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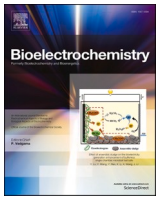

Polydopamine-immobilized yeast cells for portable electrochemical biosensors applied in environmental copper sensing

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ABSTRACT

The coupling of biological organisms with electrodes enables the development of sustainable, low cost, and potentially self-sustained biosensors. A critical aspect is to obtain portable bioelectrodes where the biological material is immobilized on the electrode surface to be utilized on demand. Herein, we developed an approach for the rapid entrapment and immobilization of metabolically active yeast cells in a biocompatible polydopamine layer, which does not require a separate and time-consuming synthesis. The reported approach allows obtaining the "electrical wire" of intact and active yeast cells with resulting current generation from glucose oxidation. Additionally, the electrochemical performance of the biohybrid yeast-based system has been characterized in the presence of $CuSO_4$, a widely used pesticide, in the environmentally relevant concentration range of 20–100 μM. The system enabled the rapid preliminary monitoring of the contaminant based on variations in current generation, with a limit of detection of 12.5 μM $CuSO_4$. The present approach for the facile preparation of portable yeast-based electrochemical biosensors paves the way for the future development of sustainable systems for environmental monitoring.

1. Introduction

There is an increasing demand for sustainable low-cost, easy to use, devices for the detection and quantification of environmental pollutants [1]. Existing traditional methods of quantification such as liquid chromatography, gas chromatography, and mass spectroscopy are highly sensitive, however, they are costly, time consuming, and require dedicated instrumentation and trained personnel, making their widespread application in the field more complex [2]. Biosensors are an alternative technology, where biological material is utilized as a recognition element to detect the presence of target analytes. More specifically, in the case of electrochemical biosensors, the biological component is "electrically wired" to an electrode, allowing an exchange of electrons that result in an electrical current. The presence of the analyte of interest influences the output of electrical current, resulting in an easy to monitor signal. [3,4]. Accordingly, electrochemical platforms have attracted particular interest due to their rapid, accurate, and sensitive response in addition to their cost-effectiveness [5,6]. An additional feature of electrochemical biosensors is that they can operate without the need for an external power source if they are utilized in a "biofuel cell" setup, meaning that the sensing electrode is used either as the anode or the cathode in a galvanic system [7]. Biological materials ranging from macromolecules, organelles, whole cells, and tissues have been explored as bioreporters in biosensor development [8–12]. Microbial unicellular organisms, including bacteria and yeast, have been well-studied for biomedical, industrial, and environmental applications [13–15]. The interest in yeast-based biosensors is growing due to yeast cell stability, high robustness, the possibility of self-regeneration and the possession of unique eukaryotic receptors [14]. Yeast are unicellular eukaryotic organisms belonging to the Kingdom Fungi. Being eukaryotes, yeasts have similar characteristics to plant and animal cells while maintaining the simplicity of manipulation of bacterial cells. Yeast

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combines the advantages of a model organism and a cell factory, supporting fundamental and applied research [13]. Among various yeast species, *Saccharomyces cerevisiae*, belonging to the family Ascomycetes, is the most widely studied thanks to its nonpathogenic characteristic and high tolerance to harsh conditions. The compartmentalization of physiological events in yeast cells has fostered their use as a platform for biosensor development. Yeast has been coupled with different transduction systems such as colorimetric, luminometric, fluorometric, and electrochemical detection systems. However, several shortcomings related to yeast biosensor development and their effective use in real-world applications remain [16,17]. Recently, *Saccharomyces cerevisiae* was engineered to develop a fluorescent biosensor achieving a low detection limit (10 nM) of bioavailable copper [11]. The implementation of microbial electrochemical biosensors represents a promising solution for the on-site detection of environmental pollutants due to their simple equipment requirement, high sensitivity, and lower costs [18,19]. Furthermore, as previously introduced another critical feature of microbial electrochemical biosensors is the possibility of using them as self-sustained devices that do not require an external power supply as the generated current is directly correlated to the presence/absence of contaminants [20–24].

One major limitation of using yeast, and microorganisms in general, as a biorecognition element in electrochemical biosensors is their poor electrical communication with an electrode [25]. The poor charge transfer could be overcome with the use of redox mediators, which can be both present as diffusible molecules in solution or immobilized in polymer backbones. However, the unwanted release of diffusible redox mediators in the environment poses a risk due to their toxicity, limiting their use in biosensors for practical application. In this regard, the use of redox polymers is more attractive thanks to the possibility of immobilizing the redox moieties on the polymer backbone or using conductive-redox polymer where the redox centers are embedded in the polymer structure [26–28]. However, the synthesis of redox polymers is usually time-consuming and complex, increasing the final cost of the biosensor. In this context, the possibility of obtaining redox/conductive polymers in situ to facilitate the charge transfer process has been recently reported by Ramanavicius and co-workers, who showed the cell-assisted synthesis of polypyrrole [29–31], and by our group for the *in vivo* formation of polydopamine on purple bacteria cells [32]. Furthermore, we recently reported an approach where dopamine is utilized in situ to obtain an adhesive polymer matrix embedding bacterial cells for the development of biophotoelectrodes with no synthetic steps involved [26]. In this regard, it was previously shown that polydopamine can be used to entrap yeast cells without affecting their activity [33]. Andriukonis *et al.* reported the possibility of utilizing polydopamine and polypyrrole to enhance current generation in yeast-based microfluidic microbial fuel cells where the biological material was suspended in solution [25]. These works underline how the use of polydopamine is attracting particular interest for the development of biosensors. Additionally, recent publications showed biosensors obtained employing this polymer for the entrapment of isolated enzymes [34] and the modification of electrode surfaces [35].

