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Modeling and characterization of an engineered microbial biosensor for high-throughput screening of arsenic in rural water

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ABSTRACT

Current arsenic analysis methods in groundwater samples rely on expensive apparatus, complicated procedures, and dangerous chemical reagents. Also, delays in detecting arsenic harm to public health, the environment, agriculture and food sectors. Therefore, in this study, a bioluminescent biosensor has been optimized and used to detect and measure arsenic concentration in groundwater. Optimum conditions for the appropriate performance of *E. coli* DH5α (pJAMA-arsR) were determined and the luminescent calibration curve was drawn. The optimization results showed that maximum luminescent light output could occur at the end of the logarithmic phase or the beginning of the stationary phase, the temperature of 37 °C, and pH between 5.5 and 7 upon adding 10 μl n-decanal (18 mM). Increasing the duration of bacterial induction by arsenic leads to elevation of biosensor luminescent light yield. Functional stability of the biosensor with 20 % glycerol (V/V) at −20 °C was verified for at least six months. Luminescence reaction of the bacterial biosensor cells to arsenic concentration in the range of 0–90 ppb was promising (R^2 = 0.948 by linear regression), but higher arsenic concentration had poisonous effect on biosensor cells. The modified Gompertz model derived here could successfully predict the bacterial biosensor growth under the optimum condition compared with experimental data. In this study critical challenges, such as technical and appropriate performance, are defined to interpret the bacterial biosensor's true perspective to promote its broad adoption and usage. The work is concluded with closing remarks and potential perspectives to emphasize the importance of the bacterial biosensor, which could detect arsenic from a wide scope in real-time, quickly, and environmentally friendly signaling tool with high sensitivity and selectivity.

1. Introduction

Arsenic contamination of natural resources is a cosmopolitan problem (Shakya and Ghosh, 2018). Countries such as Bangladesh, India and some other countries in south and south-east Asia, have encountered with chemical contaminants especially arsenic pollution in their groundwater (Organization and Organization, 2007). Arsenic often enters the rural area through water (Chen et al., 2009; Organization and Organization, 2007) which causes human diseases such as skin necrosis, cardiovascular problems, or difficulties related to the gastrointestinal tract and respiratory system (Singh et al., 2015). It has become apparent that arsenic-polluted water used for irrigation enters into soils and foodstuffs (vegetables, cereals, fish, meat, milk), resulting sever threats to human health and sustainable agricultural production in those countries (Brammer and Ravenscroft, 2009).

Arsenic is present as an inorganic species in 85–95 percent of rice as well as other vegetables, compared to 65–75 percent in many food items (Mandal and Suzuki, 2002). Arsenic deposition in crops poses a significant health threat worldwide (Kofroňová et al.,

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2018). In addition, delays in detecting toxic compound spills in the environment will result in habitat loss, harm to public health, and, finally, a rise in the cost of remediation. Therefore, in the agriculture and food sectors, early detection of pollutants is critical (Adekunle et al., 2021).

Due to the severe health effects on humans, regulatory agencies have imposed maximum permissible limits of 10 ppb for arsenic in drinking water (WHO, 2011). However, the arsenic analysis is expensive, time-consuming and requires highly skilled technical personnel (Gürkan et al., 2015), making the need for in-field arsenic detection even more urgent especially in groundwater resources of regions with the extensive arsenic contamination (Diesel et al., 2009). Hence, there is a need to develop robust and inexpensive methods as a warning system to trace arsenic concentrations and report them (Wang et al., 2021b).

Real-time toxicity tracking has been reported to have various uses in the mining, livestock, health, and food sectors (Chouler and Di Lorenzo, 2015). Biomarkers are common among real-time toxicity monitoring methods due to their high sensitivity to a wide range of toxicants, quick regeneration (recovery) rates, ease of operation, genetic homogeneity, and flexibility (Wu et al., 2020).

A biosensor is an analytical tool that displays a synergistic combination of biotechnology and microelectronics(Singh et al., 2008; Verma and Rani, 2020). Biosensors basically comprise three main constituents: a bio-component (enzyme, whole cell, antibody, DNA, etc.), a transducer (electrochemical, optical, or thermal) and an amplification system.

While purified enzymes, proteins, and antibodies can properly and efficiently detect analytes, transcription and purifying circumstances must be optimized, and active agents should be immobilized (Mehta et al., 2016). Cutting-edge DNA sequencing and gene synthesis developments have stimulated the advancement of cell-based biosensors in the age of synthetic biology. Whole cell-based biosensors have numerous advantages, including the fact that they are simple and inexpensive to develop, can be used in a variety of assays, and do not need any data analysis. Besides, whole cell-based biosensors can be used to collect information well about the cellular toxicity of analytes, and desired biosensors could be further modified by genetic engineering (Bereza-Malcolm et al., 2015; Das et al., 2016). Whole cell-based biosensors were created by combining the promoter region of metal-responsive operons with reporter genes, regulated by transcription regulators and can reflect the existence of target metal ions, accordingly (Kim et al., 2018).

There are various advancements made in the development of arsenic biosensors based either on recombinant whole cells (Luciferase-based biosensors, lacZ-based biosensors, and green fluorescent protein (gfp)-based biosensors) or certain arsenic-binding oligonucleotides or proteins (DNA-based arsenic biosensors, pH-based, Aptamers-based arsenic biosensors, and Protein-based arsenic biosensors) (De Mora et al., 2011; Kaur et al., 2015; Merulla et al., 2013b; Wang et al., 2021b).

Microbial biosensors have been developed as a supportive method to detect bioavailable heavy metals in the environment (Bereza-Malcolm et al., 2018). Recombinant luminescent bacterial strains have been investigated as possible candidates due to certain advantageous characteristics that they possess, such as high sensitivity and selectivity, short measurement times, ease of use and low costs (Lee et al., 2005; Wang et al., 2021a). When the biosensor experiences environmental stress, a signal transduction cascade takes place in which specified promoters and proteins are expressed to adjust to environmental stress (Merulla et al., 2013a). Bacterial biosensors employ these stress promoters for toxicity sensing and signal production (Kaur et al., 2015; Liao and Ou, 2005). Biosensors could have high market potential for the monitoring of environmental pollutants.

