

A green and easy-to-assemble electrochemical biosensor based on thylakoid membranes for photosynthetic herbicides detection

*Original*

A green and easy-to-assemble electrochemical biosensor based on thylakoid membranes for photosynthetic herbicides detection / Lettieri, Stefania; Battaglino, Beatrice; Sacco, Adriano; Saracco, Guido; Pagliano, Cristina. - In: BIOSENSORS & BIOELECTRONICS. - ISSN 0956-5663. - STAMPA. - 198:(2022), p. 113838. [10.1016/j.bios.2021.113838]

*Availability:*

This version is available at: 11583/2942672 since: 2021-12-03T11:55:44Z

*Publisher:*

Elsevier

*Published*

DOI:10.1016/j.bios.2021.113838

*Terms of use:*

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

*Publisher copyright*

Elsevier postprint/Author's Accepted Manuscript

© 2022. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <http://creativecommons.org/licenses/by-nc-nd/4.0/>. The final authenticated version is available online at: <http://dx.doi.org/10.1016/j.bios.2021.113838>

(Article begins on next page)

## Title

A green and easy-to-assemble electrochemical biosensor based on thylakoid membranes for photosynthetic herbicides detection

## Authors and affiliations

Stefania Lettieri<sup>a,\*</sup>, Beatrice Battaglini<sup>b</sup>, Adriano Sacco<sup>a</sup>, Guido Saracco<sup>b</sup>, Cristina Pagliano<sup>b,\*\*</sup>

<sup>a</sup> Istituto Italiano di Tecnologia, Center for Sustainable Future Technologies @ PoliTO, Via Livorno 60, 10144 Torino, Italy; stefania.letteri@iit.it; adriano.sacco@iit.it

<sup>b</sup> Applied Science and Technology Department-BioSolar Lab, Politecnico di Torino, Environment Park, Via Livorno 60, 10144 Torino, Italy; beatrice.battaglini@polito.it; cristina.pagliano@polito.it; guido.saracco@polito.it

\* Corresponding author: Stefania Lettieri, E-mail: stefania.letteri@iit.it; Tel: +39 0112257715 Istituto Italiano di Tecnologia, Center for Sustainable Future Technologies @ PoliTO, Via Livorno 60, 10144 Torino, Italy

\*\* Corresponding author: Cristina Pagliano, E-mail: cristina.pagliano@polito.it; Tel: +39 0110904626; Politecnico di Torino, Applied Science and Technology Department–BioSolar Lab, Environment Park, Via Livorno 60, 10144 Torino, Italy

## Abstract

In this study, we report on an easy-to-assemble amperometric electrochemical biosensor incorporating thylakoid membranes for the detection of photosynthetic herbicides. These molecules interfere with the light-induced photosynthetic electron transport occurring at the level of the photosystems within the thylakoid membranes, thus reducing the current of the associated bioelectrode. Thylakoid membranes isolated from pea plants were adsorbed directly on a bare carbon paper working electrode and placed in the measurement cell in the absence of any electrochemical mediator, obtaining a fully environmental-friendly biodevice capable of photocurrent densities up to 14  $\mu\text{A}/\text{cm}^2$ . Three photosynthetic herbicides inhibiting Photosystem II and belonging to different chemical classes, namely diuron, terbuthylazine and metribuzin, were detected by measuring the electrode photocurrent, which decreased reproducibly in a concentration-dependent manner in a range between  $10^{-7}$ – $5 \times 10^{-5}$  M of each herbicide. The limit of detection for the three herbicides was between  $4$ – $6 \times 10^{-7}$  M. Storage stability tests revealed for the biosensor a half-life longer than 15 days at 4 °C and full stability up to 4 months at -80 °C. This study provides a simple, environmental-friendly and cost-effective procedure for the fabrication of a mediatorless carbon paper-based electrochemical biosensor characterized by high photocurrents, long storage stability, reproducible detections and good sensitivity.

**Keywords:** electrochemical biosensors; carbon paper-based biosensors; photosynthetic herbicides; thylakoid membranes; environmental-friendly

## 1. Introduction

Water is essential for life on Earth. In the last decades, the worldwide growth of crop production has been achieved mainly through the intensive use of herbicides, which have become one of the major players in water pollution. Indeed, these agrochemicals pose risks to aquatic ecosystems, human health and productive activities, ultimately leading to a greater scarcity of water safe to use (Mateo-Sagasta et al., 2017). To preserve the water integrity, several regulations have been defined, establishing low legal limits of herbicide concentrations in water intended for human consumption (e.g. EU directive 2020/2184, limit of 0.1 µg/L for any individual herbicide and of 0.5 µg/L for total herbicides) and in surface water (e.g. EU directive 2013/39/EC, limits specific for individual herbicides with priority in the field of water policy). Photosynthesis-inhibiting herbicides represent up to 50% of the herbicides market (Fedtke and Duke 2005). Among these herbicides, there are the Photosystem II (PSII)-inhibitors that belong to different chemical classes, including triazines, triazinones, ureas, uracils, phenyl-carbamates, amides and nitriles (<https://www.hracglobal.com>; <https://wssa.net>, accessed on 30 July 2021). PSII is a multimeric enzyme embedded in the thylakoid membranes of plants, algae and cyanobacteria that, in the light-dependent reactions of photosynthesis, oxidizes water, shuttling the extracted electrons to plastoquinone molecules involved in the photosynthetic electron transport. The PSII-inhibitors bind to the Q<sub>B</sub> site of the D1 protein of PSII by competing with the native plastoquinone and block the electron transfer to this intermediate (Trebst 2007), thus interrupting the photosynthetic electron flow.