In this work, we aim to develop a dedicated approach using the monomer of dopamine for the rapid immobilization of yeast cells on electrodes to obtain portable biohybrid electrochemical sensors. Specifically, intact and metabolically active *Saccharomyces cerevisiae* cells were immobilized and electrically wired to glassy carbon electrodes employing a polydopamine matrix obtained in situ. The biohybrid system obtained withstood desiccation periods of more than 90 min, could be stored exposed to air, and enabled current generation using glucose as substrate. The electrochemical performance was also evaluated in the presence of different concentrations of copper sulfate ($CuSO_4$), an essential nutrient for the maintenance of cellular functions in all living organisms, which can become toxic at high concentrations causing oxidative stress, damage of macromolecules, and ultimately cell death [36,37]. For this reason, $CuSO_4$ is widely used as a pesticide, algicide, or fungicide and is classified as a General Use Pesticide by the U.S. Environmental Protection Agency. Our results unveil the possible use of the biohybrid system as a portable, on-demand sensing device relying on glucose oxidation for current generation and the detection of $CuSO_4$ in a range of concentrations relevant to environmental applications.

2. Materials and methods

2.1. Cell growth conditions

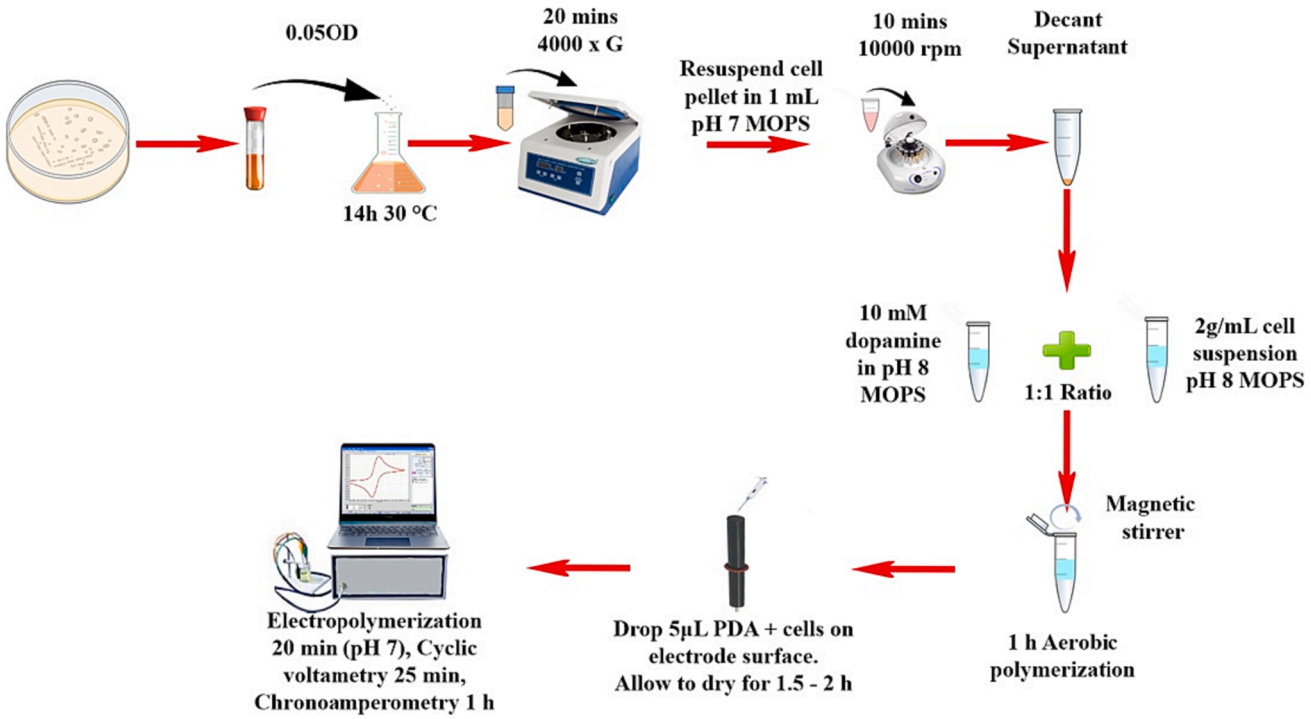
Saccharomyces cerevisiae wild type (WT) cells from W303-1B (*MATα ade2 leu2 his3 trp1 ura3*) strain were used for this study. In a typical experiment, cells were pre-inoculated overnight in 3 mL YPD medium (1 % yeast extract, 2 % bactopeptone [Gibco, Life Technologies, Waltham, MA, USA]) and 2 % glucose [Sigma-Aldrich, St. Louis, MO, USA]. The day after, a 10 mL YPD culture was inoculated using 0.05 optical density start (600 nm) in a 50 mL flask and grown in a shaking incubator at 200 rpm and 30 °C in the absence (control) and in the presence of copper sulfate ($CuSO_4$) at different concentrations 0.01, 0.1, 1, 5, 7, 9 and 10 mM. Cell growth was analyzed by measuring the optical density of the culture every 2 h using a spectrophotometer set at the wavelength of 600 nm.

2.2. Yeast cell microscopy

Yeast cell morphology was analyzed by epifluorescence microscope before and after the treatment with $CuSO_4$. An overnight culture was inoculated in YPD medium at an optical density start of 0.15 and grown in the absence (0 mM) and in the presence of 5 and 10 mM $CuSO_4$ for 4 h. 1 mL of control and treated cells were collected and resuspended in YPD. 5 μL of the resuspended cells was immobilized on a glass slide by mixing with 5 μL of 3 mM solution of low melting point agarose (Sigma-Aldrich; A-9414). Cells were analyzed at 25 °C on a Zeiss Axiovert 200 inverted epifluorescence microscope equipped with a 100X/1.30 Ph3 oil objective. Images were acquired with a CoolSNAP HQ CCD camera (Roper Scientific, Trenton NJ, USA) using MetaFluor 6.1 software (Universal Imaging Corporation, Downington, PA, USA).

