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Online Detection of Metals in Environmental Samples: Comparing Two Concepts of Bioluminescent Bacterial Biosensors

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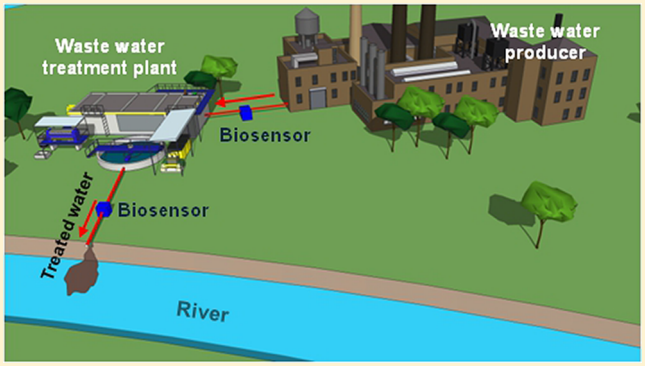
ABSTRACT: In this study, we compared two bacterial biosensors designed for the environmental monitoring of metals: Lumisens III and Lumisens IV. These two biosensors are based on the same bacterial sensors (inducible or constitutive bacterial strains) but with a different conservation mode. The results showed that the biosensor Lumisens III using immobilized cells in agarose hydrogel, allowed to detect artificial mercury contaminations on the limited period of 7 days in laboratory conditions with a reproducibility of 40%. With environmental samples, bioluminescence of the immobilized bacteria inside the biosensor was strongly limited by the environmental microflora because of the lack of oxygen, limiting the use of the biosensor to 2 days. The biosensor of the last generation, Lumisens IV, using freeze-dried bacteria in a disposable card allowed a stable detection during 10 days with 3% of reproducibility of the bioluminescence signal both in laboratory conditions and environmental samples. One analysis was performed in only 90 min against 360 min for Lumisens III. Nevertheless, the lack of specificity of the promoter, which regulates the bioluminescent reporter genes, limits the metal detection. We addressed the problem by using Lumisens IV and a data analysis software namely Metalsoft, developed in previous works. Thanks to this analytical software, Lumisens IV was a reliable online biosensor for the multidetection of Cd, As, Hg, and Cu.



1. INTRODUCTION

For the improvement of the water resource monitoring, the European Community published, in 2000, the Water Framework Directive (WFD, 2000/60/EC). One of these objectives aims at estimating and verifying the quality of these resources in particular through the monitoring of 33 priority substances (Appendix I, guideline 2008/105/CE). Several heavy metals (lead, cadmium, or mercury) are included in the compounds to be detected.

Conventional tools (physicochemical methods) used for the detection and quantification of metals in an aqueous environment are particularly sensitive and specific but do not appear to be adequate means for online environmental pollution monitoring (cost, complexity, pretreatement). So, to address the WFD's objectives, several international research teams developed innovative alternative tools dedicated to metal detection and based on biological sensors:[1,2] enzymes,[3,4] antibodies,[5] or engineered bacteria.[6−16] In this study, we were particularly interested in the last evoked category of biological sensors.

For metal detection, bacteria are engineered after the transformation of host strains (e.g., *Escherichia coli, Staphylococcus aureus*) with bioluminescence genes (e.g., *lux* genes from *Aliivibrio fischeri*) controlled by an inducible promoter, which is involved in the intracellular mechanism of metal resistance.[17] Thanks to this genetic modification, these strains are able to emit bioluminescence in the presence of some metals[10,11,18] more or less specifically.[11,19]

For online metal measurements, several biosensors were designed featuring two designs: bacteria in a liquid phase (reactor)[20−22] or immobilized (entrapped in a solid matrix) in a dedicated card or chip.[15,16,23−26] With the former, it is easy to control most of the parameters influencing the bacterial activity (temperature, pH, oxygen, etc.). Nevertheless, these systems are complex to use[27] and are not really suited for environmental applications. The systems based on immobilized bacteria seem to be easier to manage but are more difficult to control since microsensors are not yet simple to integrate. For the first time, the recent works of Elad et al.[26] demonstrated the use of entrapped bacteria for the online monitoring of several pollutants, including arsenic, during 10 days in laboratory conditions with a reproducibility of nearly 20%.

In our study, we were interested in determining the limits of the bacterial immobilization in biosensors through two major parameters: the reliability of the immobilized bacterial sensors after 10 days in the biosensor and the influence of environmental samples. For that, we compared two biosensors designed in our laboratory: Lumisens III[27,28] and Lumisens IV (this study). These systems differ in the conservation of the cells. Indeed, in Lumisens III, bacteria were maintained active and entrapped in a solid matrix of agarose hydrogel,[27,29] while

