Live, autonomous biosensor modules for environmental monitoring (SENSEI)

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ABSTRACT

In SENSEI, we have set out to prove the concept of a novel generic sensing methodology, integrating live sensor cells into a miniaturized hardware platform. The bacterial sensors were genetically engineered to bioluminesce in the presence of trace amounts of 2,4-dinitrotoluene (DNT), an indicator for the presence of buried landmines. The signal emitted by the bacteria, integrated into the biosensor module (BSM) in an interchangeable cassette, was analyzed by a specially designed optoelectronic circuit. The BSM successfully detected low DNT concentrations in soil, and a methodology was developed to quantify DNT concentration using a parallel measurement of an integrated standard.

Keywords: Biosensors; optoelectronics; landmines; keyword; environmental monitoring

1. INTRODUCTION

Importance and relevance

SENSEI is aimed at creating a novel quantitative chemical sensing methodology aimed at revolutionizing environmental monitoring, that will be made possible by several unique technological advances. The underlying principle of this methodology is an exploitation of the versatile capability of bacteria to continuously monitor their microenvironment, and emit readily measurable optical signals in response to the presence of specific compounds or to general environmental threats. The project employs bacterial strains that emit a bioluminescent signal in response to the presence of a chosen specific target material.

The project will provide a proof of concept for performing a quantitative assessment of the presence of the selected target materials in the sample to which the bacteria were exposed. The project is conducted in two interlaced tracks:

(i) Development of the procedure for extracting the quantitative information relating to the concentration of the target materials in the sample.

(ii) Construction of a miniaturized field-deployable biosensor module (BSM), designed to function as an element in a sensor network for standoff monitoring and mapping of environmental hazards. The BSM harbors encapsulated live bacterial sensor cells, acting as the core sensing element. The module detects and processes the biological signal, composes a digital record that describes its findings, and transmits the latter to a remote receiver. The module is an autonomous self-contained unit that can function either as a standalone sensor, or as a node in a sensor network.

The project constitutes a proof of concept for an innovative, generic, comprehensive and versatile chemical sensing methodology that can be deployed outdoors in large numbers.

Breakthrough character

(i) The project addresses the challenge of constructing an optoelectronic module in which live bacteria function as the core sensing element, acting in unison with a conventional optoelectronic circuit to perform chemical sensing, and- moreover- be fit to operate autonomously outdoors.

(ii) The project demonstrates for the first time the feasibility of performing quantitative chemical sensing by exploiting the capability of genetically engineered bacteria to sense the presence of a specific target material in their immediate microenvironment.

Main results

A methodology for performing quantitative chemical sensing by live bacteria as the core sensing element was demonstrated, overcoming the problem introduced by the inherent variations in measured signals emitted by different batches of bacteria, even when the measurements are carried out under the same exact conditions.

2. STATE OF THE ART

For many decades, the main and most prevalent approach for monitoring the presence of deleterious compounds in

the environment has been chemical analysis. This approach, however, while essential for regulatory purposes as well as for understanding pollution sources and processes, is almost universally limited by the need to collect a sample in the field, and transport it to a fully (and expensively) equipped central laboratory, where trained technical personnel employ an array of sophisticated analytical instrumentation. The most problematic issue inherent in this approach is not necessarily its high cost and complexity, but rather the temporal discontinuity between sample collection and availability of the results; when the target chemicals are unknown, days and weeks may pass before reliable information is available to field personnel and regulators alike. There is thus an acute need to close this spatial and temporal gap, and implement complementary in situ technologies that will report in real time, on the wellbeing of the studied environment.

The SENSEI technology aims to generate an in situ technology that will quantitatively report the presence of target materials in the monitored substances. The biosensing bacteria which act as the core sensing elements [1], are effectively minuscule "biochemical laboratories" which can be adapted by genetic engineering to sense any given target, and report their findings by emitting a bioluminescent signal. Hitherto, sensors based on bacteria were mostly limited to detecting the presence of the target. The technology described herein will have an additional feature – quantitative sensing. This technology has the potential to manifest a tremendous versatility, since in principle it can be adapted to sense any material. Moreover, the optoelectronic circuit in which the bacteria are embedded is the same, regardless of the specific material it is engineered to sense and quantify, allowing wide deployment of the systems that employ this technology.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

SENSEI strives to create a generic and comprehensive in situ chemical sensing technology that can be deployed simultaneously in many locations, in particular outdoors. The core sensing elements of the technology are live bacteria that are genetically engineered to respond to the presence of a target compound in their microenvironment by the emission of a bioluminescent signal. The bacteria are embedded in an optoelectronic circuit that is installed in a module designed to operate autonomously outdoors.

