



A low cost color-based bacterial biosensor for measuring arsenic in groundwater



Chi-Wei Huang, Chia-Cheng Wei, Vivian Hsiu-Chuan Liao *

Department of Bioenvironmental Systems Engineering, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei 106, Taiwan

HIGHLIGHTS

- A color-based As biosensor was developed.
- The As biosensor detects and accurately estimates As concentration in groundwater.
- The biosensor is cost-effective with the potential for large scale screening As.

ARTICLE INFO

Article history:

Received 31 March 2015

Received in revised form 1 June 2015

Accepted 4 June 2015

Keywords:

Biosensor

Arsenic

Color-based detection

Shelf life

Groundwater

ABSTRACT

Using arsenic (As) contaminated groundwater for drinking or irrigation has caused major health problems for humans around the world, raising a need to monitor As level efficiently and economically. This study developed a color-based bacterial biosensor which is easy-to-use and inexpensive for measuring As and could be complementary to current As detecting techniques. The *arsR-lacZ* recombinant gene cassette in nonpathogenic strain *Escherichia coli* DH5 α was used in the color-based biosensor which could be observed by eyes or measured by spectrometer. The developed bacterial biosensor demonstrates a quantitative range from 10 to 500 $\mu\text{g L}^{-1}$ of As in 3-h reaction time. Furthermore, the biosensor was able to successfully detect and estimate As concentration in groundwater sample by measuring optical density at 595 nm (OD_{595}). Among different storage methods used in this study, biosensor in liquid at 4 °C showed the longest shelf life about 9 d, and liquid storage at RT and cell pellet could also be stored for about 3–5 d. In conclusion, this study showed that the As biosensor with reliable color signal and economical preservation methods is useful for rapid screening of As pollutant, providing the potential for large scale screening and better management strategies for environmental quality control.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Arsenic (As) is a naturally occurring element which can be found widely in the environment. The release of As can occur naturally or by anthropogenic activities (Leist et al., 2000; Pott et al., 2001; Oremland and Stolz, 2003). The two main soluble forms As are pentavalent arsenate [HAsO_4^{2-} ; As(V)] and trivalent arsenite [H_3AsO_3 ; As(III)]. Inorganic As(III) is considered to be more toxic than As(V) and can be oxidized to As(V) chemically or microbiologically (Oremland and Stolz, 2003). As is a known human carcinogen and it was ranked first on the Agency for Toxic Substances and Disease Registry (ATSDR) priority list of hazardous substances in 2013 (ATSDR, 2013). Exposure to As in drinking water is associated with increased risk of skin, kidney, lung, and bladder cancers (Abernathy et al., 1999; Pott et al., 2001), which is a significant

health problem for humans around the world. Therefore, there is an urgent need to develop an accurate, fast, and inexpensive detection method for As level.

Many chemical-based As detection methods including AFS (atomic fluorescence spectroscopy), ICP-MS (inductively coupled plasma-mass spectrometry), ICP-AES (ICP-atomic emission spectrometry), GFAAS (graphite furnace atomic absorption), HGAAS (gaseous hydride generation atomic absorption spectrometry), and HPLC-ICP-MS (high-performance liquid chromatography-ICP-MS) are reliable with high sensitivity, however, these methods are usually expensive, hard to maintain the equipments, and unfavorable for rapid screening of large-scale test or applied *in situ* (Ma et al., 2014; Kaur et al., 2015).

Using biosensors to conduct the chemical detection offer an alternative as a result of rapidly screening, lower cost, and ability to measure bioavailability (Daunert et al., 2000; Girotti et al., 2008). A biosensor comprises two main components, biological sensing element and signal transducer element, which can identify

* Corresponding author.

E-mail address: vivianliao@ntu.edu.tw (V.H.-C. Liao).

and generate detectable signals (Daunert et al., 2000; Rogers, 2006). For As biosensor, the regulatory gene *arsR* from *ars* operon is often applied to compose the sensing element of biosensors for detecting As (Kaur et al., 2015). For the *in situ* applicability and convenience, color-based biosensors using *lacZ* as signaling element were developed to detect As (Stocker et al., 2003; Wackwitz et al., 2008). The use of X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactosidase) as substrate offers a simple colorimetric method detectable by eyes due to the blue color development. However, the quantitative analysis of environmental samples and applicability for As detection by color-based biosensors are not extensively studied yet.

Therefore, in this present study, the *arsR-lacZ* recombinant gene cassette in nonpathogenic strain *Escherichia coli* (*E. coli*) DH5 α was used in the color-based biosensor. The As biosensor is easy-to-use and inexpensive for As screening in groundwater samples and could be complementary to physicochemical methods. Furthermore, simple preservation methods of bacterial biosensor were also evaluated in this study.

