Anal Bioanal Chem (2011) 400:1031–1039
DOI 10.1007/s00216-011-4815-8

ORIGINAL PAPER

A pH-based biosensor for detection of arsenic in drinking water

K. de Mora · N. Joshi · B. L. Balint · F. B. Ward · A. Elfick · C. E. French

Received: 13 October 2010 / Revised: 13 February 2011 / Accepted: 16 February 2011 / Published online: 27 March 2011

Abstract Arsenic contaminated groundwater is estimated to affect over 100 million people worldwide, with Bangladesh and West Bengal being among the worst affected regions. A simple, cheap, accurate and disposable device is required for arsenic field testing. We have previously described a novel biosensor for arsenic in which the output is a change in pH, which can be detected visually as a colour change by the use of a pH indicator. Here, we present an improved formulation allowing sensitive and accurate detection of less than 10 ppb arsenate with static overnight incubation. Furthermore, we describe a cheap and simple high-throughput system for simultaneous monitoring of pH in multiple assays over time. Up to 50 samples can be monitored continuously over the desired time period. Cells can be stored and distributed in either air-dried or freeze-dried form. This system was successfully tested on arsenic-contaminated groundwater samples from the South East region of Hungary. We hope to continue to develop this sensor to produce a device suitable for field trials.

Keywords Biosensors · Groundwater · Arsenic · Assay · BioBricks

Published in the special issue *Microorganisms for Analysis* with Guest Editor Gérald Thouand.

K. de Mora · A. Elfick
School of Engineering, University of Edinburgh,
Edinburgh EH9 3JL, UK

N. Joshi
School of GeoSciences, University of Edinburgh,
Edinburgh EH9 3JW, UK

B. L. Balint
Department of Biochemistry and Molecular Biology,
University of Debrecen Medical and Health Science Centre,
Debrecen H-4032, Hungary

F. B. Ward · C. E. French
School of Biological Sciences, University of Edinburgh,
Edinburgh EH9 3JR, UK

C. E. French (✉)
University of Edinburgh,
Darwin Building, King's Buildings,
Edinburgh EH9 3JR, UK
e-mail: C.French@ed.ac.uk

Introduction

Arsenic in drinking water, in the form of arsenate or arsenite anions, is a major public health issue in a number of regions worldwide and is prevalent in South and South East Asia, especially in Bangladesh and West Bengal [1, 2]. Recent studies have shown that arsenic poisoning may be worse than previously thought, with as many as 77 million people possibly exposed to arsenic contaminated water in Bangladesh alone [3]. Studies have also shown groundwater contaminated with arsenic at concentrations up to 500 ppb in one of India's most heavily industrialised regions: the Thane region [4]. Arsenic is immediately toxic in high concentrations (~60,000 ppb), but exhibits chronic effects at lower concentrations [5]. The World Health Organization has set a maximum safe limit of 10 ppb (10 $\mu g\ l^{-1}$), but many regions still consider 50 ppb (50 $\mu g\ l^{-1}$) to be a safe target at the present time. Symptoms of inorganic arsenic poisoning can occur at concentrations varying from 300–30,000 ppb and include vomiting, stomach and intestinal irritation, nausea and diarrhoea [5]. One of the most visible signs of arsenic poisoning is skin lesions that can appear on the hands, feet and torso. These lesions can eventually result in skin cancer if untreated, but more often lead to social ostracism as arsenicosis is perceived

to be a contagious disease [6]. At lower concentrations, visible symptoms can take months or years to appear.

Current testing in the affected regions is most widely conducted using atomic adsorption spectroscopy (M. Owens, personal communication). This technique is quantitative and reliable [7] but problematic, as water samples must be shipped from contaminated sites to testing laboratories. The capital investment per system is in the order of 20,000 USD, with an additional cost for consumables and the use of a qualified technician. Various methods also exist to test for arsenic in the field, most notably the Wagtech Arsenator and paper test strips based on the Gutzeit method. The Arsenator produces a quantitative readout of the arsenic concentration based on an electronic read of the Gutzeit method [8]. The basic mechanism behind the Gutzeit-based field test strips is conversion of arsenic compounds into arsenic trihydride by zinc. The hydride then stains paper impregnated with mercuric bromide [9].

Although assays based on the Gutzeit method have been deployed in the past, researchers are looking towards enzymatic and biological approaches to detect arsenic. One approach is to use screen-printed electrodes (SPCE) with acetylcholinesterase (Ach) and to measure an amperometric response. Sanllorente-Mendez et al. have demonstrated an approach where they can detect arsenic to a limit of 1.1×10^{-8} M for their Ach/SPCE biosensor [10]. Although successful, they reported storing their electrodes at 4 °C, which may make this device difficult to transport and store in environments lacking refrigeration. Other groups have reported using the electrostatic oxidation of L-cysteine on screen-printed electrodes to determine arsenate concentration. Sarkar et al. immobilised L-cysteine on a working electrode by in situ polymerization of acrylamide and determined that their system produced a linear response to arsenic from below 1 to 30 ppb [11]. This work showed functional and sensitive arsenic detection using screen-printed electrodes; however, the most sensitive platinum variants may not be cost-efficient in terms of developing a field test device.