- Breakthrough at the fundamental level – SENSEI constitutes a merger between synthetic biology, optoelectronic engineering, materials engineering and computer engineering. It strives to combine the bacteria with optoelectronic circuitry, operating in unison as a unified digital entity. Combining a biological entity which operates subject to one set of constraints, with optoelectronic circuitry which operates subject to an entirely different set of constraints is a breakthrough in its own right. In particular, by enlisting cutting edge elements of 3D printing, the outcome is expected to be a rugged and robust system, geared to be operated by "non-specialist" users out of the laboratory.
- Breakthrough at the functional level – The technology is designated to function in two modes:
  (i) Detection of the presence of very minute traces of the target compound in the given sample. The SENSEI technology provides an unprecedented level of versatility. In principle, the sensing bacteria can be genetically engineered to respond to any target compound.
  (ii) Assessing the quantity of the target compound in the given sample. To our knowledge, the capability of employing sensing bacteria for quantitative assessment of the presence of the target compound in the given sample in situ is a unique feature of the SENSEI technology, and is demonstrated here for the first time.
- Breakthrough at the network level – The technology developed within the SENSEI framework provides a unique capability for constructing a multi-agent network of chemical biosensors that can be rapidly deployed and report their findings to the network control centre in real-time.

4. PROJECT RESULTS

SENSEI aims at providing the basis for a generic and versatile chemical sensing methodology, intended to serve as the underlying platform for a comprehensive environmental monitoring technology. The first phase of the project was devoted to address three fundamental aspects of the methodology that constitute necessary conditions for transforming it into a viable technology:

(i) Integration of live sensor bacteria into the optoelectronic circuit that measures and processes the bioluminescent signal they emit, creating the conditions for the bacteria and circuit to operate in unison.
(ii) Extend the concept of bacterial chemical sensing from the domain of a simple detection of just the presence of the target in the sample, to a quantitative evaluation of its concentration.

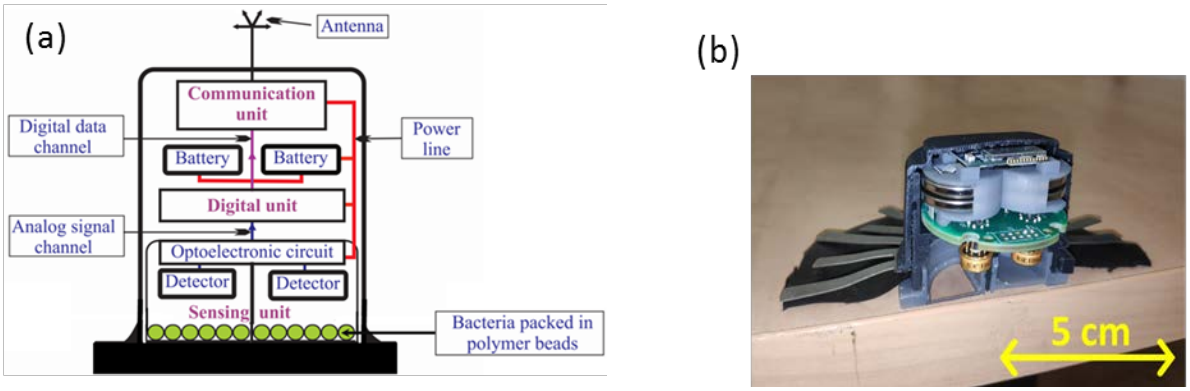

Fig. 1. The biosensor module. (a) A schematic illustration; (b) A module cut into two, to allow a view of the internal components.

(iii) Address the challenges involved in in situ operation of the prototype biosensing module outdoors, where the examined specimen is not isolated from its surrounding environment.

Our starting experimental setup was a single-channel bacteria-based biosensor module (BSM), adapted to be the platform for the SENSEI experiments, illustrated schematically in Fig.1. The BSM houses the bacteria in a special cassette attached to its bottom, allowing the bacteria to be in contact with the soil underneath its footprint. The cassette is loaded with 1.5 mm alginate beads, in which the bacteria (ca. 1.5 X 10^5 per bead) are encapsulated together with water and nutrients [2].