Luminescent microbial biosensor is a readily and commonly used tool to detect toxic events and can be used in-line and in real-time but have some shortcomings. Its signal production depends on several environmental factors including pH, substrate availability and the concentration of toxins (Farre et al., 2010). However, it could be developed into a continuous, on-line, on-site system for monitoring the quality of environmental samples. This could influence the sensitivity of biosensor toward toxic components as the changes in the resulting signal may be different depending on the potential to which microbial biosensor is exposed. It was not yet investigated what the setting of bacterial growth model needs to be to get the signal that is most sensitive to the presence of toxic components. Therefore, it was important to investigate whether adjusting the bacterial growth conditions influences on the magnitude of the change in the luminescence light output signal. Consequently, the influence of different external values on luminescence sensor sensitivity was investigated in this study. Moreover, we studied the growth curve of *E.coli* DH5α (pJAMA8-arsR), a genetically engineered luminescence bacterial biosensor for arsenic detection, with focusing on the Gompertz model to predict its growth model (Buchanan et al., 1997). In this model, the bacterial growth curve is sorted into three phases: lag, logarithmic, and stationary phase, while the death phase is not included (Halmi et al., 2015). Also, as far as we are aware, luminescent biosensors have been rarely used in West Asia to detect arsenic. Nonetheless, it appears that the difference between experimental and fieldwork when it applies to implementing these systems is still wider than it should be. In this study, a luminescent microbial biosensor has been optimized and used to detect and measure arsenic concentration in several samples collected from arsenic polluted sites of a rural area in Iran.

2. Materials and methods

2.1. The growth and luminescent characteristics of the bacteria

2.1.1. Bacterial strain and the growth medium

The *E. coli* (pJAMA-arsR) strain has been previously employed for the on-line detection of arsenic (Diesel et al., 2009). This strain was constructed after the transformation of plasmid pJAMA-arsR into *E. coli* DH5α (Stocker et al., 2003) (Fig.1). The functional mechanism of the mentioned biosensor is based on the natural resistance mechanism that encoded by the *ars* operon. In the absence of arsenic, the ArsR repressor binds to its operator/promotor site within the *ars* operon and prevents further expression of itself and the downstream *ars* genes. When arsenic enters the cell, it interacts with the ArsR repressor leading to a conformational change and dissociation of the ArsR protein from its operator. Subsequently, the *arsR* responsive promoter fused to *lux AB* gene coding for luciferase is activated and light emitted corresponding to arsenic concentration (Stocker et al., 2003). For the activation of luciferase enzyme, a long-chain aldehyde (decanal in our experiment) should be added as a substrate (Sukovataya and Tyulkova, 2001). Arsenate solutions were prepared by dilution from arsenic (V) stock solution (1 g/l) ($Na_2HAsO_4 7H_2O$, reagent grade, BDH) in distilled water at pH 7.

In the bacterial model of this study, *E.coli* DH5α (pJAMA8-arsR), the synthesis of the resistance gene to arsenic and luciferases occur by the cell (Stocker et al., 2003). The highest resistance against arsenic and luciferase production of the bacteria would be at the end of the logarithmic phase and the beginning of the stationary phase; therefore, finding the approximate time for different levels of the growth in bacteria would be exploitable and accelerating to carry out other studies. Due to the progressive rate of bacteria proliferation, time-relative logarithm curve of the rate of the population growth can be useful (***y =*** Ln ***(N/N0)***). We can describe