Whole-cell biosensors offer a potential alternative method for arsenic detection [12]. Previous arsenic bioassay systems have been developed using bioluminescence, Green Fluorescent Protein, and β-galactosidase as the reporter mechanisms [13, 14]. Previous work from van der Meer and colleagues demonstrated a system combining whole cell *Escherichia coli* biosensors with a microfluidics environment allowing arsenic concentration measurements to be taken from as few as 200 cells [15]. Bacteriological arsenic biosensors have been also been previously field tested [16]. We have previously described a biosensor based on genetically modified *E. coli* cells in which the presence of arsenate or arsenite induces expression of β-galactosidase, allowing fermentation of lactose and consequent change in pH which can be read using a simple pH indicator [17, 18]. Here, we describe further characterization of the strain, an improved formulation of assay medium that allows for static incubation of the samples and a new assay allowing continuous monitoring of up to 50 samples simultaneously.

Materials and methods

Organisms and growth conditions

Construction of our arsenic biosensor organism, *E. coli* JM109/pSB1A2-BBa_J33203, has been previously described [17]. The BioBrick assay construct, pSB1A2-BBa_J33203, is available from the Registry of Standard Biological Parts, hosted at Massachusetts Institute of Technology, as are the subcomponents BBa_J33201 (arsenic-responsive promoter) and BBa_J33202 (*lacZ'* reporter gene) used to generate it. Initial experiments were conducted in Luria-Bertani (LB) broth with 10 g/l lactose and 100 μg/ml ampicillin. Later experiments used a variant of Hugh and Leifson's OF (oxidation–fermentation) medium designated Arsenic Biosensor Medium (ABM) 6. ABM6 contains 2.0 peptone, 0.1 yeast extract, 0.3 K_2HPO_4, 2.1 $NaHCO_3$, and 0.1 g/l bromothymol blue. Lactose (10 g/l) was added if required, but in most cases was present in the dried cells used as inoculum as described below. All components were mixed prior to autoclaving. Arsenic was added as sodium arsenate from sterilised stock solutions at 1,000 or 10,000 ppb arsenic. Variants of ABM6 media were made with 1,500 mg l^{-1} of iron (II) sulphate (527 mg l^{-1} iron) and 2 mg l^{-1} zinc sulphate (0.76 mg l^{-1} zinc) concentrations, respectively, representing nine and four times the highest reported concentrations in the British Geological Survey of Bangladeshi groundwater [19]. For preparation of air-dried cells, overnight cultures were grown in LB, mixed with an equal volume of 20% *w/v* lactose, dispensed at the appropriate volume into 1.5 ml microcentrifuge tubes, and incubated with open lids at 37 °C overnight, during which the cell–lactose mixture dried to a glassy state. For freeze-drying, overnight cultures were harvested by centrifugation, resuspended in 20% *w/v* sterile lactose solution, dispensed into 1.5 ml microcentrifuge tubes, frozen at −80 °C and dried in an Edwards Modulyo freeze drier for 15 h. Dried cells were stored at room temperature.

Assay procedures

In initial experiments, assays were conducted as described previously [17, 18], with 6 ml LB plus lactose and other additives in 20 ml sealed glass vials, incubated with shaking at 37 °C, and pH was measured at intervals using

a standard semi-micro glass pH electrode. In later assays, as specified, 1 ml ABM6 was added to a tube of dried cells, and the desired concentration of sodium arsenate was added from a stock solution. Unless otherwise specified, tubes were then incubated at 37 °C without shaking. For simultaneous visual monitoring of multiple assays, up to 50 tubes were placed in a specially constructed rack in a 37 °C incubator with a clear front cover. Images were captured using a Creative Live! Vista IM webcam (model no: VF0260) and Nimisis Flix time-lapse software was used for image acquisition. Jpeg images were acquired due to the small size of the files and the .AVI time-lapse videos were made with no file compression in Flix. Due to file header and footer issues, the .AVI videos were opened and re-saved in ImageJ before being processed in Image Pro 7.0. Once parsed in Image Pro 7.0, data were processed into Excel readable spreadsheets using Mathworks Matlab.

Miller assays for β-galactosidase activity were conducted according to a standard protocol [20]. Activity of XylE (catechol-2,3-dioxygenase) was monitored by adding catechol to a final concentration of 0.1 mM to cell suspension and monitoring absorbance of the yellow reaction product (2-hydroxy-*cis*,*cis*-muconic semialdehyde) at 420 nm.

