Immobilization of fluorescent bacterial bioreporter for arsenic detection

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Abstract

Whole-cell bacterial biosensors hold great promise as a practical complementary approach for in-field detection of arsenic. Although there are various bacterial bioreporter systems for arsenic detection, fewer studies reported the immobilization of arsenic bioreporters. This study aimed at determining immobilization of specific bacterial bioreporter in agar and alginate biopolymers to measure level of arsenite and/or arsenate. To achieve sensitive detection, immobilization parameters of polymer concentration and cell density were evaluated. Moreover, by changing the culture medium, immobilized bioreporter cells in minimal medium can detect arsenite while they can detect both arsenite and arsenate in phosphate-limited minimal medium. When optimal parameters were applied, agar and alginate immobilized bioreporter systems can detect arsenite and arsenate concentrations of 10 µg/l and 200 µg/l within 5 h and 2 h, respectively. The results showed that the immobilized bacterial bioreporter systems are able to determine the concentrations of the two abundant species of arsenic; arsenite and arsenate, as opposed to other studies which reported only arsenite detection. This is the first study describe agar hydrogel and alginate bead immobilization of fluorescent arsenic bacterial bioreporter that can detect both arsenite and arsenate at the safe drinking water limit. Thus, this study will enable further steps to be taken towards developing sensitive and selective portable devices to assess environmental arsenic contamination and prevent acute arsenic toxicity.

Keywords Arsenic · Bacterial bioreporter · Fluorescence · Immobilization · Agar · Alginate

Introduction

Whole-cell biosensors (WCBs) for environmental probing have garnered significant interest as a complementary or an alternative analysis method for environmental monitoring. Heavy metal monitoring is of prime importance as they have many routes to enter water systems including direct releases or accidental spillage into waters, waste water treatment plants (effluent and sludge), industrial plants, landfilling areas, and

surface water run-off [1]. Whole-cell-based biosensors for heavy metal assessment has many advantages over currently applied laboratory techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS) or high-performance liquid chromatography (HPLC) which require skilled personnel, expensive equipments, and transportation of samples. WCBs offer cheap, simple, sensitive and rapid method of detection with the ability of multiplexed point-of-care screening [2]. For environmental monitoring of pollutants, a large number of bacterial bioreporters have been constructed and characterized [3, 4]. In general, bacterial bioreporters are obtained by introducing a reporter gene (e.g., *gfp* encoding green fluorescent protein) of which expression is controlled by the analyte sensing element consisting of operator/promoter sequence of a regulatory gene [5]. Upon exposure to their respective pollutants, they give an optical response that provides information about the type of pollutant, and insight about the toxicity of tested sample. They also provide bioavailable portion of pollutants since any pollutant has to across the cell membrane before interacting the sensing element.

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To be used as a portable and on-site monitoring device, the bioreporter cells should be preserved in a way for maintaining long-term cell viability and biological activity, preventing wash-out from flow cell while enabling efficient communication with the surrounding. Many techniques for maintenance and conservation of biosensor cells including continuous cultivation, immobilization/encapsulation in various polymers, and freeze/vacuum drying have been reviewed [6, 7].

Among heavy metals, arsenic (As) is of major health concern, because of its high degree of toxicity and presence at high concentrations in drinking water. According to the International Agency for Research on Cancer (IARC), inorganic As is classified as Group 1 carcinogen for humans [8]. Arsenic contamination is a serious worldwide problem and hundreds of millions of people have been drinking water containing arsenic at levels above the WHO provisional guideline value of 10 µg/L [9]. Arsenic is found in two dominant inorganic anions in aqueous systems as arsenite, As(III), or arsenate, As(V). It is mostly found as arsenate if the water is oxygenated (e.g., aerated and well oxygenated surface waters), and found as arsenite under reducing conditions or anoxic conditions (e.g., groundwater, deep lake sediments). These two highly toxic forms of arsenic are associated with arsenicism, a term used for arsenic related disorders including dermal lesions, skin cancers, internal cancers (bladder, kidney, lung), peripheral neuropathy and peripheral vascular disease [10]. Increased mortality rates with chronic arsenic poisoning is a serious problem particularly in regions where people can only access potable water from groundwater wells as they do not have centralized drinking water systems [11]. Thus, there is an urgent need for practical field detection of inorganic arsenic species.