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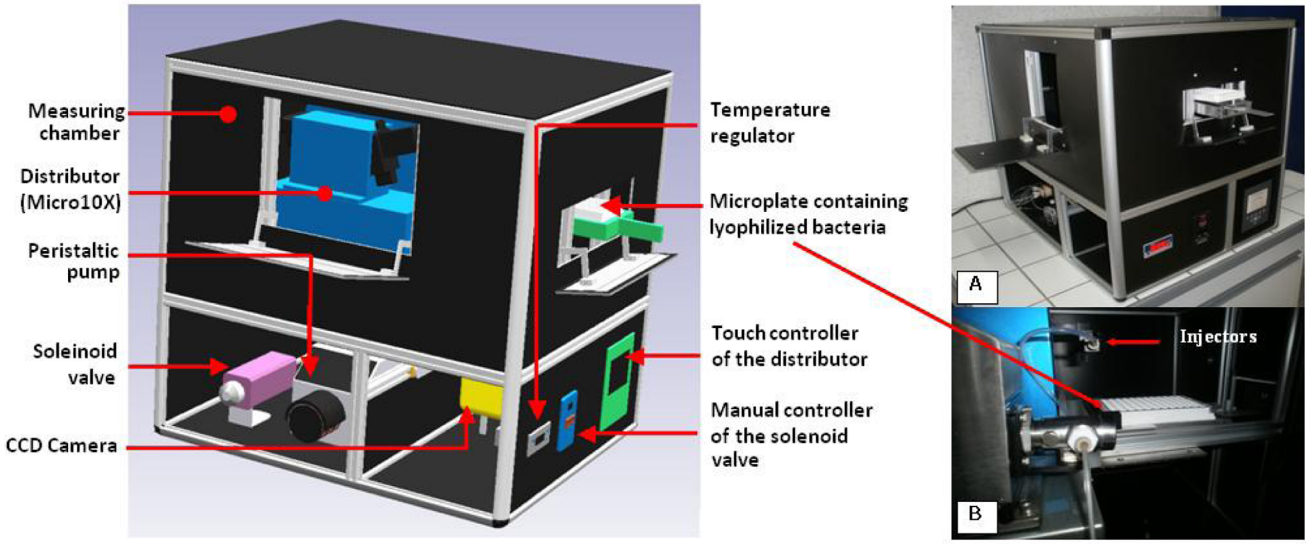

Figure 1. Tridimensional modeling performed by computer-aided design (left) and photos (right) of the biosensor Lumisens IV (A, global view; B, inside of the measuring chamber).

in Lumisens IV, bacteria were freeze-dried in a commercial microplate. Consequently, with this system, the cells are not active but require a rehydration phase. These two systems were used over a 10-days period for the detection of metals either in distilled water or in environmental samples.

2. EXPERIMENTAL SECTION

2.1. Bacterial Strain. Five strains were used for this study: *E. coli* K12 MG1655 pBMerlux, *E. coli* K12 MG1655 pBZntlux, *E. coli* K12 MG1655 pBArslux, *E. coli* K12 MG1655 pBCoplux, and *E. coli* DH1 pBTaclux described in detail by Jouanneau et al.[19] The host strain (*E. coli* K12 MG1655 or *E. coli* DH1) was transformed with plasmids harboring the ampicillin resistance gene (pBMerlux, pBZntlux, pBCoplux, pBArslux, pBTaclux) and containing the bioluminescence genes *luxCDABE* from *Aliivibrio fischeri* under the control of heavy metal-inducible promoters (*MerR-MerRp-MerTp*,[30] *ZntAp*,[31] *CopAp*,[32,33] and *ArsRp-ArsR*[34,35]) or the constitutive promoter (*Tacp*[24]), respectively.

2.2. Growth Media. An acetate medium with a C/N/P ratio of 100/10/1(w/w/w) was used for the growth of microbial cells. One liter of distilled water was supplemented with 2.835 g of sodium acetate (S2889, Sigma Aldrich), 0.1919 g of NH_4Cl (101143, Merck), 0.028 g of K_2HPO_4 (105109, Merck), 5 g of NaCl (207790010, Acros Organics), 0.5 g of yeast extract (A1202HA, Biokar Diagnotics), and 1 g of tryptone (A1401HA, Biokar Diagnotics). The pH was adjusted to 7 with a solution of HCl (0.1 M, S35584, Sigma Aldrich) or NaOH (0.1 M, 141687.1211, Panreac) and the media were sterilized by autoclaving at 120 °C for 20 min. Ampicillin solution (A9518, Sigma Aldrich) sterilized by filtration through a 0.22 μm filter (Analypore PVDF 33 mm, W2801B, Fisherbrand) was added to the media until a final concentration of 50 $\mu g \cdot mL^{-1}$ was reached.

Acetate medium supplemented with 15 $g \cdot L^{-1}$ agar type E (A1012HA, Biokar Diagnotics) was used for bacteria enumeration.

2.3. Metal Solutions. The following metals were used in this study: $CdCl_2$ (239208, Sigma Aldrich), $HgCl_2$ (83366, Fluka), $CuSO_4$ (A4778701, Labosi), and As_2O_3 (A1010, Sigma Aldrich). Concentrated metal solutions (1 or 0.1 M) were prepared in deionized water (Millipore) and stored at +4 °C in brown bottles for 12 months. Dilutions were carried out in distilled water from the stock solution and kept at room temperature for one day.

2.4. Environmental Samples. The sampling procedure used for this study respects the conditions described in standard ISO 5667-3 for all environmental samples. They were taken from the wastewater treatment plant (WWTP) outlet of Moulin Grimaud (50 000 inhabitants, La Roche sur Yon, France) in glass bottles previously washed with acid solution (HCl of 10% v/v) to limit metal contamination. The pH, conductivity, and turbidity measured in these samples were on average 7.4 and 0.9 $mS \cdot cm^{-1}$ and 4 NTU (nephelometric turbidity unit), respectively.

Some samples were artificially contaminated with metals (cadmium, mercury, arsenic, or copper).

