MARIABOX an autonomous monitoring device for marine pollution: from the laboratory to a product: design challenges and real world trade-off

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Abstract: *The MARIABOX "**MARI**ne environmental in-situ Assessment and monitoring tool **Box**" is an autonomous marine monitoring device for man made and natural pollutants under development in the frame of a project funded by the European Commission (Contract 614088). The device it is conceived for installation on free floating devices, buoys, ships, or to be used as a portable instrument. It will work fully autonomously, collecting the marine water sample, analysing it, storing the data and transmitting them via satellite link. The main, high-level user requirements for the MARIABOX system are for analytical sensitivity below the thresholds for pollutants set by the European Commission, so to enable early warning, to be portable and capable of repeating measurements over at least six months without any servicing so to diminish the costs of logistics when deployed at sea.*
These user's requirements are demanding, in terms of design choices, that must be weighed and balanced against the different technical trade-offs necessary to find a viable implementation solution.
More in details the design includes: a) a sensing and analysis box, b) a modular communication system, c) a flexible power system, d) a software platform with a web services environment, and e) a cell phone application. The box will transmit the collected data in real time through different channels according to local needs and geographical location: radio, GSM/GPRS/3G, WiFi, WiMAX or satellite link. The MARIABOX is devised in order to be fully controlled in remote and implements the OTA programming and OTA configuration features which allow the user to update the firmware of the MARIABOX unit and modify configuration parameters wirelessly. Remote updates are a key factor in deployment scalability since it offers the only possibility of easily updating or reprogramming the devices after the initial deployment without the need to sail out at sea. This approach grants that the servicing costs are very low.

Keywords: *marine pollution; biosensor; algal toxin; heavy metals; wireless analytical device.*

1. INTRODUCTION

The MARIABOX- "Marine environmental *in-situ* Assessment and monitoring tool Box" project, funded by the European Commission (Contract 614088), refers to the above quoted policy to develop an autonomous connected analytical device capable of six months remote measurements on board a buoy. MARIABOX develops a wireless marine environment analysis device for monitoring chemical and biological pollutants while installed into a buoy, a maritime means of transport or a mooring. The device, based on novel biosensors, will be of high-sensitivity, portable and capable of repeating measurements over a long time, allowing permanent deployment at sea.

The design of the system includes: a) a sensing and analysis box, b) a modular communication system, c) a flexible power system, d) a software platform with a web services environment, and e) a cell phone application. The box will transmit the collected data in real time through different channels according to local needs and geographical location: radio, GSM/GPRS/3G, WiFi, WiMAX or satellite link. The unit is designed to be remotely controlled and will implement the OTA programming and OTA configuration features which will allow the user to update the firmware of the MARIABOX unit and modify some configuration parameters wirelessly.

2. USER REQUIREMENTS AND DESIGN ITERATIVE PROCESS

The first phase of the project involved the detailed analysis of user requirements to define which analytes,

subsumed from the users and EU directives extended list, can be incorporated into the biosensors accounting for constraints of sensitivity and selectivity.

The second project step and second iteration of collaborative review of end users requirements was concerned with the mechanical layout, the power consumption, space and geometrical limitations on board sea buoys, electronics/communication systems and the water sampling system.

The third iterative step was devoted to the communication module, with two relevant data flow points to be considered in relation of interoperability of the electronics and communication, so that the MARIABOX may work also using other biosensors than those developed in the project itself. This step included an action to coordinate the development efforts in the area of interoperability and data exchange standardization between the nine EU funded projects on autonomous biosensor based analytical devices for marine water monitoring.

Biosensors generalities

In the MARIABOX project, 8 new biosensors will be developed: (a) 4 biosensors for man-made chemicals, and (b) 4 biosensors for micro-algae toxins relevant to shell fish and fish farming. The chemical pollutants defined by the end users for the MARIABOX are naphthalene, PFOS, heavy metals and Camphechlor. The biological target analytes are Saxitoxin and derivatives, Microcystin and structurally related variants, Azaspiracid and Domoic acid.
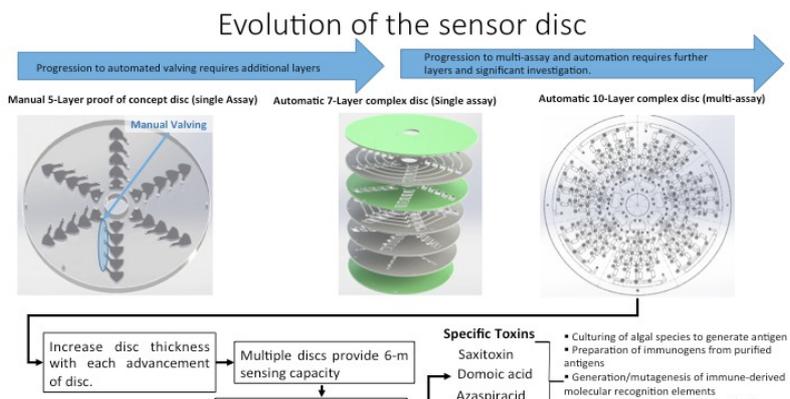
Marine toxin biosensor development