For monitoring environmental water pollution and sensing herbicides, an ever-increasing number of electrochemical biosensors have been developed (Hara and Singh 2021). Electrochemical biosensors offer several advantages over the conventional analytical techniques (i.e. high-performance liquid chromatography and gas chromatography combined to mass spectrometry) routinely used, among which portability, easiness to use, low cost, short analysis time, high sensitivity and selectivity in complex matrices are the most beneficial (Pérez-Fernández et al., 2020; Wongkaew et al., 2019). The boosted production of these biosensors has been recently linked to the extensive use of nanomaterials such as graphene with its derivatives and nano composites (e.g. graphene oxides and carbon nanotubes) (Lawal, 2019). These nanomaterials, however, display associated direct risks for health and environment (Fadeel et al., 2018) or indirect risks due to the use of hazardous chemicals (e.g. strong acids) and organic solvents (e.g. methanol, benzene, toluene, dimethylformamide, N-methylpyrrolidone) for their conventional synthesis (Eatemadi et al., 2014; Marcano et al., 2010). To these risks contribute also the toxic reagents often used either for immobilizing the biosensing element (e.g. glutaraldehyde and polyacrylamide) (Nguyen et al., 2019) or for enhancing the electrochemical performance of the biosensor (e.g. redox mediators such as quinones) (Longatte et al., 2018). As a result, for the development of novel environmental-friendly electrochemical biosensors it is of paramount importance to pay attention to the sustainability of both the material and the procedure used.

Here we report on a new methodology to fabricate an easy-to-assemble amperometric electrochemical biosensor based on an unmodified carbon paper working electrode on top of which thylakoid membranes isolated from pea plants were directly adsorbed. As material, we used carbon paper as a cheaper, largely available and ready-to-use alternative to metal-based catalyst supports or other conductive materials employed in biosensor fabrication (see SI Table-1) (Calkins et al., 2013; Lukashev et al., 2007; Noji et al., 2011; Ryu et al., 2018; Trammell et al., 2004; Tucci et al., 2019). As biosensing element, we used thylakoid membranes for their advantages for electrochemical applications over either purified PSII and reaction centers (such as higher individual protein stability, multiple electron transfer routes, and simpler and cheaper immobilization procedures), or whole cells (whose low permeability to electrolytes and low stability negatively affect the sensitivity and repeatability of the measurements) (Antonacci and Scognamiglio, 2020; Buonasera et al., 2010; Rasmussen et al., 2014; Touloupakis et al., 2005). Moreover, the thylakoid membranes need only few simple purification steps for their isolation. This leads to a double advantage, (i)

a higher stability and greater power output when compared to results obtained with purified PSII or reaction centers and (ii) a contained manufacturing cost of the biosensor (SI Table-1). We investigated the analytical performance of the novel biosensor in absence of any redox mediator testing three PSII-inhibiting herbicides belonging to different chemical classes, namely diuron, terbuthylazine and metribuzin (respectively urea, triazine and triazinone class), which have high binding affinities for the  $Q_B$  site of the D1 protein of PSII (Battaglino et al., 2021). In addition, the long-term stability of the biosensor was assessed testing its functionality under two storage conditions, at 4 °C and -80 °C. For the first time a mediatorless and long-term storable (at -80 °C) biosensor based on plant thylakoid membranes for the detection of herbicides is here proposed. It is noteworthy the avoidance of any tethering agent or other linker to immobilize the thylakoid membranes on the carbon paper electrode surface, implementing the green and easy production of the sensing device and its long-term storage stability. The here presented bioelectrode is valuable, compared to other analogous ones reported in literature, for several advantageous features mostly related to a greener and cheaper fabrication method able to guarantee high photocurrent generation and long storage stability. These features are summarized in SI-Table 1, which provides a full comparison among several bioelectrodes based on either thylakoids or other photosynthetic material (e.g. cells, isolated photosystems and reaction centers) together with their main applications including biosensors for herbicide detection, devices for photocurrent generation and photobio-electrochemical cells.

