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Microalgae fiber optic biosensors for herbicide monitoring using sol–gel technology

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ABSTRACT

Three microalgal species (*Dictyosphaerium chlorelloides* (D.c.), *Scenedesmus intermedius* (S.i.) and *Scenedesmus* sp. (S.s.)) were encapsulated in silicate sol–gel matrices and the increase in the amount of chlorophyll fluorescence signal was used to quantify simazine. Influence of several parameters on the preparation of the sensing layers has been evaluated: effect of pH on sol–gel gelation time; effect of algae density on sensor response; influence of glycerol (%) on the membrane stability. Long term stability was also tested and the fluorescence signal from biosensors remained stable for at least 3 weeks. D.c. biosensor presented the lowest detection limits for simazine (3.6 $\mu g\,L^{-1}$) and the broadest dynamic calibration range (19–860 $\mu g\,L^{-1}$) with IC_{50} $125 \pm 14\,\mu g\,L^{-1}$. Biosensor was validated by HPLC with UV/DAD detection. The biosensor showed response to those herbicides that inhibit the photosynthesis at photosystem II (triazines: simazine, atrazine, propazine, terbuthylazine; urea based herbicides: linuron). However, no significant increases of fluorescence response was obtained for similar concentrations of 2,4-D (hormonal herbicide) or Cu(II). The combined use of two biosensors that use two different genotypes, sensitive and resistant to simazine, jointly allowed improving microalgae biosensor specificity.

1. Introduction

Triazine and phenylurea herbicides (e.g. atrazine, simazine, terbutylazine, propazine) are widely used in agriculture for crop protection. These compounds are persistent in the environment and have been detected in soil, ground and surface waters as well as in tissues and body fluids (Herranz et al., 2008). Most analytical methods applied to the analysis of such herbicides include a sample clean up step, followed by liquid or gas chromatography and tandem mass spectrometry detection to achieve the low concentration limits established in the European regulations (Water Framework Directive, 2000/60/EC, European Union Brussels, 2000). Other complementary directives are applicable in different inland water systems (i.e. RD). However, the application of these methods requires the use of sophisticated equipment, skilled laboratory personnel, are time consuming and difficult to adapt for field work. Hence, a great effort is being focused by the European Union and other governmental agencies on the development of screening methods that can be applied as early warning systems for environmental monitoring.

In the last few years biosensors have shown a great potential as analytical tools for the development of rather automatic, fast and direct analysis methods that in many cases avoid sample pretreatment or require minimal sample preparation, allowing on-site field monitoring (Rodríguez Moraz et al., 2007).

Green microalgae are the main component of phytoplankton population and can survive in certain environmental conditions that could be harmful for other microorganisms (Orellana and Haigh, 2008). These microorganisms have been used in the development of biosensors that can respond to critical changes in aquatic ecosystems. For example, herbicide biosensors have been developed based on the measurement of the algal chlorophyll fluorescence at 682 nm (under 469 nm excitation light) that increases when toxicants that inhibit the algal photosystem II are present. An optical fiber based biosensor was developed for atrazine and endrine monitoring in water (Frense et al., 1998) using *Scenedesmus subspicatus* cells, immobilized on filter paper and covered with a thin alginate layer hardened with calcium chloride. *Chlorella vulgaris* was immobilized at the tip of an optical fiber bundle placed inside a homemade microcell (Naessens et al., 2000), or in a

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rotating support holding up to five different membranes to increase the number of assays (Védrine et al., 2003), and used for the detection of herbicides affecting photosystem II (PSII) such as triazines (atrazine, simazine) or phenylureas (diuron, isoproturon) herbicides at sub-$\mu g\,L^{-1}$ concentration level. Other targets, such as airborne chemical warfare agents (tabun, sarin, mustard agent, tributylamine or dibutylsulfide) were monitored using a hand-held fluorimetric detector system that measured the photochemical efficiency of *Chlorella vulgaris* entrapped together with cyanobacteria *Nostoc commune* (Sanders et al., 2001).

