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Carbon black nanoparticles to sense algae oxygen evolution for herbicides detection: Atrazine as a case study

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ABSTRACT

A novel amperometric algae-based biosensor was developed for the detection of photosynthetic herbicides in river water. The green photosynthetic algae *Chlamydomonas reinhardtii* was immobilized on carbon black modified screen-printed electrodes, exploiting carbon black as smart nanomaterial to monitor changes in algae oxygen evolution during the photosynthetic process. The decrease of oxygen evolution, occurring in the presence of herbicides, results in a decrease of current signals by means of amperometric measurements, in an analyte concentration dependent manner. Atrazine as case study herbicide was detected in a concentration range of 0.1 and 50 μM, with a linear range from 0.1 to 5 μM and a detection limit of 1 nM. No interference was observed in presence of 100 ppb arsenic, 20 ppb copper, 5 ppb cadmium, 10 ppb lead, 10 ppb bisphenol A, and 1 ppb paraoxon, tested as safety limits. A ~25% matrix effect and satisfactory recovery values of 107 ± 10% and 96 ± 8% were obtained in river water for 3 and 5 μM of atrazine, respectively. Stability studies were also performed obtaining a high working stability up to 10 h and repeatability with an RSD of 1.1% (n = 12), as well as a good storage stability up to 3 weeks.

1. Introduction

The extensive use of pesticides in agriculture over recent decades has become a major environmental concern. Among them, atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) represents one of the most photosynthetic herbicides employed for weed control in agriculture. Because of microbial biodegradation, atrazine half-life in soil is 261 days, but degradation is much longer in water due to its low solubility (Agency for toxic substances and disease registry ATSDR, 2003). Despite banned in October 2003 in several European countries, atrazine has been often described as a persistent pollutant in surface and groundwater, due of its unauthorized uses in agriculture. Many regulations have been defined for atrazine, classifying this herbicide to 3rd position in the list of 45 priority substances, thus establishing limits between 0.6 and 2 μg/L in surface water matrices (Directive, 2013/39/EU). Moreover, atrazine is also present in the Norman List of Emerging Substances "*that have been detected in the environment, but which are currently not included in routine monitoring programmes at EU level and whose fate, behaviour and (eco)toxicological effects are not well understood*" (NORMAN, 2017). The insertion of atrazine in the Norman list was due to the many health problems associated to the consumption of water rich in this herbicide, including endocrine disorders, hormonal disruption, as well as risks associated to breast and prostate cancer (Pathak and Dikshit, 2012; Supraja et al., 2019).

Several analytical techniques have been used for the detection of photosynthetic herbicides, such as gas chromatography, high performance liquid chromatography, and capillary zone electrophoresis. Nevertheless, despite their high sensitivity, these methods require high cost and prolonged time protocols, extensive sample preparation procedures, and inability to miniaturization and in-the-field analysis. In this context, biosensor technology promises a great support to the conventional analysis, providing cost-effective and in situ analysis of pollutants, and thus boosting a more comprehensive environmental monitoring. Among them, electrochemical biosensors proven their suitability for

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sensitive and affordable measurements also in complex matrices. Indeed, as asserted by Wang and co-workers (Hanrahan et al., 2004). "*Electrochemical sensing devices have a major impact upon the monitoring of priority pollutants by allowing the instrument to be taken to the sample (rather than the traditional way of bringing the sample to the laboratory)*".

Among the plethora of biosensing configurations, algal biosensors demonstrated their suitability as highly sensitive and cost-effective diagnostic tools to perform qualitative/quantitative on-site pre-selection tests of herbicides, including atrazine (Antonacci and Scognamiglio, 2019a, 2019b). Indeed, a number of optical algae-based biosensors have been described in literature for the detection of photosynthetic herbicides (Lefèvre et al., 2012; Ahmed et al., 2018; Scognamiglio et al., 2019). However, despite their high sensitivity at the picomolar level, they lack practicality when used in turbid matrices as surface waters. In this context, the purpose of this study was to develop a compact device for rapid and cost-effective tests of river waters based on amperometric detection of molecular oxygen produced by photosynthesis, which is an indirect way of measuring a photoelectric current. In particular, in the presence of photosynthetic herbicides, including atrazine as case study, a decrease of molecular oxygen occurs due to a partial and/or total block of the photosynthetic electron transport caused by the interaction between the herbicide and the D1 Q_B binding niche. This block of the electron transport determines a decrease of oxygen evolution by a herbicide-concentration dependent manner.

To this aim, an amperometric biosensor was developed by immobilizing whole cells of *C. reinhardtii* CC125 strain on a carbon black (CB) modified screen-printed electrode (SPEs) to detect herbicides in surface water. The rational relies on the ability of carbon black to sensitively sense changes in algae oxygen evolution during the photosynthetic process. Furthermore, carbon black is able to both enhance the loading surfaces for the biocomponent immobilization as well as to increase the electron transfer, resulting in a more reliable herbicides detection. Indeed, the huge advantages of carbon black as nanomaterial to modify the working surface of screen-printed electrodes was described in recent years, including its electrocatalytic properties and low cost, when compared with graphene and nanotubes, as well as the simplicity in preparing stable dispersions (Arduini et al., 2010; Cinti et al., 2015; Mazzaracchio et al., 2019).