## 2. Results and Discussion

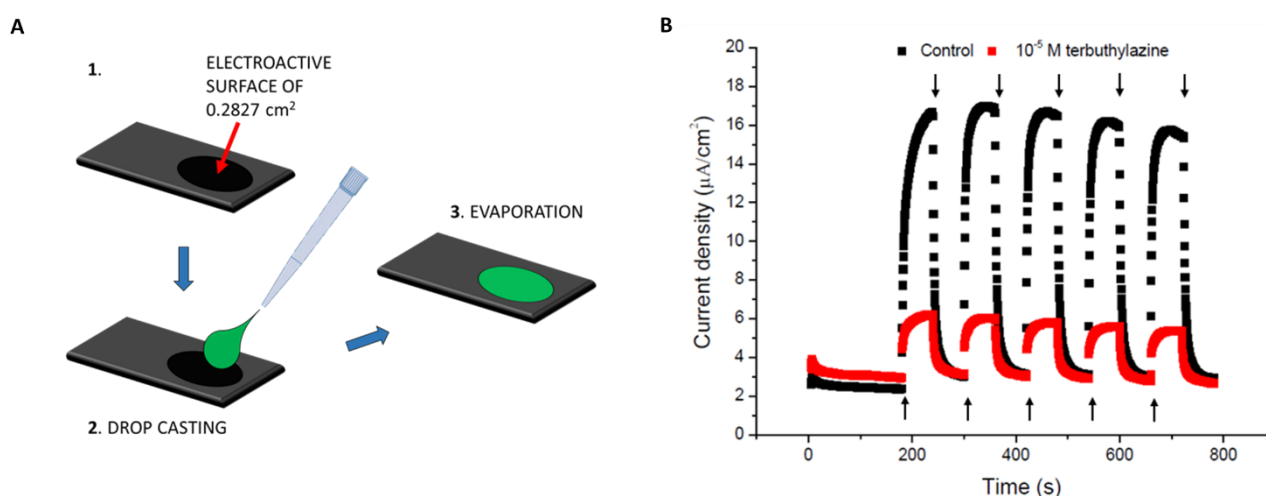
### 2.1. Bioelectrode fabrication and experimental setup

The procedure used for the bioelectrode fabrication consists of three main steps shown in Figure 1A: (i) the manual preparation of the carbon paper support with an electroactive surface of 0.2827 cm<sup>2</sup>; (ii) the drop-casting of the plant thylakoid membranes dispersed in phosphate buffer; (iii) the evaporation of the solution overnight at 4 °C in the dark (see Supporting Information for a full description of the entire procedure).

Different chemical and physical immobilization techniques can be employed to deposit the photosynthetic material on the support (Barthelmebs et al., 2011). Both methods display pro and cons. A main advantage of the chemical method is to avoid detachment of the biological material from the electrode surface, while the physical approach is superior to preserve its activity. The easiest physical approach is the adsorption method, which however can suffer from desorption phenomena, due to the weak interaction forces involved in this type of immobilisation method. To immobilize the isolated thylakoid membranes on the carbon paper support we chose a direct physical adsorption approach. Noteworthy, the immobilization was performed without any pre-treatment of either the thylakoid membranes or the carbon paper surface and avoiding the use of tethering agents or binders. These procedures are usually employed to increase the biosensor stability and/or sensitivity and consist of specific treatments of the biological sample with stabilizing agents (e.g. heavy metal salts (Giardi et al., 2001) or liposomes/polyvinylpyrrolidone (Bellemare et al., 2002)) prior to immobilization, or make use of expensive tethering agents (e.g. 1-Pyrenebutanoic acid succinimidyl ester) or other binder agents (e.g. agarose, alginate, gelatine or BSA-glutaraldehyde) to immobilize the thylakoids on the electrode surface (Calkins et al., 2013; Touloupakis et al., 2005) and avoid their leaking. Indeed, by comparing different physical and chemical immobilization procedures used for plant thylakoid membranes, a general low leaking of the biological material was observed in presence of these chemical agents, whereas a high leaking was detected in the case of adsorption (Buonasera et al., 2010). Surprisingly in our biosensor, without using any of the abovementioned strategies, but exclusively adopting a direct physical adsorption, no leaking was observed by visual inspection. This suggests that the interactions between thylakoid-thylakoid and thylakoids-carbon paper are stable and that the sum of all the non-covalent weak interactions (e.g. Van

der Waals,  $\pi$ - $\pi$  stacking, hydrogen bonds) formed between thylakoids and the support guarantees the fabrication of a stable biosensor. We attribute this high stability of the thylakoids on the support to the use of a low-salt buffer (i.e., the phosphate buffer, 10 mM  $\text{Na}_2\text{HPO}_4$ , 10 mM  $\text{KH}_2\text{PO}_4$ , pH 7.0) to wash and re-suspend the isolated thylakoid membranes before the drop-casting procedure (see Supporting Information for a full description of the entire procedure). In fact, initially, we tried with no success to immobilize the thylakoid membranes, isolated in stacked conformation according to (Pagliano et al., 2012), just re-suspending them in their final extraction buffer (i.e., 25 mM MES, pH 6.0, 10 mM NaCl, 5 mM  $\text{MgCl}_2$  and 2 M glycine betaine). Plant thylakoid membranes are organized in stacks of membrane discs called *grana* interconnected by single membrane discs. It is known that the exposure of plant thylakoids to low-ionic strength buffers, containing low amounts of monovalent cations and no divalent cations, induces *grana* unstacking (Izawa and Good, 1966). We hypothesize that, by washing the isolated thylakoid membranes with the low-salt phosphate buffer, a loss or weakening of the thylakoid *grana* structure occurs, which can favor the adhesion of the unstacked membranes on the carbon paper by enhancing the number of weak interactions possible.