Alternatively, microalgal biosensors may be based on the inhibition of the photosynthetic function (O_2 production) in the presence of a pesticide or other toxicant. Thus, *Chlorella vulgaris* photosynthetic oxygen release was monitored with a Clark electrode to detect vapors of solvents: perchloroethylene (Naessens and Tran-Minh, 1998a, 1999), and methanol (Naessens and Tran-Minh, 1998b). It has recently been described a novel fiber-optic biosensor for the analysis of simazine at $\mu g\,L^{-1}$ level, using *Dictiosphaerium chlorelloides* immobilized on a porous silicone film and oxygen transduction (Orellana et al., 2007).

One of the limiting steps in the development of microalgae biosensors is the incorporation of the biomaterial in a suitable matrix that avoids leaching, as well as the need of tedious immobilization procedures that affect stability or render a significant loss of activity (Gupta and Chaudhury, 2007). Sol–gel films are widely recognized as suitable tools for effective and low cost mass production of reversible, robust and portable optical chemical sensors and biosensors (Jerónimo et al., 2007). Silica gels exhibit chemical stability, aqueous compatibility and the size and chemical nature of the pores can be tailored to protect bioactivity. Silica is biocompatible, and living organisms may benefit from its protection and transparency exhibiting improved resistance to thermal and chemical denaturalization as well as increased resistance and operational stability (Livage and Coradin, 2006; Nguyen-Ngoc and Tran-Minh, 2007a). Some authors have proposed the preparation of sol–gel films using aqueous instead of alkoxide precursors, in order to avoid the release of alcohols during the polycondensation process, a compound toxic to the algal cells (Bhattia and Brinker, 2000; Chen et al., 2004; Coiffier et al., 2001; Nassif et al., 2003). In a recent study, a mixture of silicate and Ludox was used to trap *Chlorella vulgaris* algae (Nguyen-Ngoc and Tran-Minh, 2007a) developing a fluorescence biosensor to determine diuron (Nguyen-Ngoc and Tran-Minh, 2007b).

A second limitation of whole cell biosensors is the lack of specificity. In recent years, different toxicants were detected and identified taking advantage of the different sensitivity patterns of immobilized algal strains (Podola et al., 2004; Podola and Melkonian, 2005; Tibuzzi et al., 2007; Chaplen et al., 2007). Due to the lack of specificity of photosystem II response, further research to generate toxicant-resistant mutants seems promising (Lopez-Rodas et al., 2001; García-Villada et al., 2002, 2004). This approach was proposed using a wild-type of *Dictyosphaeriumm chlorelloides* and a TNT-resistant mutant for the detection of the explosive 2,4,6-trinitoluene (TNT) (Altamirano et al., 2004) and the detection of simazine, based on the inhibition of oxygen production (Orellana et al., 2007).

The aim of this study is to develop fiber optic biosensors for herbicide analysis, using microalgae immobilized in a sodium silicate sol–gel matrix to preserve the biological activity. Three different species of freshwater green algae have been evaluated for biosensor development: *Dictyosphaerium chlorelloides* (D.c.), *Scenedesmus* sp. (S.s.) and *Scenedesmus intermedius* (S.i.). In order to confer sensitivity, the cell strains most sensitive to lower concentrations of simazine were selected. Similar culturing of resistant cell strains provides selectivity to the analyte. Thus, ratchet cycles were used to obtain mutant resistant algae clones (not genetically modified organisms) as a result of rare pre-selective mutations.