2. Materials and methods

2.1. Construction of the recombinant plasmid

The DNA fragment comprising the promoter/operator of the *ars* operon (P_{ars}) and *arsR* gene was PCR amplified from the p1258 plasmid isolated from *Staphylococcus aureus* (NCTC 50581; National Collection of Type Cultures, Colindale, London, UK). The PCR primers used were: forward primer (5'-CCGGAATTCATAAATAACATAGACAATAATCT-3') and reverse primer (5'-CGCGGATCCCATCAACAGTCACCTGATT-3') carrying the *EcoRI* and *BamHI* restriction sites (underlined). The amplified DNA fragment was purified and digested with the restriction enzymes *EcoRI* and *BamHI*. The *EcoRI-BamHI* DNA fragment was subsequently inserted upstream of the promoterless *lacZ* gene in the vector pTZ110 (Schweizer and Chuanchuen, 2001) for creating pAs-*lacZ* fusion plasmid (Fig. 1). The recombinant plasmid pAs-*lacZ* was then transformed to *E. coli* DH5 α .

2.2. Bacterial growth and *lacZ* induction assay for As(III) detection

The bacterial growth and *lacZ* induction assays by As(III) were adapted from Wackwitz et al. (2008). Briefly, a single colony of *E. coli* DH5 α harboring pAs-*lacZ* was grown overnight in Luria-Bertani (LB) medium containing 100 $\mu\text{g mL}^{-1}$ of ampicillin at 37 °C. The overnight culture was diluted with fresh LB medium at a 1:20 ratio and incubated at 37 °C in an orbital shaker at 225 rpm until the optical density at 600 nm (OD_{600}) of 0.6 was reached. The culture was then added in 100- μL aliquots to wells of a 96-well plate (Thermo LabSystems, Helsinki, Finland), mixing up with 20 μL of the appropriate concentration of As(III), 60 μL deionized H_2O , and 20 μL X-gal substrate solution. Final X-gal concentration was 100 $\mu\text{g mL}^{-1}$.

Subsequently, the 96-well plates were covered with a lid and incubated at 37 °C in an orbital shaker at 225 rpm for 3 h. Images of blue color development were taken by digital camera (Canon PowerShot A3000 IS). In order to quantify the blue color developed

from X-gal, the optical density at 595 nm (OD_{595}) was measured in a microplate reader (Wackwitz et al., 2008) (Thermo Scientific Multiskan EX). The turbidity resulting from the bacterial cell itself was subtracted from the measured signal by measuring assay mixtures without X-gal. The induction ratio (IR) is defined as:

$$\text{IR} = \frac{\text{OD}_{595}^{\text{X-gal, As induced}} - \text{OD}_{595}^{\text{no X-gal, As induced}}}{\text{OD}_{595}^{\text{X-gal, no As}} - \text{OD}_{595}^{\text{no X-gal, no As}}}$$

The superscript “X-gal” refers to assay mixtures containing 100 $\mu\text{g mL}^{-1}$ X-gal; “no X-gal” refers to assay mixtures without X-gal; “As induced” refers to assay mixtures containing appropriate concentration of As(III); “no As” refers to assay mixtures without As(III). All experiments were carried out with quadruplicate and were repeated at least three times.

2.3. Measurement of As-spiked groundwater

The groundwater was collected and spiked with different concentrations of As(III) (0, 10, 50, 500 $\mu\text{g L}^{-1}$). These concentrations are drinking water and groundwater quality standards in Taiwan. The groundwater was measured by mixing 100 μL As-spiked groundwater sample and 100 μL *E. coli* DH5 α harboring pAs-*lacZ* at OD_{600} of 0.6 containing 100 $\mu\text{g mL}^{-1}$ X-gal to each well of a 96-well plate. The mixtures were incubated at 37 °C in an orbital shaker at 225 rpm for 3 h using the procedures described above. Images of blue color development were taken by digital camera. The OD_{595} and IR were measured and calculated by procedures described in Section 2.2. For standard curves, known concentrations of As(III) were measured in parallel with deionized distilled water replaced of the groundwater sample. Log-linear regression of the average IR at each particular As(III) concentration was used to generate a standard curve, and then the concentration of As(III) equivalent in the groundwater samples were calculated from the derived standard curve.

2.4. Liquid preservation

A single colony of *E. coli* DH5 α harboring pAs-*lacZ* was grown overnight in LB medium containing 100 $\mu\text{g mL}^{-1}$ of ampicillin at 37 °C. The overnight cell culture was diluted with fresh LB medium at a 1:20 ratio and incubated at 37 °C in an orbital shaker at 225 rpm until OD_{600} of 0.6 was reached. The culture solution was then stored at 4 °C or room temperature (RT) (about 25 °C) for further biosensor assays. The IR responded to different As(III) (0, 10, 50, 500 $\mu\text{g L}^{-1}$) after different storage periods was measured by procedures described in Section 2.2.