The thylakoids-functionalized bioelectrode (SI-Figure 1 A-B) was used for chronoamperometric measurements in a classical electrochemical cell (SI-Figure 2), with Pt wire as counter electrode and Ag/AgCl as reference electrode, using phosphate buffer as electrolyte in the absence of any redox mediator. Artificial redox mediators are often used during electrochemical measurements to overcome the issue of low photocurrent signal (Bunea et al., 2018; Buonasera et al., 2010; Calkins et al., 2013). However, these molecules can show environmental toxicity and degrading performance over time. For example, quinone-based redox mediators can cause severe cytotoxic effects, by reacting with bio-macromolecules such as proteins, lipids and DNA (Bolton et al., 2000), ultimately decreasing the photocurrents generated by exposed photosynthetic cells (Longatte et al., 2018). These mediator-induced negative effects are in contrast to our aim of implementing the biosensor performance using a greener way, thus in our system we adopted a mediatorless approach. In response to illumination, in the absence of redox mediators our system was able to generate high photocurrents (up to 14  $\mu\text{A}/\text{cm}^2$  at 1 V vs. Ag/AgCl), as visible in Figure 1B, that shows a typical chronoamperometric response curve (black curve). Here, for comparison, a typical response curve in the presence of a tested PSII-inhibiting herbicide (red curve) is reported, that shows a reduction of the current peak, attesting the biosensor sensitivity to photosynthetic herbicides.



**Figure 1. Setup and electrochemical characterization of the biosensor.** (A) Schematic representation of the procedure adopted for the fabrication of the bioelectrode. (B) Typical chronoamperometric response curve recorded with the thylakoids (chlorophyll concentration 1000  $\mu\text{g}/\text{mL}$ , 20  $\mu\text{L}$ )-functionalized carbon paper electrode in the absence (black

curve, control) or presence of  $10^{-5}$  M terbuthylazine (red curve) in the measuring buffer. Upwards arrows indicate light on and downwards arrows light off.

## 2.2. Establishing the optimal conditions

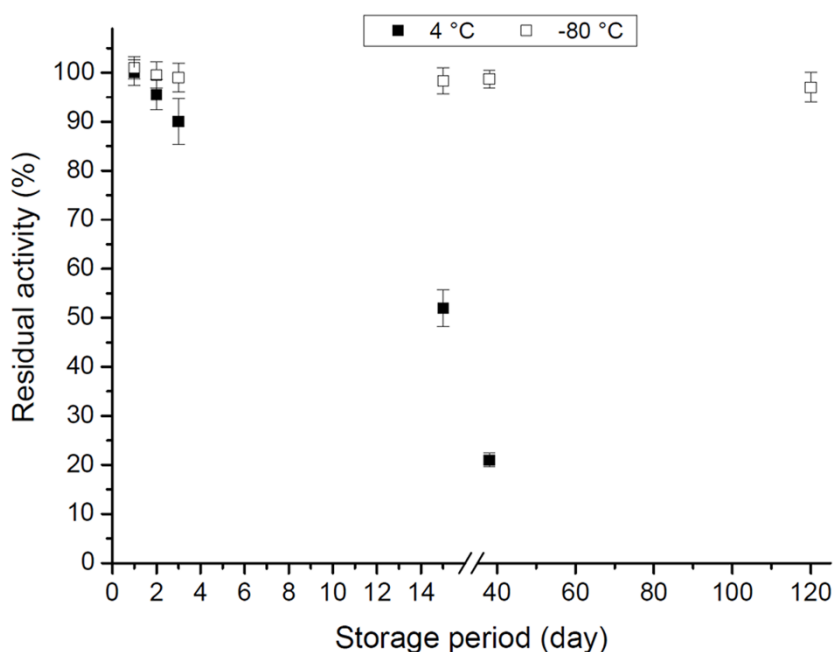
To study the biosensor by an analytical point of view, first we established the optimal operative conditions in terms of concentrations and quantities of immobilized thylakoid membranes on the carbon paper support, incubation time with the herbicides and eventual interference of the herbicides (see Supporting Information for a detailed description of the methods adopted).