2. Materials and methods

2.1. Chemicals

Sodium silicate solution (*purum* ≥10% as NaOH, ≥27% as SiO_2) was purchased from Riedel-de Häen, (Seelze, Germany). Glycerol was obtained from Sigma–Aldrich (St. Louis, MO, USA) and hydrochloric acid was purchased from Panreac (Barcelona, Spain). Herbicides (Simazin PESTANAL®, Terbuthylazine PESTANAL®, Propazine PESTANAL®, Atrazin PESTANAL®, Linuron, DCMU (*N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea)) were obtained from Riedel-de Häen (Seelze, Germany) and Sigma–Aldrich (St. Louis, MO, USA). 2,4 dichloro-phenoxyacetic acid was purchased from Sigma–Aldrich (St. Louis, MO, USA). Stocks solutions of 1000 mg L^{-1} were prepared in DMSO (Scharlau, Barcelona, Spain), except for linuron that was dissolved in methanol (Panreac PS, Barcelona, Spain). Water was purified with a Milli-Q system (Millipore, Bedford, MA). All solutions and BG11-culture media were prepared with autoclaved Milli-Q water.

2.2. Microalgal culture conditions

Three microalgal species, *Dictyosphaerium chlorelloides* (D.c.), *Scenedesmus* sp. (S.s.) and *Scenedesmus intermedius* (S.i.) (Chlorophyta) from the Algal Culture Collection of Complutense University of Madrid (Spain), were used in this study. The algae were grown axenically in culture flasks (Greiner) with 20 mL of BG-11 medium (Sigma, St. Louis, USA) at 20 °C, and under continuous light of 60 $\mu mol\,m^{-2}\,s^{-1}$ over the waveband 400–700 nm. Algae were maintained axenically in balanced growth by serial transfers of an inoculum (10^5 cells) to fresh medium once a month. The subsequent cultures (most of them 6×10^7 cell mL^{-1}) from D.c., S.s. and S.i. were encapsulated in silicate sol–gel and the increase in the amount of chlorophyll fluorescence signal was used to quantify simazine.

2.3. Biosensing layers preparation

Sol–gel membranes were prepared by mixing algae solutions with sodium silicate 2 M, at pH 9, and 2.5% of glycerol (w/w). Typically 450 μL of algae solution (6×10^7 cell mL^{-1}) was mixed with 37.5 μL of water and 12.5 mg of glycerol. Later on, 60 μL of 2 M HCl and 50 μL of 2 M sodium silicate were added. The mixture was then shaken vigorously and 30 μL were deposited on circular glass supports (1 cm diameter). After 40 min, membranes were stored in the culture medium BG-11 and kept away from the light at 4 °C.

2.4. Instrumentation

Changes in photosynthetic activity were estimated by measuring chlorophyll a fluorescence of PSII by a pulse-amplitude modulated fluorimeter PAM2000 (Walz, Effeltrich, Germany). Fluorescence measurements were carried out with a spectrofluorometer Fluoromax 2 from Horiba Jobin Yvon (Longjumeau Cedex, France), controlled with the original software (Datamax), at room temperature. Excitation was carried out at 467 nm and emitted light was collected at 699 nm (D.c. and S.s. algae) or 702 nm (S.i.).

2.5. Assay protocol

Sensing membranes containing immobilized algae were placed at the tip of a bifurcated fiber-optic cable (1 m long) in a homemade flow through cell, that has been described elsewhere (Orellana et

al., 1995), for the measurements. Algae were kept in darkness for 15 min before irradiation, during that time the sample was pumped through the flow cell. Then the biosensing layers were irradiated for 5 min with the excitation light and the fluorescence measurement was carried out immediately after the membrane was illuminated. For sensor regeneration a BG11 solution was pumped through the flow cell during 20–40 min, depending on previous simazine sample concentration.

Experimental signals were plotted as a function of the analyte concentration (in logarithmic scale) and the experimental data were fitted to a four-parameter logistic equation (Eq. (1), sigmoidal) using the Sigma Plot 9.0 datasheet (Systat Software Inc., Richmond, CA, USA) software:

$$I = \frac{I_{max} - I_{min}}{1 + ([Analyte]/IC_{50})^b} + I_{min} \quad (1)$$

where I_{max} is the asymptotic maximum (maximum emission in presence of herbicide), b represents the curve slope at the inflexion point, IC_{50} the concentration of analyte at the inflexion point (concentration giving 50% increase of I_{min}) and I_{min} is the asymptotic minimum.