2.5. Immobilization Procedure. The protocol was adapted from Charrier et al.[10] The bacterial strains were cultivated in an acetate medium with ampicillin (50 $\mu g \cdot mL^{-1}$) and taken in the exponential growth phase at an absorbance of A_{620nm} = 0.45. This bacterial suspension was diluted and mixed with a 4% (w/v) agarose solution (A0701, Sigma Aldrich) prepared in a fresh acetate medium. After homogenization, the final solution reached an absorbance of A_{620nm} = 0.075 for a final agarose concentration of 2%. Twenty-five microliters of this solution was poured into each well of the multiwell card of Lumisens III described by Affi et al.[28]

2.6. Enumeration of the Immobilized Bacteria. For the enumeration of the bacteria into the gel, the analyzed gel containing bacteria inside a well was withdrawn and introduced in a microtube of 1.5 mL (Eppendorf). The hydrogel was crushed in 500 μL of a $MgSO_4$ 10^{-2} M (M1880, Sigma Aldrich) solution with a sterile crushing piston (Cyclo-prep; AMRES-CO) and homogenized.[36] The immobilized bacteria were serially diluted in $MgSO_4$ 10^{-2} M and enumerated using the colony forming unit method on a Petri dish containing a solid acetate medium.

2.7. Freeze-Drying of Bacteria. The freeze-drying protocol was described in detail by Jouanneau et al.[19] The

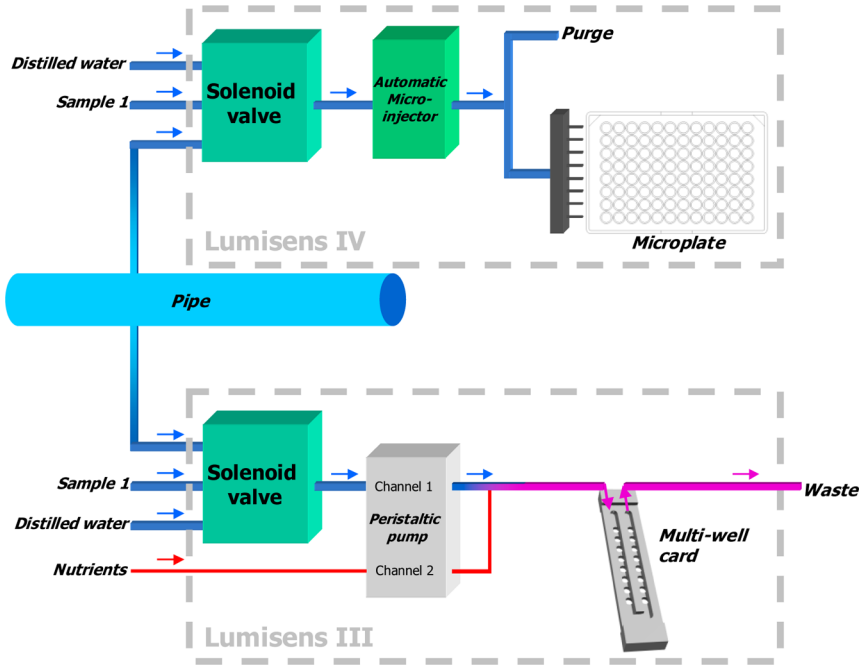

Figure 2. Comparison of the fluidic chart of the two biosensors Lumisens IV (this study) and Lumisens III.[22]

bacterial strains were cultivated at 30 °C in an acetate medium with ampicillin (50 μg.mL^{-1}) and brought to the exponential growth phase at an absorbance of A_{620nm} 0.45 or 0.18 for *E. coli* DH1 pBTaclux, because the level of the produced bioluminescence is maximized at this absorbance. Cells were then centrifuged (10 000 × g, 10 min, +4 °C) and resuspended in a sterile mixture containing one volume of fresh acetate medium and one volume of a sucrose solution (S9378, Sigma Aldrich) at 24% (w/v).[37] The final absorbance (620 nm) was adjusted to 0.075. One hundred microliters were added to the wells of a 96-well microplate with transparent bottom (Greiner Bio-One, 655088), frozen for 3 h at −80 °C and lyophilized for 36 h (−50 °C, 0.05 mbar) in a Christ Alpha 1−2 lyophilizer (Bioblock Scientific). After the lyophilization process, the microplates were hermetically closed with an aluminum adhesive film (Corning, 6570) at room temperature. The bacterial microplates were kept at −20 °C up to 1 month without any modification of the bacterial responses (results not shown). The survival rate of the bacteria after the lyophilization process was around 10% (data not shown).

2.8. Description of the Biosensors. Two generations of optical bioluminescent bacterial biosensors were compared in this study. These two systems differ through the conservation of the bacteria conditioning the fluidic network.

2.8.1. Biosensor Lumisens IV. The use of freeze-dried bioluminescent bacteria in a microplate led to specific constraints: rehydration of the bacteria, injection of samples well by well and thermoregulation. Consequently, the biosensor Lumisens IV was designed with computer-aided design software (Solid Edge V.10) to take into account these specifications and leading to the three-dimensional modeling (Figure 1). This biosensor features three compartments: the measurement (dark room), the fluidic circulation and the control. In the measurement compartment, bacteria are maintained under freeze-dried state. A distributor of 8 injectors (Micro10×, Hudson Control, United States) allows for the injection of distilled water (rehydration of the freeze-dried bacteria) and the analyzed samples in 8 wells at a time (Figure 2). This compartment is maintained at +30 °C (±1 °C) with heating resistances (Radiospares, 731-350). The emitted bacterial bioluminescence is measured with a CCD camera (Diagnostic Instruments Inc., INSIGHT, 1N18) located below the microplate in the control compartment.