It is well known the importance of working at optimal concentrations and quantities of photosynthetic biosensing material (i.e. PSII particles, thylakoid membranes, cells) used for immobilization to obtain high photocurrents, while maintaining good sensitivity to the presence of phytotoxic compounds (Attaallah et al., 2020; Laberge et al., 1999). By setting a fixed light intensity ( $\sim 1500 \mu\text{mol}/(\text{m}^2 \text{ s})$ ) and potential (1 V vs. Ag/AgCl), we tested the photocurrent generated by the biosensor prepared by drop-casting a same volume (20  $\mu\text{L}$ ) of thylakoid membranes at increasing chlorophyll (Chl) concentrations (ranging from 250 to 1250  $\mu\text{g}/\text{mL}$ ) on the electroactive surface of the carbon paper. The highest reproducible photocurrent was recorded working at 1000  $\mu\text{g}/\text{mL}$  Chl (SI-Figure 3). Preliminary inhibition tests, performed incubating the biosensor with a high concentration of herbicides ( $10^{-5}$  M), showed a rather constant sensitivity, independent from the Chl concentration used, as visible in SI-Figure 3 where the residual activity of the biosensor in presence of terbuthylazine is shown. During this inhibition test, however, we observed a significant interference, only for the diuron molecule, when working at Chl concentrations of thylakoid membranes below 1000  $\mu\text{g}/\text{mL}$ . Indeed, in these conditions (SI-Figure 4), an anomalous increased electrochemical response was observed instead of a drop of photocurrent that is expected when thylakoids are exposed to PSII-inhibiting herbicides. The same interference was detected only for diuron when we tested the bare electrode with high concentrations of the three herbicides (SI-Figure 5), suggesting an interaction of this molecule with the carbon paper, which is shielded by increasing the chlorophyll loading during immobilization. These results highlight the importance to evaluate first the influence of the analyte on the whole system, as recently reported (Wang et al., 2020). Analogous preliminary inhibition tests, incubating the biosensor with the herbicides for different times (15 min, 30 min, 1 h e 2 h), revealed a minimum incubation time of 30 min necessary to obtain a stable and reproducible inhibition (SI-Figure 6). Taken into account all these preliminary results, we decided to use thylakoid membranes at a Chl concentration of 1000  $\mu\text{g}/\text{mL}$  (corresponding to 20  $\mu\text{g}$  of Chl drop-cast on the electroactive surface of the carbon paper) and an incubation time of 30 min with herbicides as optimal conditions for the setup of the biosensor and the next analytical use.

## 2.3. Biosensor storage stability

In order to test the storage stability of the biosensor, the bioelectrodes were prepared and stored in a Petri dish in the dark at either 4 °C or -80 °C. The chronoamperometric tests were performed at defined time intervals and the recorded photocurrents compared with those generated by freshly prepared bioelectrodes. Results reported in Figure 2 show that the electrodes can be stored up to 3 days at 4 °C and up to 120 days at -80 °C without significant loss of photosynthetic activity and that after 15 days at 4 °C they retain 50% of the initial activity. Compared to other photosynthetic biosensors, whose stability was evaluated over a long-term period (> 10 days) at 4 °C, our biosensor displayed a storage stability similar to other thylakoid-based biosensors (Laberge et al., 1999) and even higher than certain whole cell-based biosensors (Ionescu et al., 2006), despite the latter generally stay active for longer times (Shitanda et al., 2009; Tucci et al., 2019).

Remarkably, our biosensor, when stored at  $-80\text{ }^{\circ}\text{C}$ , not only preserved its photosynthetic activity over months, but also did not show any loss of sensitivity to herbicides when compared to the freshly prepared counterpart (SI-figure 7), opening up new possibilities for fabrication of photosynthetic sensing devices storable for long-term in freezing conditions ( $-80\text{ }^{\circ}\text{C}$ ) and ready-to use. Such an outstanding stability has never been reported in the literature so far.



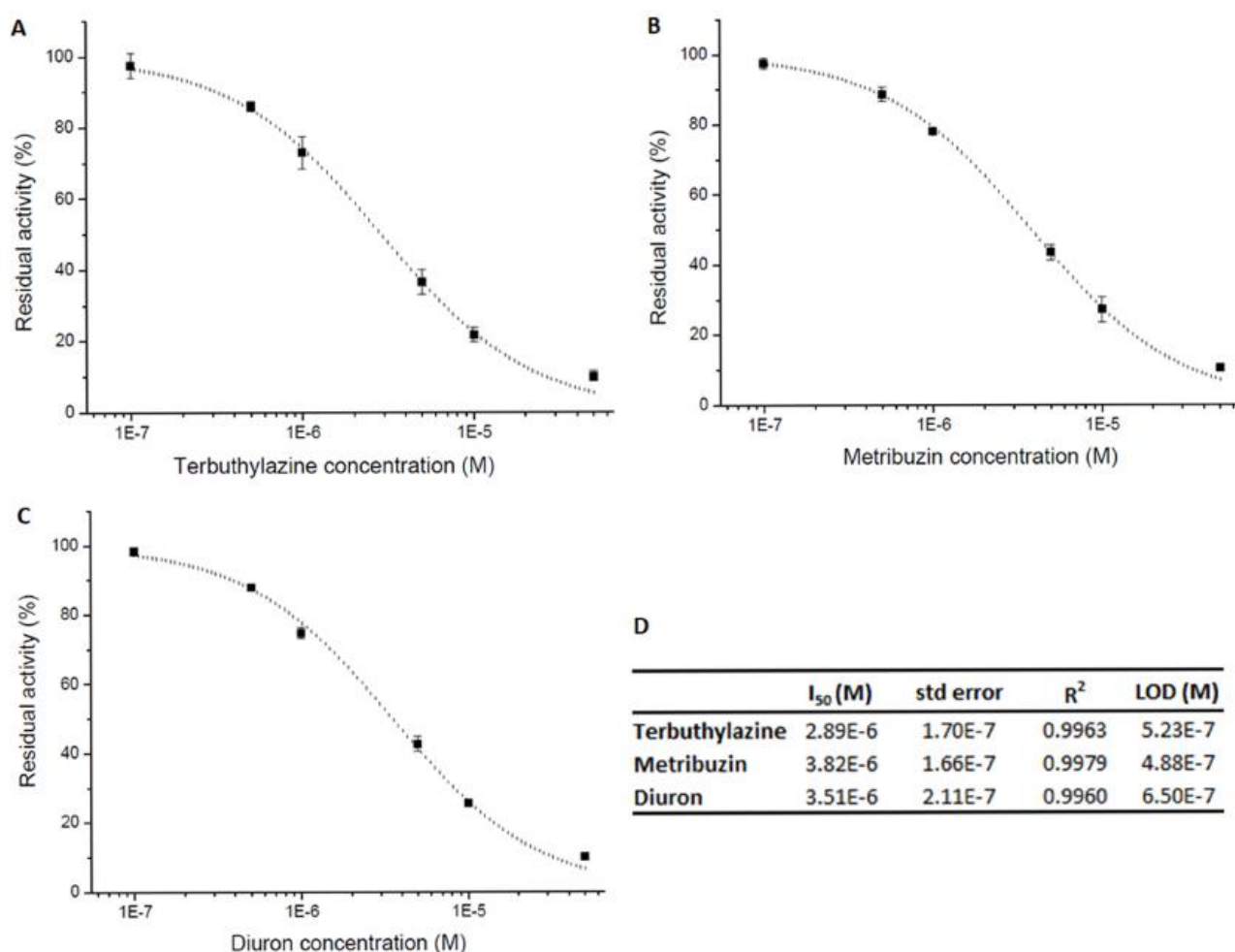
**Figure 2. Stability of the biosensor stored in the dark at 4 °C and -80 °C.** The residual activity (%) of the biosensor was calculated as the ratio of the photocurrent density obtained in each condition and the photocurrent density of the biosensor stored for 1 day at 4 °C. Points and bars are means and standard deviations of three replicates.

