Biosensors and Bioelectronics 63 (2015) 533–545

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Advances in arsenic biosensor development – A comprehensive review

Hardeep Kaur, Rabindra Kumar, J. Nagendra Babu, Sunil Mittal *

Centre for Environmental Science and Technology, Central University of Punjab, Bathinda, Punjab 151001, India

ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form
21 July 2014
Accepted 4 August 2014
Available online 7 August 2014

Keywords:
Biosensor
Arsenic toxicity
ars Operon
Aptamer
Recombinant whole cell
Reporter proteins

ABSTRACT

Biosensors are analytical devices having high sensitivity, portability, small sample requirement and ease of use for qualitative and quantitative monitoring of various analytes of human importance. Arsenic (As), owing to its widespread presence in nature and high toxicity to living creatures, requires frequent determination in water, soil, agricultural and food samples. The present review is an effort to highlight the various advancements made so far in the development of arsenic biosensors based either on recombinant whole cells or on certain arsenic-binding oligonucleotides or proteins. The role of futuristic approaches like surface plasmon resonance (SPR) and aptamer technology has also been discussed. The biomethods employed and their general mechanisms, advantages and limitations in relevance to arsenic biosensors developed so far are intended to be discussed in this review.

Contents

1. Introduction . . . 1
2. Biosensors: a convenient detection platform . . . 2
 2.1. Recombinant whole-cell-based biosensors for arsenic determination . . . 3
  2.1.1. Luciferase-based biosensors . . . 4
  2.1.2. *lacZ*-based biosensors . . . 6
  2.1.3. Green fluorescent protein (*gfp*)-based biosensors . . . 7
 2.2. Biosensors based on alternative approaches . . . 8
 2.3. Cell-free arsenic biosensors . . . 10
  2.3.1. DNA-based arsenic biosensors . . . 10
  2.3.2. Aptamers-based arsenic biosensors . . . 10
  2.3.3. Protein-based arsenic biosensors . . . 11
3. Conclusion . . . 12
Acknowledgment . . . 12
References . . . 12

1. Introduction

Arsenic (symbol As, atomic number 33) is a widespread heavy metal which has influenced human history more than any other element or toxic compound. In terms of abundance, it ranks 20th in the earth's crust, 14th in sea water and 12th in the human system (Mandal and Suzuki, 2002). The average concentration of arsenic in the earth's crust is about 3 mg/kg and in sea water is about 1–2 μg/L (Cullen and Reimer, 1989). In nature, arsenic exists in both organic and inorganic forms, having different speciations such as arsenate [As(V)] and arsenite [As(III)]. Arsenic concentration has been reported to be higher than the permissible limits in drinking

* Corresponding author. Tel.: +91 9815620186.
E-mail addresses: hardeep_kaur007@rediffmail.com (H. Kaur), rabindrabiochem@gmail.com (R. Kumar), nagendra.rd@gmail.com (J.N. Babu), sunil.cevs@gmail.com (S. Mittal).

http://dx.doi.org/10.1016/j.bios.2014.08.003
0956-5663/

water, irrigation water, variety of foodstuffs like vegetables and cereals, animal food (fish, meat, milk), etc. in many parts of the world (Chowdhury et al., 2000; Williams et al., 2006; Zhu et al., 2008; Williams et al., 2009). Arsenic contamination in the environment has aroused considerable attention due to its harmful effects on plants and animals including humans. Arsenic has been associated with mutagenic and carcinogenic potential upon prolonged exposure (IARC, 2004; Stone, 2008; Zhu et al., 2008). Due to its abundance and toxicity, monitoring arsenic in water, soil and various foodstuffs used for human consumption is becoming important. A number of techniques have been developed for this purpose in the last 5 decades and Ma et al. (2014) have reviewed these techniques very well. Most of these methods are reliable and can be used for the measurement of extremely low concentrations of arsenic. The variously approved methods used in arsenic determination along with their detection limits are listed in Table 1. However, these methods suffer from some major disadvantages like heavy and expensive instrumentation, field applicability, requirement of highly skilled technical persons, chemical processing of sample, etc. So, there is utmost need for alternate, cost- and time-efficient technologies for the real-time detection of arsenic.

