**ALGAL BIOSENSOR MODULE**

Algal photosystem reporters are robust and react very broadly to toxicity of biocides. The concept of bioreporting with living algal cells relies on measurements of photosystem II fluorescence, which is extremely sensitive to disruption by a variety of toxicants (Table 1 and Figure 1). The algal cells are small and assays using photosystem II fluorescence can be downscaled to a miniaturized format, adapted in micro-engineered fluidics units with integrated detectors, and therefore provide the currently most optimal choice to incorporate in autonomous systems for real-time and in-situ measurements of marine water quality.

It was designed, produced and tested a whole-cell biosensor based on the symbiosis between the alga *C. vulgaris* and the ciliate *T. pyriformis*.

<table>
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<tr>
<th>Biomediator</th>
<th>Target analytes</th>
<th>Target compounds</th>
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<tbody>
<tr>
<td>Microalgae</td>
<td>Pesticides</td>
<td>triazines, diazines, phenylurea, phenolic compounds</td>
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<tr>
<td><em>C. Vulgaris</em> associated with <em>T. pyriformis</em></td>
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A new microfluidic chip for immobilized algae with an automatic set-up was adopted.

Fig. 1 Symbiotic association between the alga *C. vulgaris* and the ciliate *T. pyriformis*

Fig. 2. The immobilized algal biomediator in Ca-alginate allows repeated use after regeneration with the aid of white survival led and regeneration buffer for long-time measurements.

Fig. 3 Micro-fluidic flow cell, made by Biosensor with the cages made by MicroTEC’s technology, encases the algae with special opening design.

A smart fluorimeter device was developed providing automation and transfer of data at distance to PC.

The optic module for fluorescence induction measurement consists of a microcontroller connected with series of board. It provides inputs of 500µmol photons m⁻²s⁻¹ red light for 11 s at 650nm and measures the fluorescence emission by photodiode at 680 nm (Fig. 4). The red light covered a surface of 1cm², resulting in highly reproducible measurements averaged over 1000 points.
Fig. 4 Fluorescence induction curves obtained with the algal module in presence of simazine. Inlet shows the calibration curve.

The instruments can work directly by recharged battery at 12volt. Once the command run is executed, the measurement starts and integrated fluidic pump allows automatic sampling into the flow cell with 6 parallel cavities containing the biomediator, free living or immobilized microalgae into a Ca-alginate gel. The instrument (fig. 5) has been developed for a fast from millisecond to sec fluorescence measurement of liquid or solid matrices.

Fig. 5 The sensor with the cells for static measurements and with fluidics based on gravity and on a peristaltic pump for on-line measurements

Protocol of measurement in microfluidics based on difference of the fluorescence in the same cell

Inlet in the first step cell 50% of buffer + 50% and seawater (clean control); inlet in the second step cell 50% of buffer + 50% and seawater (clean control); when there is a difference higher that 5-10% between the first measurement and the second in the same cell it means positive response; thus the system passes to measure the second cell (Fig. 6).

Fig. 6 Measurement algorithm in the cells

After analysis the data are collected either in a card or send to the central control unit of the buoy or vessel, in which the biosensor module is placed.

Device electronic in Details

Fig. 7 Electronics of the system

Main Board - Quick Processing: 4000 data for acquisition and 120 sec for data analysis. ADC - range/resolution: arbitrary units from 0 to 65535 (16 bits) x 6 cells (sequentially sampled). Sampling frequency - 15.384hz

Optical module - Survival and excitation light. Current Control drivers from 0 to 127 intensity light level by white and red far LEDs Emission light for 6 Cells sequentially sampled.

The dimension are 13x18x9 cm, the weight is 750gr. The Fig. 8 shows the instrument into the box.
The microfluidics was set by Lionix. The final configuration of the algal sensor is in Fig. 9.

LODs of this system, in the nanomolar range, are not superior to other microalgae strains that can reach LODs of picomolar, however the symbiotic biomediator has the important advantage of offering long-term resistance in marine water.