Using carefully strategized recombinant antibody technology, phage display and bio-panning techniques, we will generate antibodies with the required affinities and specificities to the proposed algal toxins. The recombinant antibodies may then be genetically tailored to enhance specificity, sensitivity and stability if required. Additionally, we will employ in-silico based homology modeling to assess antibody-toxin interactions. The antibody-toxin binding region will be identified and this will aid in enhancement of antibody selectivity and specificity. In the MARIABOX project, we are developing mammalian-derived antibodies for these analytes, to achieve the best results we adopted the strategy to covalently couple these toxins to larger more immunogenic proteins (such as Bovine Serum Albumin and/or Keyhole limphet protein) using a spacer arm to ensure no steric hinderance constraints were met. The obtained conjugates were used to produce high affinity polyclonal antibodies using a standard immunization procedure. Currently, the conjugates (analyte-carrier) for each analyte were produced and injected in the host animal (Leghorn variety chicken) for the immunization procedure. Upon completion of the immunization schedule, RNA will be extracted from B-Cells and a recombinant scFv antibody library will be generated. This library will be screened for using antibodies with directed specificity towards the analytes. Characterisation of the antibodies will be performed using Surface Plasmon Resonance (SPR) and ELISA experiments under different operating conditions.
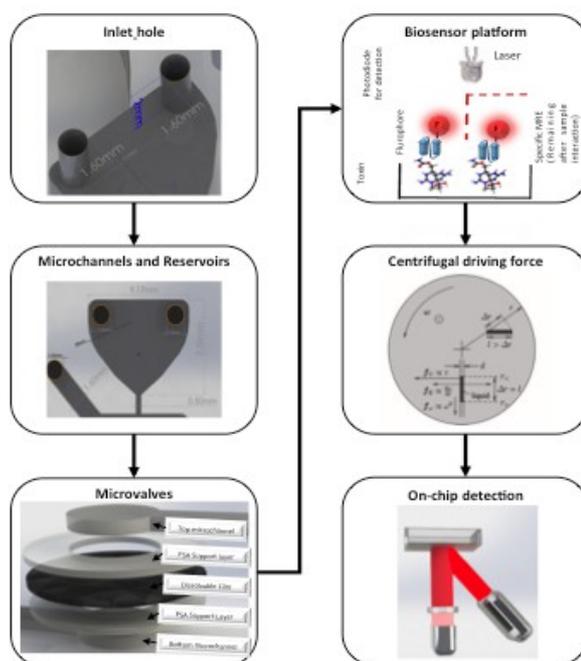
Figure 1: Schematic overview of system design components and considerations involved in platform construction and assay development and integration.

Centrifugal platform prototype development

Several considerations are required prior to development of the final centrifugal platform. Functionality of the disc was assessed by manufacturing a small-scale prototype of the final system for initial optimization studies. The design variables taken into account are shown in **Figure 1** and include; a) inlet holes to facilitate sample loading, b) microchannels and reservoirs, c) microvalves developed by layering PMMA and PSA, d) immunoassay integration, e) assay execution and progression via centrifugal force, f) on-disc detection using MRE's labeled with flurophore and photodiode detection. The optimization and integration of these stages is dependent on several design parameters for assay functionality, including laser cutting numerous reservoirs/channel sizes and widths to facilitate required flow-rates. Design of valving systems including pneumatic, hydrophobic and active valving is under investigation alongside for size. The evolution of the disc design is outlined in **Figure 2**. This includes prototype assessment using the algal toxins as preliminary targets.

Figure 2: Centrifugal sensing platform assembly and design for multiple analytes and long-term monitoring.

Heavy metal biosensor development

A different approach will be used for the development of the heavy metal biosensor. Due to their atomic structure, it is impossible to use the same strategy adopted for the other analytes. A bioinformatics approach was used and from the amino acid sequence of protein domains, that are able to bind heavy metal, several peptides have been designed. In the peptide design, additional requirements are considered, such as peptide stability and presence of amino acid residues useful for the heavy metal detection. The produced biomolecules (antibodies and peptides) will be labeled with commercial dyes with spectral characteristics (excitation and emission wavelength) in the visible region of the light spectrum (Biotium CF488 nm). They will be characterized by advanced biophysical methods using circular dichroism, Fourier-transform infrared spectroscopy and steady-state and time-resolved fluorescence spectroscopy with regard to their stability and function under different operating conditions. Advanced nano-structured surfaces will be produced for the covalent immobilization of the conjugate carrier that exposes antigens that will be bound by the labeled antibody. An optical-based method and/or a chemistry-based method will be used for the activation of surface groups of silicon wafers that will react towards the SH and/or NH residues present in the biomolecules. The biosensors developed will be a simple LED, powered by a low voltage battery, as excitation source with spectral characteristics that will match with optical properties of the fluorescent dye used for the antibody derivation (488 nm as excitation wavelength).

Marine environment challenges

The most critical marine environment challenge is the corrosion that could be expected due to the environment in which the system will be deployed. Thus, the system will be encapsulated in a box that ensures the complete isolation of the electronics and other elements susceptible to corrosion. This isolation will be imperative to guarantee successful working/operating conditions during the analysis process. These conditions, including; the temperature, humidity and light intensity must be stable during the entire process and similarly during the analysis. This box is also essential to keep the biosensors in optimal conditions while installed in the marine environment. The biosensors are developed in discs that are stored inside a cylindrical chamber which is insulated and temperature-controlled for their conservation.

Another important challenge is the mechanism that must be developed to take a water sample and prepare it for analysis; this process is hindered with the possibility of marine fouling.

Interoperability

MARIABOX aims to be interoperable both at sensor and server level, therefore different alternatives have been analysed, together with the other ongoing projects. OCG standards offer protocols both at sensor level (PUCK + Sensor ML) and at server level (SOS) that could be the solution to implement a system that can be connected to an external data collector (Ethernet interface) or in which other biosensors, developed in other projects, could be connected (RS-232 or Ethernet interface).