The detection limit (LOD) was calculated as the concentration corresponding to 10% of the zero dose and the working range (DR) of the method was evaluated 20–80% of the assay response at zero dose.

Changes in photosynthetic activity due to simazine were also estimated after 30 min of exposure, by measuring fluorescence using a PAM2000 fluorometer (Walz, Effeltrich, Germany), as explained by Schreiber et al. (1986). The percentage of inhibition of F'_m was used as an estimator of the toxic effect of simazine, according to the following formula (Eq. (2)):

$$\text{Inhibition}\,(\%) = 100 - \left[\frac{100 \times (F'_m)_{SIMAZIN}}{(F'_m)_{control}}\right] \quad (2)$$

where $F'_{SIMAZIN}$ is the maximum fluorescence after 30 min with any given simazine concentration, and $F'_{control}$ is the maximum fluorescence of the control without simazine after the same period of time. Inhibition is considered significant when it is higher than 10%.

To validate, simazine concentration in a reference solution was determined by high performance liquid chromatography (HPLC) using a HP-1100 series chromatograph from Agilent Technologies (Palo Alto, CA) equipped with a quaternary pump, autosampler, automatic injector, and a diode array DAD detector. The analytical column was a LUNA C_{18} (2) (150 mm × 4.6 mm, 5 μm) HPLC column protected by a RP18 guard column (4.0 mm × 3.0 mm, 5 μm), both from Phenomenex (Torrance, CA). A gradient program was used for the mobile phase, combining Milli-Q water and acetonitrile, the composition varying from 10% acetonitrile to 100% acetonitrile in 45 min. Analyses were performed at a flow rate of 0.42 mL min^{-1}, and the column temperature was kept at 45 °C. The injection volume was 100 μL, and the UV detector wavelength was set at 225.8 nm.

Specificity studies were carried out by measuring the dose–response curves for other chemically related and non-related herbicides, under the optimized conditions.

3. Results and discussion

3.1. Effect of pH on gelation time

The effect of pH on the gelation time was studied for sol–gels prepared using a BG-11 culture media solution or an S.c. solution (1.4×10^7 cell mL^{-1}). Gelation takes place at pH values ranging from 6 to 10. Gelation at pHs below 8 is very fast (less than one minute) therefore, pH 9 was selected to carry out cell encapsulation to allow enough time for membrane preparation (approximately two minutes).

3.2. Viability after sol–gel encapsulation

Viability of algae after immobilization in sol–gel was evaluated by fluorescence microscopy. The fluorescence emission of entrapped cells was compared with that corresponding to microalgae solutions and both profiles were very similar, confirming the survival of the immobilized algae. Blank membranes prepared with BG11 showed no fluorescence.

3.3. Effect of algae concentration on biosensor response

In order to evaluate the effect of algae concentration on the biosensor response, the different microalgae were immobilized at three different concentration levels (low: 1.9, 1.7 and 2.1×10^7 cell mL^{-1} for D.c., S.s. and S.i. respectively; medium: 3.7, 3.4 and 4.3×10^7 cell mL^{-1} and high: 5.6, 5.1 and 6.4×10^7 cell mL^{-1} for D.c., S.s. and S.i.) for biosensor development. Membranes were prepared in duplicate and the measurements were also carried out in duplicate. Average fluorescence intensity values are shown in Fig. 1.

The fluorescence signal increases with the amount of immobilized microalgae in the membranes. However, the signal reaches a maximum for medium and high concentrations, especially for S.i. and S.s. So, high concentrations (5.6, 5.1 and 6.4×10^7 cell mL^{-1} for D.c., S.s. and S.i., respectively) were selected to carry out following experiments.