As a benchmark, the BSM was loaded with sensor bacteria genetically engineered to respond to DNT (2,4-dinitrotoluene [3,4]), a volatile manufacturing by-product of TNT that serves as a signature chemical for the presence of buried landmines [5]. A representative example of the BSM's response to different DNT concentrations in the soil underneath its footprint is presented in Fig. 2. A second demonstration of the BSM operation is presented in Fig. 3, where two BSMs were placed over lumps of DNT buried in the sand. As can be clearly seen, both BSM units detected the presence of the buried DNT.

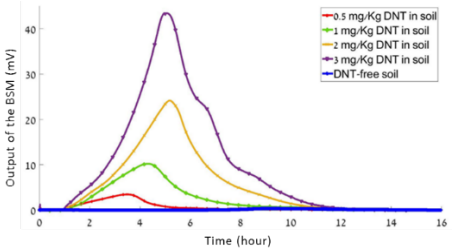

Fig. 2. Dose-dependent response of the BSM to different concentrations of DNT in soil.

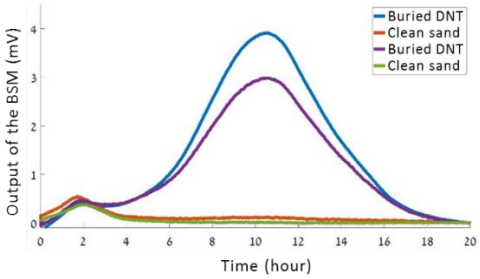

Fig. 3. Outdoor operation of the BSM. Two BSMs were placed over buried DNT (50 g of DNT under 8 cm of sand), and two BSMs over DNT-free sand.

These experiments are a sampler of a wide survey carried out for establishing the possibility of a viable biosensing technology for outdoor environmental monitoring. An important additional element that needed to be augmented was the capability of quantifying the target concentration in the sample. To our knowledge, hitherto, bacterial-based sensing was limited to the detection of the presence of the target material and did not assess its quantity.

Within the framework of SENSEI, we were able to demonstrate the possibility of quantitative sensing based on bioluminescence bacteria, and outline a roadmap that will lead into making it a viable technology. The basic hurdle that had to be removed is the variability of the bioluminescent signal emitted by different batches of bacteria. To solve this, we have performed a standard measurement in parallel to the sensing operation. The ratio between the two measurements remained constant, irrespective of either the bacterial batch or other parameters that differed between assays. Preliminary results are presented in Fig. 4, which demonstrates that the bioluminescent signal varied significantly between the different experiments, while the "standard ratio" (SR) between the bioluminescent signal of the sample and that of the standard remained constant.

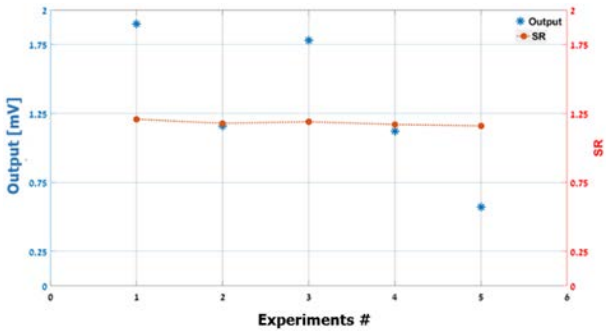

Fig. 4. Typical measurement in which the SR (in red) remains stable despite the large variety of the absolute output of the BSM (in blue) for the same concentration of DNT (30mg/L).

This achievement opens the way for quantitative assessment of the target material in the examined sample. Exploring this possibility is demonstrated in Fig. 5, in which we present the calibration curve for measuring the concentration of DNT in solutions.

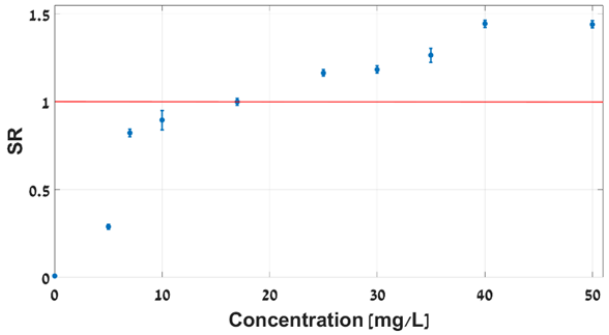

Fig. 5. Quantitative measurement of DNT using the SR. The mean value of the SR and its standard deviation (denoted by the error bars) is presented. The red line denotes the value SR=1, equal to a DNT concentration of 17 mg\L.