WCBs hold great promise in rapid in-field bioassays for arsenic alternative to abiotic sensors and conventional laboratory techniques. There are various arsenic bioreporter strains developed combining different reporter genes with different regulatory genes. Frequently used sensing elements are the promoter/operator region of *ars* operon and *arsR* gene that are generally derived from *E. coli* arsenic resistance plasmids. The reporter elements are commonly based on the optical techniques that can be detected by color development, bioluminescence or fluorescence [12, 13]. However, a small number of studies have been reported about their immobilization to be later integrated in a biosensor device. In this study, fluorescent arsenic bacterial bioreporter strain that we formerly constructed [14] was immobilized in agar and alginate biopolymer matrices to convert the liquid assay into reproducible solid assay format. In literature, there are agar and agarose immobilized arsenite bioreporter systems [15, 16] while there is no alginate bead immobilization of arsenic bioreporter cells. To our knowledge, in this study, agar hydrogel and alginate bead immobilization of fluorescent bacterial bioreporter systems are firstly characterized that can detect both arsenite and

arsenate at a safe drinking water limit. The immobilization parameters were optimized for better fluorescence response, and different media were used to evaluate bioreporter performance in terms of sensitivity and selectivity.

Materials and methods

Bacterial strains and media

E. coli MG1655 (pBR-arsR773) strain harboring the sensor plasmid which was constructed with a transcriptional fusion between operator/promoter region of *ars* operon with its *arsR* regulatory gene and reporter gene, *gfp*, was used as fluorescent bioreporter strain [14]. M9 supplemented medium (6 g Na₂HPO₄/l, 3 g KH₂PO₄/l, 0.5 g NaCl/l, 1 g NH₄Cl/l, 0.5 g MgSO₄·7H₂O/l, 0.00015 g CaCl₂·2H₂O/l, 1 g casamino acids/l, 4 g glucose/l, pH 7) and MOPS supplemented medium (9.84 g MOPS sodium salt/l, 0.047 g Na₂HPO₄/l, 0.045 g KH₂PO₄/l, 0.5 g NaCl/l, 1 g NH₄Cl/l, 0.5 g MgSO₄·7H₂O/l, 0.00015 g CaCl₂·2H₂O/l, 1 g casamino acids/l, 4 g glucose/l, pH 7) were used. MOPS is 3-(*N*-morpholin-*o*) propanesulfonic acid. Ampicillin (100 µg/ml) was added to bacterial culture to maintain plasmid stability.

Chemicals

NaAsO₂ (sodium arsenite) and Na₂HAsO₄ (sodium arsenate) were used as inducers. Agar (05039) and alginic acid sodium salt (71238-for immobilization of microorganisms) were purchased from Sigma-Aldrich.

Bacterial cell preparation

The bioreporter cells grown in M9 supplemented medium overnight was used for inoculation into fresh M9 or MOPS supplemented medium at a 1:50 (v/v) ratio. Exponentially growing cells were centrifuged at 3000×g for 5 mins at 25 °C. Cell pellets were resuspended in a calculated amount of fresh medium to adjust cell concentration. For optimization of gel concentration, final cell density of OD₆₀₀ of 0.1, and for optimization of cell density, final OD₆₀₀ of 0.1, 0.4 and 0.7 were used. For optimization experiments, all bacterial cultures were prepared in M9 supplemented medium. The optical cell density measurements were done by using Multiskan GO UV/Vis Microplate and Cuvette Spectrophotometer (Thermo Fisher Scientific, USA) at 600 nm.

Agar gel immobilization

To assess the effect of agar concentration, three aqueous solutions of agar with different percentages (1, 2 and 3% (w/v)) were autoclaved at 121 °C for 15 min and kept at 70 °C.

Before immobilization procedure, temperature of agar solutions was decreased to 42 °C in a water bath. Bacterial culture prepared in M9 supplemented medium at $OD_{600} = 0.2$ was mixed with agar solution in 1:1 (v/v) (cells:agar) ratio, and gently pipetted for homogenization of agar-cell matrix. The procedure was carried out in temperature-controlled heat-block at 40 °C. The matrix was immediately pipetted into wells of the 96-well microplate in 100 µl aliquots and left to solidify at room temperature for 30 mins. The microplate was then covered and stored at 4 °C in an airtight container to prevent evaporation until use, which was always the next day. For agar gel optimization experiments, immobilized cells were induced with final 25 and 100 µg/l of As(III) concentrations.