3.4. Influence of glycerol on membrane stability

The use of glycerol is recommended because affects cell membrane permeability and decreases stress caused by encapsulation with the resultant increase in the sol–gels viability (Nassif et al., 2003). The influence of this additive in long term membrane stability and robustness was evaluated measuring the response of the biosensing layers prepared with 6.4×10^7 cell mL^{-1} S.i. solutions in the presence of: 0, 2.5, 5, 10 and 15% (w/w) glycerol. Four measurements were carried out for each concentration level (two membranes were examined in duplicate). A 2.5% glycerol concentration was selected because fluorescence intensity was maximized and showed a good reproducibility (<3% vs. 15% in the absence of glycerol).

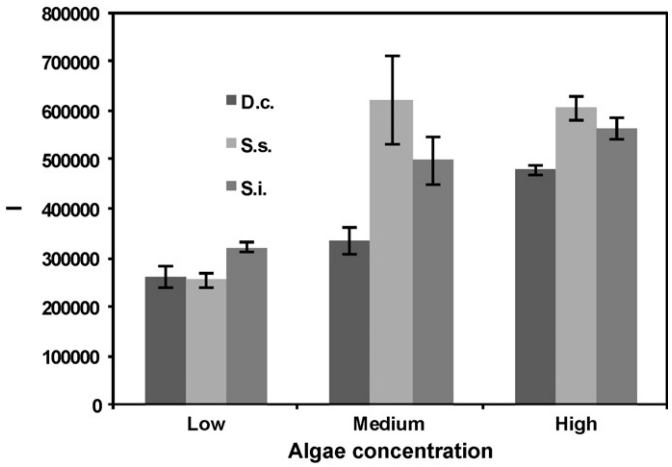

Fig. 1. Effect of algae density on sensor response ($n = 2$ biosensors), (low: 1.9, 1.7 and 2.1×10^7 cell mL^{-1}; medium: 3.7, 3.4 and 4.3×10^7 cell mL^{-1} and high: 5.6, 5.1 and 6.4×10^7 cell mL^{-1} for D.c., S.s. and S.i., respectively). λ_{exc}: 467 nm, λ_{em}: 699 nm.

Table 1
Optical biosensor response to different herbicides.

Herbicide	Detection limit ($\mu g L^{-1}$)	IC_{50} ($\mu g L^{-1}$)[a]	Dynamic range ($\mu g L^{-1}$)	Reversibility
Simazine	3.6	125 ± 14	19–860	Yes
Atrazine	13.5	88.2 ± 0.7	28–282	Yes
Propazine	7.6	127 ± 2	20–540	Yes
Terbuthylazine	3.3	18 ± 3	6–55	Yes
Linurón	4.1	37 ± 6	9–149	Yes

[a] Average value ± SD.

3.5. Analytical characteristics

The effect of sample pH was evaluated by measuring the response of the biosensors to simazine (400 $\mu g L^{-1}$) at different pH values in the 6–9 range. Each biosensor was measured in duplicate. Results are collected in Fig. 1S (supplementary material). Signals were slightly higher for lower pHs for the three microalgae. Therefore, hereafter herbicide solutions were prepared in BG11 culture media (pH 6.3).

For calibration purposes, D.c., S.s. and S.i. biosensors were first equilibrated, at least during two hours, with BG-11, in the absence of toxic compounds, and cycles of darkness/irradiation (15 min darkness/5 min irradiation). Response to the BG-11 blank sample showed a good repeatability with RSD (%) values lower than <6% (D.c.: 5.6 %, S.s.: 3.3 %, S.i.: 2.5%).