In this context, the analytical performances of the proposed biosensor were evaluated, investigating two novel aspects: i) the combination of CB with algae whole cells, never explored before; ii) the use of CB-SPEs for oxygen, never reported in literature.

2. Materials and methods

2.1. Chemicals

All reagents were purchased as high purity grade. Tris-acetate-phosphate, tricine, sucrose, methanol, sodium alginate, sodium chloride, calcium chloride, atrazine, catechol, bisphenol A were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Copper (Cu^{2+}) and arsenic (AsIII) were purchased from Carlo Erba.

2.2. Algae growth conditions and characterization

C. reinhardtii CC125 was photoheterotrophically grown in Tris-acetate-phosphate (TAP) medium pH 7.2 prepared according to Harris protocol (Hoober, 1989), under continuous light at 50 μmol photons m^{-2} s^{-1} (OSRAM Fluora) and with stirring at 150 rpm at 25 °C (Certomat BS-1 incubator, Sartorius). All the experiments were performed exploiting cell cultures in early mid-exponential growth phase, with Abs_{750} ~ 0.5 optical density (O.D.), corresponding to ~0.9 × 10^6 cells/mL and ~ 8 μg/mL chlorophyll content. Cell growth was evaluated spectrophotometrically by quantifying the absorbance O.D at 750 nm wavelength. Cell number was assessed by a Bio-Rad TC-10 automated counter (Hemel Hempstead, UK). Pigment content was calculated by the Lichtenthaler method once extracted with 80% acetone (Kong et al., 2016). Algae photosynthetic profile was estimated by recording the chlorophyll fluorescence induction curves (Kautsky curves) by a Plant Efficiency Analyzer (PEA, Hansatech Instr. Ltd., Kings Lynn, Norfolk, UK), at room temperature after 10 min of dark adaptation and with a 5 s saturating pulse excitation light (600 W/m^2) provided by an array of six red light emitting diodes (650 nm peak). Algae capability of atrazine detection was expressed as variable fluorescence 1-V_J. *C. reinhardtii* strains cultures in triplicates were incubated with atrazine in a concentration range from 10 to 150 nM for optical detection for 15 min under continuous light (50 μmol photons m^{-2} s^{-1}), room temperature and continuous stirring (150 rpm). Control cultures were grown under physiological conditions and carried out in parallel in all trials.

2.3. CB-SPE production

Graphite-based SPEs were delivered by SENSE4MED, Italy. SPEs were modified by drop casting using a CB dispersion.The dispersion of CB was prepared by adding 10 mg of CB powder to 10 mL of solvent (a mixture dimethylformamide (DMF): water (1: 1)) sonicated for 60 min at 59 kHz. Then 2 μL of CB dispersion was drop cast on a SPE working electrode surface for 3 consecutive times (CB-SPE).

2.4. C. reinhardtii CC125 immobilization on CB-SPEs

C. reinhardtii CC125 strain was exploited for the immobilization on screen-printed electrodes (SPEs) modified with carbon black nanoparticles (CB). A volume of ~ 14 mL of cell cultures in early mid-exponential growth phase (with Abs_{750} ~ 0.5 O.D., corresponding to ~ 0.9 × 10^6 cells/mL concentration and ~ 8 μg/mL chlorophyll) were harvested by 10 min centrifugation at 2000×*g* and 22 °C to obtain a final amount of cells equal to 1.2 × 10^7. The cell pellet was re-suspended in 50 μL of 50 mM tricine pH 7.2, and mixed with 100 μl of a 1% (w/v) sodium alginate solution in the same buffer, obtaining a final cell concentration of 0.08 × 10^6 cells/μL. 5 μl of this suspension, containing ~ 4 × 10^5 cells, were deposited over a carbon black modified working electrode surface (diameter 3.0 mm) of a three-electrodes SPE at the University of Rome "Tor Vergata", with a carbon counter electrode and an Ag/AgCl reference electrode. Thus, 5 μl of the mixture were dropped on the CB-SPE hosting algae/alginate suspension allowing the physical gelation of alginate and the consequent entrapment of cells on the working electrode. Finally, the algae immobilized electrode (hereafter referred as CC125/CB-SPE) was immersed 20 min into 50 mM Tricine, 20 mM $CaCl_2$, 5 mM $MgCl_2$, 70 mM sucrose pH 7.2 and incubated for 24 h under continuous light (50 μmol photons m^{-2} s^{-1}) and room temperature.

