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Creation of a fiber optic based biosensor for air toxicity monitoring

Evgeni Eltzov[a], Vladislav Pavluchkov[b], Matan Burstin[c], Robert S. Marks[c,d,e,*]

[a] Department of Environmental Engineering, Faculty of Engineering Science, Ben-Gurion University of the Negev, Beer-Sheva, Israel
[b] Department of Chemistry Engineering, College of Engineering, Sami Shamoon, Ashdod, Israel
[c] Department of Biotechnology Engineering, Faculty of Engineering Science, Ben-Gurion University of the Negev, Beer-Sheva, Israel
[d] National Institute for Biotechnology in the Negev Ben-Gurion University of the Negev, Beer-Sheva, Israel
[e] The Ilse Katz Center for Meso and Nanoscale Science and Technology, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

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ABSTRACT

Most people spend a greater amount of time indoors rather than outdoors, so it follows that it's composition could directly affect occupants, thereby providing a new demand for sensors that will monitor indoor air pollution. In this paper, bioluminescent bacteria were used as bioreporters for the determination of air toxicity. Bacteria were immobilized in 96 well plates with matrices of different types and volumes, and exposed to a selection of model toxic compounds in air. TV1061 strain immobilized in alginate in wells of a microtiter plate showed the best response to a low concentration of chloroform (6.65 ppb), while US permission limits stands now at 50 ppm. This system was then adapted to the end face of a portable fiber optic biosensor, and we predict that one day it could be used as an air pollution alert system in an indoor environment.

1. Introduction

Air pollution is a serious problem in many developed countries. But little attention is paid to air pollution in homes and office buildings and for many years, such environments were assumed free of health damaging pollutants. Recently, the tendency to accept this view has changed. Poor urban air quality may be hazardous and may pose a risk to people in indoor areas [1] inducing many ill effects on health, mainly related to the exposure to low-level air pollutants, generally not identified as a specific cause or compound [2]. Most pollutants encountered indoors are: particulate matter (Pm) including fungal spores; gases, such as ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO), and sulfur dioxide (SO_2); microbial and chemical volatile organic compounds (VOCs); and passive smoke. They differ in their toxic effect (health symptoms) and active concentrations, while these effects (e.g. eye, nose, and throat irritation, headache and fatigue, dry or irritated skin, and breathing problems) are usually referred to as sick building syndrome (SBS). Poor indoor air quality affects workers and also visitors negatively so that work productivity is decreased. Statistics records work performance affected to be as high as 6–9%, the higher value being obtained in field validation studies [3].

It follows that a demand for continuous, real-time, monitoring techniques for indoor air pollution would be useful. Despite the many advantages of conventional methods (e.g. highly accurate and sensitive determination of tested air samples), these applications have many disadvantages. The main disadvantage is the inability to test real-time air, as samples must be collected on-site and tested in a central lab using such tests as gas chromatography (GC), fluorometry or liquid chromatography (HPLC) [4–6]. In most cases the results may take a few days to obtain, while skilled personnel is needed as well as expensive equipment. Smart gas sensing systems, i.e. electronic noses, comprising an array of gas sensors [7–9], quartz-crystal resonators [10] and other sensor techniques may meet these requirements for indoor air monitoring. However, the main disadvantage of all these applications is their inability to measure the biological effects of tested air pollutants for various types of toxicities (e.g. cyto/neuro/geno toxicities or endocrine disrupting effects), owing to the fact that it may be more important to know the effect of indoor pollutants than their actual compositions. Developing biosensors based on living organisms may therefore be found to be of use.

Bioluminescent assays gained increased attention thanks to advancements in genetic manipulation techniques, which offered the possibility to modify non emitting organisms, isolated from different habitats, into both luminescent and specifically responsive reporters [11]. These methods suggested that activation of the reporter luciferase genes will produce a readily detectable light signal that can monitor the bacterial response in real-time, while using simple luminometry (e.g. fiber optic) [12]. Many different biore-

* Corresponding author at: Department of Biotechnology Engineering, Faculty of Engineering Science, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel.
E-mail address: rsmarks@bgu.ac.il (R.S. Marks).

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porter bacteria, each carrying unique stress promoters that activate different regulatory circuits, were created and used for environmental monitoring [13]. Included are the bacterial heat-shock and cold-shock responses to monitor protein damage [14] and the SOS regulatory network involved in protection against DNA damage [15,16]. Despite the fact that there were some studies that used bacteria for air toxicity monitoring (e.g. using bioluminescence [17–19] and electrochemistry [20,21]), most reported applications concerned the determination of water toxicity exclusively.

The aim of this study was to develop a real-time air toxicity monitoring system for indoor environments. The present research was divided into two parts. First were shown the capabilities of the bioluminescence bacteria to sense the presence of the toxic compounds in air and also shown was the influence of the immobilization method on the response of these microorganisms, tested with two different bacterial strains separately and several gaseous chemical compounds. In the second part, this method was applied to the fiber optic mode. The observed simplicity of usage (self-contained) and maintenance makes this application an attractive system for close to real-time monitoring in the indoor air quality monitoring field.

2. Experimental

2.1. Materials

LB Agar Difco (244520) and LB Difco (244620) were purchased from Becton Dickinson. Chloroform (03080521) was purchased from Bio Lab Ltd. Toluene (108883) was purchased from Gadot. Kanamycin (K1377), alginate (A2158) and mitomycin C (M0530) were of analytical grade and purchased from Sigma. All stock solutions were diluted with double distilled water (ddW), and stored at temperatures suggested by the manufacturers' instructions.