Results

Effect of medium and inoculum level

Previously, we have described assays in continuously shaken LB cultures inoculated from a previous liquid culture, with pH monitored at intervals using a pH electrode [18]. Clearly, this assay configuration is not ideal for field use. We therefore sought to develop a system with more suitable characteristics for use by non-experts under field conditions with minimal laboratory equipment. To clarify the effect of different growth parameters, initially, we investigated the effect of using a more dilute growth medium. Use of LB diluted by a factor of 2 or 4 showed the higher dilution factor medium led to less pH decrease in arsenic-free controls while maintaining a strong pH response to 60 ppb arsenic, but further dilution led to a decreased pH response in the presence of arsenic. Use of different levels of inoculum was also investigated. A larger inoculum led to more rapid response, but where samples were incubated for longer periods, the level of initial inoculum made little difference to the final pH over the range tested (data not shown).

Effect of bicarbonate

In our previous characterization tests, we sought to determine the effect of common groundwater buffer ions on pH response [18]. Phosphate at relevant levels was found to have no effect on the assay but bicarbonate was unexpectedly found to apparently increase the sensitivity of the assay, leading to enhanced pH response at low arsenic conditions. To determine the basis for this effect, we constructed a modified version of the biosensor by adding a second reporter gene, *xylE* (encoding catechol-2,3-dioxygenase; BioBrick BBa_J33204) to the BioBrick biosensor plasmid, giving the new biosensor construct pSB1A2-BBa_J15501. Biosensor organisms were incubated in the presence of differing levels of bicarbonate and arsenate and were assayed for pH, β-galactosidase activity (Miller assay) and XylE activity. As previously observed, the pH assay showed an enhanced response to low arsenate concentrations in the presence of bicarbonate, whereas no difference was seen at higher arsenate concentrations (Fig. 1a); thus, the presence of bicarbonate appeared to increase the sensitivity of the assay. However, Miller assays showed that β-galactosidase activity was increased in the presence of bicarbonate at both low and high arsenate concentrations (Fig. 1b); thus, it appears that the apparent lack of effect of bicarbonate on the pH-based assay at high arsenate concentrations is an artefact caused by saturation of the pH response above a certain β-galactosidase activity. Furthermore, despite a high background activity, XylE activity was also seen to increase in the presence of arsenate (Fig. 1c), suggesting that the effect was due to increased induction of the reporter genes rather than some direct effect on the activity of β-galactosidase. It therefore seems likely that bicarbonate is increasing the interaction of arsenate with the ArsR repressor protein, perhaps by increasing entry of arsenate into the cell or modifying arsenate speciation. Further experiments may clarify this. For practical purposes, our previously described experiments [18] have indicated that the effect of bicarbonate appears to saturate at a concentration below 10 mM bicarbonate; thus, addition of sodium bicarbonate to all assays at a concentration above this should both enhance sensitivity and reduce variability due to varying bicarbonate concentrations in water samples.

Development of an assay medium suitable for static incubation

The effect of different incubation conditions on assays in LB was also investigated, using a variety of different containers and liquid–air ratios (data not shown). The most significant result was that in unshaken cultures, a large pH drop always occurred in arsenic-free controls, presumably due to the decreased aeration, making the test unreliable. Since we aim to develop an assay suitable for field use, this led us to investigate alternative formulations of assay medium. The OF (oxidation–fermentation) test is a standard