2.5. Biosensor set-up

The biosensor measuring set-up (Fig. 1A) included a potentiostat (DRP-STAT400 model, Dropsens Instruments Ltd., Llanera, Asturias, Spain) and a fluorimeter (Plant Efficiency Analyzer, PEA, Hansatech Instr. Ltd., Kings Lynn, Norfolk, UK). The measurement chamber is constituted by a Hansatech plastic clip (30 mm in diameter and 15 mm height) hosting the slit for SPE insertion, defining a 10 mm diameter measuring area (200 μL volume), and performing electrochemical analysis in a static mode. On the top of the clip is placed a light unit consisting of an array of 6 ultra-bright red LEDs optically filtered to a peak wavelength of 650 nm (readily absorbed by chlorophyll) at a light intensity of 315 μmol photons m^{-2} s^{-1} to periodically stimulate the photosynthetic activity of immobilized algal cells during electrochemical tests. The light intensity is neither photoinhibitory nor limiting for the light-dependent photosynthetic reactions for algal cells entrapped into the alginate/calcium chloride matrix on the working electrode surface of the CB-SPE. Measurements of the maximum photochemical efficiency of Photosystem II (F_V/F_M) were performed after a 10 min dark

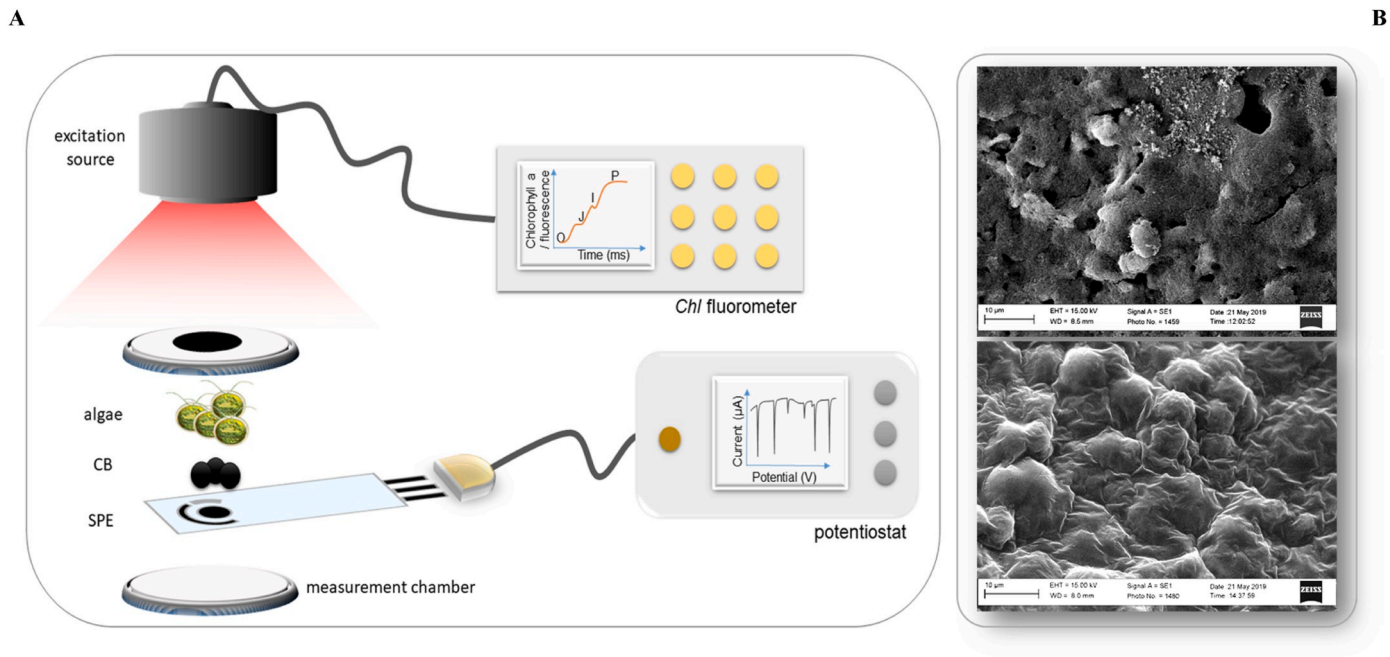

Fig. 1. A) Scheme of the CC125/CB-SPE biosensor set up. B) Scanning electron microscopy image of carbon black modified screen-printed electrode in absence (top image) and in presence (bottom image) of immobilized *C. reinhardtii* CC125.

adaptation, during that all reaction centres are fully oxidized making them available for photochemistry and any latent chlorophyll fluorescence yield is quenched.

2.6. Assessment of biosensor performance

Oxygen evolution capacity of the CC125/CB-SPE biosensor in absence and in presence of the atrazine was measured at RT with an applied potential of −0.6 V and an acquirement interval of 0.5 s, exploiting the capability of carbon black to sense the light-induced oxygen production. The oxygen evolution rate was determined under illumination (light intensity of 315 μmol photons m^{-2} s^{-1}), performing repeated cycles of 30 s light excitation and 10 min dark. 50 mM Tricine, 20 mM $CaCl_2$, 5 mM $MgCl_2$, 50 mM NaCl, 70 mM sucrose, pH 7.2 was used as measuring buffer for electrochemical analysis. Atrazine was added into the electrochemical cell in a concentration range from 0.1 to 41.6 μM. Working/storage stability and interferences were also studied in the same conditions, as well as matrix effect (using surface water samples from Sebou river diluted 1:2 with 2X measuring buffer) and recovery studies. In details, Interference studies were performed after a 15 min incubation of CC125/CB-SPEs with 200 μL of measuring buffer and a solutions 100 ppb arsenic, 20 ppb copper, 5 ppb cadmium, 10 ppb lead, 10 ppb bisphenol A, 1 ppb paraoxon, and 5 μM atrazine as well as with a solution spiked with all the above listed compounds. The biosensor stability instead was evaluated by amperometric measurements run for many hours at room temperature and under repeated cycles of 10 min dark and 30 s light of red LEDs (intensity 315 μmol photons m^{-2} s^{-1}) in measuring buffer. For the recovery, CC125/CB-SPEs were incubated in 200 μL of measuring buffer diluted 1:2 (v:v) in surface water (Sebou River, Morocco) with atrazine.