2.5. Cell pellet preservation

A single colony of *E. coli* DH5 α harboring pAs-*lacZ* was grown overnight in LB medium containing 100 $\mu\text{g mL}^{-1}$ of ampicillin at 37 °C. The overnight cell culture was diluted with fresh LB medium at a 1:20 ratio and incubated at 37 °C in an orbital shaker at 225 rpm until OD_{600} of 0.6 was reached. The culture was then harvested by centrifugation (3000g) and the supernatant was removed. Afterward, the cell pellets in 1.5 mL microcentrifuge tubes were stored at 4 °C or RT for further biosensor assays. The cell pellet was resuspended by LB medium prior to *lacZ* induction assays. The IR responded to different As(III) (0, 10, 50, 500 $\mu\text{g L}^{-1}$) after different storage periods was measured by procedures described in Section 2.2.

2.6. Data analysis

The experiments were conducted at least three times for error analyses. The results are presented as the mean $\pm$ standard

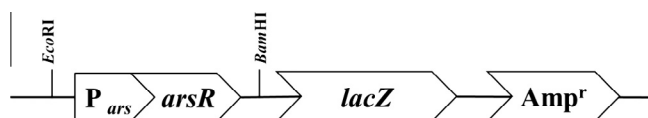


Fig. 1. Schematic organization of the pAs-*lacZ* biosensor plasmid. Arrows represent genes and their direction. *EcoRI* and *BamHI* are restriction sites used for cloning. Amp^r is an abbreviation of ampicillin resistance gene.

deviation (SD). Statistical significance was defined as $P < 0.05$ by student's *t*-test analysis. Standard curves were fitted by a log-linear regression analysis.

3. Results and discussion

3.1. Characterization of the color-based As bacterial biosensor

In order to construct color-based biosensor for detecting As, the P_{ars} and *arsR* sequences in the plasmid pI258 of *S. aureus* were inserted into the promoterless pTZ110 vector which has low *lacZ* background activity (Schweizer and Chuanchuen, 2001). The resulting plasmid designated pAs-*lacZ* was shown in Fig. 1. Some studies have shown that high *lacZ* background causes false-positive and misleading the results of biosensors (Stocker et al., 2003; Wackwitz et al., 2008). In the present study, *E. coli* DH5 α harboring pAs-*lacZ* showed an obvious blue color when exposed to As(III) relative to that of control bacterial cells with no As(III) (Fig. 2).

Biosensor assays were performed in 96-well plates to allow visual signal detection by digital imaging as well as spectrophotometrical measurements. Color development from X-gal by the *lacZ*-based biosensor strain as response to As(III) was tested by exposing biosensor cells to different concentrations of As(III) (0,

10, 50, 100, 500, 1000 $\mu\text{g L}^{-1}$) at 37 °C. The blue color started to develop after 2.5 h induction and the visible blue color could be obviously distinguished after 3-h induction time. Strain pAs-*lacZ* produced visible blue color from the lowest As(III) concentration (10 $\mu\text{g L}^{-1}$) with a linear signal increase up to 500 $\mu\text{g L}^{-1}$ and a plateau above 1000 $\mu\text{g L}^{-1}$ (Fig. 2). Fig. 2A shows that increasing As(III) concentration was associated with increasing intensity of blue color. The background value (cells exposed to no As(III)) was low; hence, the color intensity could be distinguished from it (Fig. 2A). To further quantify the blue color, OD₅₉₅ was measured by spectrophotometer (Wackwitz et al., 2008). The concentration of 10 $\mu\text{g L}^{-1}$ As(III) was able to induce statistically increased color intensity comparing to that of control (no As(III)) ($P < 0.05$) (Fig. 2B). In addition, Fig. 2B shows a log-linear relationship from 10 to 500 $\mu\text{g L}^{-1}$ As(III). We also tested the response of pAs-*lacZ* biosensor to As(V) and the dose-response patterns were similar to that of As(III) whereas the IR of As(V) was lower than that of As(III) (data not shown). Given As(III) is more toxic and predominantly present in As-contaminated groundwater in many regions around the world, As(III) was chosen for biosensor assays in the present study.

It has been suggested that the specificity/selectivity of the bacterial biosensor depends upon the sensing promoter and the sensitivity depends upon the proper selection of the reporter (Köhler et al., 1999; Daunert et al., 2000). In the pAs-*lacZ* recombinant plasmid (Fig. 1), the P_{ars} and *arsR* sequences have been previously characterized showing specificity to As(III), As(V), and Sb(III) yet no response to other metals at 1 μM , including Sb(V), Cd(II), Co(II), Cu(II), Fe(II), Hg(II), Mn(II), Mn(VII), Ni(II), Pb(II), Sn(II), Zn(II), Cr(VI), Se(IV), and Se(VI) (Liao and Ou, 2005). Therefore, the pAs-*lacZ* biosensor could not differentiate between concentrations of As(III), As(V), and Sb(III). Other studies reported that the bioluminescent As biosensors responded to other metals (e.g. Cd, Pb, Sn) with various detection limits (Hakkila et al., 2004; Charrier et al., 2011). This might attribute to different P_{ars} and *arsR* sequences and *lux* reporter for the biosensors as it has been previously reported that the bioluminescent bacteria were not specific to only one metal but rather to a range of metals (Binet and Poole, 2000; Riether et al., 2001; Charrier et al., 2011).