2.3. Preparation of the biohybrid electrodes

Scheme 1 summarizes the protocol utilized to prepare the biohybrid electrodes with wild type (WT) *Saccharomyces cerevisiae*. The cells were pre-inoculated overnight in 3 mL YPD medium (1 % yeast extract, 2 % bactopeptone [Gibco, Life Technologies, Waltham, MA, USA]) and 2 % glucose [Sigma - Aldrich, St. Louis, MO, USA]. The day after, pre-culture was inoculated in 20 mL YPD using 0.05 optical density start (600 nm) in a shaking incubator at 200 rpm and 30 °C for 14 h to reach an optical density of about 3.5–4.0. After this time, the culture was centrifuged at 4000 g for 20 min. The cell pellet was resuspended in 1 mL of 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 50 mM glucose and further concentrated by centrifugation at 10000 rpm for 10 min. Thereafter, a cell suspension of 2 g mL^{-1} was made using 20 mM MOPS buffer (pH 8) + 10 mM $MgCl_2$ + 50 mM glucose. The 2 g mL^{-1} cell suspension was further mixed with a 10 mM solution of dopamine hydrochloride (in MOPS buffer at pH 8.0) in a 1:1 ratio for a final concentration of 1 g mL^{-1} of yeast cells and 5 mM dopamine hydrochloride. Following this, we modified the procedure previously developed for microbial electrodes by our group [26] to adapt it for the preparation of the yeast-based biosensor. Specifically, the mixture was stirred using a magnetic stirrer under aerobic conditions at 250 rpm for 1 h. After this time, 5 μL of the obtained mixture was dropped on a glassy carbon electrode (3 mm diameter) and was left to dry for 90 min. The electrochemical polymerization was then performed by 20 repeated cyclic voltammetry between −0.1 and + 0.5 V with a scan rate of 20 mV s^{-1} (Palmsens 4).



Scheme 1. Protocol for the entrapment of *Saccharomyces cerevisiae* yeast cells in PDA matrix and immobilization on the glassy carbon electrode.

2.4. Electrochemical characterization

After the electrochemical polymerization, the obtained yeast electrodes were characterized by cyclic voltammetry and chronoamperometry in a three-electrode setup. For the best comparison of the cyclic voltammetry, only the current densities obtained during the second anodic scan were considered. The counter electrode was a Pt wire, and Ag|AgCl (3 M NaCl, Basi MF2052) electrode was utilized as a reference. All the potentials reported in this work refer to this reference electrode. Two types of control experiments were performed (i) by utilizing heat-treated yeast cells with polydopamine (metabolically inactivated cells), and (ii) by preparing electrodes with only polydopamine immobilized on the glassy carbon electrode. All the experiments were performed in the presence and absence of different concentrations of $CuSO_4$ at room temperature (24 ± 1 °C) in 25 mL of 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$ + 50 mM glucose with the electrolyte exposed to air (aerobic conditions). $CuSO_4$ at different concentrations ranging from 20 µM to 100 µM was always added to the electrolyte after electrochemical polymerization and before starting the electrochemical characterization. The electrolyte was continuously stirred with a magnetic bar during the experiments. For all the different conditions investigated, at least three independent replicate experiments were performed, and values are reported with one standard deviation.

Furthermore, electrochemical impedance spectroscopy (EIS) was performed to study the charge transfer process in the yeast-PDA system in a three-electrode mode. The EIS analyses were performed in a frequency range from 500 kHz to 5 mHz using an applied sinus signal of 10 mV at + 0.32 V. The impedance spectra are presented as Nyquist plots. The complex nonlinear least square fitting of the obtained impedance data was performed using equivalent electric circuit models fitted with the PSTrace software of PalmSens.

3. Results and discussion

3.1. Growth analysis of Saccharomyces cerevisiae cells in the presence of copper sulfate

Firstly, the effect of different concentrations of $CuSO_4$, namely 0.01, 0.1, 1, 5, 7, 9, 10, and 20 mM, on the continuous growth batch culture of *S. cerevisiae* WT cells was analyzed. Fig. 1 reports the optical density at 600 nm for *S. cerevisiae* growth as a function of time. It is interesting to note that low concentrations of $CuSO_4$, from 10 µM to 1 mM did not significantly influence the growth. Only starting from 5 mM $CuSO_4$ a significant growth inhibition of 13.4 % could be obtained after 4 h of incubation (Fig. 1A). Accordingly, the effect of $CuSO_4$ concentrations above 5 mM was investigated, as shown in Fig. 1B. It was observed that as the concentrations of $CuSO_4$ increased, there was a proportional decrease in growth of the cells along time with 7, 9, and 10 mM causing 24.0, 45.9, and 58.1 % growth inhibition at 4 h of incubation, respectively. Concentrations of $CuSO_4$ above 10 mM completely inhibited the growth of *S. cerevisiae* cells (data not shown).

Based on the growth curve obtained, we investigated if exposure to high concentrations of $CuSO_4$ has morphological effects on the yeast cells. Fig. 2 shows representative images of yeast cells observed in brightfield treated with $CuSO_4$ concentrations ranging from 0 to 10 mM. The microscopy analysis did not reveal significant morphological differences among the cells after 4 h of exposure to the contaminant. It should be noted that based on the current results it cannot be excluded that minor morphological changes took place but could not be identified due to the resolution of the utilized microscope.