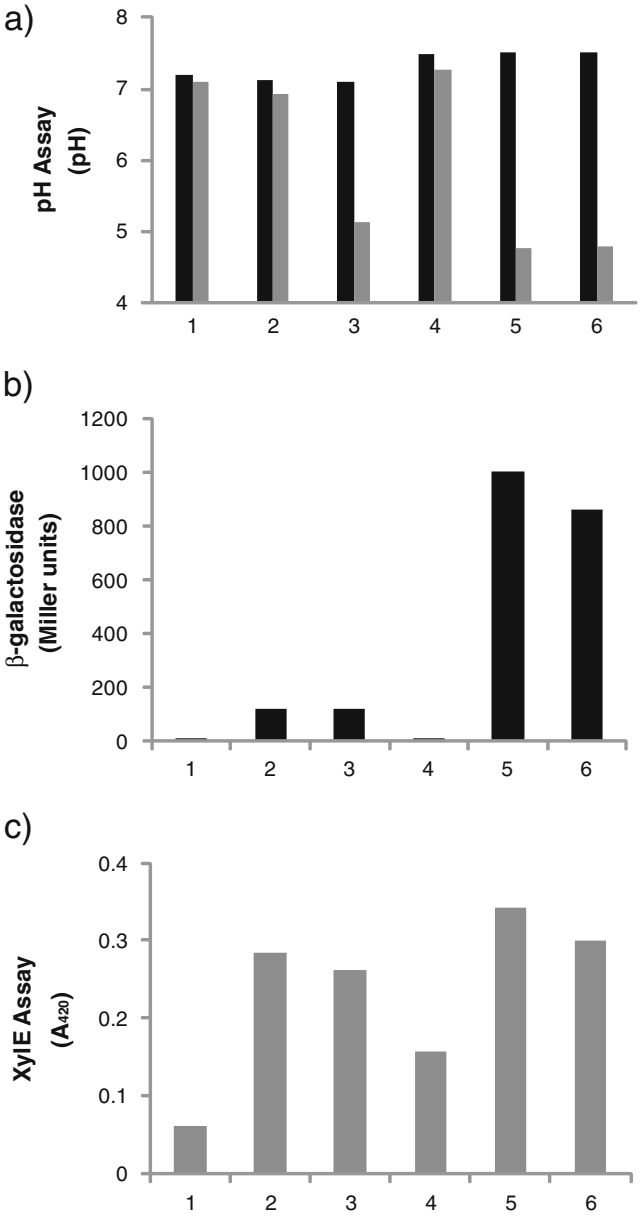

Fig. 1 Effect of bicarbonate on reporter activity. **a** Initial and overnight pH, **b** β-galactosidase (Miller assay), **c** XylE (catechol-2,3-dioxygenase) assay. Sample identification: *1* no bicarbonate, 0 ppb arsenic as arsenate. *2* no bicarbonate, 7.5 ppb arsenic as arsenate. *3* no bicarbonate, 15 ppb arsenic as arsenate. *4* 12.5 mM bicarbonate, 0 ppb arsenic as arsenate. *5* 12.5 mM bicarbonate, 7.5 ppb arsenic as arsenate. *6* 12.5 mM bicarbonate, 15 ppb arsenic as arsenate

and widely used test for the production of acid from carbohydrates and is commonly used in the identification of medically important bacteria [21]. This assay is normally conducted without shaking and uses the pH indicator bromothymol blue to generate a visual response. Various modifications of OF medium, with the addition of bicarbonate, were tested, eventually resulting in the development of ABM6 (see Materials and methods for composition), which was found to give a good colour response in less than 24 h in the presence of arsenate concentrations as low as 5 ppb arsenic but not to change colour over 24 h in the absence of arsenic, provided that a very low inoculum level was used (data not shown). This medium was used as the basis for further experiments.

Drying of cells for storage and distribution

Development of this assay for field use will require that cells be dried for storage and distribution. We have previously reported that lactose is a suitable cryoprotectant for freeze-drying of cells, resulting in as high as 30% survival under the conditions tested. This also provides lactose required for the assay. Since our previous experiments had shown that a small inoculum provides the best results for overnight assays under unshaken conditions, we investigated the possibility of air-drying as an alternative to freeze-drying, as this would remove the requirement for an expensive piece of equipment. Cells (initial volume of 20 to 100 μl per 1.5 ml microcentrifuge tube) were air-dried at 37 °C from a suspension containing 10% *w/v* lactose and were found to dry overnight to a glassy consistency. Figure 2 shows survival of cells dried from initial volumes of 20 or 100 μl of cell suspension (Fig. 2). Thus, the tubes containing 100 μl of cells initially showed higher survival but later showed more rapid die-off. This may be simply due to less effective drying leading to higher moisture content when sealed. Nevertheless, tubes of biosensor cells dried in this way showed reliable response to arsenic for at least 2 weeks after drying. Unless this can be improved with further development, freeze-drying will probably be required for longer-term storage.

Development of a colorimetric assay for the high-throughput quantification of pH measurements

To facilitate characterization of our biosensor system, we have developed a technique to measure the pH of multiple samples simultaneously over any desired period with high temporal resolution (Materials and methods). In order to calibrate the image acquisition system, we measured the absorbance of water samples with bromothymol blue adjusted to pH values in the range 10.91 to 3.24 (Fig. 3). The greatest change in absorbance was found to occur at 625 nm.

In the multi-tube system, assays are conducted by inoculating a series of 1.5 ml microcentrifuge tubes with ABM6 medium supplemented with arsenate, then placing them on a rack in a 37°C incubator in front of a Web camera. Images are acquired over the period of the experiment and compiled into a time lapse video which is read into Image Pro 7.0 and analysed using a custom-designed script. As the colour of the blue tubes stands out