## 2.4. Detection of herbicides

To evaluate the biosensor sensitivity to the selected PSII-inhibiting herbicides, chronoamperometric measurements were performed to detect the photocurrents generated in the presence of increasing herbicide concentrations (which decrease proportionally to the herbicide content), and the photocurrent in the absence of herbicide (used as reference).

The photocurrent generated in control condition by our bioelectrode in the optimized conformation was in the order of  $14\text{ }\mu\text{A}/\text{cm}^2$  (Figure 1B, SI-Figure 3). This value is comparable or even higher than those displayed by other thylakoid-based bioelectrodes prepared with precious metals as support (e.g. Au,  $0.28\text{-}1.1\text{ }\mu\text{A}/\text{cm}^2$  (Lam et al., 2006; Ryu et al., 2018)), even if lower than those generated by analogous bioelectrodes modified with conductive carbon nanomaterials (e.g. Au–multi-walled carbon nanotube,  $68\text{ }\mu\text{A}/\text{cm}^2$  (Calkins et al., 2013)). Nonetheless, in our cheaper system, made up only of thylakoid membranes adsorbed on bare carbon paper, the observed photocurrent of  $14\text{ }\mu\text{A}/\text{cm}^2$  is high enough, when used as reference, to allow sensing the inhibitory effects in the presence of photosynthetic herbicides at different concentrations. The sensing capabilities of our biosensor for terbutylazine, metribuzin and diuron, expressed as percentage of residual activity, calculated as ratio between the photocurrent obtained in the presence of herbicide with respect to that in the absence, are shown in Figure 3A-C. By plotting the residual activity against herbicide concentrations between  $10^{-7}\text{ M}$  and  $5 \times 10^{-5}\text{ M}$ , high reproducible curves were obtained for each herbicide, even when considering assays performed with bioelectrodes stored at  $-80\text{ }^{\circ}\text{C}$  thawed before use and freshly

prepared bioelectrodes (SI-Figure 7). All herbicides displayed a comparable dose-dependent inhibition curve and similar  $I_{50}$  (concentration causing a 50% inhibition of the activity) and limit of detection (LOD) values (Figure 3D). Overall, the biosensor was sensitive to concentrations as low as  $5.23 \times 10^{-7}$  M for terbuthylazine,  $4.88 \times 10^{-7}$  M for metribuzin and  $6.50 \times 10^{-7}$  M for diuron (respectively corresponding to 0.12 mg/L, 0.10 mg/L and 0.15 mg/L). Despite these LOD values are insufficient to directly reach the concentrations imposed by the stringent environmental regulations on the water quality (i.e. maximum concentration allowed 0.1  $\mu\text{g/L}$  for a single herbicide and 0.5  $\mu\text{g/L}$  for total herbicides), our biosensor can be used for herbicide detection after a step of pre-concentration of the water sample (Alahmad et al., 2021; Cheng et al., 2021), procedure already successfully adopted with other thylakoid-based biosensors (Giardi et al., 2005; Touloupakis et al., 2005). Our bioelectrode is not selective towards a single photosynthetic herbicide. However, since it is able to detect the presence of this group of herbicides as warning hazard, it could be used as tool for prescreening of a large number of (pre-concentrated) water samples, to select the ones to be sent to specialized laboratories for further investigations with more advanced analytical techniques. Immobilization of thylakoid membranes isolated from mutants hypersensitive towards specific photosynthetic herbicides (Rea et al., 2009) could help to overcome the relative low sensitivity and improve the selectivity of our system in the future.