2.7. Statistical analysis

Data from three separated experiments were analysed and mean values ± standard deviation reported. For a given experiment, each condition was tested in triplicate. Detection limits were calculated on the basis of 99% confidence interval, which, assuming the normal distribution, corresponds to 2.6 × standard error of the measurements (σ), exploiting the modified relationship for the Langmuir absorption isotherm, LOD = 2.6 × σ × I_{20}/(100–2.6 × σ) where I_{20} value is the 20% inhibitory concentration (Scognamiglio et al., 2019).

3. Results

3.1. Immobilization of C. reinhardtii CC125 on CB-SPEs and SEM analysis

Whole cells of *C. reinhardtii* CC125 strain were immobilized on the working electrode surface of a SPE modified with CB. To preserve the algae photosynthetic functionality, CC125 strain cells were entrapped in a calcium/alginate matrix for its immobilization. Thus, 1.2 × 10^7 cells were suspended in 50 μL of tricine and 100 μL of 1% sodium alginate. Then, 5 μL of this suspension were drop cast on CB-SPE and crosslinked using 5 μL of 200 mM $CaCl_2$ in the same buffer, to obtain a final number of cells on the working electrode of 4 × 10^5. The CC125/CB-SPEs were thus stored in 200 mM $CaCl_2$ in measuring buffer under continuous light at 50 μmol photons m^{-2} s^{-1} for successive measurements. The CC125/CB-SPEs were then inserted into the measurement chamber and associated to a chlorophyll fluorescence dedicated fluorometer to provide light excitation, as well as to a potentiostat for the amperometric measurements (Fig. 1A). The calcium/alginate matrix was able to furnish a biocompatible environment for algae entrapment, without collateral effect on their metabolism; at the same time, its porous network guaranteed a good diffusion of the target analyte.

In order to deeply evaluate algae immobilization on the working electrode, the CB-SPEs was observed at Scanning Electron Microscopy (SEM) before and after algae immobilization. Fig. 1B shows SEM microphotographs at a magnification of 10 μm of a CB-SPE (top side) and a CB-SPE with immobilized algae (bottom side), highlighting the presence of algae cells entrapped into the calcium/alginate matrix.

3.2. Electrochemical characterization of CC125/CB-SPE biosensor

In the attempt to study the biosensor by an analytical point of view, an electrochemical characterization was provided to optimise the best working conditions. To identify the applied potential for monitoring the

oxygen reduction signal generated by the algal activity, in response to light exposure (λ = 650 nm, 315 μmol m^{-2} s^{-1} intensity) in the absence and in the presence of atrazine, CC125/CB-SPE was incubated for 10 min in dark in a reaction volume of 200 mL of measuring buffer. Then, a light flash of 30 s of red LEDs, optically filtered to a peak wavelength of 650 nm, was used for stimulating algae photosynthetic activity and provide light-induced oxygen production, which resulted in peak current signals from 0.3 to 1.5 μA depending on the potential applied in the range from −0.8 to −0.3 V. As highlighted in Fig. 2A, the *ad hoc* applied potential for the measurements light-induced oxygen evolution was equal to −0.6 V, which resulted in peak currents of 1.5 μA, with light intensity, immobilized cell number, and acquisition time interval were set as at 315 μmol photons m^{-2} s^{-1} intensity, 4 × 10^5 immobilized cell number, and 0.5 s, respectively.

Further investigated parameters were the light intensity exploited to illuminate algae, the number of algae cells used for immobilization on the electrode working surface, and the time interval for the acquisition of the current signals. Results indicated that:

i) the optimal light intensity was 315 μmol photons m^{-2} s^{-1} (Fig. 2B), with 4 × 10^5 immobilized cell number and 0.5 s acquisition time interval; on the contrary mid (620 μmol photons m^{-2} s^{-1}) and high-light (from 1025 to 2030 μmol photons m^{-2} s^{-1}) capable to elicit algae cells;
ii) 4 × 10^5 cells were the ideal algae amount to obtain the higher current signals (Fig. 2C), with 315 μmol photons m^{-2} s^{-1} light intensity and 0.5 s acquisition time interval;
iii) 0.5 s interval was the best compromise to achieve a good current signal with the higher repeatability (Fig. 2D), with 4 × 10^5 immobilized cell number and 315 μmol photons m^{-2} s^{-1} light intensity; indeed, with 0.25 and 0.1 s scan rate, higher current signals were obtained but the repeatability was very low.