Several As bacterial biosensors have been previously described using different reporter genes including *lacZ*, *luxAB*, *luxCDABE*, *luc*, and *gfp* with various sensitivities for detecting As(III) (Tauriainen et al., 1997; Stocker et al., 2003; Liao and Ou, 2005; Wackwitz et al., 2008; Cortés-Salazar et al., 2013; Sharma et al., 2013). In particular, the sensitivities of the bacterial biosensor for As developed in this study are comparable to other studies using *lacZ* as reporter gene to detect As (Stocker et al., 2003; Wackwitz et al., 2008; Cortés-Salazar et al., 2013). The electrochemical As(III) biosensor on a biochip could detect 7.5 $\mu\text{g L}^{-1}$ As(III) in 25–50 min (Cortés-Salazar et al., 2013). Stocker et al. (2003) used a *lacZ*-based biosensor which was able to produce a visible blue color at As(III) concentrations above 8 $\mu\text{g L}^{-1}$. In 96-well plate assays, *lacZ*-based bioreporter strains showed various sensitivities to As(III) with 0.2, 10, 50 $\mu\text{g L}^{-1}$ for strain 2245, strain 1595, and strain 2066, respectively (Wackwitz et al., 2008). Despite of same reporter gene and regulatory gene used, ability of biosensor to detect As varies as a result of many factors including culture medium, host cell, exposure time, substrates.

3.2. Measurement of As concentration in As-spiked groundwater by biosensor

Several color-based As biosensors have been previously reported (Stocker et al., 2003; Wackwitz et al., 2008). However, limited studies have examined color quantification for estimating As concentration quantitatively in environmental samples. To

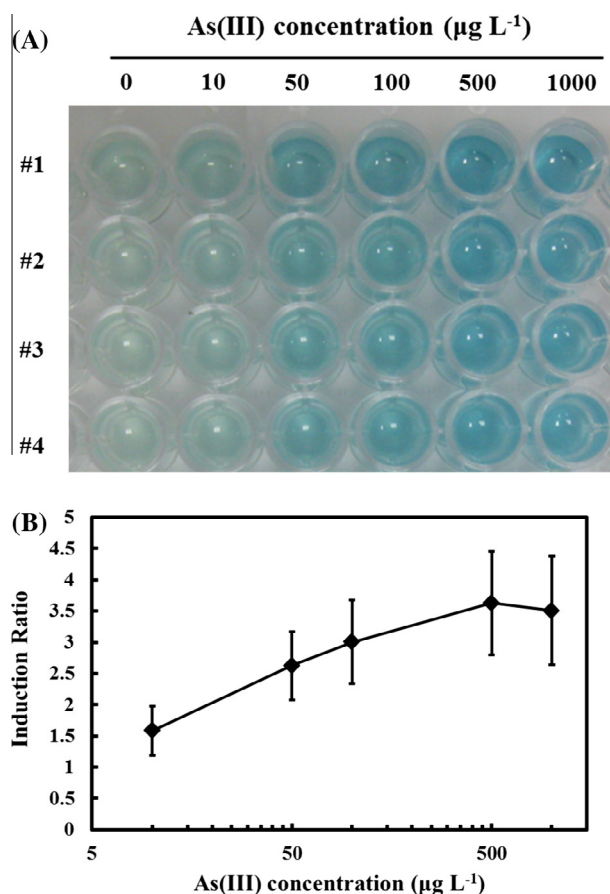


Fig. 2. The dose-response relationship of color-based pAs-*lacZ* biosensor. (A) Color development from X-gal as response to various As(III) concentrations. The assay was carried out in a 96-well plate. Image was taken after 3-h incubation time at 37 °C. The image presented here is the results of 4 colonies of the biosensor cells. (B) Color development from X-gal measured by spectrophotometer as response to As(III) concentrations. Blue color was quantified by measuring OD₅₉₅ after 3-h incubation time at 37 °C. The induction ratio (IR) was described in Section 2.2 in detail. The data presented here are the mean values of at least three independent experiments with standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

verify the applicability in quantitative measurement of color-based As biosensor, the groundwater spiked with different concentration of As(III) was used in *lacZ* induction assay.

The groundwater sample was previously analyzed by ICP–MS and found that the natural content of As was very low. As a result, different concentrations of As(III) (0, 10, 50, 500 $\mu\text{g L}^{-1}$) were spiked into the groundwater sample. These concentrations referring to drinking water (10 $\mu\text{g L}^{-1}$) and groundwater quality standards (50, 500 $\mu\text{g L}^{-1}$ for drinking and non-drinking, respectively) in Taiwan were used to test whether the color-based biosensor comply the standards of current regulation.