Sensors are one of the alternatives, comprising two parts, namely the recognition unit and the signal transducer. The recognition unit may comprise a biocomponent or a chemical receptor. In the case of arsenic, few chemosensors for naturally occurring arsenate (Lohar et al., 2013; Sahana et al., 2013) and arsenite ions (Ezeh and Harrop, 2012) have been reported (Table 1). The major hurdles in designing a chemosensor for arsenic species are the control of selectivity particularly over phosphate, sensitivity, strong interference of other ions and the high solvation energies of anions (Dietrich, 1993; Beer and Gale, 2001; Ezeh and Harrop, 2012; Du et al., 2014). So, biosensors have earned a special niche as analytical devices and offer services in environmental monitoring (Baeumner, 2003), food safety (Mello and Kubota, 2002) and clinical diagnosis (Wang, 1999). Biosensors have the advantage of sensitivity, specificity, simplicity, low manufacturing cost, better limit of detection, fast response time, ease of use, portability, and ability to furnish continuous real-time signals. They also circumvent the need for any sample pretreatment and expert handling. Also, the extent of bioavaliable toxicity which could not be detected by conventional analytical methods can be measured by biosensors as the biocomponents used in the system are part of the living world.

Only a limited number of papers have been published in this field. A search using the PubMed central engine and Scopus database using the words “arsenic biosensors” shows 60 and 147 research publications, out of which only 50 deal with arsenic biosensors. The current review focuses on the general mechanism of biosensor working and detailed account of various types of arsenic biosensors developed in the past. The present review is an effort to highlight the various advancements so far in the development of arsenic biosensors. The study has revealed various advantages and limitations associated with recombinant bacterial, DNA- and protein-based arsenic biosensors.

2. Biosensors: a convenient detection platform

A biosensor is an analytical device that presents a synergistic combination of biotechnology and microelectronics (Singh et al., 2008). Biosensors mainly comprise three main components: a biocomponent (enzyme, whole cell, antibody, DNA, etc.), a transducer (electrochemical, optical, or thermal) and an amplification unit. Biosensors could be developed for any molecule, relevant to xenobiotics, human health or environmental protection.

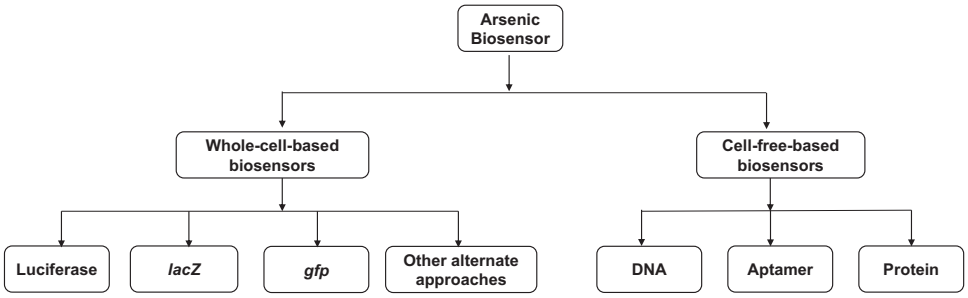
Table 1
Analytical methods used for determination of arsenic with their detection limits.