Finally, to better highlight the good choice of using carbon black nanoparticles for the modification of the working electrode surface of screen-printed electrodes, amperometric measurements of bare and CB modified SPEs were performed at an applied potential of −0.6 V. A 1.5-fold higher current signals produced by light-induced oxygen evolution in the case of CB-SPEs in comparison with the bare electrode (Fig. S1), demonstrating the capability of carbon black to enhance the electrochemical performances of algae in terms of increased current signals. In addition, this current signal resulted more than 1.8-fold higher than the signal obtained using *C. reinhardtii* IL strain immobilized on a Multi-Walled Carbon Nanotube (MWCNT) working electrode surface of a graphite SPE, which provided a current of 0.8 μA at an applied potential of −0.7 V, in static mode (Husu et al., 2013). This underlines the ability of carbon black both to enhance the current signals by increasing the electron transfer and to lower the applied potential thus avoiding interferences.

3.3. Study of CC125/CB-SPE biosensor analytical performances

3.3.1. Herbicide detection: atrazine as case study

The CC125/CB-SPE biosensor was optimized for atrazine detection by amperometric analysis, measuring the inhibition of the photosynthetic activity in the presence of the herbicide as a decrease of oxygen production and current signal reductions (Fig. 3A). Thus, the CC125/CB-SPE was incubated for 15 min in dark with atrazine in a concentration range from 0.1 to 50 μM in a reaction volume of 200 μL of measuring buffer; then, a light flash of 30 s of red LEDs, optically filtered to a peak

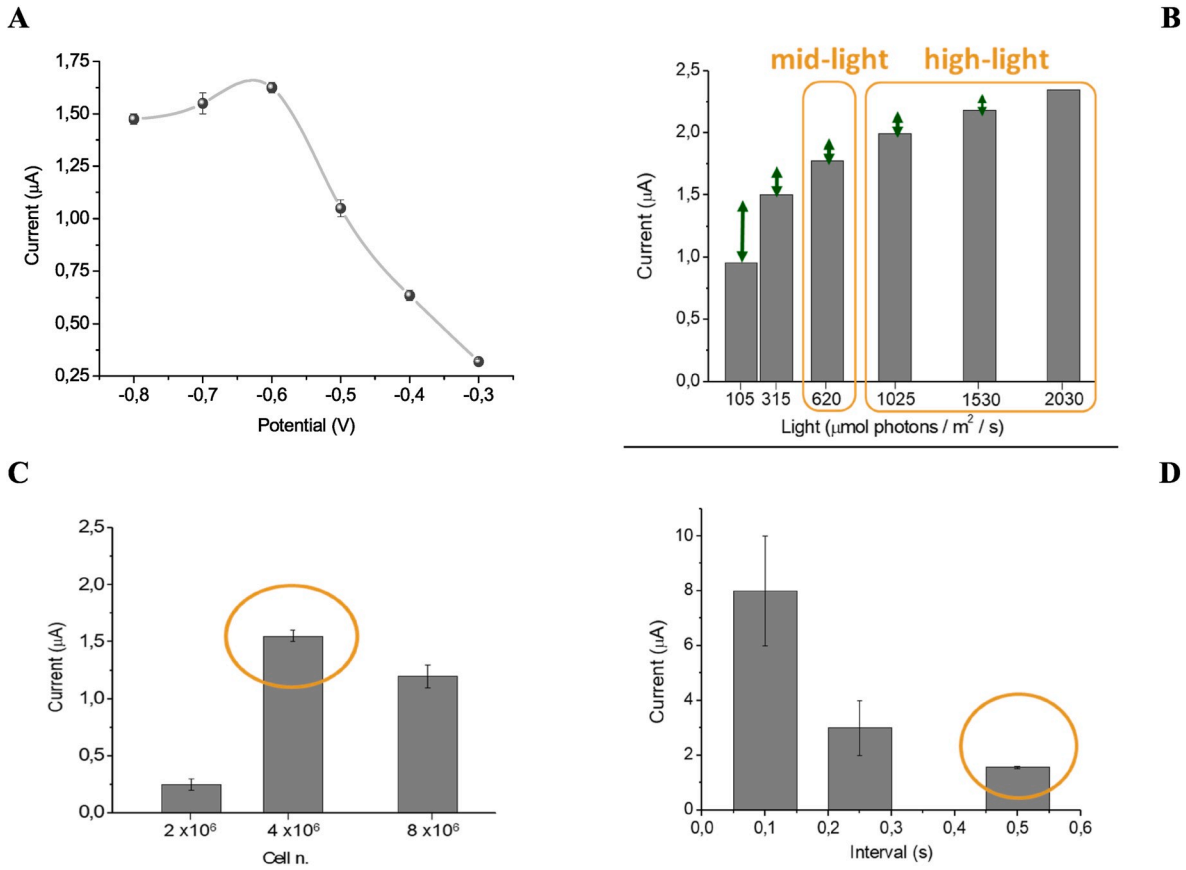

Fig. 2. A) Current intensity as a function of the applied potential. B) Current intensity as a function of light intensity. C) Current intensity as a function of immobilized cell number. D) Current intensity as a function of acquisition time interval. Applied potential −0.6 V, repeated cycles of 30 s light and 10 min dark, n = 3. Measurement volume: 200 μL of measuring buffer.Other parameters are at their optimum values.