The fluidic compartment contains a peristaltic pump (Medorex Peristaltic Pump TL) and a multiposition solenoid valve (VICI Valco, Interchim) to select the liquid to analyze.

Software were used to control these elements: Vcom V-1.1.01. for the solenoid valve, SPOT Advanced V-4.6. for the CCD camera and Softlink V-3.14 for the Micro10× distributor.

Lumisens IV was used as follows: first, the microplate containing the freeze-dried bacteria was inserted inside the measuring room. Each microplate column (8 wells) can be used to perform only one analysis due to the number of injectors. Consequently, a 96-well microplate allows for the analysis of 12 samples in a sequence decided by the user. Before the assay, the freeze-dried bacteria were reconstituted for 30 min with 100 μL per well of distilled water (injected by the distributor). Twenty-five microliters of sample were added to all the 8 wells and the microplate was then incubated for 60 min in the Lumisens IV's temperature-regulated measurement chamber.

For the purposes of our experiment, bacteria were exposed daily to contaminated samples in the same conditions as Lumisens III (mercury, 500nM; distilled or environmental waters). The bioluminescence emitted by the bacteria was measured with the CCD camera after the incubation time with an integration period of 60 s.

2.8.2. Biosensor Lumisens III. The technological details of the biosensor Lumisens III were described by Charrier et al.[27] Contrary to Lumisens IV, bacteria are immobilized in an agarose hydrogel (2%) in a multiwell card designed by Affi et al.[28] In these hydrogels, the initial bacterial density was identical to that of Lumisens IV (A_{620nm} = 0.075).

Immobilized bacteria are constantly fed with the sample to analyze (distilled water or environmental samples) at a flow rate of 2 mL·min^{-1} thanks to a multichannel peristaltic pump (Meredos TL, Medorex). This pump also allows the feeding