**Figure 3. Concentration-response curves for the herbicides terbuthylazine, metribuzin and diuron.** The residual activity (%) of the biosensor was calculated as the ratio of the photocurrent density obtained in the presence and in the absence of terbuthylazine (A), metribuzin (B) and diuron (C). Points and bars are means and standard deviations of three replicates. The experimental points were fitted using the Langmuir adsorption isotherm (fit curves in dotted line) according to (Kobližek et al., 2002) and the  $I_{50}$  dose and LOD values were derived together with the  $R^2$  and standard errors (D) (for  $I_{50}$  and LOD determination and statistical analysis see details in the Supporting Information).

### **3. Conclusion**

A green, storable and mediatorless biosensor made of only carbon paper and plant thylakoid membranes was developed and tested for detection of photosynthetic herbicides. The biosensor generates photocurrent densities up to 14  $\mu\text{A}/\text{cm}^2$ , relying on the only connection between the thylakoid membranes and the electrode support, avoiding the use of redox mediators or other strategies to enhance the current. The remarkable storage stability displayed by the biosensor, up to 120 days at  $-80\text{ }^\circ\text{C}$ , is one of the main advantages of this device, since it demonstrates the possibility of fabrication of photosynthetic biosensors storable for long-term in freezing conditions and ready-to use upon thawing. Moreover, it is also noteworthy that in absence of tethering agents or other linkers for thylakoid immobilization, the biological material did not detach from the carbon paper surface, allowing a green, easy and cheap fabrication procedure. Although the aforementioned characteristics make it an “ideal biosensor”, the drawback is the detection limit that needs optimization to allow detecting the low legal limits of herbicide concentrations directly in water sample without a pre-concentration step. Overall, we achieved our goal of developing a new environmental-friendly biosensor for herbicide detection satisfying these aspects: high photocurrents, long storage stability, and highly reproducible detections.

### **Experimental section**

A detailed description of the materials, procedures and methods used is available in the Supporting Information.

### **Credit authorship contribution statement**

Stefania Lettieri: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. Beatrice Battaglino: Investigation, Formal analysis. Adriano Sacco: Conceptualization, Methodology, Writing – review & editing. Guido Saracco: Funding acquisition. Cristina Pagliano: Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

### **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.

### **Acknowledgments**

The authors would like to thank Dr. Marco Paleari (IIT-CSFT) and the Mechanical Workshop (IIT-CCT) for the design and fabrication of the holder employed for the electrochemical measurements.

## References

- Alahmad, W., Sahragard, A., Varanusupakul, P., 2021. *Biosens. Bioelectron.* 194, 113574.
- Antonacci, A., Scognamiglio, V., 2020. *Trends Biotechnol.* 38, 334–347.
- Attaallah, R., Antonacci, A., Mazzaracchio, V., Moscone, D., Palleschi, G., Arduini, F., Amine, A., Scognamiglio, V., 2020. *Biosens. Bioelectron.* 159, 112203.
- Barthelmebs, L., Carpentier, R., Rouillon, R., 2011. Physical and chemical immobilization methods of photosynthetic materials, in: Carpentier R. (Eds.), *Photosynthesis research protocols. Methods in molecular biology (Methods and Protocols)*, vol 684. Humana Press, Totowa, NJ, pp. 247-256.
- Battaglino, B., Grinzato, A., Pagliano, C., 2021. *Plants* 10, 1501.
- Bellemare, F., Boucher, N., Lorrain, L., 2002. Method for testing photosynthetic activities. 2412206.
- Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., Monks, T.J., 2000. *Chem. Res. Toxicol.* 13, 135–160.
- Bunea, A.I., Heiskanen, A., Pankratova, G., Tesei, G., Lund, M., Åkerlund, H.E., Leech, D., Larsen, N.B., Sylvest Keller, S., Gorton, L., Emnéus, J., 2018. *ACS Appl. Energy Mater.* 1, 3313–3322.
- Buonasera, K., Pezzotti, G., Scognamiglio, V., Tibuzzi, A., Giardi, M.T., 2010. *J. Agric. Food Chem.* 58, 5982–5990.
- Calkins, J.O., Umasankar, Y., O'Neill, H., Ramasamy, R.P., 2013. *Energy Environ. Sci.* 6, 1891-1900.
- Cheng, H., Yi, L., Wu, J., Li, G., Zhao, G., Xiao, Z., Hu, B., Zhao, L., Tian, J., 2021. *Biosens. Bioelectron.* 189, 113266.
- Eatemadi, A., Daraee, H., Karimkhanloo, H., Kouhi, M., Zarghami, N., Akbarzadeh, A., Abasi, M., Hanifehpour, Y., Joo, S.W., 2014. *Nanoscale Res. Lett.* 9, 1–13.
- Fadeel, B., Bussy, C., Merino, S., Vázquez, E., Flahaut, E., Mouchet, F., Evariste, L., Gauthier, L., Koivisto, A.J., Vogel, U., Martín, C., Delogu, L.G., Buerki-Thurnherr, T., Wick, P., Beloin-Saint-Pierre, D., Hischier, R., Pelin, M., Candotto Carniel, F., Tretiach, M., Cesca, F., Benfenati, F., Scaini, D., Ballerini, L., Kostarelos, K., Prato, M., Bianco, A., 2018. *ACS Nano* 12, 10582–10620.
- Fedtko, C., Duke, S., 2005. Herbicides, in: Hock, B., Elstner, E.F. (Eds.), *Plant Toxicology*. Marcel Dekker, New York, pp.247-330.
- Giardi, M.T., Guzzella, L., Euzet, P., Rouillon, R., Esposito, D., 2005. *Environ. Sci. Technol.* 39, 5378–5384.
- Giardi, M.T., Leonardi, C., Esposito, D., Margorelli, A., Mattoo, A., Angelini, G., 2001. Biosensor and method for monitoring herbicides. EP1134585A2.
- Hara, T.O., Singh, B., 2021. *ACS ES&T Water* 1, 462–478.
- Ionescu, R.E., Abu-Rabeah, K., Cosnier, S., Durrieu, C., Chovelon, J.M., Marks, R.S., 2006. *Electroanalysis* 18, 1041–1046.
- Izawa, S., Good, N.E., 1966. *Plant Physiol.* 41, 544–552.
- Koblížek, M., Malý, J., Masojídek, J., Komenda, J., Kučera, T., Giardi, M.T., Mattoo, A.K., Pilloton, R., 2002. *Biotechnol. Bioeng.* 78, 110–116.
- Laberge, D., Chartrand, J., Rouillon, R., Carpentier, R., 1999. *Environ. Toxicol. Chem.* 18, 2851-2858.