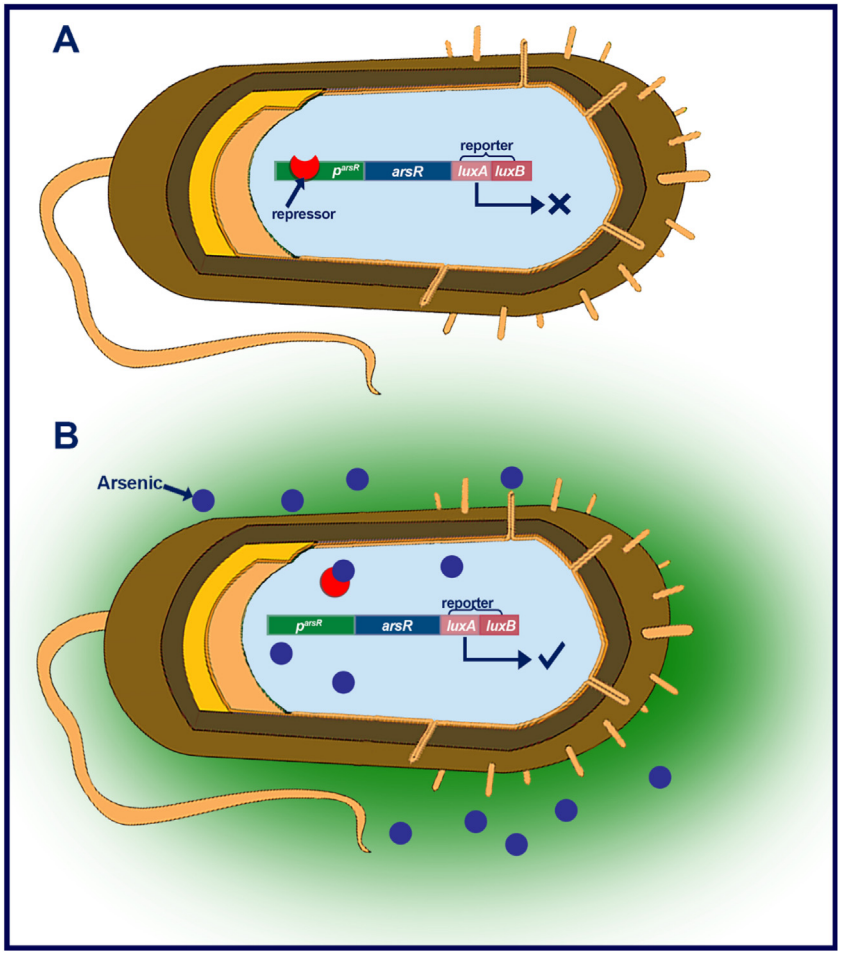

Fig. 1. Schematic drawing of the arsenic biosensor *E. coli* (pJAMA-arsR). (A) In the absence of arsenic, transcription of *luxAB* reporter gene is repressed by ArsR. (B) When arsenic enters the cell, it interacts with the ArsR repressor leading to the dissociation it from promoter. Therefore *luxAB* reporter genes are de-repressed and luciferase enzyme produced in which luminescence light output can be monitored.

the three phases of the growth curve by three parameters, each of which acts on different aspects (Zwietering et al., 1990):

1 The maximum growth level (μ_m): which is defined as the slope of the tangent at the inflection, and usually by the mental imagery that one part of the curve is almost linear and the slope of the line is the maximum growth rate.
2 t_L or lag time: which is introduced as an intersection of the tangent with the X-axis.
3 The maximum amount of cells: which can be obtained in the asymptotic A, which represents the ultimate value of $Ln\ (N/N_0)$ (Zwietering et al., 1990).

This theory creates a precise relationship between mathematical modeling aspect and biological and fundamental science. The Gompertz model contains the following equation:

$$\ln\left(\frac{N}{N_0}\right) = ae^{-e^{(B-ct)}} \quad (1)$$

While a, B and c are the constants of the formula and can be extracted from the fitting laboratory data analysis.

Luria-Bertani (L.B.) medium (Merck) was used for the growth of the microbial cells. The pH was adjusted to 7, and ampicillin with a final concentration of 100 ppb was added to the medium. An overnight culture of *E. coli* luminescent biosensor stocks was prepared at a dilution of 1:50 into LB medium supplemented with 100 ppb of ampicillin. The cells were grown in a shaker at 37 °C until turbidity at 600 nm was reached between 0.5 and 0.6. The culture was then placed on ice for 15 min, and 10 mL of ice-cold sterile glycerol (87 % (v/v)) was added and mixed. The mixture was kept on ice while it was divided into micro-tubes and samples were then stored at −20 °C (Stocker et al., 2003).

Stored *E. coli* (pJAMA-arsR) strain (0.5 mL) was cultured in LB broth which was supplemented with 100 ppb of ampicillin at pH 7. The bacteria were grown in a shaking incubator with a speed of 150 rpm at 37 °C. Then 200 μL of overnight culture was added to each well of an ELISA microplate and optical density at 630 nm was determined using a microplate reader (Awareness technology, USA). Assay mixtures of the luminescent light emissions were prepared in 0.5 mL micro-tubes. First, 30 μL of biosensor cell suspension was pipetted into each micro-tube following by addition of 170 μL of arsenate standard solution which mixed thoroughly with the sampler. Second, the mixture was covered with parafilm and incubated at 37 °C in a rotary shaker at 150 rpm for 1 h. After incubation, 10 μL of n-decanal substrate solution (18 mM in 50 % v/v ethanol in water) was added to each micro-tube mixed again by pipetting. The luminescent light emission was subsequently measured using a luminometer (Berthold Technologies, Germany).

2.2. Optimization of parameters essential for the arsenic detection

Bioluminescence production was optimized using various parameters including incubation time in the presence of arsenic and n-decanal solution, temperature, n-decanal levels, glycerol, pH,

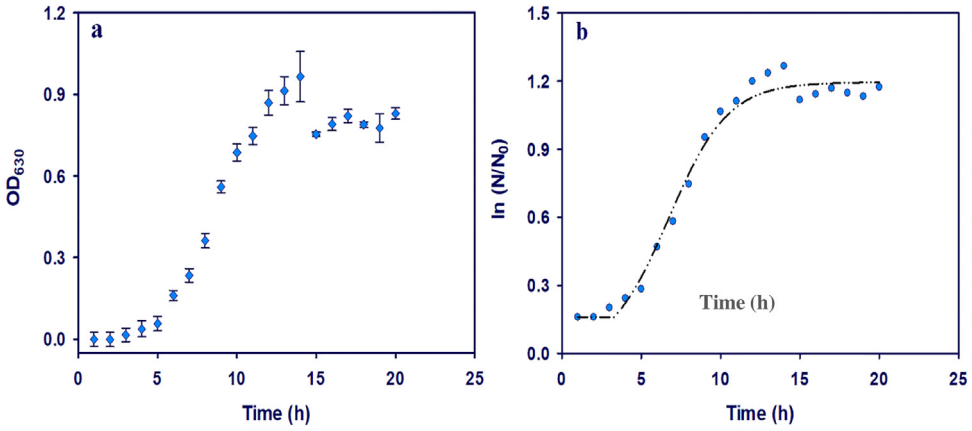

Fig. 2. The growth curve with respect to experimental data (a), and using the Gompertz model (b).

and aeration. The time of the mixtures incubation with the arsenic and n-decanal were optimized at different times (15, 30, 60, and 90 min) and (30-sec intervals for 13 min), respectively. The bioluminescent yield was optimized at different ranges of temperature (20, 30, and 37 °C), n-decanal levels (5, 10, 15, and 20 μL) and glycerol (75 %, 80 % and 85 % v/v). The bioluminescence response to pH and aeration of the medium were determined with varying pH (5.5, 6, 6.5, 7 and 7.5) and setting a rotary shaker to 25, 50, 100, 150 and 200 rpm. The experiments were designed so that all factors would remain constant with the exception of one factor, all tests were performed in triplicate. Each test started with the thawing of frozen samples in a water bath at 25 °C for 2 min immediately prior to the assay. Testing mixtures containing 100 μL of cell suspension and 100 μL of arsenate (75 ppb) were prepared in a 0.5 mL microtube. Bioluminescence production was measured in an identical manner to the method outlined in the aforementioned tests.