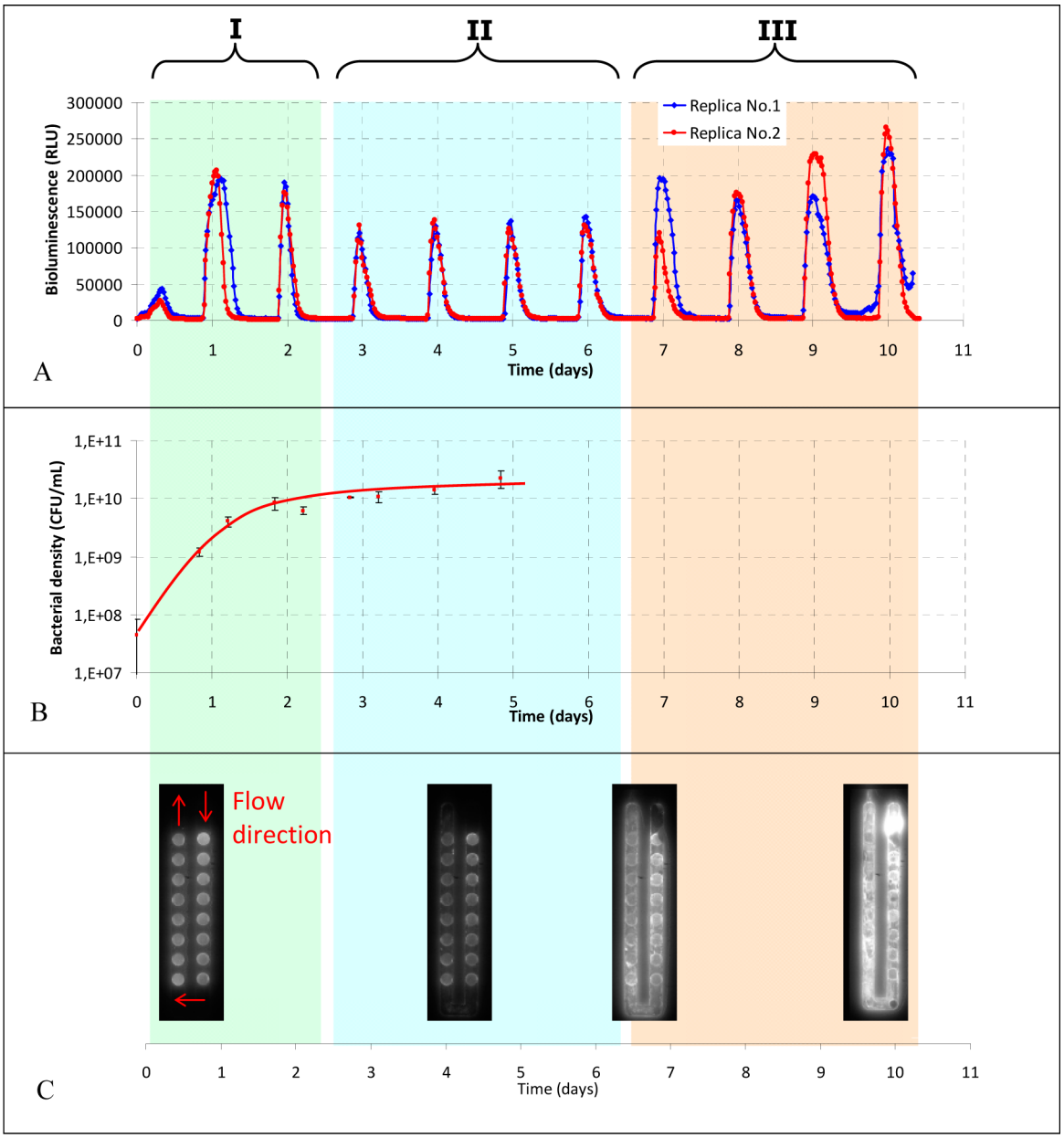

Figure 3. Bioluminescence monitoring (RLU: Relative Light Unit) of *E. coli* K12 MG1655 pBMerlux induced by daily exposures to mercury (500 nM). (A) Laboratory conditions (distilled water); (B) Growth curve of the cells (CFU = colony forming unit) in the microwells; (C) CCD camera imaging of the immobilized bacteria in the multiwell card. I, stabilization phase; II, stationary phase; III, collapsing phase. Each point is the average of three measurements. Standard errors are 5% and hence are not shown.

with a concentrated nutritional solution at a flow rate of 0.02 $mL{\cdot}min^{-1}$ (final concentration in the circulating media: 0.2835 $g{\cdot}L^{-1}$ of sodium acetate, 0.01919 of $g{\cdot}L^{-1}$ NH_4Cl, 0.0028 $g{\cdot}L^{-1}$ of K_2HPO_4, 5 $g{\cdot}L^{-1}$ of NaCl, 0.05 $g{\cdot}L^{-1}$ of yeast extract, 0.1$g{\cdot}L^{-1}$ of tryptone, and 50 $\mu g{\cdot}mL^{-1}$ of ampicillin) to maintain the cell activity (Figure 2). To maintain a stable temperature in the biosensor Lumisens III, the system (biosensor, media, and samples) was kept at 20 °C. This temperature value was lower than the one used in Lumisens IV and helped to limit the bacterial colonization of the multiwell card and fluidic network.

Immobilized bacteria were exposed daily to a sample spiked with mercury (500 nM) for 100 min either in distilled water or in a raw effluent of a WWTP outlet followed with a washing step of 22 h before a new induction. The emitted bioluminescence was measured in a black chamber with a CCD camera (SPOT RT SE 6, Optilas). The images were collected every 30 min with an integration time of 60 s.

The Vcom V-1.1.01. and the SPOT advanced V-4.6. software were used to control the solenoid valve and the CCD camera, respectively.

The oxygen available for the bacteria immobilized in the microwell card was monitored with a 50 μm-diameter microprobe (OX-50, Unisense) integrated in a tube just upstream from the card. The dissolved oxygen measured by the microsensor was recorded with the SensorTrace BASIC software (Unisense).

2.9. Data Analysis. The pictures of the bacterial bioluminescence of both biosensors taken with the CCD camera were processed with the Kodak image analysis software