3.2. Bioelectrocatalysis study

With the aim to develop biosensors operating at concentrations lower than 1 mM, which are of interest for agricultural and environmental monitoring, and based on our previous experience with the use of polydopamine as a redox matrix, we decided to explore the electrocatalytic features of *S. cerevisiae* cells immobilized on a glassy carbon electrode with PDA. When preparing the biohybrid polydopamine-yeast

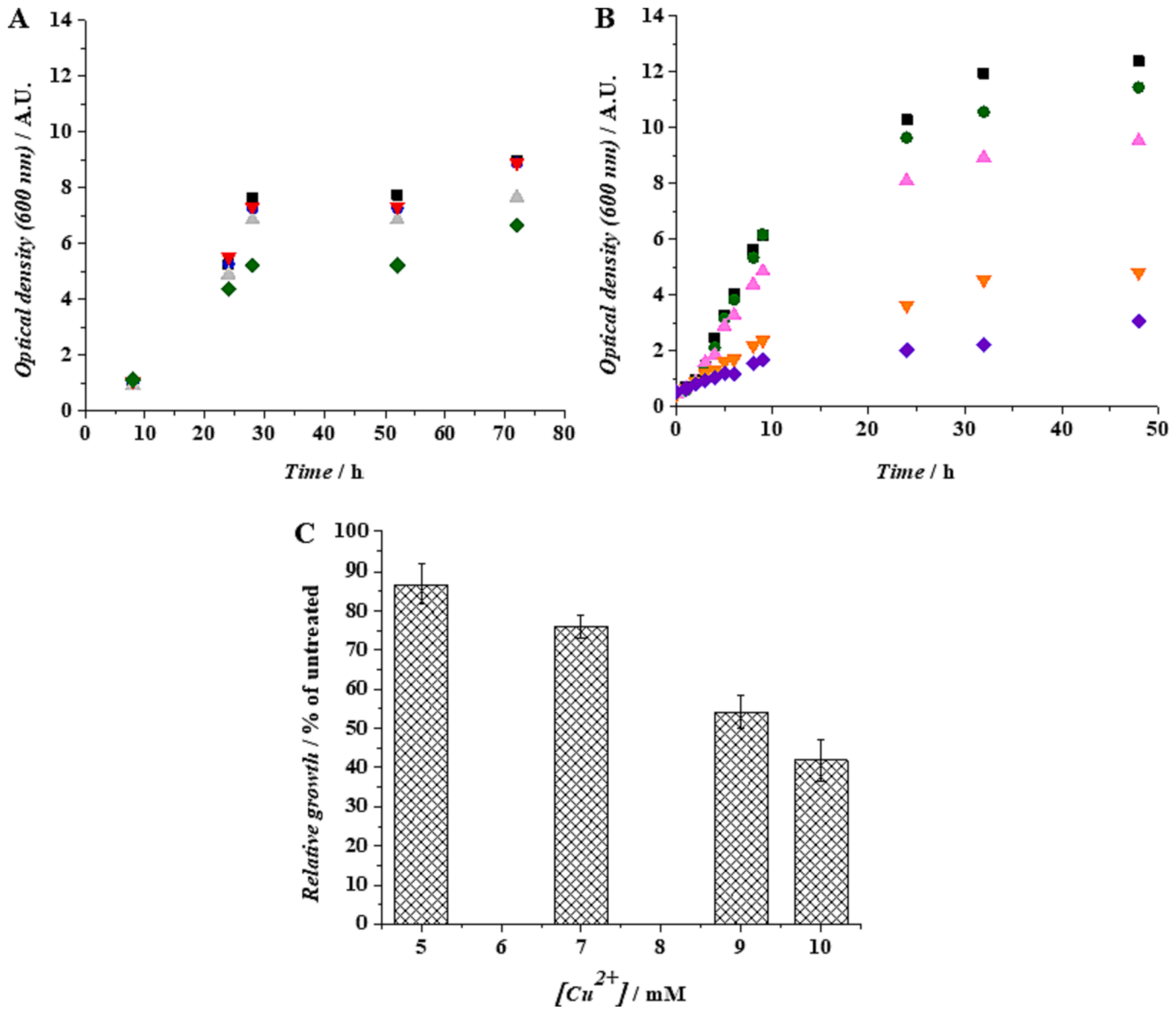


Fig. 1. (A and B) Growth curves of *S. cerevisiae* cells treated with $CuSO_4$. Wild type cells were grown overnight and then inoculated in YPD under constant shaking at 30°C in the presence of 0 (black), 0.01 (blue), 0.1(grey), 1 (red), 5 (green), 7 (pink), 9 (orange), and 10 mM $CuSO_4$ (purple). Optical density was measured at intervals using a spectrophotometer and (C) shows the percentage relative growth of the cells in the presence of different concentrations of $CuSO_4$ after 4 h of incubation. % relative growth was calculated against the control cell growth. R^2: 0.9815. Error bars indicate one standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