Fig. 2S (supplementary material) shows a representative dose response curve obtained with the D.c. biosensor, in the 5×10^{-4}–10 mg L^{-1} simazine range. Fluorescence intensity (I), after 5 min irradiation time, vs. logarithm of simazine concentration, has been plotted for calibration purposes and the experimental points were fitted to a four parameters logistic curve (Eq. (1)) ($r > 0.990$). The limit of detection was lower for the D.c. (3.6 $\mu g L^{-1}$) than for the S.s. (48.5 $\mu g L^{-1}$) and S.i. (31.0 $\mu g L^{-1}$) biosensors (Table 1). Fig. 2 depicts the mean calibration curve obtained for three different membranes corresponding to three different wild-type simazine sensitive D.c. algae cultures, and measured in different days, showing the excellent precision of the biosensor.

D.c. microalgae showed also more sensitivity than S.s. and S.i. in experiments performed using pulse amplitude modulated (PAM) fluorometry. Throughout recent years, PAM detection has been developed to monitor the influence of stress factors, such as herbicide presence, on microalgae photosynthesis (Schmitt-Hansen and Altenburger, 2007). Changes in photosynthetic activity due to simazine were estimated after 30 min of exposure; afterwards, inhibition was calculated. Inhibition is considered significant when higher than 10%. The inhibition values obtained in this case were ($n = 4$: 10.8 ± 0.4% for 18 $\mu g L^{-1}$ of simazine (D.c.); 9.4 ± 0.3% for 36 $\mu g L^{-1}$ of simazine, and 11.5 ± 0.6% for 50 $\mu g L^{-1}$ of simazine (S.s.); 8.6 ± 0.4% for 26 $\mu g L^{-1}$ and 16.2 ± 0.5% for 36 $\mu g L^{-1}$ of simazine (S.i.).

D.c. biosensors showed a broader working range (19–860 $\mu g L^{-1}$; $IC_{50} = 125 \pm 14 \mu g L^{-1}$ ($n = 3$ different membranes from different algae cultures) than S.s. (83–70 $\mu g L^{-1}$; $IC_{50} = 219 \pm 58 \mu g L^{-1}$) or S.i. (63–20 $\mu g L^{-1}$; $IC_{50} = 197 \pm 38 \mu g L^{-1}$) Moreover, D.c. biosensor was reversible (baseline recovery time <40 min) therefore we decided to continue our experiments with this microalgae specie.

Accuracy was tested by analyzing solutions prepared from a solid simazine high purity certified analytical standard used in residue and environmental analysis (Catalogue reference C16950000, Dr. Ehrenstorfer GmbH, Augsburg, Germany). Solutions of the certified analytical standard (four replicates; 122 $\mu g L^{-1}$ (±3 $\mu g L^{-1}$) were prepared in BG-11 culture media.

Biosensor was stabilized with the BG-11 solution and afterwards reference material solutions were pumped through the system. Four independent measurements were performed, each one with a different solution, using BG-11 to recover the baseline between measurements. The analysis reported a simazine concentration of 112 ± 13 $\mu g L^{-1}$ ($P = 0.05$).

For validation purposes, the simazine samples were analyzed by high performance liquid chromatography (HPLC). The confidence interval for the obtained concentration value was 106 ± 13 $\mu g L^{-1}$, calculated for $P = 0.05$.

Values for both methods were statistically compared (t-test, $P = 0.05$) and no significant difference was found. Therefore, the microalgal biosensor was accurate for simazine determinations.

Biosensor long term stability was evaluated measuring the D.c., S.s. and S.i. sol–gels prepared with 4.0×10^7 cell mL^{-1} (medium concentration level) and 6.0×10^7 cell mL^{-1} (high concentration level) solutions, and 2.5% glycerol. Four membranes were prepared for each type of microalgae and concentration level, and one measurement was performed with each sensing membrane. Fluorescence intensity remains stable at least for three weeks after preparation (Fig. 3).