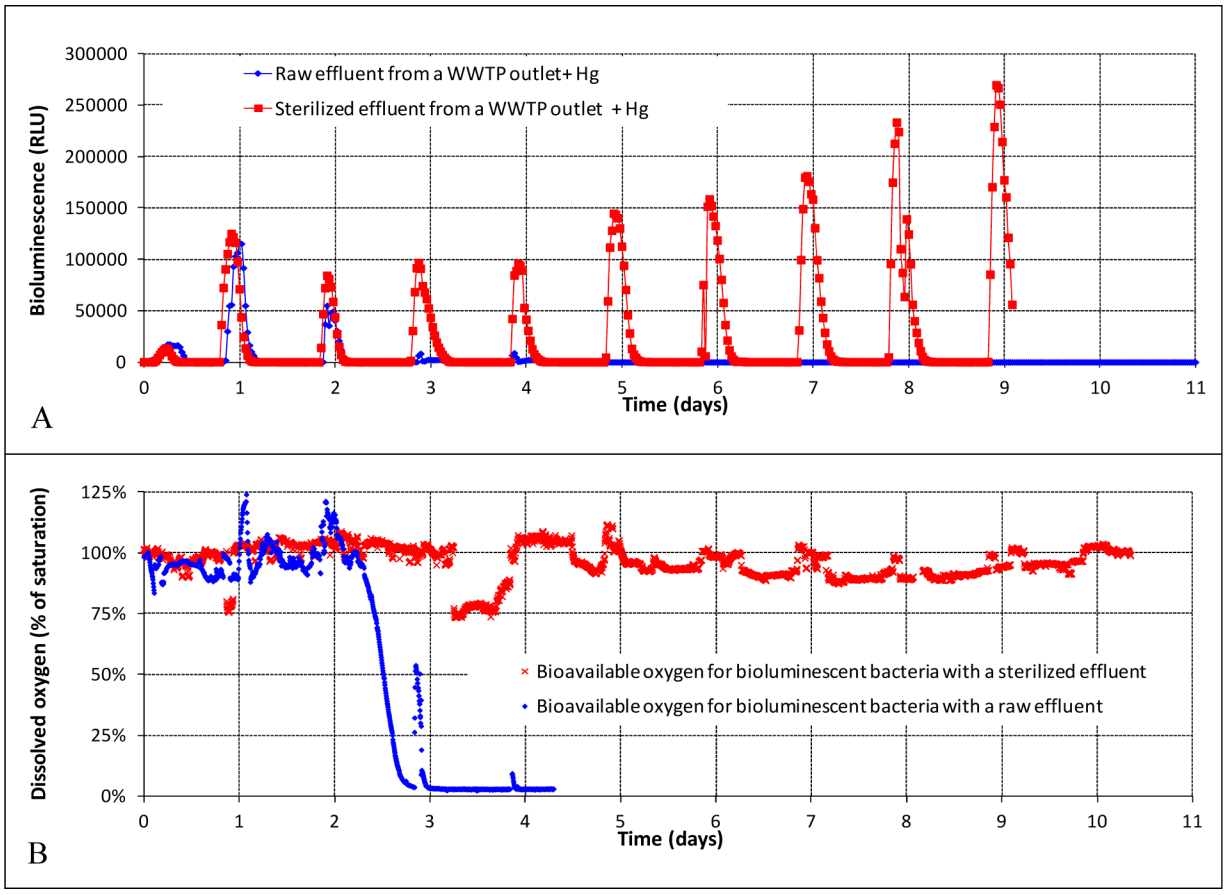

Figure 4. Bioluminescence of *E. coli* K12 MG1655 pBMerlux induced by daily exposures of mercury (500 nM). (A) Wastewater effluent samples. (Each point is the average of three measurements. Standard errors are 5% and hence are not shown.) (B) Measurement of the bioavailable oxygen for immobilized bacteria in the microwells of the biosensor Lumisens III (standard errors of the measurement are 11% and were not added for clarity).

(Scientific Imaging System, Eastman Kodak Company, Version 3.6) and converted into numerical bioluminescence values.

Some of the bioluminescence data was analyzed with Metalsoft[19] to predict the metal concentration in the tested samples. The heart of this software is a predictive model that helps to identify and semiquantify metals (cadmium, arsenic, mercury, and copper) in unknown samples from the data of bioluminescence obtained with the bacterial set.

Shortly, the prediction algorithm deals with the classification of samples according to the bioluminescence intensity emitted by the five bioluminescent bacterial strains via four CHAID-type decision trees specific to each analyzed metals. Each tree allows the prediction of a concentration range for one metal. Metalsoft was detailed in Jouanneau et al.[19]

3. RESULTS

3.1. Lumisens III versus Lumisens IV. The first objective of this research was to compare two analytical devices for the detection of metal in environmental water samples according to the Figure 2 setting. For this purpose, only the mercury-inducible bioluminescent bacterial strain *E. coli* K12 MG1655 pBMerlux was used.

3.1.1. Long-Term Monitoring of Mercury with Immobilized Cells in Lumisens III. For this first stage, bacteria were exposed to a sterilized distilled water sample spiked with mercury (500 nM) for 100 min (2 mL·min^{-1}) a day over a ten-day period. The rest of the time, bacteria were exposed to sterilized distilled water (2 mL·min^{-1}) supplemented with a concentrated nutritional solution (0.02 mL.min^{-1}) (cf., section 2.8.2). The duplicated results obtained in these conditions are presented in the Figure 3A.

As expected, each mercury exposure (100 min) was correlated with a significant increase in the emitted bioluminescence level; the total measurement delay was about 6 h including the bioluminescence induction and the return to the background level. Nevertheless, the bioluminescence levels were unstable during this ten-day period. The variation rate of the bioluminescence was nearly 40% during the measurement period divided into three phases namely the stabilization phase (I), the stationary phase (II) and the collapsing phase (III) (Figure 3A). Between days 0 and 2 (included) (Figure 3A, I), reproducible variations were observed between the different inductions explained by the bacterial growth of *E. coli* K12 MG1655 pBMerlux within the multiwell card (Figure 3B). The increase of the cellular density and the physiological state of the bacteria[10,38] during this phase was probably at the origin of the differences in bioluminescence emissions measured between the daily inductions by the mercury. From days 3−6 (included) (Figure 3A, II), the bacteria were in a stable phase depicted by a reproducible and repeatable bioluminescence signal bordering 5% in variation. The growth study showed that the immobilized bacteria were in the stationary growth phase. After day 7 (Figure 3A, III), the bacteria entered a phase in which the bioluminescence signal was unstable. To understand this last