5. FUTURE PROJECT VISION

5.1 Technology Scaling

Three complementary components need to be considered in the design of a biosensor-based environmental monitoring network: hardware, software and bioware. SENSEI has made considerable progress in all three aspects, but in order for the developed technology to be brought forward in the TRL ladder, additional headway needs to be made in all three directions. To reach at least TRL6, the following steps will be taken:

- Construct an advanced version of the BSM, designed to process in parallel a set of samples, assayed by a panel of different bacterial sensors
- Design and implement communication hardware and protocols for turning the individual SENSEI modules into a functional network
- Construct bacterial sensor panels tailored towards specific applications (e.g. agricultural, environmental quality, security).
- Develop a drone-based technique for deployment and collection of the modules (Fig. 6), and preparing them for repeated use after the reloading of fresh sensor cassettes.

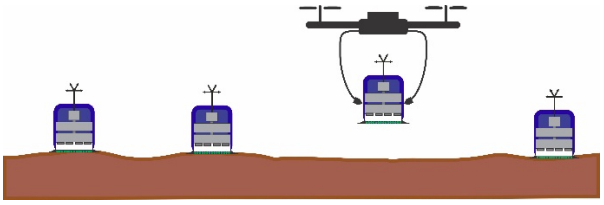

Fig. 6. Schematic illustration of the envisaged drone-mediated BSM deployment and collection.

5.2 Project Synergies and Outreach

The main strengths of the current SENSEI partners are in applied physics/engineering on the one hand and synthetic biology/environmental sciences on the other hand. The SENSEI Phase 2 consortium will have to include additional fields of expertise, including imaging specialists and information technology experts, as well as partners responsible for device production and commercialization. Out of the current ATTRACT Phase 1 projects, RE-SENSE seems to be an ideal partner for Phase 2. Other potential partners will be sought over the next few months, inside and outside of the ATTRACT community.

Envisioned dissemination activities will include a public website and proactive involvement in community outreach activities, with a particular focus on high schools.

5.3 Technology application and demonstration cases

Whole-cell biosensors, packaged in the SENSEI envelope, may provide a simple and cost-effective solution for diverse monitoring applications. In Phase 1, we have proven the ability of the basic module to reliably provide accurate quantitative information on the presence of a target chemical under its footprint. In Phase 2 we will concentrate on soil pollution monitoring, demonstrating two different implementations: (a) an interconnected network of stationary monitors for continuous monitoring of soil quality; (b) a rapidly drone-deployable set of senor modules, to be deposited on demand, for example, in limited access regions. In both cases, the targets to be monitored could include specific pollutants (e.g. pesticides), agricultural nutrients (e.g. nitrates), or overall toxicity/genotoxicity. For each

set of targets, a dedicated panel of bacterial sensors will be employed, and a specific demonstration site will be prepared.

To reach this objective, we envision a partnership that will include research groups across Europe, end-users in the water quality industry, and commercial entities for the production and marketing the developed systems.

5.4 Technology commercialization

As indicated above, the consortium that will be put together in Phase 2 will include at least one entity dedicated to the commercialization of the technology.

5.5 Envisioned risks

The main risk we see is in the public acceptance of the field use of genetically engineered microorganisms. To mitigate this risk, we will introduce molecular modifications that will prevent the bacterial sensors from (a) surviving outside the environment of the monitoring device and (b) transfer antibiotic resistance genes to other organisms. In addition, in our outreach and dissemination activities we will emphasize the non-existent actual risks involved in the use of our sensor microorganisms, as well as our intention to fully abide with all relevant laws and regulations.

5.6 Liaison with Student Teams and Socio-Economic Study

The Hebrew University Faculty of Science and its Business School lead several entrepreneurship programs, in which graduate student teams join forces with a specific research group, providing insights/ideas as well as contributing to the actual research efforts and to the formation of the final product. SENSEI Phase 2 will be integrated into one of these programs, and hopefully to at least one additional program in one of the consortium's partner institutions. A mid-level researcher will be appointed as a mentor to the students and a liaison to their parent program.

SENSEI Phase 2 will gladly contribute to all socio-economic studies initiated by the ATTRACT initiative.

6. ACKNOWLEDGEMENTS

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