

MARIABOX an autonomous sea water pollution monitoring device for natural and man-made pollutants: challenging user requirements and tackling design criticalities

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Abstract

The MARIABOX project, funded by the European Commission (Contract 614088), is developing an autonomous, analytical device based on novel biosensors to monitor chemical and biological pollutants in seawater. The device will be suitable for installation on free floating devices, buoys, ships, or to be used as a portable instrument. The main, high-level user requirements for the system are for the device to be highly sensitive, portable and capable of repeating measurements over a long time, allowing permanent deployment at sea.

All of these 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

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

1. INTRODUCTION

The European Union considers that seas and oceans are a relevant economical resource for Europe and therefore included marine research in the FP7 Research program and more recently Horizon2020. Monitoring the water quality of the seas and ocean is an essential part of the European Maritime strategy and agenda with 9 projects currently ongoing on the development of novel autonomous sensing devices for marine water quality.

The protection of the marine environment is endorsed by the following directives: Article 16 of the Water Framework Directive (2000/60/EC) that sets out the “Strategies against pollution of water” (outlining the steps to be taken),

- Decision 2455/2001/EC that

establishes the First list of priority substances

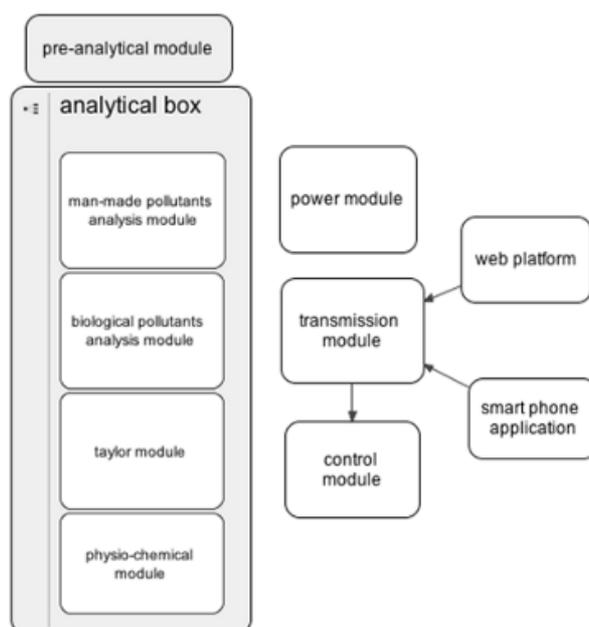
- The Commission Directive 2009/90/EC that lays down the technical specifications for chemical analysis and monitoring of water status.

Additionally, the organic pollutants on which the MARIABOX focuses are listed in Annex 6 of the “Review of organic pollutants found in coastal waters, JRC Marine Strategy Framework Directive - Task Group 8 Report Contaminants and pollution effects -April 2010”.

The MARIABOX- “Marine environmental in situ Assessment and monitoring tool Box” project, funded by the European Commission (Contract 614088), is intended as an instrument to support the European strategy for the sea and ocean protection and exploitation. It refers to the

above quoted references to develop an autonomous connected analytical device capable of six months of 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 word “Maria” is the plural of the Latin “mar” (sea) and expresses the wide applicability that this system offers in multiple locations where low-cost and real-time in situ analytical monitoring devices are required.



The approach 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 will be 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. 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. Therefore, the maintenance costs are significantly reduced.

MARIABOX follows the concept of modular design. It includes four sensing modules that can either be combined together or can be completely removed if not necessary for the use case scenario:

MARIA chemical-POD: for monitoring chemical pollutants

MARIA biological-POD: for monitoring biological pollutants (such as Algae toxins)

MARIA tailored-POD: an add-on module for an optional set of biosensors or environmental sensors

MARIA routine-POD: physicochemical routine parameters (Temperature, pH, Conductivity and dissolved Oxygen)

An advanced bio-sensing approach based on the use of nanostructured silver nanoparticles will be developed in the frame of MARIABOX. Biosensors will be developed for 5 man-made chemicals

and for 4 categories of micro-algae toxins relevant to shell fish and fish farming. The novel MARIABOX biosensors will contribute to new standards for environmental analysis

2. FROM CONCEPT TO USER REQUIREMENTS AND FUNCTIONAL DESIGN

Initially, the project underwent a first phase of detailed analysis of user requirements.

These had to undergo iteration with collaborative discussion with expert in biosensors to define for which analytes, subsumed from the users and EU directives extended list, the biosensors can be built within the needed 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 and communication systems, the water sampling system.

This second step had now to take also into account the biosensors functional and operational constraints, such as the temperature working range, the storage conditions (temperature, humidity) for long term measurements campaigns, the choice of the source of light for biosensors.