2.3. Drawing of calibration standard curve

The relationship between arsenate concentrations and bioluminescence production was determined by plotting standard curve. The bacterial cells (100 μL) and the arsenate solution (50 μL) were mixed. The calibration series was found to contain the following: 0, 15, 30, 50, 75, and 90 ppb arsenate. The detection limits for the biosensors were determined. Thus, the correlation between concentrations of arsenate in the range of 0–30 ppb (0, 2, 4, 6, 7.5, 8, 15 and 30 ppb) and bioluminescence production was established. To verify the effectiveness of the biosensors as a detector, response to samples with known arsenic concentrations (8, 15 and 30 ppb) was compared with a linear interpolation of the standard curve.

2.4. Analyzing arsenic-affected groundwater

2.4.1. Groundwater sampling

Cluster random sampling was used to take groundwater samples. Rivers, springs and water wells were sampled at KoohSorkh Township (North of Kashmar in NE Iran) including Rivash city, the Chelpu region and rural areas of Akbarabad. The groundwater samples were immediately filtered through 0.45 μm filters and were transferred to acid-washed 500-mL polyethylene bottles. For quality assurance/quality control (QA/QC), the samples were then acidified to a pH of about 2 by the addition of HNO_3 (65 %, 14 M, Merck) (see Supplementary information). The water bottles were transferred to the lab, stored at 4 °C, and analyzed for the amount of arsenic concentration using both the biosensor method and Atomic Absorption Spectroscopy (Analyst 800 in Asra Shargh Mashhad, Iran). Groundwater samples from Water & Sewage Co. Khorasan Razavi were taken, and arsenic concentrations were then measured using DC polarography (797 VA Computrace in Water & Sewage Co. Khorasan Razavi, Iran). During groundwater sampling procedures, concentrated nitric acid was added to preserve the sample composition. Samples were then acidified to a pH of approximately 2. In the case of these acidified samples, 250 μL of a 1 M potassium phosphate solution ($KHPO_4.3H_2O$, $K_2HPO_4.3H_2O$, Sigma) was added per 50 μL groundwater sample to the test vial for the purpose of raising the pH and buffering the sample at approximately pH 7.

2.5. Freeze-drying of E. coli (pJAMA-arsR) strain

An overnight culture was diluted 1:50 into 50 mL of LB medium which was supplemented with 100 ppb of ampicillin and grown in a shaker at 37 °C until the bacteria cells had entered the stationary phase, which is the phase of maximum bioluminescence production. Cells were harvested by centrifugation (7000 × *g*, 20 min, 4 °C) and suspended in the same volume of LB medium which was supplemented with 10 % sucrose solution. Cells were freeze-dried in 200-μl aliquots following standard procedures (Janda and Opekarová, 1989). Freeze-dried cells were tested for arsenate. Cells were reconstituted in an overnight culture, and luminescence measurements were taken in the same way as with the fresh cells. All experiments were done in triplicate. Excel and Sigmaplot were used to analyze data.

3. Results

3.1. The growth characteristics of the bacteria

The bacterial growth curve was plotted according to the experimental data (Fig. 2a). However, after calculating ($\ln \frac{N}{N0}$) toward time and fitting the growth curve according to the Gompertz model, the growth curve was then replotted (Fig. 2b).

The first step in predicting the growth curve is deriving the Gompertz model constants. Microsoft Excel and Sigma Plot were used for data entry and estimating the three constants. The next step in analyzing the growth curve was finding the asymptotic and the lag phase. The lag time (t_L) happened at 4.6657 h after inoculation. Horizontal asymptote was determined by the given function equal to 1.1575 (t tends to infinity). The maximum growth level (μ_m) was equal to 0.225744.

3.2. Luminescent emissions

Escherichia coli DH5α cells containing the plasmid pJAMA-arsR were cultivated at 37 °C in LB medium supplemented with 100 μg

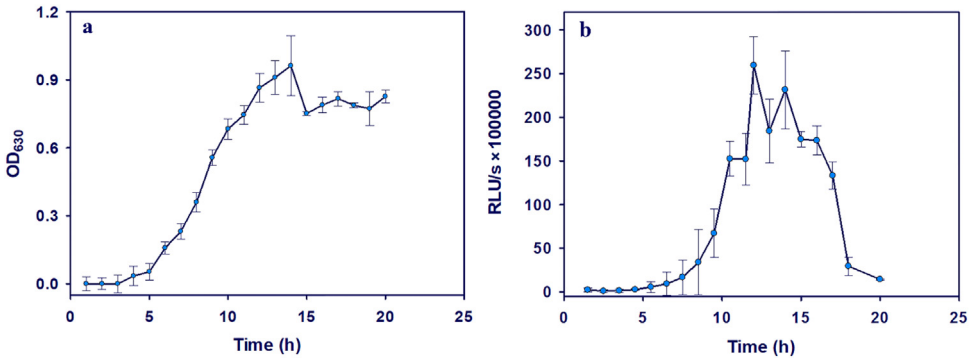

Fig. 3. Growth curve of *E. coli* DH5α (pJAMA-arsR) in semi-logarithmic coordinates (a), and luminescent light output of *E. coli* DH5α (pJAMA-arsR) in the presence of arsenate (b). RLU = relative light units.