Alginate bead immobilization

To assess effect of alginate concentration, three aqueous solutions of alginate with different percentages (2, 3 and 4% (w/v)) were prepared one day before procedure for polymer stabilization. Bacterial cultures prepared in M9 supplemented medium at $OD_{600} = 0.2$ were mixed with the alginate matrix in 1:1 (v/v) (cells:alginate) ratio and stirred gently for complete homogenization of cell-alginate matrix. With a syringe pump (Goldman AR-03, Turkey) connected to a 10 ml syringe with a 2 mm inner diameter spout, the bacteria-alginate solution was dripped at a flow rate of 3 ml/min into a 0.2 M $CaCl_2$ solution in a beaker glass, spontaneously forming spherical beads. The beads were gently stirred for additional 30 min for matrix hardening and to assure the completion of the gelling process. After that beads were rinsed with sterile dH_2O to remove excess calcium chloride and then placed into wells of the 96-well microplate. The microplate was then covered and stored at 4 °C in an airtight container to prevent evaporation until use, which was always the next day. For alginate bead optimization experiments, immobilized cells were induced with final 25 and 100 µg/l of As(III) concentrations.

Bioassays for arsenic detection

For arsenic time-dependent dose response experiments, bioreporter cells were cultured and prepared in either M9 supplemented or MOPS supplemented medium at optimal cell concentrations respectively for agar and alginate immobilizations. Agar and alginate solutions were also prepared at respective optimal gel concentration. The microplates were removed from 4 °C storage and incubated at room temperature for one hour to allow the cells to recover. Dilution series of As(III) and As(V) were prepared in sterile deionized water to obtain final As ion concentrations of 10, 25, 50, 75, 100, 150 and 200 µg/l (ranging from 0.133 µM to 2.66 µM of elemental As). These arsenic dilutions including an arsenic-free control (deionized water) were added in 25 µl and 100 µl aliquots for agar and

alginate matrices, respectively. Fluorescence measurements were performed by using Varioskan Lux Multimode microplate reader (Thermo Fisher Scientific, USA) which was programmed to measure fluorescence for every 30-min during 16 h with continuous incubation at 30 °C ambient temperature. For the reporter protein, sGFP, excitation/emission wavelengths of 395 nm/509 nm were selected.

Metal specificity test

The specificity of the immobilized bioreporter systems by various metal ions including Ag(I), Cd(II), Co(II), Cu(II), Fe(II), Hg(II), Mn(II), Ni(II), Pb(II) and Zn(II) were investigated. As(III) and As(V) were added in 50 µg/l, whereas all the other chemicals were added in 250 µg/l of final metal ion concentration. Both agar and alginate immobilized bioreporter cells were prepared in MOPS supplemented medium employed with their respective optimal parameters. The fluorescence measurements were taken using the procedure described above.

Data analysis

Raw fluorescence intensities were expressed in the instrument's arbitrary relative fluorescence units (RFU). The stable background fluorescence emitted from *E. coli* carrying promoterless plasmid (as blank) was subtracted from all averages of fluorescence values at the corresponding time points. The response of bioreporter cells to different concentrations of As(III) or As(V) was indicated by fluorescence intensity. Paired student's t test with a significance level of 0.05 was used between induced samples and uninduced sample (negative control) to determine the detection limit. The data presented are the average values of a triplicate of each induction presented with standard deviations shown as error bars.

Results

Optimization of the agar gel immobilization-percentage and cell density

Three final agar percentages of 0.5, 1 and 1.5 (w/v) with final cell density of $OD_{600} = 0.1$ were tested to assess the effect of agar concentration on the fluorescence response. Figure 1a shows that increasing agar concentration resulted in an increase of fluorescence response to arsenite. Hence, final agar concentration of 1.5% (w/v) was employed for further experiments. Final optical cell densities (OD_{600}) of 0.1, 0.4 and 0.7 (0.1 unit of $OD_{600} = 1 \times 10^8$ cells/ml) with final agar concentration of 1.5% (w/v) were also tested. When the cell concentration increased within agar matrix, the fluorescence response of uninduced cells drastically increased (Fig. 1b). The sensitivity

Fig. 1 Effect of different parameters on the fluorescence response of agar-immobilized arsenic bioreporter, **a)** agar percentages (w/v) with $OD_{600} = 0.1$, **b)** optical cell density (OD_{600}) with agar percent of 1.5 (w/v). Agar-cell matrix was prepared in M9 supplemented medium. The average value from triplicates of each induction is presented for 6-h assay time with standard deviation

of cell-agar matrix decreased with increasing cell density such that 25 µg/l of As(III) could not be distinguished from uninduced sample. The detection limit increased to 100 µg/l of As(III) which is not useful. Thus, final OD_{600} of 0.1 was chosen for further experiments due to the best sensitivity and dose-dependent fluorescence increase towards arsenite.