- Lam, K.B., Johnson, E.A., Chiao, M., Lin, L., 2006. *J. Microelectromechanical Syst.* 15, 1243-1250.
- Lawal, A.T., 2019. *Biosens. Bioelectron.* 141, 111384.
- Longatte, G., Sayegh, A., Delacotte, J., Rappaport, F., Wollman, F.A., Guille-Collignon, M., Lemaître, F., 2018. *Chem. Sci.* 9, 8271–8281.
- Lukashev, E.P., Nadtochenko, V.A., Permenova, E.P., Sarkisov, O.M., Rubin, A.B., 2007. *Dokl. Biochem. Biophys.* 415, 211–216.
- Marcano, D.C., Kosynkin, D. V., Berlin, J.M., Sinitiskii, A., Sun, Z., Slesarev, A., Alemany, L.B., Lu, W., Tour, J.M., 2010. *ACS Nano* 4, 4806–4814.
- Mateo-Sagasta, J., Zadeh, S.M., Turrall, H., Burke, J., 2017. Executive Summary. Rome, Italy: FAO; Colombo, Sri Lanka: International Water Management Institute (IWMI). CGIAR Research Program on Water, Land and Ecosystems (WLE)
- Nguyen, H.H., Lee, S.H., Lee, U.J., Fermin, C.D., Kim, M., 2019. *Materials* 12, 1–34.
- Noji, T., Suzuki, H., Gotoh, T., Iwai, M., Ikeuchi, M., Tomo, T., Noguchi, T., 2011. *J. Phys. Chem. Lett.* 2, 2448–2452.
- Pagliano, C., Barera, S., Chimirri, F., Saracco, G., Barber, J., 2012. *Biochim. Biophys. Acta-Bioenergetics* 1817, 1506–1515.
- Pérez-Fernández, B., Costa-García, A., De La Escosura- Muñiz, A., 2020. *Biosensors* 10, 32.
- Rasmussen, M., Wingersky, A., Minteer, S.D., 2014. *Electrochim. Acta* 140, 304-308.
- Rea, G., Polticelli, F., Antonacci, A., Scognamiglio, V., Katiyar, P., Kulkarni, S.A., Johanningmeier, U., Giardi, M.T., 2009. *Protein Sci.* 18, 2139–2151.
- Ryu, D., Kim, Y.J., Kim, S. Il, Hong, H., Ahn, H.S., Kim, K., Ryu, W., 2018. *Nanomaterials* 8, 189.
- Shitanda, I., Takamatsu, S., Watanabe, K., Itagaki, M., 2009. *Electrochim. Acta* 54, 4933–4936.
- Touloupakis, E., Giannoudi, L., Piletsky, S.A., Guzzella, L., Pozzoni, F., Giardi, M.T., 2005. *Biosens. Bioelectron.* 20, 1984–1992.
- Trammell, S.A., Wang, L., Zullo, J.M., Shashidhar, R., Lebedev, N., 2004. *Biosens. Bioelectron.* 19, 1649–1655.
- Trebst, A., 2007. *Photosynth. Res.* 92, 217–224.
- Tucci, M., Bombelli, P., Howe, C.J., Vignolini, S., Bocchi, S., Schievano, A., 2019. *Microorganisms* 7, 630.
- Wang, P., Zhao, F., Hartmann, V., Nowaczyk, M.M., Ruff, A., Schuhmann, W., Conzuelo, F., 2020. *Bioelectrochemistry* 136, 107597.
- Wongkaew, N., Simsek, M., Griesche, C., Baeumner, A.J., 2019. *Chem. Rev.* 119, 120–194.