Talanta 132 (2015) 583–590

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

On-line biosensor for the detection of putative toxicity in water contaminants

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ARTICLE INFO

Article history:
Received 22 June 2014
Received in revised form
17 September 2014
Accepted 20 September 2014
Available online 13 October 2014

Keywords:
Bioluminescence
Sensor
Water pollution
Flow-through monitoring

ABSTRACT

Potential threat on drinking water requires monitoring solutions, such as the one proposed herein, as a real-time, wide ranged, water monitoring system to detect the presence of toxicants in water. We studied the role of a selected number of parameters affecting performance and, thus, improved the prototype into an optimized next-generation device, resulting in enabling increased measurement duration, coupled with increased sensitivity. The chosen parameters in question were the peristaltic flow system, the fiber probe matrix stability through a re-design of the fiber probe holder and flow unit cell, as well as the modulation of bacterial medium concentration to increase bioreporter performance while keeping biofouling in check. Measurements were made with spiked samples and validated with polluted field-collected samples.

1. Introduction

Increasing population and activities thereof, produce large varieties and quantities of waste chemicals [1], which oftentimes affect drinking water quality [2–4]. In order to safeguard our water supplies, it must be monitored constantly to prevent its contaminated form from reaching the consumers [5–7], while being diverted to processing centers for its remediation.

There are two main forms of water monitoring. One requires the tester to go to the field and make an on-site test or alternatively taking the sample to an accredited test laboratory. The main disadvantage is that the user must go to the place in question. However, the advantage of the first possibility is that the dispatchable equipment includes disposable elements and the answer is quasi-immediate enabling a response. The second form involves use of a continuous monitoring system, with near real-time monitoring, that will give the user continuous feed on the particular point, where the tests are being conducted, such as a chosen river site, which is advantageous as only infrequent human dispatch would then be required. However, the sophisticated mechanics involved complicates the overall system from a number of engineering parameters.

There are two main analytical categories in monitoring water [8], the classical chemical chromatographic ones and the bioassay-based ones. The former (HPLC, GC, GC/MS [9]) is aimed at both identification and quantification. Their disadvantage is their specificity: an unfamiliar toxicant will not be detected. In addition, these are costly and require both sophisticated instrumentation as well as qualified personnel. They are nonetheless very useful and remain today the workhorse of the chemical identification industry. On the other hand, bioassays assess a potential toxic effect on a living organism, however, they lack the ability to measure a specific concentration or even provide most of the times an identification of the monitored toxicant. Despite these disadvantages, those sensing systems have one great advantage: the toxicant monitoring range is very broad [8,10–14]. In this particular area, bacterial-based bioassays, have shown promising applicative results. These consist in using genetically modified bioreporter organisms that generate light as a response to toxic compounds (see Section 2.2).

Bioassays using bacterial bioreporters are used either in their classical liquid-phase bacterial suspension [15–17] or immobilized in biosensor systems [18–20]. In both cases, the tested water

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http://dx.doi.org/10.1016/j.talanta.2014.09.032
0039-9140/

samples must be disposed of via a septic step according to licensed (MLI or II) laboratory regulations,, so as to prevent contamination of the environment, which is protected by stringent regulations. Disposable bioassays are usually autoclaved, although one may imagine chemical neutralization could also be used, while immobilized-phase on-line biosensors require asceptization of the test effluent, despite the fact that one can create stable immobilized probes with virtual non-leaching of the said bioreporters [10,13,20], while still retaining good levels of sensitivity.

The aim of this study was to improve on some of the limitations found in the earlier system [13]. It was then discovered that the bacterial on-line system required that the user provide growth medium continuously, so as to sustain the immobilized bacteria in a continuous stream of water. Indeed, the signal improved, however, an increase in biofouling occurred, which could affect the overall sensor performance. Therefore, we checked two types of such growth media at different concentrations so as to obtain that concentration of either growth medium, which is really necessary to reduce increased biofouling potential while helping the system increase its performance. We also then noticed that increased sample flow rate had a detrimental mechanical effect on the hydrogel structure that was used to entrap the bacteria, thus we designed herein, a protective proprietary fiber optic probe flow cell. This study therefore looked at improving both sensor sensitivity and increasing measurement time, while retaining simplicity of operation, size, and real-time monitoring capability.