matrix, the PDA-cells ratio and the desiccation time on the electrode were varied to define the best adhesion to the electrode surface. The optimal conditions were found to be a mixture composed of 1 mg mL^{-1} of yeast cells and 5 mM dopamine undergoing aerobic self-polymerization for 1 h before being spotted on the electrode and allowed to dry for at least 1.5 h under air. Following, an electrochemical polymerization step was performed by a series of 20 cyclic voltammetry at 20 mVs^{-1} in an electrolyte at pH 7, completing the preparation of the biohybrid electrode. The obtained biohybrid system was characterized by performing cyclic voltammetry at 2 mVs^{-1}. First, we evaluated bio-electrocatalysis of the biohybrid electrodes obtained by entrapping *S. cerevisiae* – PDA (yeast-PDA) through CV experiments (Fig. 3A). From the CV of the bioelectrode prepared with live yeast cells (black) a clear catalytic response can be noted, with an onset for the oxidative reaction at + 0.2 V in agreement with previous literature where PDA was used as a redox mediator [26]. Conversely, no catalytic response was obtained from the electrode containing only PDA, and only the redox peak due to the oxidation of the redox matrix could be observed at + 0.2 V. For a comparison of the different electrodes, the current density at + 0.4 V during the anodic scan was utilized. The control electrode with only PDA achieved a low current density (0.3 ± 0.1 mA cm^{-2}) while the biohybrid electrode with immobilized live yeast cells achieved a current density of 1.5 ± 0.2 mA cm^{-2}. In addition, live yeast-PDA achieved a two-fold higher catalytic activity compared to the heat-treated yeast-PDA (0.8 ± 0.1 mA cm^{-2}). The current generation obtained from the heat-treated yeast-PDA system could be due to some residual active yeast in the PDA matrix. Notably, the improvement of the current density for the hybrid system compared with the electrode containing heat-treated yeast with PDA or only PDA provided essential information about the biocatalytic role of active yeast cells. It is important to remark that the biohybrid electrode tolerated the 90 min of desiccation while being exposed to air, with no major negative effects on the bioelectrocatalytic response, allowing its facile storing and transportation given its use in the field. The catalytic activity of the biohybrid electrode was compared in the

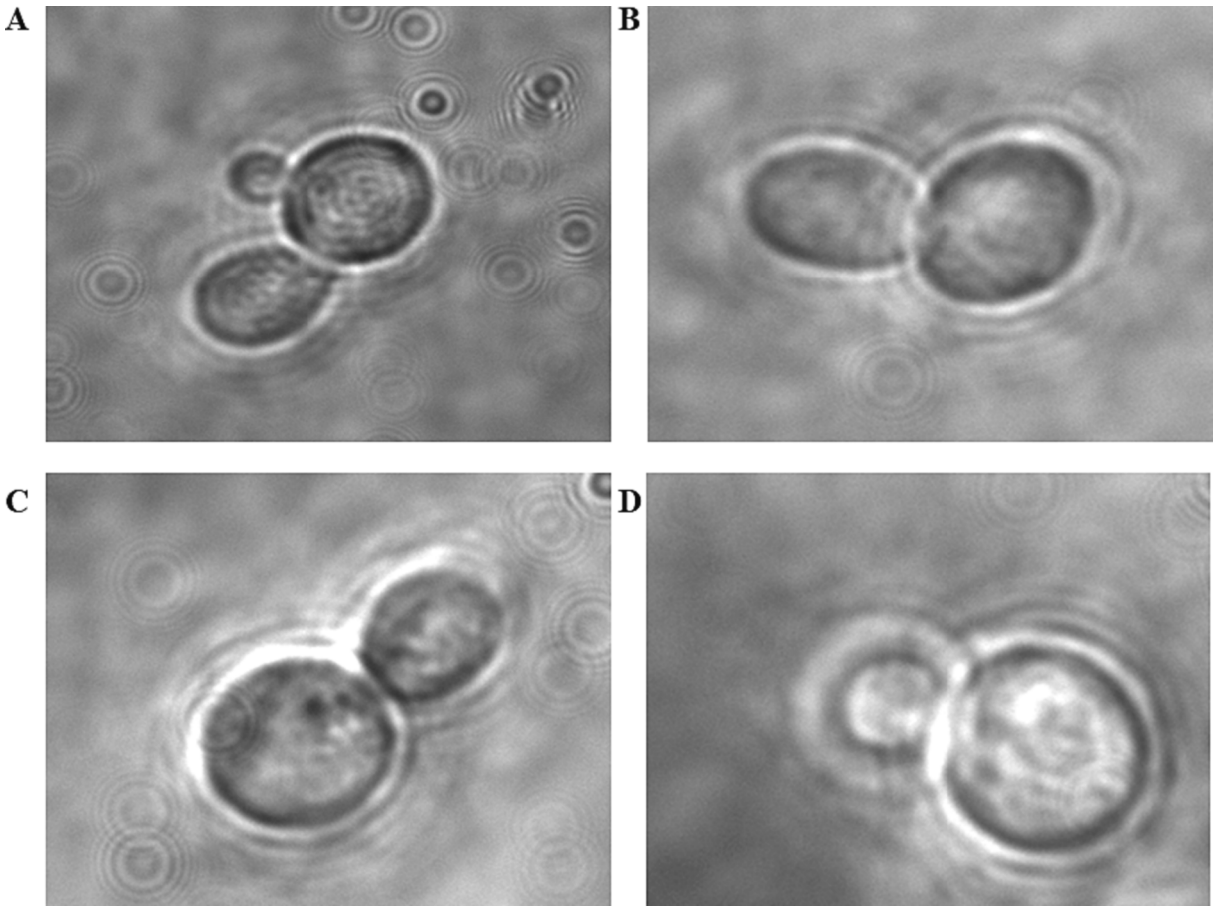


Fig. 2. A: Representative pictures of yeast cells (W303-1B) grown in YPD medium in the presence of 0 (A), 5 (B), and 10 mM $CuSO_4$ (C &D). After 4 h, 1 mL of each sample was centrifuged and resuspended in 1 mL YPD. 5 μL of the resuspended cells was immobilized on a glass slide by mixing with 5 μL of 3 mM solution of low melting point agarose (Sigma-Aldrich; A-9414). Cells were analyzed at 25 °C on a Zeiss Axiovert 200 inverted epifluorescence microscope equipped with a 100X/1.30 Ph3 oil objective.