Optimization of alginate bead immobilization-percentage and cell density

Three final alginate percentages 1, 1.5 and 2 (w/v) with final cell density of $OD_{600} = 0.1$ were tested to assess the effect of alginate concentration on bioreporter fluorescence response. With increasing alginate concentration, the background fluorescence owing to uninduced sample increased (Fig. 2a), and fluorescence values of induced samples compared to that of uninduced samples decreased. Thus, final alginate concentration of 1% (w/v) was found to be best due to higher fluorescence response and sensitivity. Final optical densities (OD_{600}) of 0.1, 0.4 and 0.7 with

final alginate concentration of 1% (w/v) were also tested. When cell concentration was increased within alginate beads, fluorescence signal increased accordingly (Fig. 2b). Although cell density of $OD_{600} = 0.7$ had the highest fluorescence response, its huge standard deviation would disrupt the reliability of the test results. Thus, final cell density of OD_{600} of 0.4 was used in further experiments producing higher fluorescence response than that of $OD_{600} = 0.1$ and having much lower standard deviation than that of $OD_{600} = 0.7$ (Fig. 3).

Metal specificity of agar and alginate immobilized bioreporter cells

When the specificities of the immobilized arsenic bioreporter systems were evaluated, both agar and alginate immobilized arsenic bioreporter cells did not give any significant change of fluorescence response to tested metal ions (Fig. 4). This demonstrates the high specificity of immobilized arsenic bioreporters towards arsenite and arsenate.

Fig. 2 Effect of different parameters on the fluorescence response of alginate-immobilized arsenic bioreporter, **a)** alginate percentages (w/v) with $OD_{600} = 0.1$, **b)** cell density (OD_{600}) with alginate percent of 1 (w/v). Alginate-cell matrix was prepared in M9 supplemented medium. The average value from triplicates of each induction is presented for 6-h assay time with standard deviation

Fluorescence response of the agar immobilized bioreporter system to arsenite and arsenate

After determination of optimal agar concentration (1.5% (w/v)) and cell density ($OD_{600} = 0.1$), these parameters were employed for kinetic assays with agar immobilized arsenic bioreporter cells. A concentration range of arsenite and

Fig. 3 Images of arsenic bioreporters immobilized in calcium-alginate beads with different alginate percentages **a)** 1(w/v), **b)** 1.5 (w/v), and **c)** 2 (w/v)

arsenate between 0 and 200 µg/l were added to obtain time- and dose-dependent responses. Fluorescence emission curves were shown up to 8 h for clarity.

When bioreporter cells prepared in M9 supplemented medium were immobilized in agar matrix, arsenite concentration of 200 µg/l was detected after 1 h incubation. The detection limit decreased to 10 µg/l after 2.5 h of incubation period with a statistically significant change ($p < 0.05$) (Fig. 5). Arsenate concentrations between 0 and 200 µg/l did not yield any significant fluorescence response within assay time (data not shown).

For agar immobilized bioreporter cells prepared in MOPS supplemented medium, arsenite concentrations of 200 µg/l and 10 µg/l were detected after 1.5 h and 5.5 h of incubation time, respectively (Fig. 6a). Arsenate concentrations of 200 µg/l and 10 µg/l were detected after 1 h and 3.5 h incubation, respectively, with a statistically significant change ($p < 0.05$) (Fig. 6b).

Fluorescence response of the alginate immobilized bioreporter system to arsenite and arsenate.

Alginate bead immobilized arsenic bioreporters were induced with a broad concentration range of arsenite and

Fig. 4 Metal specificity of agar immobilized (filled symbol) and alginate immobilized (stripped symbol) arsenic bioreporter cells prepared in MOPS supplemented medium. Arsenite and arsenate were added in

50 $\mu\text{g/l}$, and other metal ions were added in 250 $\mu\text{g/l}$. Control refers to no-metal addition. The average value from triplicates of each induction is presented for 6-h induction time with standard deviation

arsenate between 0 and 200 $\mu\text{g/l}$ for determination of time- and dose-dependent responses. Optimal alginate concentration (1% (w/v)) and cell density ($\text{OD}_{600} = 0.4$) parameters were employed. Fluorescence emission curves were shown up to 8 h for clarity.