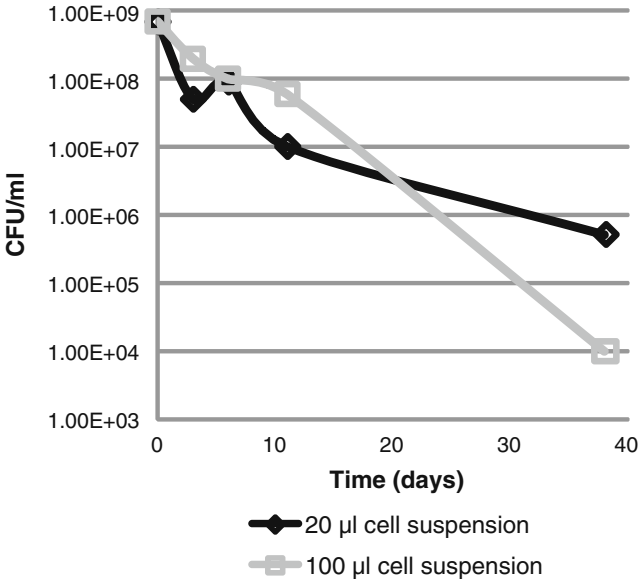

Fig. 2 Survival of air-dried cells following storage at room temperature

relative to the background, a colour threshold is applied to create regions of interest. For each region of interest, the red, green and blue colour channel intensity levels are averaged over the area of the tube and recorded in an Excel spreadsheet. The script applies the same regions of interest to each frame in the time-lapse creating RGB numerical values for each tube at each time step. The Excel file is read into MathWorks Matlab to parse the data into a three-dimensional matrix of the red, green and blue colour intensities. Initial examination of the raw data confirmed that the greatest colour change occurred on the red channel as seen at 625 nm in Fig. 3. Plots showing the colour change over time with bromothymol blue therefore used data from the red channel.

Calibration of the pH scale in the plot was achieved by imaging pH-adjusted samples of bromothymol blue. The suppliers of bromothymol blue (Sigma) specify a colour

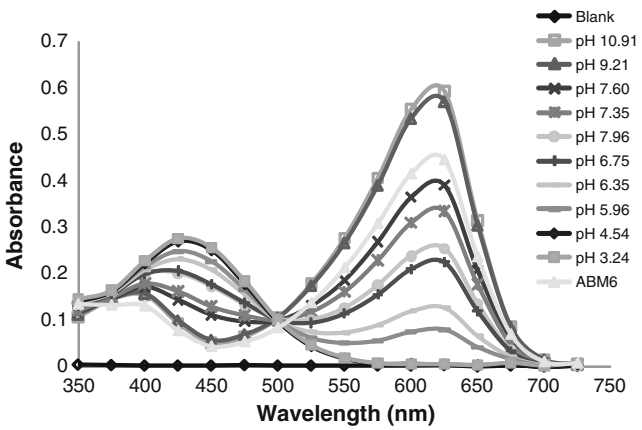

Fig. 3 Absorbance spectrum of bromothymol blue at varying pH values

change over the pH range of 7.6 (blue) to 6 (yellow) with shades of green in the transition region. Figure 4 shows a plot of illumination against pH for the same pH-adjusted samples used in Fig. 3. These data confirm that we can measure the colour change in samples over the 7.6–6 pH range specified by the supplier.

Once the assay configuration had been established and calibrated, the first trial of the system was performed using air-dried cells (dried from 50 µl of liquid consisting of 25 µl overnight culture plus 50 µl 20% *w/v* lactose; see Materials and methods above) following the addition of 1 ml ABM6 amended with various concentrations of arsenic (as sodium arsenate). Figure 5 shows the colour development over 60 h. The tubes are arranged in groups of three replicates in increasing concentration on the *X* axis and time on the *Y* axis. The general trend in the image shows that the higher concentration samples change from blue to yellow more rapidly than the samples with lower arsenic concentration. Note that one tube in the 10 and the 50 ppb arsenic concentrations did not change colour, indicating that further attention must be paid to quality control during preparation of assays.

In Fig. 5, colour changes can be observed over time. The exact moment of this change can be plotted if a quantitative measure of the colour of the tube can be obtained. We measured the tube colour using the quantitative method previously described in the Materials and methods section. Figure 6 shows the quantified change in colour of the pH samples over time. The data were processed by first normalising the cell-free controls to a pH of 7.6 as these tubes do not change colour and are therefore suitable for use as a datum and for colour correction. The tubes are adjusted to the same starting pH value, which corresponds to a pH of 7.6 or above. As the experiment is conducted

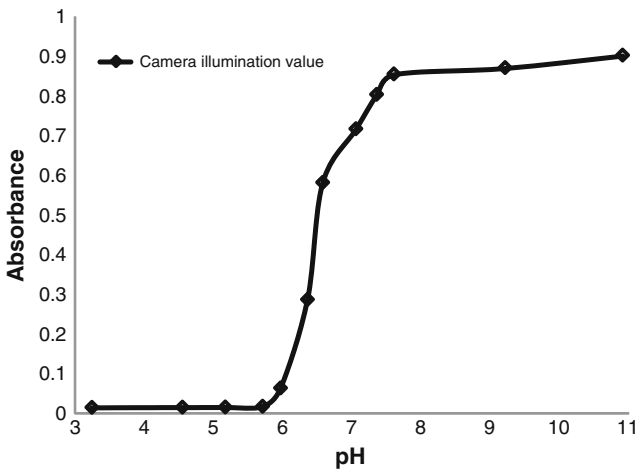

Fig. 4 Numerical illumination value against pH for a range of pH-adjusted samples. Each data point represents a software processed illumination value for a pH calibrated 1 ml tube of water and bromothymol blue