Analytical technique	Analytical tools	Detection limit	Disadvantages	Reference
Spectroscopy	UV–vis spectroscopy	1.0 μg/L	Tedious sample preparation, sample preparation prone to false positive and false negative readings, significant interference of other ions like Pb, Ni, S, P, etc.	Tahir et al. (2008)
	Electrothermal Atomic Absorption Spectroscopy (ETAAS)	0.02 μg/L	Strong interference and costly matrix modifiers	Anawar (2012)
	Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP–OES/AES)	0.05 μg/L	Expensive analysis	Chen et al. (2009)
	Inductively Coupled Plasma-Mass Spectrometry (ICP–MS)	0.4 ng/L	Expensive analysis and requires trained manpower, ArCl polyatomic species interference	Petursdottir et al. (2012)
	Gas Chromatography–Mass Spectrometry (GC–MS)	5.8 ng/L	Difficult sample preparation	Richter et al. (2012)
Electrochemical voltammetry	Anodic Stripping Voltammetry (ASV)	0.8 ng/L	Expensive analysis, Cu interference, tedious analysis and requires trained manpower	Gao et al. (2013)
	Cathodic Stripping Voltammetry (CSV)	37.5 ng/L	Expensive analysis, Cu interference, tedious analysis and requires trained manpower	Gibbon-Walsh et al. (2010)
	Chronopotentiometry	7.5 ng/L	Tedious analysis and requires trained manpower	Salaun et al. (2012)
Chemical Sensors	Thiol compound based SPR sensor	3.0 ng/L	Non-specific sensing and significant redox interference	Forzani et al. (2007)
	Dosimetric Fluorescent probe	0.24 μg/L	Strong interference by Cu^{2+} and Co^{2+} ions and analysis only in Non-aqueous media	Ezeh and Harrop (2012)
	Dosimetric Fluorescent probe-ArsenoFluors	0.14 μg/L	Analysis only in Non-aqueous media	Ezeh and Harrop (2013)
	Antipyrine Based Schiff Base	225 μg/L	Poor detection limit and pH dependent fluorescence	Lohar et al. (2013)
	Ag@Fe_3O_4 SERS sensor	10 μg/L	Non-specific sensing	Du et al. (2014)