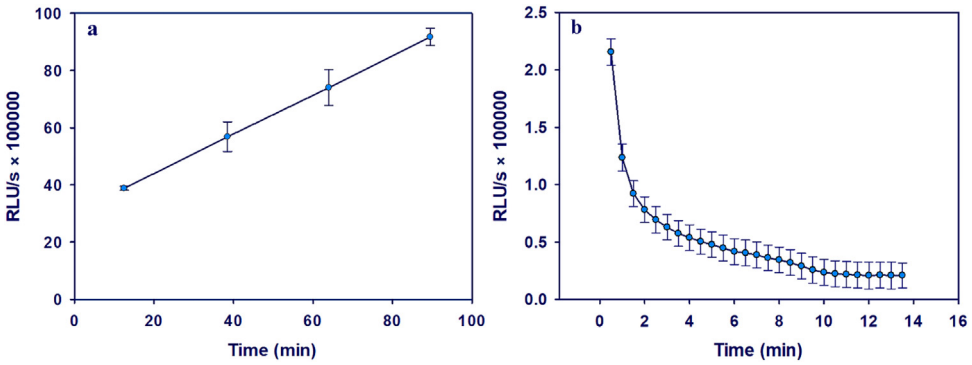

Fig. 4. Effects of the cell incubation time on the luminescent light yield of *E. coli* DH5α (pJAMA-arsR) at presence of 75 ppb arsenate (a), and n-decanal (18 mM) solution (b).

of ampicillin to find suitable optical density for the later experiments (Fig. 3a). Growth in the experimental system was used to characterize the 4.5 h lag phase of *E. coli* DH5 α (pJAMA-arsR). The curve exhibited no measurable light emission during the lag phase, and the cells subsequently entered the exponential growth (Fig. 3b). During the log phase, there was a significant change in the bioluminescent light yield and this was followed by the stationary phase. The maximum light emissions occurred at the end of the exponential phase and the beginning of the stationary phase. This was reflected by a significant decrease in light output during the stationary phase.

3.3. Stability of protocol factors for detection of arsenic

The cells incubation time with the respective arsenate solution was tested for different times in the range of 15–90 min. The light emission from *E. coli* DH5 α (pJAMA-arsR) cells increased dramatically when the incubation with arsenic increased (Fig. 4a). Also, the n-decanal solution was examined in the range of 0–13 min. Luminescent light yield from *E. coli* DH5α (pJAMA-arsR) cells significantly decreased when the incubation with n-decanal increased (Fig. 4b). In order to evaluate the impact of temperature on the light emission values, a shaker incubator was set to 20, 30 and 37 °C. Results showed that 37 °C was the optimum temperature for the maximum light emission rates, and increasing the arsenic concentration caused the elevation of light output emission (Fig. 5).

The effect of n-decanal concentrations on the light emission was measured in the range of 5–20 μl (18 mM) showing that at 10 μL, light emission rate was significantly higher than the other samples (Fig. 6a). In the case of glycerol, the light emission rate was notably decreased when the value of glycerol was increased to more than 20 % (Fig. 6b). In terms of pH, the results showed that the light

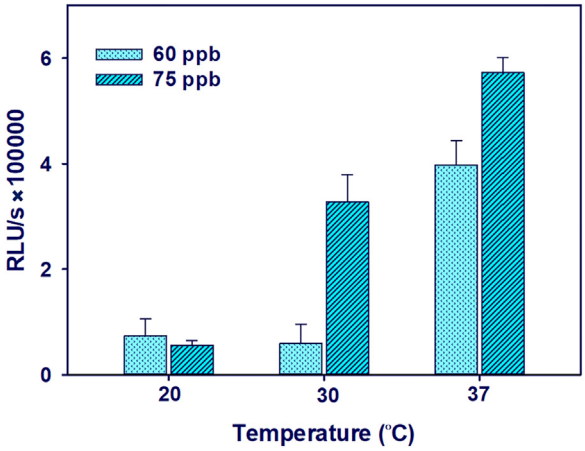

Fig. 5. Temperature optimization for a suitable light yield of *E. coli* DH5 α (pJAMA-arsR) in the presence of arsenic.

emission rate was higher at optimal pH range for *E. coli* DH5 α (pJAMA-arsR) (pH 7), and at pH 5.5 (Fig. 6c). The effect of aeration on bacterial luminescence emission was also evaluated. Results indicated that in the range of 50–200 rpm light emission did not change significantly (Fig. 6d).

3.4. Fitting of the calibration curve

The calibration standard curve depicts the relationship between light emission from *E. coli* DH5 α (pJAMA-arsR) and arsenic concentration. The slope of the curve measures the sensitivity of the quantity of light emission to changes in the arsenic concentration. Therefore, arsenic concentrations in unknown samples were deter-