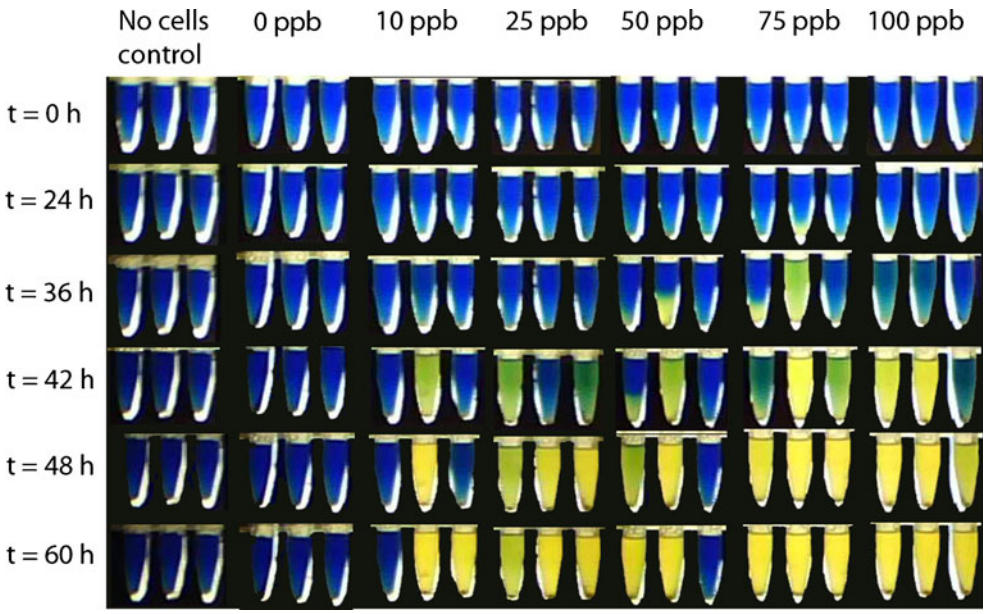

Fig. 5 Arsenic assays using bromothymol blue as a pH indicator. Each arsenic concentration was tested in triplicate on two separate days. Samples (50 µl inoculum) were statically incubated at 37 °C for a period of 65 h. *Blue* represents a safe level of arsenic while *yellow* indicates contamination. This figure shows the biosensor tubes in increasing arsenic concentrations at t=0, 24, 36, 42, 48 and 60 h where the error represents the standard error of six replicates

over a period of several days, these tubes are also used for illumination correction as minor intensity variations are observed due to the day–night cycle.

The data shown are the average of five or six replicates, as any tubes that failed to grow were removed for the purpose of data processing. The 50-ppb samples begin to change colour first followed by the 75 and 100 ppb samples. Finally, the 10 and 25 ppb samples change colour at approximately 30 h, about 10 h after the other samples. The arsenic-free control does not exhibit any colour change during the course of the experiment, as also seen in Fig. 5. Thus the time of colour change gives an approximate indication of the relative arsenic concentration.

Figure 7 shows the relative pH change over time for freeze-dried and air-dried cells. For freeze-dried cells, the pH change begins after 8 h in the samples containing 50 and 100 ppb arsenic and is complete after approximately 15 h. The freeze-dried arsenic-free control begins to show a colour change after 12 h, but does not complete this change until nearly 40 h. For the air-dried cells, assays with 50 and 100 ppb arsenic begin to show a change in colour after approximately 12 h, but do not complete the transition until nearly 35 h. The air-dried arsenic-free control begins to change colour after 18 h but does not finish this colour transition until nearly 60 h. This experiment shows an additional cell-free control with 100 ppb arsenic that showed no pH change during the period of the test. If the duration of the test were limited to 24 h, the freeze-dried tubes would have indicated a positive result at both the 50 and 100 ppb arsenic concentrations while showing almost no change in the absence of arsenic.

In previous experiments, a change in pH has been observed in absence of arsenic. A false-positive result is not acceptable in a field test. With the aim of eliminating colour change in arsenic free samples, we performed the same

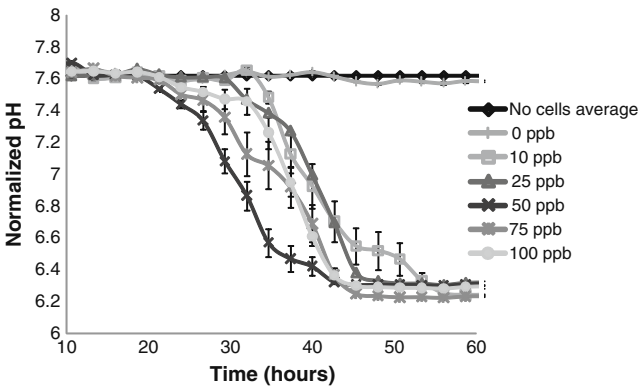

Fig. 6 pH change over time for air-dried cells with varying concentrations of arsenate using bromothymol blue as the indicator. The *error bars* show the standard error calculated from six replicates, where the experiments conducted using three replicates were performed on two separate days