After 3-h incubation at 37 °C with As(III)-spiked groundwater or deionized water, obviously visible blue color was developed when the *lacZ*-based biosensor cells were exposed to As(III) comparing to that of no As control (Fig. 3A). Moreover, the blue color developed in groundwater has similar pattern with that in deionized water (Fig. 3A), suggesting that the response to As(III) of biosensor cells might not be interfered by other elements in the groundwater. Hence, the results suggest that the *lacZ*-based As biosensor could be used as a semi-quantitative measurement for future *in situ* application.

In order to quantitatively estimate As concentrations in groundwater, a standard curve was generated from known concentrations of As(III) in deionized water by log-linear analysis ($R^2 = 0.99$), and the resulting equation was used to evaluate the equivalent As(III) concentrations in groundwater sample. Fig. 3B shows that As(III) concentrations could be accurately estimated in the groundwater sample by the *lacZ*-based biosensor (correlation coefficient = 0.99).

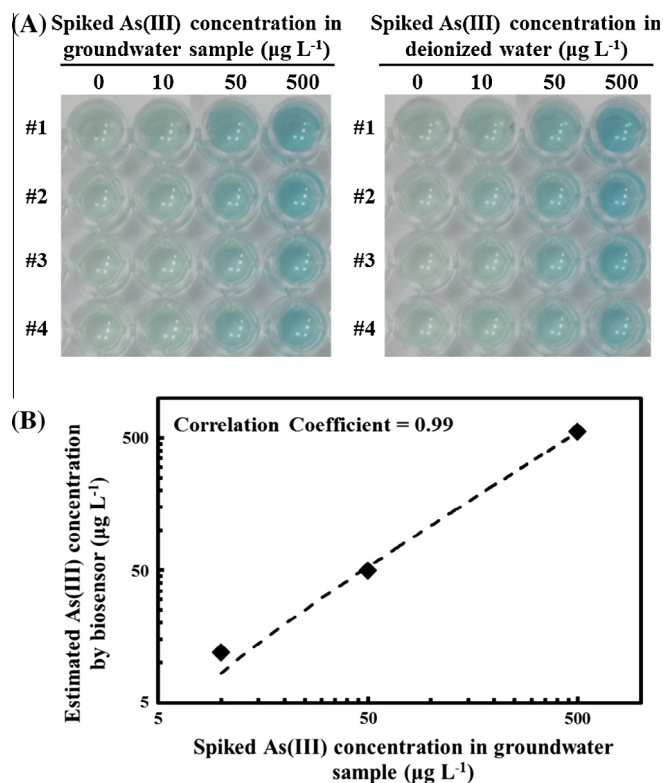


Fig. 3. Measurement of As concentration in As-spiked groundwater by pAs-*lacZ* biosensor. The groundwater sample was spiked with various As(III) concentrations and measured by pAs-*lacZ* biosensor. (A) Representative images were taken after 3-h incubation at 37 °C and then the blue color was quantified by measuring OD₅₉₅. As(III)-spiked deionized water was used as reference and standard curve. (B) Correlation between the estimated As(III) concentrations by biosensor and spiked As(III) concentrations in groundwater samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The estimated equivalent concentrations of As(III) in the spiked-groundwater sample are presented in Table 1. It is noted that a higher standard deviation was observed in higher As(III) concentration (e.g. 500 $\mu\text{g L}^{-1}$). Dilution of samples might provide a solution to this problem but it might not be practical in screening large amount of samples. Despite this, the result indicated that the *lacZ* quantification by measuring OD₅₉₅ could successfully apply in estimating the bioavailable As(III)-equivalent concentration in groundwater sample.

3.3. Effects and comparison of simple preservation methods

Drying and encapsulation have been widely used in long-term preservation of bacteria (Bjerketorp et al., 2006). Encapsulation in gel could preserve the bacterial cells for several weeks or months (Premkumar et al., 2002; Kim and Gu, 2003). Freezed or vacuum drying could preserve the bacteria for months (Stocker et al., 2003; Shin et al., 2005). However, these preservation techniques not only require additional devices but also result in high cost from electricity and instruments. Therefore, simple preservation methods such as liquid and cell pellet at 4 °C and RT (about 25 °C) were evaluated in this study.

For liquid storage at 4 °C, the response activity of the biosensor to each As(III) concentration was slightly declined in the first 3 d, yet the IR for higher As(III) concentrations (50 and 500 $\mu\text{g L}^{-1}$) still remained high (IR > 1.5) (Fig. 4A). Afterward, an increase of IR was observed, which might be due to cell adaptation to cold shock (storage at 4 °C), and reached the maximum IR at d 5. Finally, the response activity of the biosensor to As(III) was totally lost at d 10 (Fig. 4A). The dose–response curves remained high log-linear relationship ($R^2 > 0.90$) during the storage except for d 3 ($R^2 = 0.80$), d 9 ($R^2 = 0.83$), and d 10 ($R^2 = 0.65$) (Fig. S1). Taken together, the result shows that the biosensor cells were able to maintain the response activity for about 9 d in liquid storage at 4 °C.