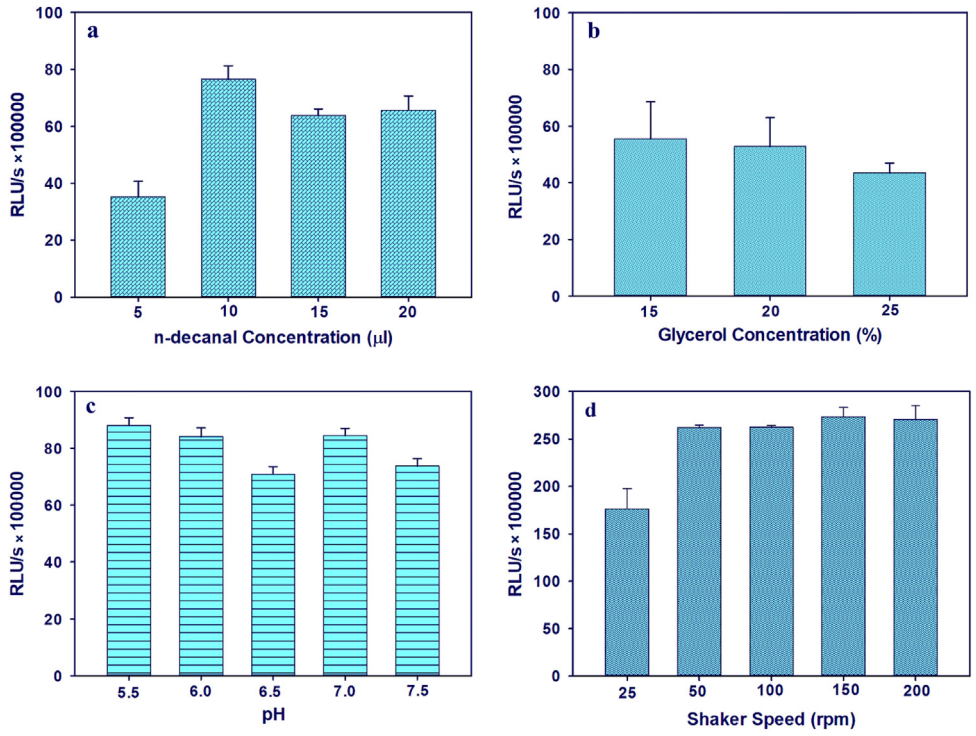

Fig. 6. Effects of various factors on *E. coli* DH5 α (pJAMA-arsR) luminescent light output. Light emission from the bacterial cells after adding different volumes of n-decanal (a) glycerol (b) different pH (c) and aeration (d).

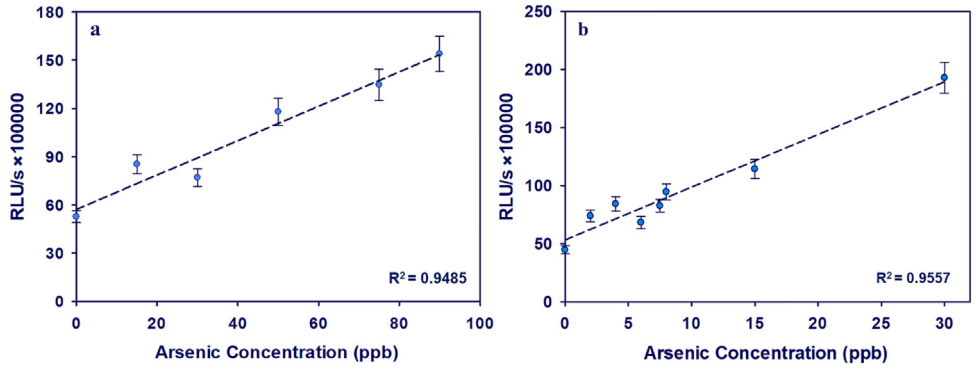

Fig. 7. Calibration standard curve for the *E. coli* DH5 α (pJAMA-arsR) light emission in related to 0-90 ppb (a) and 0-30 ppb (b) arsenic concentration. R^2 values result from linear interpolation of the averages from three replicates.

mined by linear interpolation of the calibration standard curve. A valuable numerical measure of association between the variables is the correlation coefficient. The correlation coefficient is a valuable numerical measure of association between the variables. Since more than 90 ppb of arsenate caused strength of the association to decrease (data was not shown), the standard curve was fitted in the range of 0–90 ppb As. The light emissions produced and total arsenic concentration readings were perfectly in agreement (R^2 = 0.948 by linear regression), hence confirming that the biosensor applied would give a proper calibration for measurements of arsenic in the concentration range of 0–90 ppb (Fig. 7a). The limitations of biosensor detection in the range of 0–30 ppb of arsenate were determined. The light emissions produced, and total arsenic concentration readings were perfectly in agreement (R2 = 0.955 by linear regression). Since the concentration of less than 10 ppb of arsenate displayed some margin of error. Therefore, limitation detection was definite at this concentration (Fig. 7b).

3.5. Testing of arsenic-polluted groundwater

Arsenic concentrations measured using the chemical techniques and biosensor method in the water samples were shown at Table 1 . All the wells were categorized as safe or not-safe on the basis of the WHO guideline value for arsenic in drinking water (10 ppb). The data showed a total of 13.04 % false negative and 13.04 % false positive results. Many other nations still adopt a level of 50 ppb (Bahrain, Bangladesh, Bolivia, China, Egypt, India, Indonesia, Oman, Philippines, Saudi Arabia, Sri Lanka, Vietnam, Zimbabwe) (Punshon et al., 2017). The results showed a total of 13.04 % false negative and 4.34 % false positive blow under 50 ppb arsenic.

3.6. Analyzing of arsenic detection after freeze-drying of the biosensor

Correlation of luminescent light and arsenic concentration in the range of 0–90 ppb after freeze-drying was shown at Fig. 8. The sta-

Table 1
Comparison of the chemical techniques with the biosensor in the detection of arsenic.

As concentration (ppb)	50<	50>	50 – 10	10<	10>
Percentage %	4.34	95.65	52.17	56.52	43.47
False negative	1 (4.34 %)	3 (13.04 %)	4 (17.39 %)	0	3 (13.04 %)
False positive	3 (13.04 %)	1 (4.34 %)	3 (13.04 %)	3 (13.04 %)	0

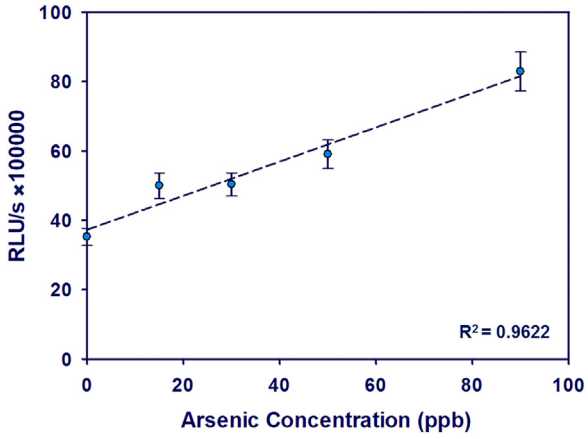

Fig. 8. Light emission from the freeze-dried *E. coli* DH5 α (pJAMA-arsR) biosensor.

bility of freeze-dried bacteria was tested as compared to the original culture and the light emission produced and total arsenic concentrations measurements were perfectly in agreement (R^2 = 0.962 by linear regression), therefore the freeze-dried biosensor remained intact storage.