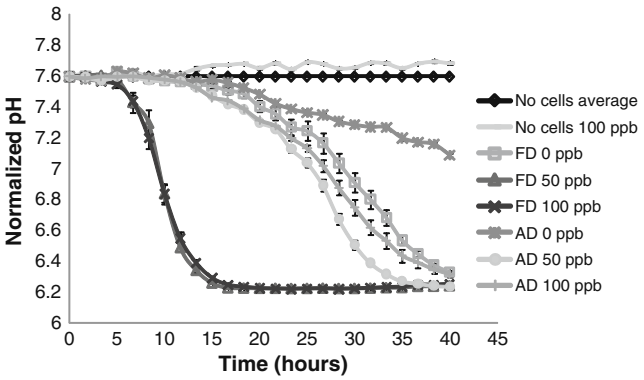

Fig. 7 pH change over time comparing freeze-dried (FD) with air-dried (AD) samples in ABM6 using bromothymol blue as the indicator. Error is the standard error of six replicates

assays using an alternative indicator, bromocresol purple, which has a lower transition pH. Assays with 50 and 100 ppb arsenic responded within 14 and 10 h, while the negative control shows little change until 30 h (Fig. 8). The pH change in the arsenic-free control also appears to halt at around 50 h, before the colour change is complete, suggesting that further modifications to the medium and indicator formulation may yield an assay which does not give a complete colour change in the absence of arsenic even after prolonged incubation.

Effect of other groundwater components

The British Geological Survey [19, 22] report on arsenic contamination in the example region of Bangladesh examined groundwater composition including several ions which could potentially affect the sensor. The *arsR* promoter/operon used in our system is known to be affected by antimonite [23]. As antimonite is also toxic to humans, detecting this compound would not necessarily be considered a false-positive. However, 90% of the samples tested in Bangladesh for antimonite by the British Geological Survey had 0.1 ppb or less, far below the WHO standard and far below the concentration of arsenic [19]. Therefore, antimonite is not thought to be of concern.

Iron (II) and zinc were also considered as possible sources of interference. The BGS report listed the maximum concentrations as 60 and 0.20 mg l^{-1}. Variants of ABM6 media were made with 1,500 mg l^{-1} iron (II) sulphate (527 mg l^{-1} iron) and 2 mg l^{-1} zinc sulphate (0.76 mg l^{-1} zinc) concentrations, respectively, representing nine times and four times the highest reported concentrations. These ions were tested with three air-dried cell replicates on two separate days, and the data are shown in Fig. 9. The data show that there are no noticeable changes in the sensor performance in the presence of zinc and iron (II).

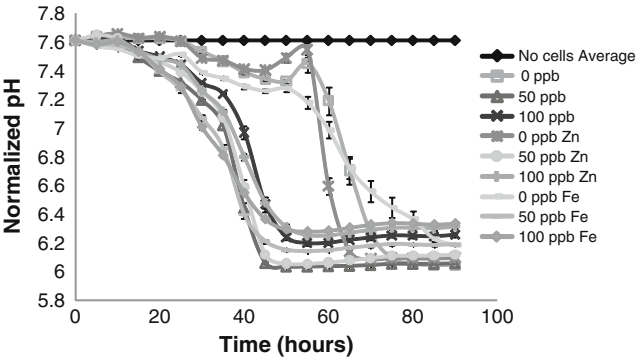

Fig. 9 Effects of iron (II) and zinc on sensor performance using air-dried cells. Samples contained 0, 50 or 100 ppb arsenic as sodium arsenate, in the presence or absence of 0.76 ppm zinc or 527 ppm iron (II) as indicated. Error shown is the standard error of the six replicates

Sensor performance using contaminated groundwater samples from Hungary

To test the biosensor system in a real world situation, we acquired several water samples from wells in Hungary that are known to be contaminated with arsenic [24]. We tested three samples of known concentrations, 1, 48 and 98 ppb arsenic. ABM6 medium at twice the normal strength was diluted 1:1 with filter sterilised water samples to give final arsenic concentrations of 0.5, 24 and 49 ppb arsenic and added to tubes containing air-dried cells. Figure 10 shows results obtained using the high-throughput colorimetric assay. The 24 and 48 ppb samples begin to transition at about 24 h, while the 0.5 ppb sample starts to change colour after 48 h. The arsenic-free control did not significantly change colour during the experiment.