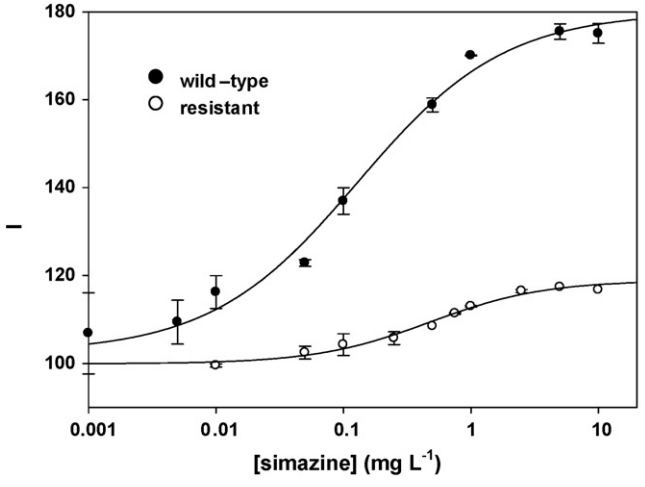

Fig. 2. Dose–response curves for sensitive wild-type and simazine resistant D.c. membranes. Simazine concentrations ranging from 1×10^{-3} (wild-type, $n = 3$ biosensors obtained from different microalgae cultures) or 0.01 mg L^{-1} (resistant type, $n = 2$ biosensors) to 10 mg L^{-1}. λ_{exc}: 467 nm, λ_{em}: 699 nm.

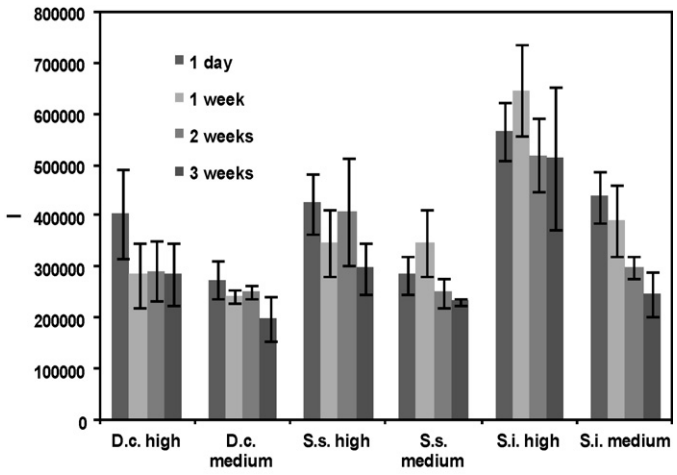

Fig. 3. Microalgae in sol–gels: long term stability ($n = 2$ biosensors), (medium: 3.7, 3.4 and 4.3 × 10^7 cell mL^{-1} and high: 5.6, 5.1 and 6.4 × 10^7 cell mL^{-1} for D.c., S.s. and S.i., respectively). λ_{exc}: 467 nm, λ_{em}: 699 nm.

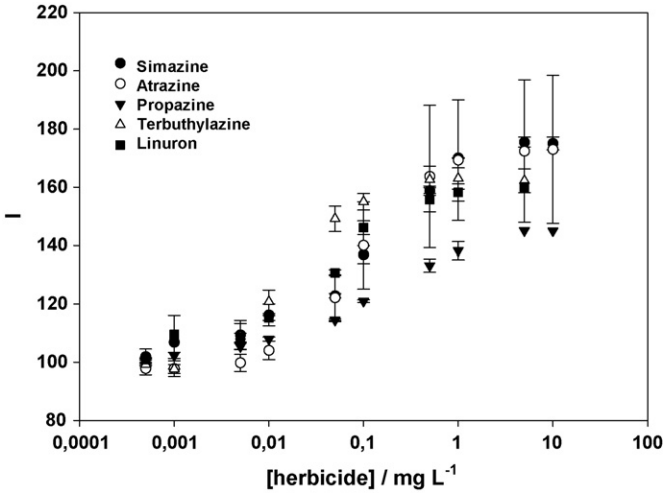

Fig. 4. Dose–response signals for five herbicides (D.c. silicate sol–gel membranes). Herbicide concentrations ranging from 5×10^{-4} to 10 mg L^{-1}. λ_{exc}: 467 nm, λ_{em}: 699 nm ($n = 2$ biosensors).