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 biosensors based analytical devices for marine water monitoring.

2.1 User requirements, choice of analytes

Identifying user requirements was a significant task, allowing the development of a system with high value and immediate applicability. Initially, the possible MARIABOX end-user categories were identified and then the participating end-users were asked to provide their requirements for the system, through a targeted questionnaire. Several categories have been identified as the main MARIABOX end-user categories: Governmental agencies, responsible for marine water quality monitoring, Research institutes, oil/gas companies. Additionally, the aquaculture industry and NGOs have been identified as possible users of the MARIABOX measurements and open data.

In the framework of MARIABOX project, 9 biosensors will be developed: (a) 5 for man-made chemicals, and (b) 4 for micro-algae toxins relevant to shell fish and fish farming (Table xx). The pollutants selected for the MARIABOX demonstration are naphthalene, PFOS, Hg, Cd and Camphechlor, standardization The specific target analytes selected are Saxitoxin and derivatives, Microcystin and structurally related variants, Azaspiracid and Domoic acid. The novel MARIABOX biosensors will contribute to new standards for environmental analysis.

Table xxx. List of chemical pollutants and algal toxins to be detected by MARIABOX

Chemical pollutants for which biosensors will be developed

Category	Analyte
PAHs	Naphthalene
Fluorinated surfactants	PFOS
Heavy metals	Hg
Heavy metals	Cd
Pesticides	Campechlor

Algal toxins for which biosensors will be developed

Algae	Risk	analytes
Dinoflagellate, Alexandrium	Fish kills, Contaminated Shell fish eating causes human toxic	Saxitoxin and derivatives

Cyanobacteria – Microcystis, Nodularia, Anabaena, Oscillatoria, Nostoc.	syndrome PSP Found in contaminated shellfish (bivalve mollusks particularly) and fish, kills livestock and is a high risk to humans from contamination of drinking water and eating contaminated shellfish	Microcystin and structurally related variants.
Azadinium spinosum	Contaminated fish eating causes human toxic syndrome AZP	Azaspiracid
Pseudo nitzschia sp.	Contaminated fish eating causes human toxic syndrome ASP	Domoic acid

2.2 Biosensors

Biosensors are defined as compact analytical devices that incorporate a biological derived sensing element (molecular recognition element, MRE), integrated within or intimately associated with a physicochemical transducer. In frame with the MARIABOX project, biosensors able to detect in the in real-time and with high specificity chemical pollutants and microalgal toxins molecules will be developed.

Once identified the target analytes we assessed the feasibility of the biosensor development for chemical pollutants Naphthalene, Perfluorooctaic acid (PFOA), Camphechlor and of the biological pollutants: micro-algal toxins Saxitoxin (and its derivatives), Microcystin (different structural variants), Azaspiracid and Domoic acid. We selected the best MRE for the biosensor development and antibodies were preferred choice due to their high selectivity and stability in operative condition.

In the MARIABOX project, we are developing antibodies for these analytes, to achieve the best results we adopted the strategy to chemically modify their structure with a bi-functional linker in order to obtain an amino reactive derivative. A retro-synthetic analysis for each compound was done. The presence of the amino reactive group in the structure of the derivative compound allow to covalently attach the molecule to an immunological protein carrier (such as Bovine Serum Albumin and/or Glutamine Binding Protein). The obtained conjugates were used to produce high affinity polyclonal antibodies using a standard immunization procedure. At the moment the conjugates (analyte-carrier) for each analytes were produced and injected in the host animals (two rabbits for each analyte) for the immunization procedure.

At the end of antibodies production mono-specific antibodies will be purified and the specificity against the analytes will be evaluated in Surface Plasmon Resonance (SPR) and ELISA experiments under different operating conditions

A different approach will be used for the development of heavy metal biosensor (cadmium and mercury). Due to their atomic structure 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 peptides design additional requirements are considering, such as peptide stability and presence of amino acid residues useful for the heavy metal detection.

The produced biomolecules (antibodies and peptides) will be labelled 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.

We will produce advanced nanostructured surfaces for the covalent immobilization of the conjugate carrier that expose antigens that will be bind from 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 characteristic that will match with optical properties of the fluorescent dye used for the antibody derivation (488 nm as excitation wavelength).

During the project the biosensors interface will be defined and developed. In particular, the analytical performance of the biosensors will be evaluated in terms of dynamic range of the response, sensitivity, reproducibility, selectivity, response time, stability in operational and storage conditions, sample matrix influence, life-time and spectral response to the analyte binding.