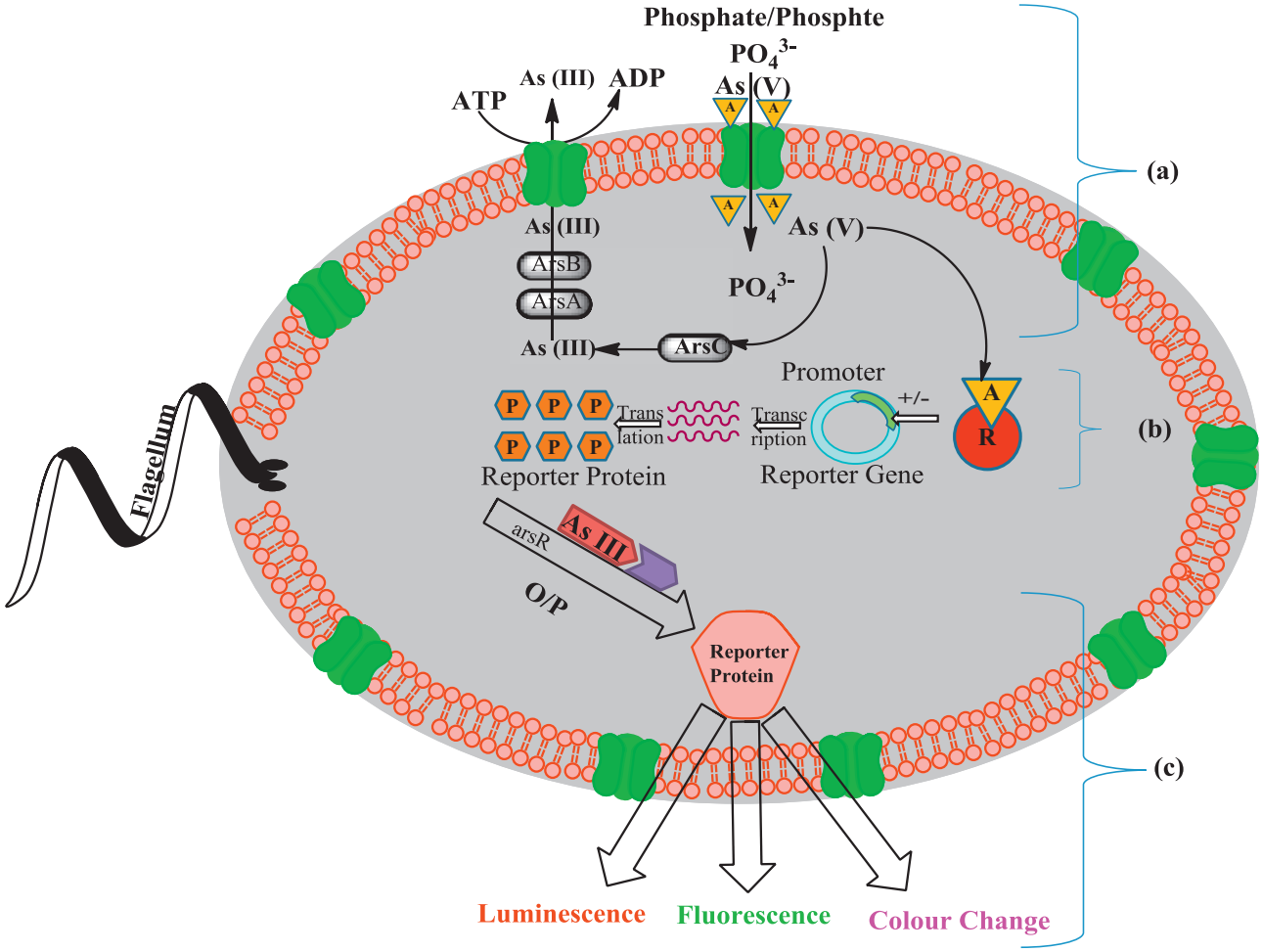
Pesticides, heavy metals and other food contaminants have been mainly focused as target moieties for biosensor construction (Saleem, 2013). Analytes interact with the biorecognition element either by inhibiting some metabolic activity (growth or enzyme activity) or by distorting the original conformation of certain vital biomolecules (DNA and Ag–Ab complex). Both the strategies work towards the fundamental aim of a reduced or an enhanced biochemical signal, which is recognized by the biorecognition component and converted to a comprehensible signal by the transducer. The present review has divided the biosensing approaches on the basis of the biological component employed for arsenic biosensor development as depicted in Scheme 1. With the increased development in material science and understanding of various arsenic-binding mechanisms, novel biosensors have been developed for arsenic determination. This review provides extensive up to date findings on arsenic biosensors, with an emphasis on the mechanistic details.

2.1. Recombinant whole-cell-based biosensors for arsenic determination

Most bacteria demonstrate resistance to arsenic and maintain a number of cellular responses in terms of metabolic processes to remediate it (Cervantes et al., 1994). The resistance against arsenic is provided by the *ars* operon of plasmid or chromosomal origin. The operon acts as a transport system to pump toxic arsenite out



Scheme 1. Biological component based classification of arsenic biosensors.



Scheme 2. Schematic representation of whole-cell-based biosensor for (a) Arsenic [As(V) and As(III)] transport by phosphate channel (b) Working mechanism of signal transducer for arsenic biosensor, and (c) Detection of As(III) by luminescence, fluorescence and color change. (For interpretation of the references to color in this scheme legend, the reader is referred to the web version of this article.)