4. Discussion

Biosensors are generally an analytical device that is used for detection of an analyte (Rotariu et al., 2016) and has potential application in detection of heavy metals in the environment (Maghsoudi et al., 2021; Zhou et al., 2016). Attempts in genetic manipulation on bacterial regulatory circuits and expression regulators led to a rise in the amount of whole cell biosensors with enhanced metal selectivity and sensitivity (Kang et al., 2018). Genetically modified strains of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* and *Rhodopseudomonas palustris* as biosensor–reporter strains have been used for arsenic detection (Diesel et al., 2009). In response to the presence of arsenic, such bacteria produce a reporter protein, the amount or activity of which is measured in the bioassay (Diesel et al., 2009). Arsenic is rapidly bonded to inorganic or organic compounds, therefore incubation, temperature and other factors can affect the arsenic monitoring and assessment (Jouanneau et al., 2011). In the present study, *E. coli* (pJAMA-arsR) strain as luminescent biosensor was used to detect arsenic in arsenic-polluted water in the west of Asia. As far as we are aware, this is the first application of a microbial biosensor assay to measure arsenic in Iran. Different factors in the procedure were tested for their effectiveness in preserving the activity of biosensor bacteria during detection of arsenic. Activity preservation is essential when the development of a user-friendly, reliable biosensor-based arsenate test is planned and quickness (response time) and robustness (response stability during storage) are important criteria (Grieshaber et al., 2008).

One of the significant outcomes from curve fitting in a growth model is the potency to apply a model of growth that has a firm underlying mechanistic function based on the theoretical knowledge of the system (Shukor, 2014). One of the best of such model is the Gompertz kinetics, a sigmoid function, that models growth as being slowest at the start and end of a given time period. In this study, the growth of *E.coli* DH5α (plasmid pJAMA8-arsR) was modeled exploiting the Gompertz curve model at 30 °C. The lag time of the bacterial growth in this study occurs at 4.6657 h. An endeavor which used *Lactobacillus plantarium* bacteria showed the asymptote and the lag time before the bacteria reached its maximum growth rate (Carey, 2009). The highest growth rate of *E.coli* DH5α (plasmid pJAMA8-arsR) was found at 0.22574. It means approximately that two-tenths of the bacterial population is increased during the fastest period of the growth per hour. The data from other studies predicted the fastest bacterial growth, which is why it is applied directly to medicine and biology (Huang, 2011; Lo, 2007). This model hypothesizes that the highest number of bacterial population was achieved in $Ln\ (N/N_0)$ which was equal to 1.1575. Furthermore, it predicted the results of the bacterial lag phase before getting the maximum level of the growth. These results are vital to extend the using of models and to understand the relationship between growth and calculations. In a study, the impact of different levels of a natural antimicrobial agent on the growth kinetics of common food spoilage (Pseudomonas aeruginosa, Enterococcus faecalis) and food pathogenic microorganisms (Listeria monocytogenes and Salmonella abony) was evaluated. The R^2 value was greater than 0.95 for most of the cases indicating that the model could provide a good fitting to the experimental data (Gupta et al., 2012). By incorporating variability, the resulting model is suitable for pathogen behavior applications. A study demonstrated that overall predictions using the Gompertz showed good agreement with the experimental values, indicating success at providing reliable predictions of the growth of L. monocytogenes (Ding et al., 2010). In a study, the effect of a nanoparticle has been studied quantitatively on two common pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*, and the growth curves were modeled. The modified Gompertz model, using a death term, was found to be useful in explaining the non-monotonic nature of the growth curve (Chatterjee et al., 2015). And in another study, which biohydrogen production by dark fermentation in batch reactors was modeled using the Gompertz equation showed the statistical quality of fit by the Gompertz equation was high (Gadhamshetty et al., 2010).

Maximum response to arsenic was found at the end of the logarithmic phase which used for stocks preparation because the variation of cell number was lowest. Other studies have shown the same results, but the maximum response to arsenic in different strains can vary (Guo et al., 2011; Joshi et al., 2008). The luminescence response with different induction times of arsenic was directly linear over a wide range from 15 min to 90 min. It should be noted that values produced by biosensors can only be compared to standards with known arsenic concentrations carried out under the same conditions (Koo and Li, 2016). Therefore, an induction time of 60 min was used in order to obtain a reproducible signal in a shorter time. Since living systems were applied, continuing incubation of the cells with the arsenic would lead to increased gene expression, and thus, absolute light yield values are meaningless descriptors for arsenate concentration (Stocker et al., 2003). It was shown that the amount of light emitted was still increasing after 2 h of incubation, however, the induction coefficient did not increase considerably after 70 min, and sensitivity did not improve (Huang et al., 2015; Jouanneau et al., 2011).

Our study has shown that temperature had a major impact on the emission of bioluminescence and that, as expected, maximum response was at 37 °C. Similarly, Jawhara and Mordon, have shown

that the bioluminescence of *E. coli* bacteria in the growth phase is temperature dependent (Jawhara and Mordon, 2004). The effect of different volumes of n-decanal, initially, on the intensity of luminescent bacteria was a direct relationship, but the volume of 10 μL is the peak of the graph. N-decanal is a substrate of bacterial luciferase and to keep this reaction ongoing, the fatty aldehyde must be continuously regenerated (Gregor et al., 2018). It penetrates well through the cell membrane and releases light in the presence of bacterial luciferase in the live cells providing the basic conditions for the reaction. This compound has been reported to be an effective inhibitor (Kim and Spiegel, 2013; Suominen et al., 2020).

It was found that the strain can be stable in 20 % (v/v) glycerol for at least 6 months at -20 °C. By increasing the glycerol concentration luminescence intensity decreased, which is probably due to the reduction of the number of bacteria. In similar studies, the *arsB*::*luxAB* gene fusion strain has been found to be persistent and stable at −20 °C for at least 6 months when stored in 25 % (v/v) glycerol (Cai and DuBow, 1997) which proved its valuable efficiency to act as a stable and simple arsenic bioreporter (Kaur et al., 2015). In 2015 glycerol was used as a carbon source and showed that it has had great influence on the luminescence of microorganisms (Kumar et al., 2015).