For liquid storage at RT, the response of the biosensor to As(III) differs from that of liquid storage at 4 °C. The response activity of the biosensor to each As(III) concentration remained almost constant in the first 3 d storage, but the response activity was dramatically decreased at d 4 (Fig. 4B). The dose–response curves remained high log-linear relationship ($R^2 = 0.99$) during the storage (Fig. S2). Taken together, the result indicates that the response activity of cells could last for 3 d in liquid storage at RT.

For cell pellet storage at 4 °C, the response activity of the biosensor to each As(III) concentration dropped immediately at d 1 (Fig. 4C). Although log-linear relationship stayed high at d 2–4, the low IR of As(III) induction (10, 50 $\mu\text{g L}^{-1}$ As(III)) made the quantitative measurement unreliable (Fig. S3). In contrast, 500 $\mu\text{g L}^{-1}$ As(III) inductive response retained to d 4, whereas 10, 50 $\mu\text{g L}^{-1}$ As(III) inductive response completely lost at d 2 and 3 (Fig. 4C), suggesting that the decline of response activity might be due to the weak detection of the biosensor rather than the lethal toxicity of As(III).

For cell pellet storage at RT, the biosensor shows different response activity pattern to As(III) from that of cell pellet storage at 4 °C (Fig. 4D). A longer period (about 5 d) of response activity to As(III) was observed (Fig. 4D). However, similar to that of cell pellet storage at 4 °C (Fig. S3), the biosensor cell pellet was unable

Table 1
Estimation of As(III) concentration in As(III)-spiked groundwater by biosensor.

	Spiked As (III) concentration ($\mu\text{g L}^{-1}$)		
	10	50	500
Estimated by biosensor ($\mu\text{g L}^{-1}$)	11.9 $\pm$ 2.4	49.3 $\pm$ 22.9	557.1 $\pm$ 303.2

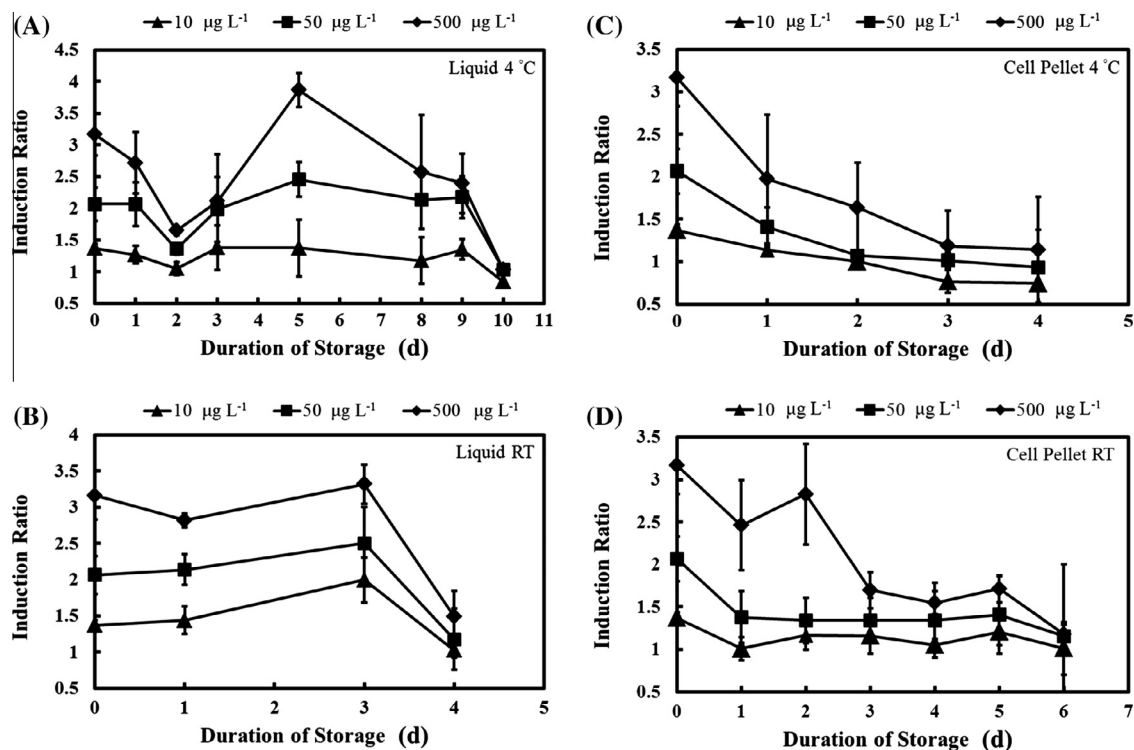


Fig. 4. Response activity of pAs-lacZ biosensor to As(III) in different preservation methods. (A) The biosensor cells were stored in LB broth at 4 °C. (B) The biosensor cells were stored in LB broth at room temperature (about 25 °C). (C) The biosensor cell pellets were harvested by centrifugation and stored at 4 °C. (D) The biosensor cell pellets were harvested by centrifugation and stored at room temperature (about 25 °C). All the response activity was examined by measuring OD₅₉₅ after various duration of storage. Different As(III) concentrations were used in response activity test and presented by triangles (10 µg L⁻¹), squares (50 µg L⁻¹), and diamonds (500 µg L⁻¹). All data presented here are the mean values of at least three independent experiments with standard deviation.