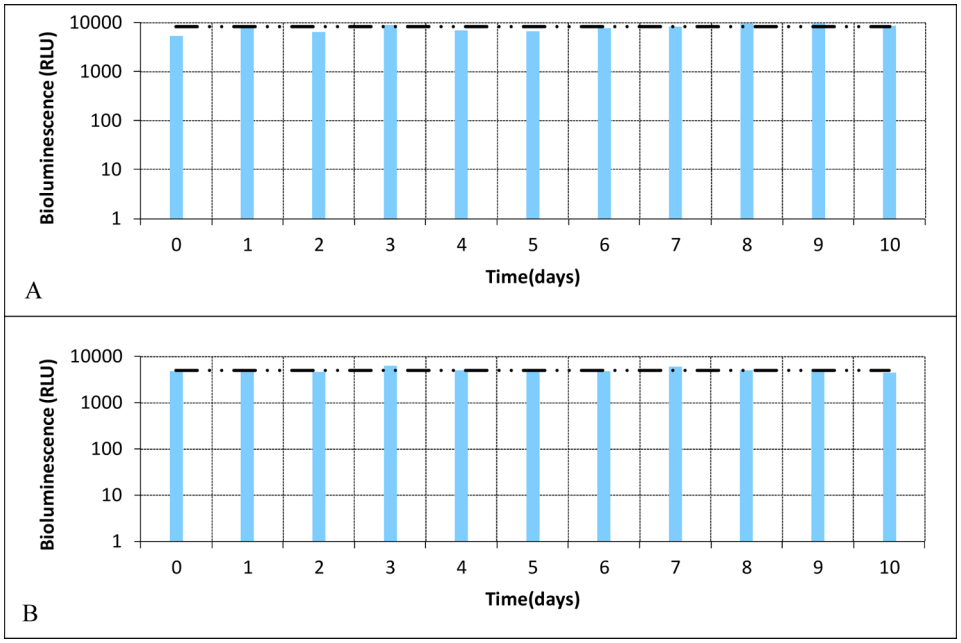

Figure 5. Bioluminescence of freeze-dried *E. coli* K12 MG1655 pBMerlux induced by daily exposures of a sample with mercury (500nM) and reproduced ten times. (A) Laboratory conditions (distilled water) and (B) environmental samples (raw wastewater). Standard errors of the measurements are 3% in both cases and hence are not shown.

phase (III), images were taken by the CCD camera (Figure 3C) showing a colonization of the card from the bacteria initially confined inside the agarose matrices. Consequently, the bioluminescence measured from day 7 was the sum of the bioluminescence emitted by the entrapped bacteria and by the bacterial colonization explaining the variations of the observed bioluminescence levels.

This study helped to determine the duration time in the laboratory conditions of the biosensor Lumisens III to 7 days including three days needed for the growth of the immobilized bacteria.

Following the same protocol as above, Lumisens III was used to detect the daily exposure to mercury (500nM) in water from a WWTP effluent. With a sterilized environmental sample, the induction profile (Figure 4A) was similar to the results observed with laboratory samples with the three phases (Figure 3A). Consequently, the sterilized environmental samples did not significantly disrupt the use of the Lumisens III biosensor.

Conversely, with the raw effluent of the WWTP outlet, the results were in accordance with other profiles (sterilized environmental samples or laboratory samples) only during the first two days. Afterward, the bioluminescence dropped sharply until it totally disappeared. This phenomenon can be attributed to the microbial flora of the raw sample, which colonized all the pipes of the fluidic network and consequently consumed all the oxygen necessary for the bioluminescence reaction (Figure 4B).

3.1.2. Long-Term Monitoring of Mercury with Freeze-Dried Bacteria in Lumisens IV. The biosensor Lumisens IV was designed to encounter the weaknesses of its predecessor. With this new concept, bacteria are kept in commercial microplates in a freeze-dried state. To compare the results obtained with these two biosensors, Lumisens IV was tested in the same conditions as above (initial cell density, samples, contamination level with mercury).

With daily exposures to mercury (500 nM) spiked in distilled water, the new biosensor presented neither a stabilization phase nor a collapsing phase of the signal during all 10 days of the experiment (Figure 5A). Thanks to the biosensor's bacteria preservation mode, the data was very stable with a reproducibility bordering 3%. Moreover, the monitoring time does not seem to be a limiting factor anymore contrary to the previous system Lumisens III, which was limited to 7 days (including a stabilization period of 3 days).

As with Lumisens III, Lumisens IV was tested with raw water samples from a mercury-spiked WWTP effluent (Figure 5B). Contrary to the Lumisens III, the measured bioluminescence levels remained very stable (reproducibility = 3%). This important difference between both systems is also attributed to the bacteria preservation mode. In the Lumisens IV, bacteria were isolated in the wells of the microplates providing independent analyses. On the contrary, in Lumisens III, bacteria were influenced by the previous exposures: chemical toxicity, biological damages because of phages or protozoa of the samples or competition for nutrients (environmental microflora).

3.2. Online Monitoring of Multiple Metals. The first step of this study allowed for the selection of the best online mercury monitoring system that would provide good reproducibility throughout the use of the biosensor.

Given that, in environment, metals are usually in a mixture, we addressed the problem of their online detection with bioluminescent bacteria. The major difficulty with the use of bacterial sensors relates to their lack of specificity.[11,19] Consequently, we addressed the problem by using our software, Metalsoft, described by Jouanneau et al.[19] for bioassays. This software, now applied to a biosensor, is dedicated to data mining and allows us to estimate the metal concentration in environmental samples spiked with several mixed metals from the bioluminescence levels emitted by 5 strains (*E. coli* K12 MG1655 pBMerlux, *E. coli* K12 MG1655 pBZntlux, *E. coli* K12 MG1655 pBArslux, *E. coli* K12 MG1655 pBCoplux, and *E. coli* DH1 pBTaclux).