2. Materials and methods

2.1. Chemicals

Tryptone (T7293), Yeast Extract (92144), NaCl (S7653), alginate (B25266), $CaCl_2$ (C1016), p-chlorophenol, copper sulfate (209198), kanamycin (K1377), and ampicillin (A0797) were obtained from Sigma. All stock solutions were diluted with distilled water (dW) and stored at temperatures as suggested by the manufacturers' instructions.

2.2. Bacterial strain

The bioluminescent *Escherichia coli* bioreporter strain TV1061 [21] used in this study is sensitive to metabolic changes, such as with cytotoxic substances. It harbors plasmid-borne fusions with a specific heat-shock *grpE* promoter adjacent to the *luxCDABE* reporter operon, whose activation is detected by light emission (bioluminescence). The *Lux* operon has five promoterless 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) [22]. Strain stocks were stored at −80 °C with 20% (v/v) glycerol as a cell cryoprotectant additive [12]. Stock bioreporter strains 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^{-1} kanamycin and, thereafter, incubated at 37 °C in a rotary thermo-shaker overnight (MaxQ 4450, ThermoScientific, USA). These were then stored at 4 °C for future experimentation.

2.3. Bacterial growth

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}) supplemented with 50 μg mL^{-1} kanamycin for TV1061. Cells were grown overnight at 37 °C in a rotary thermo-shaker (MaxQ 4450, ThermoScientific, USA) 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 and without antibiotics, to an early exponential phase (OD 600_{nm} of 0.2) as determined by an Ultrospec 2100 pro spectrophotometer (Amersham, England).

2.4. Fiber optic probe bioreporter immobilization procedure

The harvested cells were mixed 1:1 (v/v) with a filter-sterilized 2% (w/v) low viscosity sodium alginate solution. Multimode optical fibers, PUV 400 BN (CeramOptec, GmBH, eramOptecBonn), 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 proximal tip, which was then used for the immobilization of the bioluminescent bioreporter bacteria [23]. 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 [20], thus entrapping the bacteria onto the fiber proximal tip within a hardened calcium alginate matrix. Six to seven layers have been shown to be the optimal number of layers when previously tested [13]. Thereafter, the optical fiber probe, with immobilized bioluminescent bacteria at its tip, was immediately used after preparation for experimentation (Fig. 1C).

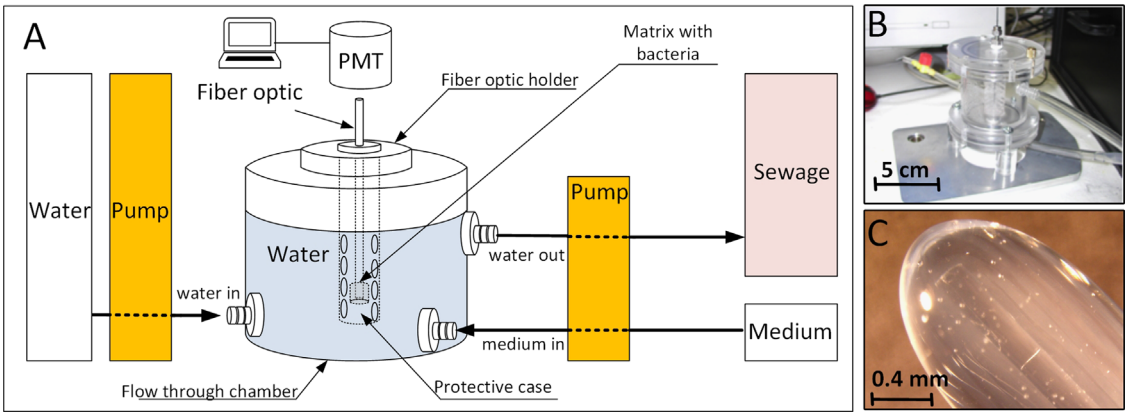

Fig. 1. A. Scheme of the perspex "protective" flow unit holding the fiber optic probe and its connected peristaltic pumps; B. Photo of perspex flow unit; C. Calcium (2% v/v)-polymerized alginate with six adlayers forming the fiber optic probe.