to quantitatively measure low concentration As(III) (10 µg L⁻¹) due to low IR by As(III) induction (Fig. S4). The inductive response of the biosensor to 500 µg L⁻¹ As(III) began to decline at d 2, whereas others declined at d 1 (Fig. 4D).

Other As biosensors with different preservations have been previously described. *E. coli* bioreporter cells carrying *gfp* were encapsulated in agarose beads and incorporated into a microfluidic device and cell-beads frozen at -20 °C in the chip retained the inducibility to As for up to one month (Buffi et al., 2011). However, storage at 4 °C resulted in a rapid decrease in 10 µg L⁻¹ As(III) inductive response within 5 d (Buffi et al., 2011). In addition, Kuppardt et al. (2009) evaluated different preservation methods to optimize the storage condition of biosensor cells. Vacuum drying and encapsulation in gels were evaluated and the cells were stored at 4 °C and the inductive response was observed to retain for at least 60 d (Kuppardt et al., 2009). In this study, liquid storage at 4 °C shows the shelf life about 9 d (Fig. 4A), and liquid storage at RT and cell pellet could retain the activity (Fig. 4B–D) for about 3–5 d. Nevertheless, the simple preservation methods presented in this study could keep biosensor cells available to As(III) induction for several days without complex and expensive preservation procedures.

4. Conclusions

In summary, this study constructed a color-based As biosensor that demonstrates both a semi-quantitative measurement for As through visual blue color and a quantitative range from 10 to 500 µg L⁻¹ As(III) in 3-h reaction time by measuring OD₅₉₅. Color-based biosensor developed in this study detects and accurately estimates As concentration in groundwater sample to comply the groundwater quality standards. Therefore, the reliable

color signal produced by the As biosensor could be further used for *in situ* screening in future study. Different simple preservation methods were tested in this study. Liquid storage at 4 °C showed the longest shelf life about 9 d. Liquid storage at RT and cell pellet could also be stored for about 3–5 d. Moreover, the As biosensor is cost-effective to measure As (~1 USD per sample) comparing with that of physicochemical methods (e.g. ~50 USD per sample by ICP-AES). Our study shows that the color-based biosensor is useful for rapid screening of As pollutant, providing the potential for large scale screening and better management strategies for environmental quality control.

Conflict of interest

The authors declare that no competing interests exist.

Acknowledgements

This study was funded in parts by the research project supported by the Taiwan EPA. The views or opinions expressed in this article are those of the writers and should not be construed as opinions of the Taiwan EPA. Mention of trade names, vendor names, or commercial products does not constitute endorsement or recommendation by Taiwan EPA.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2015.06.011>.