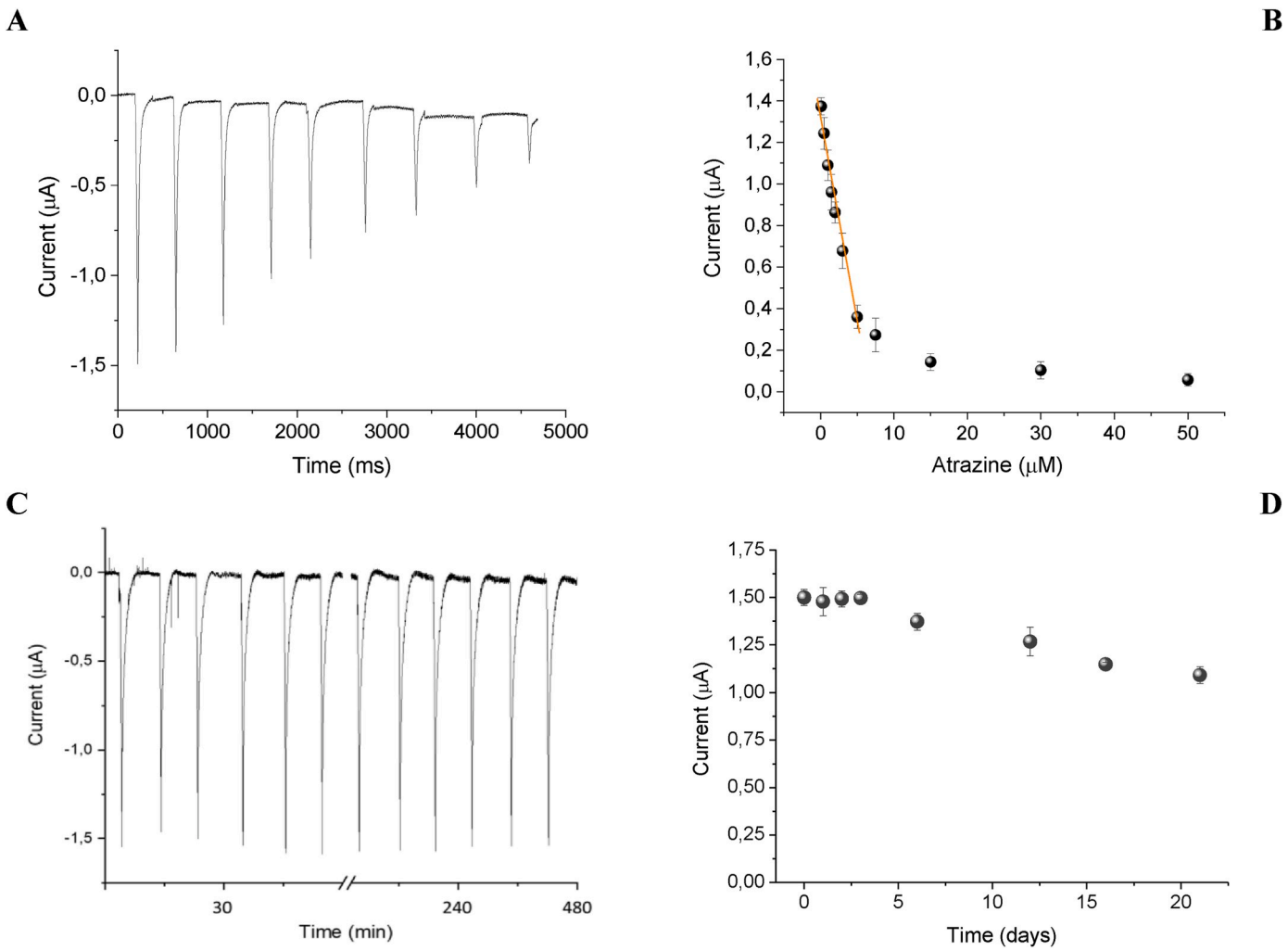

Fig. 3. CC125/CB-SPE amperogram (A) after addition of increasing amount of atrazine and corresponding calibration plot (B). Working stability (C) and storage stability (D) of CC125/CB-SPE expressed as light-induced oxygen evolution. Applied potential −0.6 V, repeated cycles of 30 s light and 10 min dark, n = 3. Measurement volume: 200 μL of measuring buffer.

wavelength of 650 nm at an intensity of 315 μmol photons m^{-2} s^{-1}, was applied to periodically stimulate the photosynthetic activity and provide light-induced oxygen evolution. Results evidenced that oxygen production and thus current signals decreased proportionally to the increase of herbicide concentrations. A linear response was obtained by serial additions of atrazine in the concentration range from 0.1 to 5 μM allowing the construction of a calibration curve using the linear regression reported by the equation y = 1.31 (±0.03) - 0.19 (±0.01) x, with an $R^2 = 0.977$. A detection limit of 1 nM was obtained for I_{20} value, which is the 20% inhibitory concentration (LOD is calculated as 2.6 × σ × I_{20}/100–2.6 × σ) (Fig. 3B). The linear range and the LOD found can be considered coherent with the Maximum Residue Level (MRL) set by the EU Directive 2013/39/EU for pesticides in surface water (from 0.6 to 2 μg/L); indeed, 1 nM of atrazine corresponds to 0.22 μg/L.

Despite atrazine response is the result of algal oxygen variation signals observed under illumination, control experiments of the current change caused by atrazine addition was performed. Atrazine was added to the measuring buffer in the absence of algae and the current was recorded both in the dark and in the light. The result showed that no current signal was observed (Fig. S2).