3.6. Specificity study

Biosensor response to other pollutants was tested measuring the dose–response curves to other pollutants that inhibit the photosystem II. The calibration plots corresponding to atrazine, linuron, propazine and terbutilazine are presented in Fig. 4 (two membranes from two different cultures, $n = 2$). The detection limits, the IC_{50}, are collected in Table 1. The herbicides are classified according to their mechanism of action. Thus, the D.c. microalgal sensor shows response to those herbicides which inhibit the photosynthesis at photosystem II (triazines: simazine, atrazine, propazine, terbuthylazine; urea based herbicides: linuron). IC_{50} is recommended to perform an interspecies comparison of sensitivity to different pollutants (Juneau et al., 2007). Thus, our IC_{50} values for simazine and atrazine are very similar to those obtained by Védrine et al. (2003) for *Chlorella vulgaris* (simazine: 127 ± 4 μg L^{-1}; atrazine: 96 ± 4 μg L^{-1}). However, their detection limits are bellow μg L^{-1}, probably due to the longer measuring times they apply (14 h to get a stable fluorescence signal). In other studies carried out by Podola and Melkonian (2005) it has been shown that simazine toxicity for biosensors produced with nine different microalgae strains was much lower than that obtained for other herbicides such as diuron and isoproturon. They reported Lowest Observed Effect Concentration values (LOCV) for simazine between 2 and 50 μg L^{-1}, depending on the microalgae strains.

No significant increase of fluorescence was obtained for 2, 4-diclorophenoxy acetic acid (2,4-D), a weed killer whose mechanism of action resembles hormones regulating plant growth up to 1 mg L^{-1}. Copper (II) showed no effect on the biosensor response up to concentrations higher than 0.5 mg L^{-1}.

3.7. Comparison between wild-type and resistant algae

In order to improve selectivity a first attempt was made to obtain simazine resistant mutants by using fluctuation analysis as previously described (Lopez-Rodas et al 2007). This system maintains large cell populations under selection pressure, enabling favorable mutations to be conserved during selection, resistant algae were preserved in a 1 mg L^{-1} solution before encapsulation. The response curve for the resistant algae can be seen in Fig. 2. The detection limit for the resistant mutants was increased 17 times in comparison to the wild-type microalgae (64.6 μg L^{-1} vs. 3.8 μg L^{-1}). However, resistant algae exhibit a similar response toward atrazine or DCMU than those of sensitive algae (IC_{50} (μg L^{-1}): 111 ± 12 vs. 88.2 ± 0.7 and IC_{50} (μg L^{-1}): 50 ± 11 vs. 52 ± 14, for atrazine and DCMU, respectively). Thereafter, the comparison of the response of the wild-type and the resistant microalgae biosensors would allow increasing the selectivity of the devices, as it will be possible to identify groups of compounds affecting in different ways photosystem II.

4. Conclusions

Three species of microalgae cells were encapsulated in a silicate matrix and fluorescence response to simazine was evaluated. *Dictiosphaerium chlorelloides* biosensor presented the lowest detection limits, the broadest dynamic calibration range, and an accurate response and reversibility. The new developed microalgae biosensor does not reach the European Community directive for herbicide detection (0.1 μg L^{-1}) in drinking water, when working with short irradiation times (15 min darkness/5 min irradiation). However, in comparison to other microalgae-based biosensors reported in the literature for herbicide analysis, it has been demonstrated that the use of resistant strains can be a useful tool to improve biosensor specificity. In the algal biosensor wild-type sensitive cells exhibit a decrease in their biological response in presence of simazine, while resistant algae work as control exhibiting a significant smaller decrease. In contrast under other herbicides, such as atrazine or DCMU, resistant algae exhibit similar response than those of sensitive algae. This pattern demonstrates that the use of simazine-resistant cells is an appropriate procedure to improve biosensor specificity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.05.013.

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