Furthermore different parameters will be considered during the project as well as the temperature working range and the storage conditions such as temperature and humidity for long-term measurements campaigns. At the end the results obtained using the developed biosensors will be compared with standard analysis methodologies present on the market.

Once the analytes have been selected, it is time to start defining the complete MARIABOX architecture, ensuring the correct operation of the system while deployed on a real environment. From a mechanical point of view, there are several challenges that must be considered during the design phase.

The most critical one is the corrosion that could be expected due to the marine 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 also key to guarantee the working operation conditions during the analysis process, since these conditions must be stable during all the process and the same in all the analysis, that is, the temperature, humidity and darkness must be constant and the same in each measurement. This box is also essential to keep the biosensors in optimal conditions while installed in the real environment. The biosensors are developed in discs that are stored inside a cylindrical chamber which is insulated and temperature-controlled for their conservation.

Other important challenge is the mechanism that must be developed to take a water sample and prepare it for the analysis. The salinity and possible objects or organic matter that could obstruct the system must be considered during the design process. According to this, the system must include cleaning mechanisms and also a waste tank to avoid any contaminant leak to the environment.

The electronics (MARIABOX-CORE) must be designed in a modular way to allow only the connection of the required modules. This flexible solution makes possible to take advantage of the infrastructure and resources already available in those scenarios where will be installed, like the power supply or the communication system.

The power supply must ensure the system autonomy till the discs stored in the cylindrical chamber must be replaced. The correct estimation of the power consumption will guarantee the success of the project, even more in a scenario where the system dimensions are limited by the infrastructures in which must be installed. The power system will integrate renewable energy solutions to recharge the batteries that feed up MARIABOX and also some energy efficient algorithms to manage the power consumption.

2.3 From users interoperability concept to detailed data formats

MARIABOX system will integrate three different wireless technologies that could be enabled/disabled when required. These technologies have been selected according to user requirements and based on real scenarios needs: WiFi, for short-range communication; 3G, for medium/large-range communication, and satellite, for medium/large-range communication if there

is not 3G coverage. The data collected after the analysis is sent from MARIABOX-CORE to a central server (MARIABOX-NET) or to an authorized mobile device (MARIABOX-MOB).

MARIABOX aims to be interoperable both at sensor and server level, therefore different alternatives have been analysed, together with the other ongoing projects in OCEAN call, in order to ensure the interoperability with third-party solutions. 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). shows the communication architecture and the required interfaces that MARIABOX system aims to implement to ensure the data interoperability.

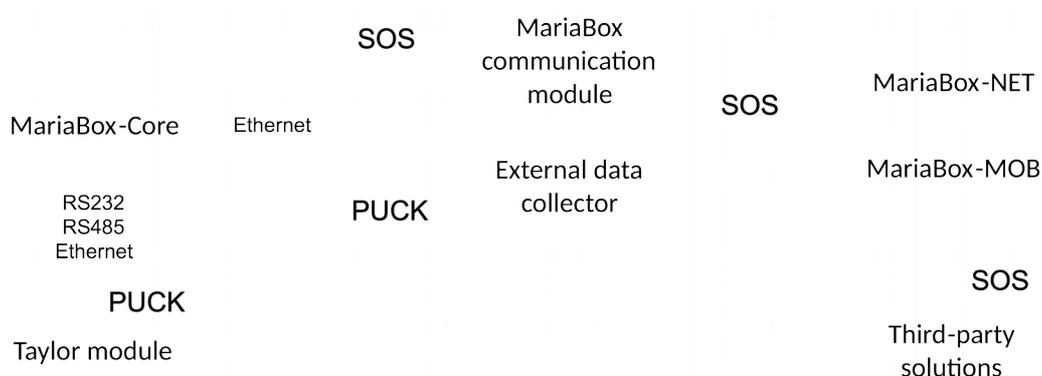


Figure. MARIABOX communication system – Data interoperability

4. CONCLUSIONS

The scientific developments at the core of the project are novel biosensors.

Nevertheless the entire MARIABOX system is confronted with a host of implementation challenges for which a detailed analysis of risk and benefits of the branching possibilities is required.

Beyond the scientific and engineering progress, the use of such a device is worthy only because of the improved and timely, widespread availability of the data and information related to environmental pollution, that will be exploited for aquaculture, environmental and health protection actions by the civil authorities, for citizen awareness and information, for research, providing a wide distributed ubiquitous network of sensors capable of providing real-time data and to support an improved spatiotemporal picture of the trends of the environmental variables considered.

Without the capability of almost real-time data distribution, the benefit of such a device will not supersede existing practices in marine pollutant monitoring.

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