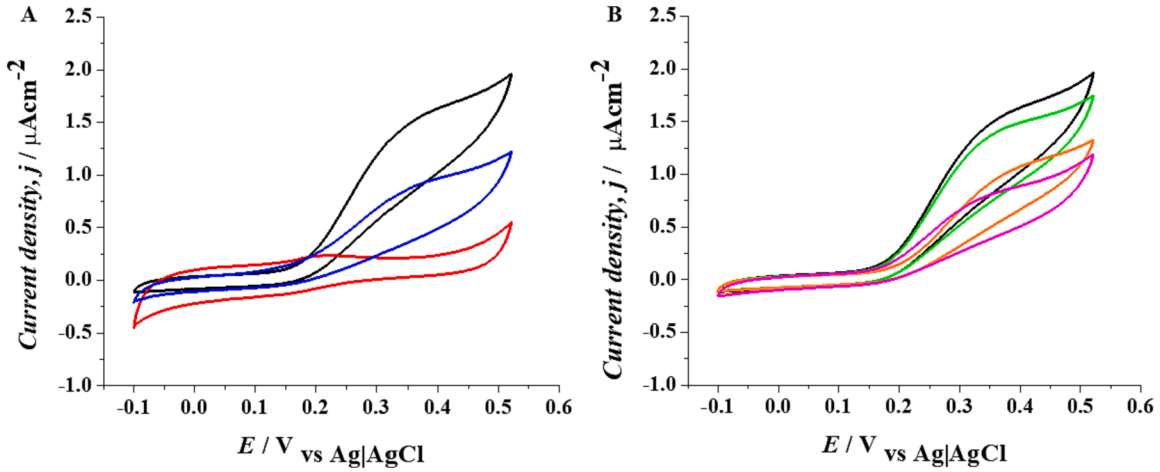


Fig. 3. (A) Cyclic voltammetry for the bioelectrodes prepared with live yeast cells in the PDA matrix (black), heat-treated yeast cells (blue), and sterile electrodes prepared with polydopamine alone (red). (B) Cyclic voltammetry showing the effect of 0 (black), 20 (green), 50 (orange), and 100 μM $CuSO_4$ (purple) on the electron transfer ability of live yeast cells immobilized with PDA on glass carbon electrode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

absence and the presence of $CuSO_4$ (Fig. 3B). The results showed that the current density decreased considerably as the metal concentration increased. Remarkably, the addition of the highest concentration (100 μM) of $CuSO_4$ decreased the catalytic activity of the system by two-fold, confirming the effect of the $CuSO_4$ on the electron transfer ability of live yeast cells immobilized with PDA on the glassy carbon electrode. As previously mentioned, $CuSO_4$ is a widely used pesticide/fungicide that can be found in high concentrations in agricultural environments. Thus, for this study, we did not investigate the effects of other contaminants envisioning the application of the biohybrid system in environments

where only $CuSO_4$ is expected. However, it is known that biosensors based on intact organisms can be influenced by various contaminants and selectivity is a major limitation of intact organisms-based biosensors [38]. On one side, this aspect could be an advantage, allowing for the early detection of environmental hazards even if a detailed contaminant is not identified. On the other side, if selectivity is required, the engineering of the biocatalyst can be envisioned to express preferred metabolic pathways and increase selectivity. Our group is directing future studies utilizing engineered yeast cells toward this goal.

To clarify the role of PDA in facilitating the extracellular electron transfer process, EIS analysis was performed on electrodes prepared with only yeast cells, only PDA, and the biohybrid system yeast-PDA. Fig. 4 shows the obtained EIS spectra for the three electrodes. The biohybrid system (black) showed a lower impedance compared to both the system with only PDA (red) and the one with only yeast (blue). The experimental data were fitted to equivalent electrical circuit models composed of resistances and capacitance reported in the Supplementary Material (Figure S1, S2, and S3).

For all the circuits, R_1 represents the resistance of the solution, while the couple R_3 and C_2 represent the double-layer capacitance and the charge transfer resistance. For the case of the electrode prepared with only yeast, R_2 represents the resistance for the diffusion of the electrolyte in the cells deposit in parallel to a capacitance (C_1). For the case of the electrode prepared with PDA only and the one prepared with yeast-PDA, R_2 represents the resistance of the pores in the encapsulating PDA matrix that allows diffusion of the electrolyte connected in parallel to a capacitance (C_1). The third couple R_4 and C_3 (present only for the biohybrid system yeast-PDA) represent the PDA layer entrapping the yeast cells on the electrode. A similar model has been recently used to fit EIS spectra obtained for bacterial cells entrapped in an alginate layer on an electrode surface [39]. Accordingly, in this system, the electrons obtained from the oxidation of glucose must cross various interphases that include the yeast cell membranes, the PDA matrix, and the electrode. The various components of the circuit are further influenced by the presence of metabolically active yeast cells in the PDA matrix. Table 1 reports the values obtained for the fitting, highlighting that the redox mediation system in the complete biohybrid electrode allowed reducing the charge transfer resistance (R_3), enabling current generation during glucose oxidation with the biohybrid electrode.

Table 1
Parameters obtained for the equivalent circuit elements of the control electrodes prepared with only yeast and only PDA, and for the complete biohybrid electrode prepared with yeast and PDA. The values are calculated based on the fitted impedance spectra. The equivalent electric circuit models are presented in the Supplementary Material (Figure S1, S2, and S3).