The maximum intensity of light luminescence is observed at pH between 5.5 and 7.5. Jawhara and Mordon indicated that pH has significant effects on bioluminescence *E. coli* (pRB474), a slightly acid pH changes the luminescence in the logarithmic phase. The maximum light emission of these cells was at pH 5.0 (Jawhara and Mordon, 2004). Also at physiological pH, the substrate (for luciferase reaction) simply does not cross the cell membrane of prokaryotes and eukaryotes. However, effectively in slightly acidic conditions combined and penetrated into cells. Graper observed high luminescence of *E. coli* when the bacteria was placed at neutral pH 7 (Graper, n.d.).

The standard curve was designed to extrapolate the unknown values of natural samples. The results have shown that the optical response of the biosensor to different concentrations of arsenic (range of 0−90 ppb) with a regression of 0.948 was relatively linear. The higher concentrations of arsenic had a toxic effect on the biosensor (a decrease in number) and thus the corresponding optical response. The limitation of biosensor detection in the range of 0−30 ppb of arsenate was examined. Since the concentration of less than 10 ppb of arsenate had a little error so, limitation detection was definite at this concentration. Trang, et al. conducted natural water sources to measure arsenic concentrations using *E. coli* and showed that the performance of the bacterial cells was well in the range of 7−100 ppb (Trang et al., 2005).

After optimization and calibration, the biosensor was used for arsenic detection in contaminated natural samples. Biosensor had a detection range of 10−90 ppb, which is commensurate with the permissible detection limit stated by WHO (10 ppb) (Cotruvo, 2017). The analyses performed on water samples showed that data collected from the chemical techniques and biosensor differed by 26.08 %. However, chemical methods often provide a measurement of the total concentration of potentially toxic arsenic, they do not provide a true evaluation of bioavailability or its toxic effect toward living organisms (Czub et al., 2021; Rodriguez-Mozaz et al., 2005). The presence of inorganic compounds can be another confounding variable (Jouanneau et al., 2011). Chemical processes, such as the adsorption of arsenic to iron (hydro) oxides from groundwater samples might significantly reduce the amount of arsenic available to the cell and lead to a lower detection of total arsenic in the sample (Park et al., 2018). Moreover, the increase in iron content above a certain level causes failure to detect the arsenic in the environment (Teixeira et al., 2020). The iron itself is not toxic to bacterial cells and this creates the hypothesis that arsenic may have created a complex with iron and have been released from the cell (Trang et al., 2005). In 2005, comparing the results of the arsenic biosensor with AAS showed a total of 10.4 % difference results. These findings have indicated that the arsenic biosensor is superior to chemical field kits in accordance with the standards defined by the WHO (10 ppb) (Trang et al., 2005) and can complement chemical methods since they are able to distinguish between bioavailable and unavailable forms of arsenic (Hassan et al., 2016).

The strong and verifiable relationship was found between the results of the AAS method and the biosensor by removing the two maximum and minimum values measured by the AAS. These results suggest that the biosensor seems to be one of the most commonly used biological methods in the measurement of toxic and bioavailable arsenic (Hassan et al., 2016). Additionally, complex chemical methods, including AAS and ICP-OES, are not available anywhere, have high maintenance costs and require expertise to use (Bereza-Malcolm et al., 2015).

Due to their ease of use and low cost, laboratory methods for measuring arsenic using biosensor technology have more commercialization potential in Asia and other areas with groundwater resources contaminated with arsenic (Rampley et al., 2020; Trang et al., 2005) and can be a good alternative to detect the bioavailability of arsenic in the water and plant samples (Chen and Rosen, 2014).

In contrast to the intact cells, the luminescent light output of the freeze-dried strain was half that of the intact cells. In a previous analysis, the freeze-dried strain showed low bioluminescence intensity and a different activity order than the subculture strain in the existence of pure inducer chemicals (Ko and Kong, 2017). Freeze-dried KG1206 was used to test the bioluminescence behavior of five different inducer compounds before adding the freeze-dried strain to the groundwater samples. Compared to the behavior of the subcultured strain (before freeze drying), the freeze-dried strain had very low bioluminescence output (Ko and Kong, 2009). The explanation of the freeze-dried strain's low output is unknown at this time, although it might be linked to cell membrane damage caused by the deep-freezing, freeze drying, or reconstitution stages (Ko and Kong, 2017).

5. Conclusions

The modified Gompertz model used in this study could successfully predict the growth under the optimum condition when compared to experimental data. This model can be applied to a broad range of prokaryotic cells and eukaryotic cells for future growth kinetics endeavors. The results presented in this work emphasize the importance of detection of bioavailable arsenic in the groundwater by biosensors. This study evaluated the effects of different factors on the protocol of arsenic detection by *E. coli* DH5α (pJAMA-arsR), a genetically modified biosensor. The results indicated that bioluminescent emissions could be enhanced during the end of the log phase and the beginning of the stationary phase of the growth, incubation time, optimal temperature and pH, thereby the procedure was sat based on stable amounts of factors. The standard curve between arsenic concentrations (7.5−90 ppb) and bioluminescent emissions was relatively linear, which therefore used for the detection of arsenic in groundwater samples collected from rural areas of Iran. The findings of this study support the potential of the bioluminescent strategy for the detection of arsenic in groundwater that can be monitored using *E. coli* DH5α (pJAMA-arsR) as a luminescent reporter assay. Furthermore, the bacterial biosensor used in this study was properly optimized to detect arsenic in rural groundwater samples, which can be used as a fast, safe and green signaling tool for prevention and further cleaning up of bioavailable-arsenic contaminated waters.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psep.2021.07.019.

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