Alginate immobilized bioreporter cells in M9 supplemented medium, arsenite concentration of 200 $\mu\text{g/l}$ was detected after 2 h while detection limit decreased to 10 $\mu\text{g/l}$

after 4.5 h of incubation time with a statistically significant change ($p < 0.05$) (Fig. 7). Arsenate concentrations between 0 and 100 $\mu\text{g/l}$ did not yield significant fluorescence emission within assay time; however, concentrations of 150 and 200 $\mu\text{g/l}$ resulted in a slight induction after 11 h (data not shown).

When bioreporter cells prepared in MOPS supplemented medium were immobilized in alginate beads, arsenate

Fig. 5 Kinetic profile of fluorescence emission of agar immobilized arsenic bioreporters formulated with optimal parameters in M9 supplemented medium induced with different arsenite, As(III), concentrations. Error bars are shown only when they exceed the size of the symbols

Fig. 6 Kinetic profiles of fluorescence emission of agar immobilized arsenic bioreporters formulated with optimal parameters in MOPS supplemented medium induced with different **a)** arsenite, As(III), and

b) arsenate, As(V), concentrations. Error bars are shown only when they exceed the size of the symbols

concentrations of 200 µg/l and 10 µg/l were detected after 1 h and 4 h of incubation period, respectively (Fig. 8b). Arsenite concentrations of 200 µg/l and 10 µg/l were detected after 2 h and 4 h incubation, respectively, (Fig. 8a) with a significance level of 0.05. Fluorescence values have increased over time proportionally to the added arsenic concentrations.

Discussion

Over the last two decades, recombinant *E. coli* bioreporters for arsenic have been intensively developed by exploiting the presence of different sensing and reporter elements. On the other hand, irrespective of its sophisticated genetic

Fig. 7 Kinetic profile of fluorescence emission of alginate immobilized arsenic bioreporters formulated with optimal parameters in M9 supplemented medium induced with different arsenite, As(III), concentrations. Error bars are shown only when they exceed the size of the symbols

engineering, in-field applications of arsenic bacterial bioreporters outside the laboratory boundaries is only possible when they become integrated into suitable platforms to be used as portable sensing devices. To this end, studies for immobilization of arsenic bacterial bioreporter cells include lyophilization [17–19], agar [15] and agarose entrapment [16, 20] and alginate embedded fibre-optics [21]. The aim of this study was to improve a previously described arsenic detection [14] by conversion of the liquid assay to an immobilized format. We investigated both agar hydrogel and alginate bead immobilization to optimize their sensitivity and selectivity. Moreover, as opposed to many reports, we investigated bioreporter's response to arsenate which is the second most abundant ionic form of arsenic. We used cell entrapment method for immobilization since it is one of the least disruptive process of cell immobilization [22]. The arsenic bioreporter cells were entrapped in the interstices of naturally occurring biocompatible gel polymers, namely agar and alginate, to obtain functional systems. Bioreporter cells were grown to exponential phase since they showed higher fluorescence performance both in M9 and MOPS supplemented media than other phases, in the previous study [14]. All bioassays were performed at 30 °C in order to slow the growth of bacteria in the matrix (optimum growth at 37 °C) to keep cell number constant and to mimic outdoor conditions.

Optimization procedures

Agar hydrogel was chosen as one of the immobilization matrices since it is an advantageous well-known biopolymer for

entrapment of bacterial cells into hydrogel due to its ease of preparation, low cost, biocompatibility, good mechanical and acid stability [23]. The agar percentages of 0.5, 1 and 1.5 (w/v) were chosen since final agar percent higher than 1.5 was too viscous to mix with the cell culture and required high working temperature (higher than 42 °C) while agar percentages lower than 0.5 was not solid enough to support the cells. The results for agar gel optimization showed that final agar gel concentration of 1.5% (w/v) had highest fluorescence response compared to lower agar concentrations (Fig. 1a) which also had better mechanical rigidity. Similar findings were found in the study that reported the best agar concentration for their stress-responsive bioluminescent bacterial strains was 1.5% [24].