2.2. Bacterial strains

Two different *Escherichia coli* strains were used in this study: DPD2794 [22–24] and TV1061 [25], both obtained as a gift from S. Belkin (Hebrew University, Jerusalem, Israel). Each strain harbors plasmid-borne fusions of the specific promoters to a reporter gene. The *recA* [22] promoter in strain DPD2794 activates DNA repair systems due to DNA damage. The heat-shock *grpE* [26] promoter in strain TV1061 is sensitive to metabolic changes, such as with cytotoxic substances. All these promoters are plasmid integrated to the *lux* CDABE reporter operon, which has five promotorless structural genes. These are responsible for both the heterodimeric luciferase units (*lux* A and B) and the synthesis of the luciferase substrate, tetradecanal, by an ATP- and NADPH-dependent multienzyme complex composed of fatty acid reductase, transferase, and a synthetase (*lux* C, D and E) [27]. Strain stocks were stored at −80 °C with 20% (v/v) of glycerol as a cell cryoprotectant additive [28]. The bioreporter strains from stock solution were placed on LB-agar plates (NaCl 5 g × L^{-1}, yeast extract 5 g × L^{-1}, tryptone 10 g × L^{-1}, agar 15 g × L^{-1}) supplemented with 50 μg/mL kanamycin and after growth overnight at 37 °C in a rotary thermo-shaker (Gerhardt, Germany) and were stored at 4 °C for future experiments.

2.3. Growth conditions

Bacterial cultivation prior to measurements was performed in 10 mL LB-medium (NaCl 5 g × L^{-1}, yeast extract 5 g × L^{-1}, tryptone 10 g × L^{-1}) [29] supplemented with 50 μg/mL kanamycin for strains TV1061 and DPD2794. Cells were grown overnight at 37 °C in a rotary thermo-shaker (Gerhardt, Germany) at 120 rpm in the presence of the antibiotic. Cultures were then diluted to approximately 10^7 cells/mL and re-grown in 25 mL LB at 26 °C without shaking antibiotics, until an early exponential phase (OD_{600} of 0.2) as determined by an Ultrospec 2100 pro spectrophotometer (Amersham, England).

2.4. Immobilization procedures

2.4.1. Fiber optic

The harvested cells were mixed 1:1 with a filter-sterilized 2% (w/v) low viscosity sodium alginate solution. Multimode optical fibers, PUV 400 BN (CeramOptec, GmBH, Bonn), were used in these experiments. They present a pure silica core diameter of 400 μm, with a refractive index of 1.4571 (at 633 nm) and a cladding diameter of 440 μm, with a refractive index of 1.4011 (at 633 nm). Their black nylon jacket was stripped away from a 1-cm long optical fiber tip which was then used for the immobilization of bioluminescent cells [30]. The 1-cm optical fiber tip was first exposed (for a few seconds) to the bacterial alginate suspension, and then dipped (for a few seconds) into a sterile 0.5 M calcium chloride solution, thus entrapping the bacteria onto the fiber within a hardened calcium alginate matrix. Repeating these steps thickened the ad-layer, thus increasing the number of bacterial sensor cells attached to the optical fiber transducer [31]. Six to seven layers were shown to have been the optimum number of layers when water was tested [32]. But for air toxicity measurement a new optimum of layer numbers was determined. Thereafter, the optical fiber, with immobilized bioluminescent bacteria at the end tip, was ready for monitoring of the various contaminants in air and was used immediately after preparation.

2.4.2. 96 well plates

As in the fiber optic immobilization procedure, harvested cells were mixed 1:1 with a filter-sterilized sodium alginate solution (diluted to approximately 3.6 × 10^6 cfu/mL) and placed in different volumes (n = 3 for each volume), 50, 75 and 100 μm^3, into 96-well microtiter plates (Dynatech). Then equal volumes of a calcium chloride solution were added to the wells. Similar volumes of a liquid bacterial suspension were placed in the same plates. For the agar based immobilization a suspension of agar (Agar-agar, Sigma, St. Louis, Mo, USA) in sterile deionized water was heated to 100 °C. Then the solution was cooled to 40 °C and inoculated with a suspension of reporter *E. coli* cells in LB medium to get the same concentration as in the alginate case. Then the prepared solution was placed in the same volume into 96 well plates.

2.5. Instrument set-up

We have previously designed a field-operable fiber-optic photodetector device [32]. In order to monitor toxicity in air, the device was modified by Neobionics Ltd., Israel, so as to include the components allowing such a feature (Fig. 1). The original instrument set-up was placed in a light-tight box. To prevent damage to the photon-counting unit by environmental light, a manual shutter (71430, Oriel) was placed in front of the detector (Fig. 1B3). To move the slide shutter, a workshop-made lever was placed outside the box (Fig. 1C). The output signal, in analog measurements, was the mean value of the signals that included a.c. components (pulses) generated after multi-anode magnification in the photomultiplier tube. A Hamamatsu HC135-01 PMT Sensor Module was used for bioluminescence measurements (490 nm), combining the sensitivity of a photomultiplier tube with the intelligence of a microcontroller (Fig. 1B1). The detector was optimized to the blue light region and included a 21-mm diameter active area convenient to gather light radiation without any optical focusing elements [32]. Several components exhibiting various features were then added to this device as follows. A special chamber for air toxicity monitoring was created (Fig. 1). Bacteria were immobilized on