3.3.2. *Working and storage stability studies*

The CC125/CB-SPE biosensor working stability was evaluated by means of amperometric measurements run for many hours at room temperature, under repeated cycles of 10 min dark and 30 s light of red LEDs, optically filtered to a peak wavelength of 650 nm, at an intensity of 315 μmol photons m^{-2} s^{-1} in a measurement volume of 200 μL of measuring buffer. Results showed 100% of light-induced oxygen evolution activity up to ca. 8–9 h, with a progressive decrease of signal later on. Fig. 3C reports 8-h amperometric analysis, indicating not only a very good operational stability but also a high intra-electrode repeatability with RSD of 1.1% (n = 12).

Storage stability analysis was also assessed on CC125/CB-SPEs kept in measuring buffer at room temperature under continuous light at 50 μmol photons m^{-2} s^{-1} for 21 days. Amperometric tests were performed at defined time intervals and the current signal of each SPE recorded in a volume of 200 μL of measuring buffer. Results reported in Fig. 3D shows that any detectable loss of the photosynthetic activity did not occur up to 4 days, expressed as percentage of light-induced oxygen evolution current, while a 90% of their initial activity was retained within 6 days and 70% within 21 days.

3.3.3. *Interference study, matrix effect, recovery study, and reversibility test*

With the aim to evaluate the capability of the proposed biosensor in surface water samples, some chemicals including metals, pesticides, and phenolic compounds were analysed as interferents at legal limits established by the European legislations for surface water (where

present) (EU Directive, 2013/39/EU).

In detail, CC125/CB-SPEs were incubated in 200 μL of measuring buffer fortified with standard solutions of 100 ppb arsenic, 20 ppb copper, 5 ppb cadmium, 10 ppb lead, 10 ppb bisphenol A, 1 ppb paraoxon, and 5 μM atrazine as well as with a solution spiked with all the above listed compounds, to evaluate the synergistic effect of different chemicals in a mixture. Results reported in Fig. 4A highlights that the interfering species did not affect the analysis of atrazine at the tested concentrations. It should be noted here that the proposed biosensor is free from both algae and electrochemical interferences. Indeed, in the last case we did not observe any electrochemical interference because the atrazine response is the result of oxygen variation signal (peak) observed under illumination (as highlighted in Fig. 3A). In this way, if electroactive species are present in solution, the current due to their electrochemical reduction is included in baseline signal, without any effect on the response decrease due to the atrazine presence in the sample.

To investigate the suitability of the proposed biosensor in real samples, matrix effect and recovery studies were performed. To estimate matrix effect, CC125/CB-SPEs were incubated in 200 μL of 2X measuring buffer diluted 1:2 (v:v) in surface water (Sebou River, Morocco) fortified with atrazine in a concentration range from 0.1 to 50 μM. A calibration curve was obtained with a linear range from 0.1 to 5 μM atrazine, described by the equation y = 1.01 (±0.003) - 0.16 (±0.01) x (R^2 = 0.995) (Fig. 4B). The ratio between slopes of the calibration curves obtained in standard solutions and real samples was equal to 1.3, indicating a ~25% dependence from the waste water matrix. The calibration curve obtained for the algal biosensor in real samples was further used to calculate the recovery values of the surface water samples. Recovery values of 108 ± 10% and 95 ± 8% were obtained for 2.5 and 4 μM of atrazine, respectively, highlighting a satisfactory capability to detect atrazine also in surface water.

In order to gain further insights, reversibility tests were performed to evaluate the nature of atrazine inhibition (Fig. 4C). These tests consisted in washing CC125/CB-SPEs with measuring buffer after the measurement of the inhibition at a 5 μM atrazine concentration, and recording the recovered current. As reported in figure, a complete recovery of the algae oxygen evolution occurs.

4. Discussions

Thanks to this novel configuration, which combine algae with carbon black, the proposed biosensor demonstrated to be able of revealing atrazine as a case study herbicide with detection limit in the nanomolar range (1 nM corresponding to 0.22 μg/L), meeting the requirements of herbicide limits between 0.6 and 2 μg/L in surface water (EU Directive, 2013/39/EU). The biosensor was also tested in river water, a vulnerable matrix that can be subject to pesticide pollution due to agricultural practices. Results indicated its suitability for herbicide analysis in real samples, without interferences from compounds which can be present at safety limits provided by the EU Directives. A ~25% matrix effect and satisfactory recovery values were also achieved. In addition, being this biosensor based on a whole-cell bioreceptor, no sample pre-treatment was required.

A critical aspect, however, was evidenced in the immobilization procedure of algae cells on the carbon black modified screen-printed electrodes. Indeed, a number of immobilized CC125/CB-SPEs was not able to furnish any electrochemical response, which were, therefore, not included in the analysis. This procedural issue can be ascribed to critical steps of the immobilization method (e.g. cell harvesting), as well as to the intrinsic complexity of a whole biological system, thus requiring further investigations. Furthermore, it is noteworthy that algae whole cells, despite their high sensitivity, lack in specificity, being capable to sense the presence of herbicide classes (i.e. triazines and ureas) and unable to discriminate single compounds. Indeed, such systems can act as on/off alarm for in-the-field analysis, providing a general overview about the presence of such targets in water matrices. To this aim, further studies entail the exploitation of array of interchangeable bioreceptors projected *via* rational design (Antonacci and Scognamiglio, 2019a), with desired features in terms of higher selectivity towards punctual targets, to face drawbacks related to low selectivity and specificity.