Besides polymer concentration, bioreporter cell density is also an important parameter for biosensor device development since it may yield either positive or negative effects. For cell concentration optimization within agar matrix, final optical cell density, $OD_{600} = 0.1$ was found to provide better fluorescence performance compared to higher cell densities. Increase in the cell concentration resulted in much higher background fluorescence leading to poor sensitivity as the fluorescence increase of induced samples were less significant. The high background fluorescence of uninduced cells could be attributed to 'leakage' of the *ars* promoter, i.e., incomplete binding of the ArsR repressor protein in the absence of metal. Although it could be seen as a slight leakage at low cell densities, it became more prominent when the cell concentration was increased (Fig. 1b). This result could also be explained by the negative effects of the crowded cell population such as shading, quenching, and limited oxygen availability to the deeper

Fig. 8 Kinetic profiles of fluorescence emission of alginate immobilized arsenic bioreporters formulated with optimal parameters in MOPS supplemented medium induced with different **a)** arsenite, As(III), and

b) arsenate, As(V), concentrations. Error bars are shown only when they exceed the size of the symbols

bacterial layers in the agar matrix. Moreover, some cells may die in the immobilization matrix becoming a barrier to the mass transfer of small molecules [25].

Alginate gel was chosen as another immobilization matrix for arsenic bioreporter cells. It is also commonly used and attractive biopolymer for cell entrapment due to its ionic gelation property in presence of divalent cations, especially Ca^{2+} . Alginate solution forms spherical beads spontaneously when it is dripped into calcium chloride solution, making the

process rapid and mild. Calcium alginate beads are biocompatible, transparent, stable, permeable, and they have high-water absorption capacity and high mechanical strength [26]. In our procedure, the average number of beads formed from 1 ml of alginate-cell matrix mixture was 30. Into the wells of 96-well microplate, three beads were placed for measurements since they could be arranged better than a higher number of beads, and they provided reproducible results than a smaller number of beads.

For the tested alginate percentages 1, 1.5 and 2 (w/v), the size of the beads increased as the concentration of alginate increased such that 1% (w/v) alginate matrix solution resulted in beads of average diameter of 3-mm while 2% (w/v) alginate matrix solution resulted in average diameter of 4-mm (Fig. 3). Moreover, the beads lost their transparency and appeared to be white as the alginate concentration increased, probably due to more tightly crosslinked network at higher concentrations with more calcium binding sites [27]. The optimal alginate concentration was found to be 1% (w/v) in terms of fluorescence response and sensitivity (Fig. 2a). As final alginate concentration increased, the background fluorescence and standard deviation increased possibly due to increased number of cells, since the size of beads increased which can accommodate higher number of cells. It was reported that higher alginate concentration results in fewer number of pores with smaller pore size [28]. Hence, it can be concluded that 1% concentration allowed higher diffusion rate of arsenite inside the bead matrix leading to higher arsenic bioavailability and induction of GFP expression.

Unlike agar gel matrix, increase in cell concentration in alginate beads resulted in increasing fluorescence response (Fig. 2b), emphasizing the importance of optimization of bioreporter cell density for biosensor development. However, increased cell concentration had a negative impact on sensitivity and reproducibility by resulting in higher standard deviation. This high deviation from the mean may result from the variation of cell numbers in beads and uneven diffusion of oxygen and arsenic solution to cells to deeper bacterial layers in alginate bead.

Metal specificity of the immobilized bioreporter systems

In natural environments, other metal ions are found in combination with arsenic, which may produce a non-specific induction. As metal specificity of agar and alginate immobilized bioreporter is concerned, 10 metals including Ag(I), Cd(II), Co(II), Cu(II), Fe(II), Hg(II), Mn(II), Ni(II), Pb(II) and Zn(II) were tested along with As(III) and As(V). These tested metals were chosen since they are listed as primary interest for their potential to harm human health [29] and they are generally found in soils and groundwaters. The results showed that both agar and alginate immobilized bioreporter systems were only responsive to arsenite and arsenate showing higher specificity for arsenic species (Fig. 4).

Fluorescence response of the immobilized bioreporter systems to arsenite and arsenate

The applicability of immobilized bioreporters were assessed by inducing the cells with arsenite and arsenate in different media. The concentrations of arsenic ions between 0 and

200 µg/l were chosen in relation to provisional guideline value of WHO and also Turkey's current regulation of drinking water quality standard of 10 µg/l. Currently, countries employ different quality standards for arsenic such that the safe limit for arsenic is 50 µg/l in India, China and Bangladesh [30].