References

- Abernathy, C.O., Liu, Y.P., Longfellow, D., Aposhian, H.V., Beck, B., Fowler, B., Goyer, R., Menzer, R., Rossman, T., Thompson, C., Waalkes, M., 1999. Arsenic: health effects, mechanisms of actions, and research issues. *Environ. Health Perspect.* 107, 593–597.
- Agency for Toxic Substances and Disease Registry (ATSDR), U.S. Department of Health and Human Services, P.H.S., 2013. Priority list of hazardous substances.
- Binet, M.R., Poole, R.K., 2000. Cd(II), Pb(II) and Zn(II) ions regulate expression of the metal-transporting P-type ATPase ZntA in *Escherichia coli*. *FEBS Lett.* 473, 67–70.
- Bjerketorp, J., Hakansson, S., Belkin, S., Jansson, J.K., 2006. Advances in preservation methods: keeping biosensor microorganisms alive and active. *Curr. Opin. Chem. Biol.* 17, 43–49.
- Buffi, N., Merulla, D., Beutier, J., Barbaud, F., Beggah, S., van Lintel, H., Renaud, P., van der Meer, J.R., 2011. Development of a microfluidics biosensor for agarose-bead immobilized *Escherichia coli* bioreporter cells for arsenite detection in aqueous samples. *Lab Chip* 11, 2369–2377.
- Charrier, T., Durand, M.J., Jouanneau, S., Dion, M., Pernetti, M., Poncelet, D., Thouand, G., 2011. A multi-channel bioluminescent bacterial biosensor for the on-line detection of metals and toxicity. Part I: Design and optimization of bioluminescent bacterial strains. *Anal. Bioanal. Chem.* 400, 1051–1060.
- Cortés-Salazar, F., Beggah, S., van der Meer, J.R., Girault, H.H., 2013. Electrochemical As(III) whole-cell based biochip sensor. *Biosens. Bioelectron.* 47, 237–242.
- Daunert, S., Barrett, G., Feliciano, J.S., Shetty, R.S., Shrestha, S., Smith-Spencer, W., 2000. Genetically engineered whole-cell sensing systems: coupling biological recognition with reporter genes. *Chem. Rev.* 100, 2705–2738.
- Girotti, S., Ferri, E.N., Fumo, M.G., Maiolini, E., 2008. Monitoring of environmental pollutants by bioluminescent bacteria. *Anal. Chim. Acta* 608, 2–29.
- Hakkila, K., Green, T., Leskinen, P., Ivask, A., Marks, R., Virta, M., 2004. Detection of bioavailable heavy metals in EILATox-Oregon samples using whole-cell luminescent bacterial sensors in suspension or immobilized onto fibre-optic tips. *J. Appl. Toxicol.* 24, 333–342.
- Kaur, H., Kumar, R., Babu, J.N., Mittal, S., 2015. Advances in arsenic biosensor development – a comprehensive review. *Biosens. Bioelectron.* 63, 533–545.
- Kim, B.C., Gu, M.B., 2003. A bioluminescent sensor for high throughput toxicity classification. *Biosens. Bioelectron.* 18, 1015–1021.
- Köhler, S., Belkin, S., Schmid, R.D., 1999. Reporter gene bioassays in environmental analysis. *Fresenius J. Anal. Chem.* 366, 769–779.
- Kuppardt, A., Chatzinotas, A., Breuer, U., van der Meer, J.R., Harms, H., 2009. Optimization of preservation conditions of As(III) bioreporter bacteria. *Appl. Microbiol. Biotechnol.* 82, 785–792.
- Leist, M., Casey, R.J., Caridi, D., 2000. The management of arsenic wastes: problems and prospects. *J. Hazard. Mater.* 76, 125–138.
- Liao, V.H., Ou, K.L., 2005. Development and testing of a green fluorescent protein-based bacterial biosensor for measuring bioavailable arsenic in contaminated groundwater samples. *Environ. Toxicol. Chem.* 24, 1624–1631.
- Ma, J., Sengupta, M.K., Yuan, D., Dasgupta, P.K., 2014. Speciation and detection of arsenic in aqueous samples: a review of recent progress in non-atomic spectrometric methods. *Anal. Chim. Acta* 831, 1–23.
- Oremland, R.S., Stolz, J.F., 2003. The ecology of arsenic. *Science* 300, 939–944.
- Pott, W.A., Benjamin, S.A., Yang, R.S.H., 2001. Pharmacokinetics, metabolism, and carcinogenicity of arsenic. *Rev. Environ. Contam. Toxicol.* 169, 165–214.
- Premkumar, J.R., Rosen, R., Belkin, S., Lev, O., 2002. Sol-gel luminescence biosensors: encapsulation of recombinant *E. coli* reporters in thick silicate films. *Anal. Chim. Acta* 462, 11–23.
- Riether, K.B., Dollard, M.A., Billard, P., 2001. Assessment of heavy metal bioavailability using *Escherichia coli zntAp::lux* and *copAp::lux*-based biosensors. *Appl. Microbiol. Biotechnol.* 57, 712–716.
- Rogers, K.R., 2006. Recent advances in biosensor techniques for environmental monitoring. *Anal. Chim. Acta* 568, 222–231.
- Schweizer, H.P., Chuanchuen, R., 2001. Small broad-host-range *lacZ* operon fusion vector with low background activity. *Biotechniques* 31 (1258, 1260, 1262).
- Sharma, P., Asad, S., Ali, A., 2013. Bioluminescent bioreporter for assessment of arsenic contamination in water samples of India. *J. Biosci.* 38, 251–258.
- Shin, H.J., Park, H.H., Lim, W.K., 2005. Freeze-dried recombinant bacteria for on-site detection of phenolic compounds by color change. *J. Biotechnol.* 119, 36–43.
- Stocker, J., Balluch, D., Gsell, M., Harms, H., Feliciano, J., Daunert, S., Malik, K.A., van der Meer, J.R., 2003. Development of a set of simple bacterial biosensors for quantitative and rapid measurements of arsenite and arsenate in potable water. *Environ. Sci. Technol.* 37, 4743–4750.
- Tauriainen, S., Karp, M., Chang, W., Virta, M., 1997. Recombinant luminescent bacteria for measuring bioavailable arsenite and antimonite. *Appl. Environ. Microbiol.* 63, 4456–4461.
- Wackwitz, A., Harms, H., Chatzinotas, A., Breuer, U., Vogne, C., van der Meer, J.R., 2008. Internal arsenite bioassay calibration using multiple bioreporter cell lines. *Microb. Biotechnol.* 1, 149–157.