5. Conclusions

A highly sensitive algal biosensor was realised for the analysis of photosynthetic herbicides in surface water. This system highlighted the capability of carbon black to sense algae oxygen evolution during the photosynthetic process and monitor its changes in the presence of environmental pollutants. Many advantages can be highlighted for the proposed biosensor, including high sensitivity, repeatability, and stability, also in comparison with similar biosensors reported in literature (Table 1). To further advance this work, additional studies will be performed to obtain an algal sensor platform based on an array of bioreceptors with higher selectivity towards selected herbicides.

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.

CRediT authorship contribution statement

Raouia Attaallah: Methodology, Investigation. **Amina Antonacci:**

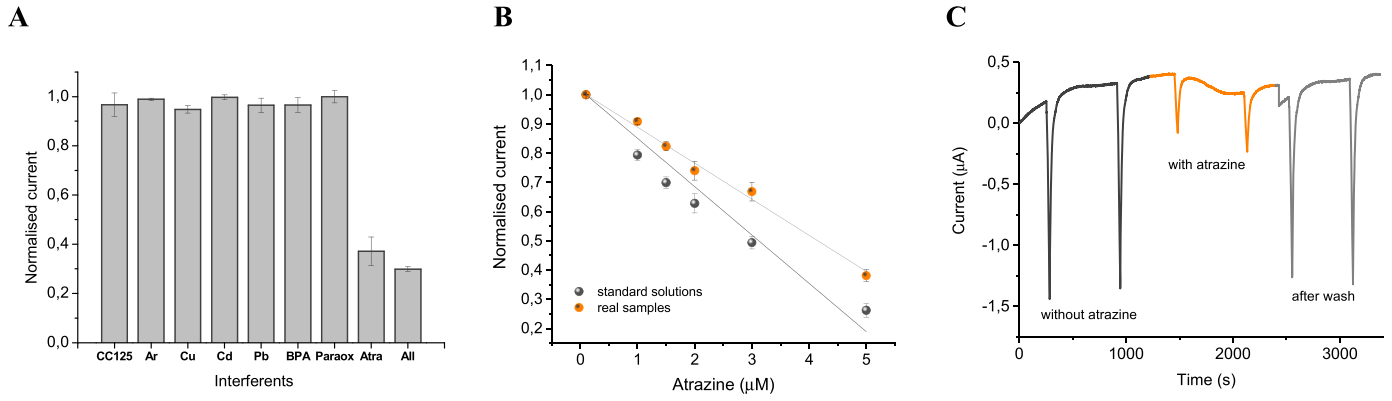

Fig. 4. CC125/CB-SPE interference study using 100 ppb arsenic, 20 ppb copper, 5 ppb cadmium, 10 ppb lead, 10 ppb bisphenol A, 1 ppb paraoxon, and 5 μM atrazine as well as a solution spiked with all the above listed compounds (A), matrix effect using river water samples (B), and reversibility test (C). Applied potential −0.6 V, repeated cycles of 30 s light and 10 min dark, n = 3. Measurement volume: 200 μL of measuring buffer.

Table 1
Selected optical and electrochemical algae-based biosensors for herbicide detection.

Bioreceptor	Transductor	Support	Detection limit	Storage stability	Response time	Real matrix	Ref.
Chlamydomonas reinhardtii	Chlorophyll fluorescence	Silicon septum	21 μg/L	3 weeks RT	15 min	n.d.	Scognamiglio et al. (2009)
Chlamydomonas reinhardtii	Chlorophyll fluorescence	Plastic substrate	21 μg/L	n.d.	40 min	n.d.	Ferro et al. (2012)
Chlorella vulgaris	Fibre optics	Glass microfiber membrane	1 mg/L	1 week	30 min	n.d.	Naessens et al. (2000)
Chlorella vulgaris	Fibre optics	Quartz microfibre filter	0.25 μg/L	4 weeks	10 min	n.d.	Védrine et al. (2010)
Chlorella vulgaris	Amperometric	SPEs	200 mg/L	4 weeks 4 °C retaining 80% activity	4 min	n.d.	Shitanda et al. (2009)
Chlorella pyrenoidosa	Amperometric	SPEs	0.1 mg/L	n.d.	n.d.	n.d.	Alsu et al., 2011
Chlamydomonas reinhardtii	Amperometric	CB-SPEs	1 nM = 0.22 μg/L	3 weeks RT retaining 70% activity	15 min	river	*Our developed biosensor*

Methodology, Investigation. **Vincenzo Mazzaracchio:** Methodology. **Danila Moscone:** Data curation. **Giuseppe Palleschi:** Writing - review & editing. **Fabiana Arduini:** Validation, Data curation. **Aziz Amine:** Writing - review & editing. **Viviana Scognamiglio:** Conceptualization, Supervision, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2020.112203.

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