Sample	Circuit component						
	R_1/Ω	R_2/MΩ	R_3/MΩ	R_4/MΩ	C_1/μF	C_2/μF	C_3/μF
Only yeast	867	10.7	32	–	1.05	1.35	–
Only PDA	867	3.35	6.80	–	1.85	4.05	–
Yeast-PDA	875	0.79	0.72	0.88	1.9	13.5	0.24

3.3. Chronoamperometric assays for the yeast-PDA biohybrid electrode

While the CV experiments revealed that the hybrid system with live yeast-PDA efficiently provided high catalytic activity, and the EIS analysis confirmed the role of the PDA matrix in decreasing the charge transfer resistance, we aimed to further evaluate the variation in current generation over time of the biohybrid system when exposed to $CuSO_4$. Accordingly, we evaluated chronoamperometric assays (CA) of live yeast-PDA, dead yeast-PDA, and only PDA (Fig. 5A) also in the presence of different concentrations of $CuSO_4$ (20, 50, and 100 μM) for 60 min at 0.32 V *vs* Ag|AgCl. This potential was selected to perform the CA study as it provides a sufficient overpotential relative to the anodic oxidation peak of the redox matrix that maintains the mediator in its oxidized state.

Corroborating the results obtained by CV, the bioelectrode furnished higher current density than heat-treated yeast-PDA and control electrodes with only PDA (Fig. 5A). In addition, after 1 h of chronoamperometry, the live yeast-PDA electrode produced a three-fold higher charge (2,104 μC) than heat-treated yeast-PDA and PDA-only systems (790 μC and 647 μC, respectively), confirming the bioelectrocatalytic role of yeast. Following, the amperometric i-t trace of the biohybrid system was evaluated under increasing additions of $CuSO_4$ as shown in Fig. 5B. Notably, the current density decreases with successive additions of $CuSO_4$, which affects the biocatalytic performance compared to the biohybrid electrode in the absence of the pollutant. When adding 100 μM $CuSO_4$, the bioelectrode showed a remarkable

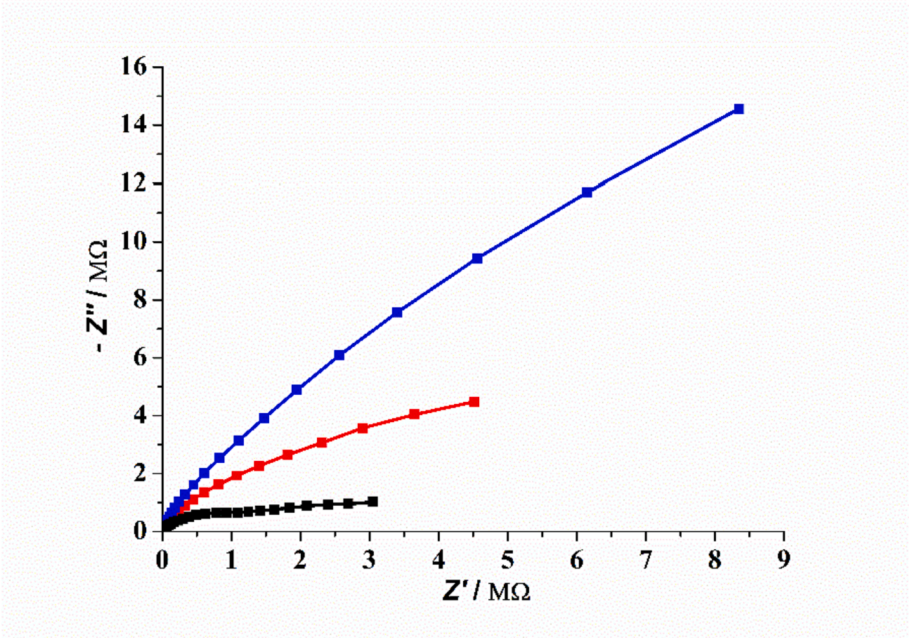


Fig. 4. EIS spectra for only yeast (blue), only PDA (red), and yeast cells in the PDA matrix (black). Frequency range 500 kHz – 5 mHz, potential amplitude 10 mV, applied potential + 0.32 V. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