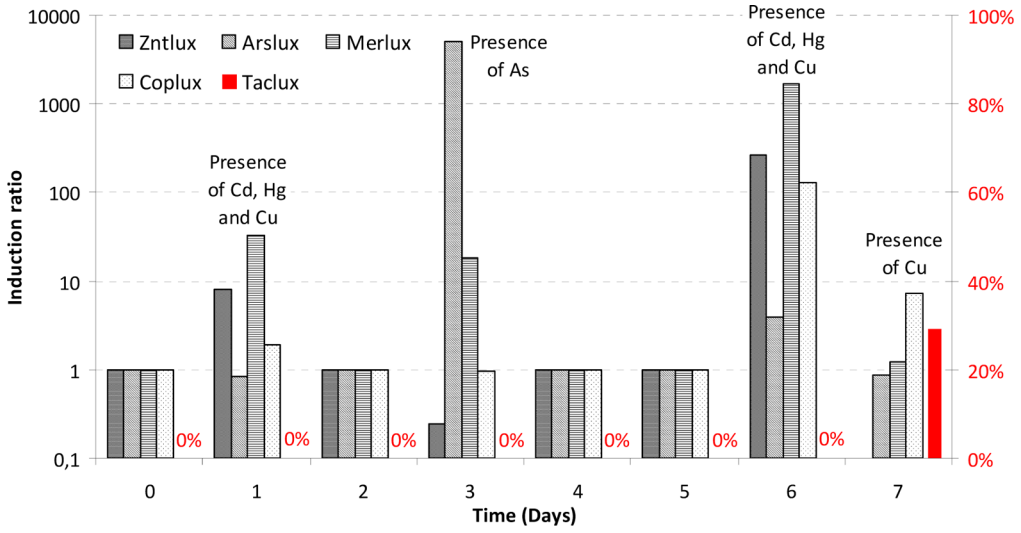

Figure 6. Identification of metals with Lumisens IV associated with the data analysis software Metalsoft applied to the online monitoring of one raw wastewater spiked or not with different metals. Standard errors of the measurements are 3% in both cases and hence are not shown. The results were expressed with the induction ratio for the inducible strains or the inhibition rate for the constitutive strain. The induction ratio corresponds to the ratio between the bioluminescence emitted with the sample and the background bioluminescence of the strains. The inhibition rate corresponds to the percentage of bioluminescence inhibited because of the sample's toxicity in relation to the control for the constitutive strain (*E. coli* DH1 pBTaclux). Zntlux, Arslux, Merlux, Coplux, and Taclux are respectively, *E. coli* K12 MG1655 pBZntlux, *E. coli* K12 MG1655 pBArslux, *E. coli* K12 MG1655 pBMerlux, *E. coli* K12 MG1655 pBCoplux, and *E. coli* DH1 pBTaclux.

Several pollution events were simulated with a metal-spiked (cadmium, arsenic, mercury, or copper) WWTP effluent and monitored daily with the biosensor Lumisens IV. The bioluminescence data provided by the bacterial set was analyzed with the analytical software Metalsoft and plotted in Figure 6 with the metal identified with the software. Thanks to Lumisens IV, 4 pollution events were detected and confirmed with standard chemical methods for the day 1 (Cd = 142.5 nM, Hg = 109.9 nM, and Cu = 49.52 μM), the day 3 (As = 8.46 μM), the day 6 (Cd = 3.45 μM, Hg = 107.4 nM, and Cu = 47.44 μM), and the day 7 (Cu = 171.35 μM).

To the best of our knowledge, the link between Lumisens IV and Metalsoft appears to be the first trustworthy system dedicated to online water pollution monitoring and is able to provide reliable information about the bioavailable metals and their concentrations in the samples.

4. DISCUSSION

The main asset in using a bacterial biosensor is its ability to monitor online the bioavailable fraction of pollutants in environmental effluents and to estimate their acute toxicity on living organisms.[16,24,26,27,39]

Recent poineering works published by Elad et al.[26] showed that it is possible to use a biosensor with immobilized bacteria over a long period (10 days) to detect several chemicals or stresses thanks to three strains, *E. coli* RFM443 recA, *E. coli* DH5α micF and *E. coli* MG1655 arsR induced by DNA damage, oxidative stresses and heavy metals (As, Sb) respectively. Conversely, our biosensor Lumisens III, which is also based on bacteria immobilized in a hydrogel matrix, was unable to monitor effectively the levels of metals present in the environmental samples over 10 days because of the evolution of the bacteria inside the card and disturbances related to the environmental microflora.

This study showed the interest of the freeze-drying procedure to preserve bacteria in an online biosensor versus immobilized bacteria. Indeed, this preservation stage allowed for the improvement of the stability, sensitivity (results not shown) and reproducibility of bacterial sensors, the deletion of the stabilization phase (3 days) observed with biosensor Lumisens III (immobilized bacteria) and the effective analysis of environmental samples. The analysis time was significantly decreased with Lumisens IV (from 6 h with Lumisens III to 1.5 h), and the number of analyzed samples is only limited by the size of the 96-well microplate. Indeed, we estimated that with the current system, one measurement (hence one sample) could be done every minute.

On the other hand, the scientific literature dedicated to the evaluation, characterization or use of these sensors underlines the lack of specificity of the bioluminescent bacteria for metal detection.[10,11,19] To resolve this task, Elad et al.[40] and Jouanneau et al.[19] used statistical models dedicated to data analysis. These models, based on the Bayesian decision theory and decision trees (CHAID algorithm[41]), respectively, exploit the crossing of the biological responses of a bacterial set to identify pollutants present in analyzed samples. In this study, the use of the data analysis software Metalsoft with the data provided by Lumisens IV helped to monitor and estimate heavy metal levels (cadmium, arsenic, mercury, and copper) in environmental samples online with great reliability. We believe that this is the future of biomonitoring.

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Notes

The authors declare no competing financial interest.

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