodes, yielding current density.

Electrochemical analysis was conducted through voltammetry experiments using EPS and potassium ferri/ferrocyanide in a phosphate buffer solution with a pH of 7.2. The analysis involved subtracting capacitive currents from the obtained voltammograms. The analysis of theoretical potential was conducted using the onset values from half-cell experiments for both the cathode and anode. This approach allowed for the estimation of the maximum theoretical potential achievable by the bio-battery.

**S4. Bio-battery assembly and measurements**

The bio-battery assembly process involves three key stages: 1) *Bioelectrode placement*: Initially, the bioelectrodes are positioned with a separator membrane between hydrogels. This step likely ensures proper alignment and spacing of the components within the battery. 2) *Connection of poles*: Following electrode placement, the negative and positive poles are connected using copper adhesive tape. This connection is essential for establishing the electrical circuit within the battery. 3) *Fixation onto petri dish:* Once the electrodes are connected, the bio-battery is fixed onto a petri dish. This fixation likely provides stability to the assembly for subsequent measurements and experimentation.

After assembly, various measurements are collected, including power curves, open circuit voltage (OCV), galvanostatic charge-discharge curves, polarization curves and potentiostatic charge-discharge curve. The measurements are collected using a potentiostat from PalmSense (model PalmSense4).

The weight of the bio-battery varies depending on the type of membrane used, ranging between 300 and 500 mg. This variation suggests that different membranes have different compositions or thicknesses, impacting the overall weight of the assembled bio-battery. Three types of membrane separators underwent testing: a home-made 210-micrometer thick carrageenan membrane, a home-made fibroin membrane obtained via electrospinning, and a sandwich-shaped membrane comprising two commercial Whatman membranes (Whatman ® glass microfiber filters, Grade GF/A) filled with iota-carrageenan. The Whatman membranes, known for their

high-quality filtration properties, provided structural support. The incorporation of iota-carrageenan within the Whatman membranes contributed to reducing crossover, thus improving the overall efficiency and stability of the bio-battery setup. While all configurations facilitated adequate battery function, the use of a carrageenan membrane in conjunction with Whatman membranes resulted in a comparatively low crossover rate.

Current collectors were prepared by silk-screening carbon ink onto recycled PET substrates, chosen for their feasibility and eco-friendliness. Initial studies on crossover revealed that the bio-battery operated effectively for 2 to 3 hours. Subsequent experiments were limited to a 2-hour timeframe, after which a new bio-battery was fabricated for reproducibility studies. Given the study's primary objective to demonstrate EPS-enabled battery charging and discharging, crossover occurring after extended usage periods was not a major concern. The experimental procedures involved the setup and characterization of a microbial bio-battery. Firstly, a detailed schematic representation of the bio-battery setup was created, highlighting the integration of iota-carrageenan hydrogels in both compartments. This was followed by capturing photographic depictions of the assembly process, showing the components such as PET with carbon-based charge collectors printing, hydrogel with potassium ferricyanide, and the finalized bio-battery configuration.

To investigate the microbial colonization on the carbon electrodes for each bio-battery device, both optical microscopy and scanning electron microscopy were utilized systematically. These techniques provided information of the Saccharomyces cerevisiae colonization and the formation of EPS on the electrode surfaces for each device.

The charge-discharge performance of the bio-battery was evaluated. Different current values were applied to generate charge-discharge curves, with a focus on low currents of 1 μA and 2 μA. Additionally, charge curves using significantly higher currents were investigated to assess performance under varied conditions. The bio-battery performance was assessed through various experiments. This included studying the variation of voltage used in bio-battery charging *versus* time using

different current values, examining the long-term stability of the OCV over 2 hours, and analyzing current density versus voltage curves with different quantities of EPS in the anodic compartment. OCV measurements were taken after specific interventions, such as adding 300 microliters of EPS and completing 6 cycles of charge and discharge. Potentiostatic charge-discharge experiments were also conducted at +1.0V, and the resulting current *versus* time data was analyzed. During the quasi-steady state polarization after a 200-second charge and discharge cycle, the charge obtained was utilized to generate a power curve. This analysis was used into the performance characteristics of the bio-battery.