The detection limit of agar hydrogel immobilized bioreporters prepared with M9 supplemented medium for arsenite was of 10 µg/l after 2.5 h of induction. Our detection limit was lower than similar studies by Elad et al. [15] and Charrier et al. [17]. Elad et al. reported minimal response ratio for 0.02 mg/l arsenite (As(III)) after 2 h for their bioluminescent *arsR* reporter, immobilized in 1.5% LB-agar and assayed at a microtiter plate at 37 °C. Charrier et al. reported 1 µM (75 µg/l) detection limit for arsenite after 2 h for their immobilized bioluminescent *E. coli* DH1 (pBarslux) strain in final 2% agarose and OD₆₂₀ of 0.1 at a microtiter plate at 30 °C.

For the detection limit of alginate bead immobilized bioreporter prepared with M9 supplemented medium, any of the tested arsenate concentrations could not be detected within the assay time but arsenite concentrations of 50 µg/l and 10 µg/l were detected after 3 h and 4.5 h of induction, respectively. After microplate assay was completed, the alginate beads were taken out and excited under UV channel. While uninduced beads (Fig. 9a) did not give any GFP expression and only reflecting the blue light, arsenite induced beads (Fig. 9b,c) gave green fluorescent emissions in a dose dependent manner that higher arsenite concentration resulted in more intense green light.

In most of the studies about arsenic biosensors, bacterial bioreporters' response to environmentally relevant concentrations of arsenate has not been reported. However, among inorganic arsenic species after arsenite, arsenate is the next most toxic form. Arsenite and arsenate are readily interconverted and therefore often found together, and the speciation between these might take place depending on the conditions such as the redox potential, pH, oxygen enrichment status. Furthermore, biotransformation of inorganic arsenic has been shown to occur in both animals and humans. Reduction of arsenate and oxidation of arsenite in vivo have been demonstrated in experimental animals [31].

The effect of medium used in immobilization procedure was tested by using MOPS supplemented medium. Since bioreporter cell culture and aqueous agar gel solution were mixed with 1:1 (v/v) ratio, the medium that cells were resuspended influenced the bioreporter's sensitivity and selectivity. When bioreporter cells were prepared in MOPS supplemented medium which has limiting inorganic phosphate content, they became highly sensitive towards arsenate ions. This phenomenon was explained by the chemical similarity between arsenate and phosphate ions. When cells were assayed in phosphate-rich environment, arsenate uptake become limited due to competitive phosphate uptake into the cells [32]. In

Fig. 9 The photos of alginate beads with immobilized bioreporters **a)** induced with water, **b)** induced with 25 $\mu\text{g/l}$ of arsenite, **c)** induced with 150 $\mu\text{g/l}$ of arsenite, in M9 supplemented medium after 16 h of incubation. Images were taken by smartphone camera while the beads were placed on EVOS Fluid Imaging Station (Thermo Fisher Scientific, USA) under illumination of blue channel with excitation 390 ± 40 nm and emission 446 ± 33 nm

MOPS supplemented medium formulated agar matrix, bioreporter cells showed remarkable change of response to arsenate that arsenate concentration as low as 10 $\mu\text{g/l}$ could be detected after 3.5 h of induction. Similar response was also seen in MOPS supplemented medium formulated alginate beads, the bioreporter cells sensitivity towards arsenate increased and arsenate concentration of 10 $\mu\text{g/l}$ can be detected after 4 h of induction.

Conclusion

This work details the two different immobilization methods for arsenic specific bacterial bioreporter cells for sensitive quantification of inorganic arsenic ions. These agar and alginate immobilized systems can be used as both arsenite and/or arsenate biosensors simply by changing the medium composition. Both forms of immobilized bioreporters have detection limits at environmentally relevant range which includes two typical permissible arsenic concentrations in drinking water of 10 $\mu\text{g/l}$ (for most European countries and the United States) and 50 $\mu\text{g/l}$ (for China and other countries). The 96-well microplate format described in this work displayed promising results that will lead to further investigations for developing specific, sensitive, and low-cost portable biosensor device for monitoring arsenite and arsenate content of naturally polluted water samples.

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Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

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