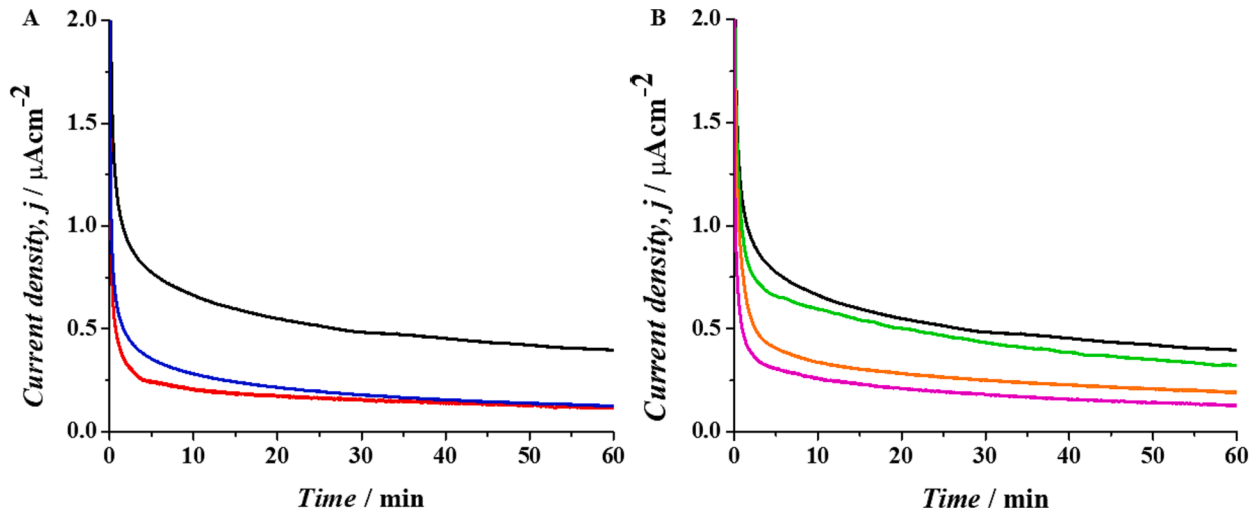


Fig. 5. (A) Amperometric i-t traces of PDA – Yeast electrodes (black), PDA – Heat-treated yeast cells (blue), and PDA alone (red), (B) Chronoamperometry showing the effect of 0 (black), 20 (green), 50 (orange), and 100 μM $CuSO_4$ (purple) on the electron transfer ability of live yeast cells immobilized with PDA on glassy carbon electrodes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

decrease in current density for a corresponding charge of only 779 μC. To better correlate the catalytic activity of the biohybrid electrode and the presence of $CuSO_4$ in the electrolyte, we evaluated the relationship between the current density obtained from the amperometric i-t tests at 2500 s *vs* $CuSO_4$ concentration (Fig. 6).

As the $CuSO_4$ concentration increased, the current density decreased linearly for the hybrid system with live yeast-PDA. Remarkably, the response of the electrochemical biosensor is affected by the presence of $CuSO_4$ starting from low concentrations (20 μM), which are relevant for environmental monitoring. Linear fitting of the experimental data presented in Fig. 6 gives a calibration curve with a slope, corresponding to the sensitivity, of $-4.8 \pm 0.6 \times 10^{-3}$ μA*cm^{-2}*L*μmol$^{-1}_{CuSO4}$ and a limit of detection of 12.5 μM $CuSO_4$. While it should be underlined that the linear relation between $CuSO_4$ concentration and current density could be improved, the obtained R^2 (0.956) is promising since the system has yet to be optimized to maximize sensitivity. As previously mentioned, our group is focusing future studies on engineered yeast cells to obtain a biohybrid system that shows both improved selectivity and sensitivity.

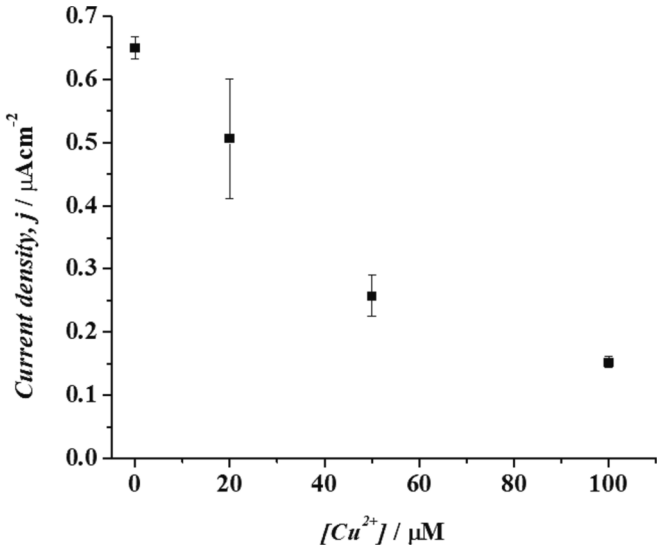


Fig. 6. Relationship between $CuSO_4$ concentrations and current density during chronoamperometry. E_{APP}: +0.32 V. R^2: 0.956. Error bars indicate one standard deviation.

To the best of the author's knowledge, this is the first report of a portable biohybrid electrode based on active yeast cells immobilized on an electrode surface allowing current generation and the on-demand monitoring of $CuSO_4$. Accordingly, the present study paves the way to new opportunities for developing self-powered biosensors and performing in situ monitoring of critical pollutants for the agriculture field and the environment.

4. Conclusions

The use of bioelectrochemical systems for the monitoring of environmentally relevant pollutants is of great interest for the preliminary and low-cost detection of contaminants. For on-demand use, the biohybrid system must be portable, having the biological catalyst immobilized on an electrode that can be dried and transported to a specific location. Accordingly, the use of a bioinspired polymer such as polydopamine is particularly relevant thanks to its adhesive properties, biocompatibility, and redox mediation features. In this work, we have shown that the embedding of metabolically active yeast cells in polydopamine allowed for obtaining a bioelectrode resistant to more than 90 min of desiccation exposed to air. The biohybrid system generated electricity from glucose oxidation, opening up the opportunity to use the biosensor in self-sustained/powered mode, without the need for an external power supply. Furthermore, the catalytic response of the biohybrid electrode was influenced by the presence of $CuSO_4$, with decreasing current and charge densities, enabling a preliminary evaluation of the presence of the pollutant in the range 20–100 μM. These results provide a sustainable approach to overcome the limitations of yeast-based biosensors in real-world applications and pave the way for the future development of electrochemical yeast-based biosensors.

CRediT authorship contribution statement

Ohiemi Benjamin Ocheja: Formal analysis, Investigation, Writing – original draft. **Ehthisham Wahid:** Investigation, Validation. **Jefferson Honorio Franco:** Formal analysis, Data curation, Writing – review & editing. **Massimo Trotta:** Conceptualization, Supervision, Writing – review & editing. **Cataldo Guaragnella:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Enrico Marsili:** Conceptualization, Supervision, Validation. **Nicoletta Guaragnella:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Matteo Grattieri:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2024.108658.

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