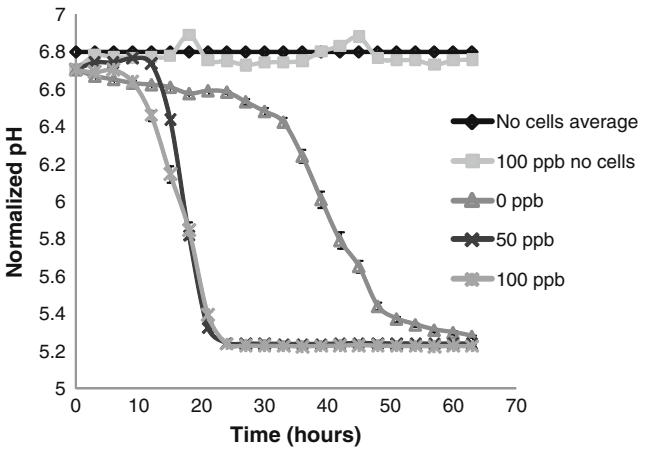

Fig. 8 Freeze-dried cells grown in ABM6 over time in absence and presence of arsenic using bromocresol purple as the indicator. Error is the standard error of three replicates

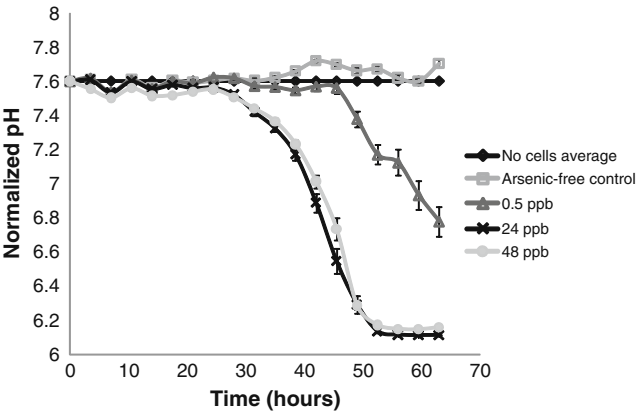

Fig. 10 Responses to arsenic contaminated groundwater samples from Hungary using air-dried cells. Effective concentrations of arsenic were 0.5, 24 and 48 ppb. *Error bars* represent the standard error of the six samples

Discussion

There is a clear need for a cheap, simple, sensitive and reliable assay for monitoring of arsenic concentrations in groundwater in rural areas. Ideally, this should be easy to manufacture, should not require expensive laboratory equipment and should allow a non-expert user to obtain reliable results. A well-designed whole-cell biosensor system could fulfil these requirements. Other groups have described whole-cell biosensors using GFP- and carotenoid-based systems. Tani et al. [25] have described a system using recombinant *E. coli* transformed with plasmids with copies of the *ars* promoter/operator using GFP as a reporter. Although they reported some success obtaining a detection limit of 7.5 ppb, when translated into a field device, a UV excitation and visualisation system is necessary to read the result. Yoshida et al. have also developed a whole-cell arsenite biosensor using *Rhodopseudomonas palustris* with the novel function of having a result that is readable using the human eye [26]. They successfully demonstrated the evaluation of bacterial colour change in the 1.0 to 500 ppb range using 30 volunteers during a double-blind test. Our pH-based biosensor system has the advantage that it gives a clear visual response instead of a relative colour change and shows high sensitivity, responding reliably to arsenate concentrations below the recommended WHO limit of 10 ppb arsenic. We have shown that buffer ions likely to be present in relevant groundwater do not interfere with the assay results and have demonstrated that our system functions with contaminated groundwater samples from Hungary.

One potential issue with a biosensor of this type is that contamination with other lactose-degrading organisms, such as coliforms, may lead to false-positive responses. If the assay is to be used in regional laboratories, this can easily be resolved by autoclaving or filter sterilisation of the samples. For field use, we are designing a disposable device incorporating a sterile filter, so that assay medium and water sample are filter sterilised as they are added to the dried cell pellet. The device can then simply be left in a warm place overnight to allow colour development.

Clearly, before any such device is put into large-scale use, it will be necessary to thoroughly investigate the effects of various parameters such as fluctuations in incubation temperature and presence of other dissolved substances. Our new multi-tube visual monitoring system will allow us to conduct large numbers of assays simultaneously, greatly facilitating our characterization experiments. Since the hardware required is relatively inexpensive, such a system may also be useful for performing large numbers of simultaneous routine assays in regional laboratories. In the current configuration, it appears that the time at which colour change occurs is the most useful parameter for estimating arsenic concentration and that these changes take place within 24 h when freeze-dried cells are used. Our system allows this information to be extracted by processing of the image data. Further development of the imaging protocols could be conducted in an open-source environment and potentially implemented as a plug-in for ImageJ or as a smart phone application.

In terms of storage and distribution, we have demonstrated that the assay works well with cells freeze-dried from lactose solution, and even air-dried cells are usable for at least several weeks following storage at room temperature. We are currently undertaking tests of stability under different storage conditions and have demonstrated successful detection of arsenic in groundwater samples from Hungary. Ultimately, we hope that an assay of this type will allow cheap and simple routine monitoring of arsenic concentrations in groundwater, reducing the burden of illness associated with this widespread problem.

Acknowledgments The authors would like to thank Jovana Radulovic for discussions and advice regarding the analysis of the numerical data, William Edwards for Matlab programming advice, David Kelly for help with Image Pro 7.0 and Steve Mowbray for assistance with lyophilisation. We would also like to thank the Biotechnology and Biological Sciences Research Council and the Engineering and Physical Sciences Research Council for funding.

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