ID37- 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 "MARIne 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.

MARIABOX is designed in a modular way in order to be able to include additional biosensors or novel improved biosensors so to be ready to satisfy the evolving market requests. It consists of four sensing modules that can either be combined together or each of them can be removed if not necessary for the use case scenario or substituted with another one useful for other analytes.

The four analytical modules are:

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)

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 specific biological target analytes are Saxitoxin and derivatives, Microcystin and structurally related variants, Azaspiracid and Domoic acid.

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 allows 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. 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. Besides, 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.

Advanced nano-structured surfaces will be produced for the covalent immobilization of the conjugate carrier that exposes 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).

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