of the cell (Owolabi and Rosen, 1990; Diorio et al., 1995; Rosen, 1999). The Gram-positive bacterial plasmid (*Staphylococcus aureus* p1258) carries an *ars* operon with one regulatory gene, *arsR*, and two structural genes, *arsB* and *arsC* (Ji and Silver,1992). The *arsR* gene product acts as a transcriptional repressor, *arsB* acts as an efflux pump for arsenite and *arsC* transcribes an arsenate reductase, which is responsible for As(V) reduction to As(III) and further extrusion or sequestration of the latter by the cell. Similarly, the Gram-negative bacterial plasmid (*Escherichia coli* R773) has one regulatory gene, *arsD*, and a structural gene, *arsA*, in addition to the other three genes. The *arsAB*–ATPase complex carries out the extrusion process in this case (Chen et al., 1986). The development of whole cell biosensors employ these metabolic processes for their design. In the case of arsenic, the well-established ability of As(III) or As(V) to bind the *arsR* protein and undo its repressor function on the *ars* promoter leads to subsequent synthesis of reporter genes. Thus, whole-cell-based biosensors devised using the above approach have been further subdivided, based on their signal transduction mechanisms like luciferase, *lacZ* and green fluorescent protein (*gfp*), whereby the signal relay is either in terms of fluorescence, luminescence or colorimetry as depicted in Scheme 2. The whole-cell biosensors developed for arsenic have been reviewed by Diesel et al. (2009) and Merulla et al. (2013). The present review is an all-embracing study of whole-cell- and cell-free arsenic biosensors developed until now.

2.1.1. Luciferase-based biosensors

A number of recombinant strains have been reported based on the luminescent activity of the luciferase gene. Luciferase provides the most sensitive and simple measurement of gene expression and regulation. It also offers the option of either firefly or bacterial origin of the gene to ensure host compatibility. Initial gene constructs containing luciferase (luxAB) in fusion with the arsenic resistance operon and their regulation were reported by Corbisier et al. (1993) and Ji and Silver (1992), respectively. Afterwards, a luminescent recombinant bacterial strain for arsenic analysis was reported by Tauriainen et al. (1997). The firefly *Photinus pyralis*' luciferase gene (*lucFF*) was expressed under the regulatory control of the *ars* operon of the *S. aureus* plasmid P1258 in a shuttle vector plasmid pT0021 in three different host strains, namely *S. aureus* RN 4220, *B. subtilis* BR151 and *E. coli* MC1061. *S. aureus* was found to be most sensitive among the three host strains and it demonstrated a detection limit of 7.5 μg/L, 4.03 μg/L and 37 μg/L for arsenite, antimonite and cadmium, respectively. Another luciferase bioreporter strain was developed and investigated for arsenic in copper arsenate (CCA), sodium arsenate and chromate copper solution (CC) (Cai and DuBow, 1997). *E. coli* strain LF20012 was genetically modified to bear the *arsB*: *luxAB* gene for an arsenic-induced luciferase response. It was observed that the three compounds had a synergistic toxic effect on cells, which was elevated in dephosphorylated medium conditions. The recombinant strain was stable for 6 months at −20 °C in 25% (*v*/*v*) glycerol, which proved its valuable efficiency to act as a stable and simple arsenic bioreporter. Tauriainen et al. (1999) reported two more luminescent microbial reporters, *E. coli* MC 1067 (pT0031) and AW3110 (pT0031), both with the shuttle vector pT0031, harboring *lucFF* under the expression control of the *ars* promoter and *arsR* gene from *E. coli* pR773. *In vivo* and *in vitro* studies were conducted to prove the luminescence efficiency. Tauriainen et al. (2000) demonstrated the applicability of a freeze-dried luminescent bacterial strain on arsenic-spiked water samples. Bioreporter strains generated in their laboratory, namely *S. aureus* RN4220 (pT0021), *B. subtilis* BR151 (pT0021), *E. coli* MC0061 (pT0031) and *E. coli* AW3110 (pT0031), were tested for their efficacy. The *E. coli* strain MC1061 (pT0031) was found to be most sensitive with a detection limit of 7.5 μg/L. Petanen (2001) constructed a special metal-induced expression system utilizing different host and shuttle vector combinations. Arsenite-sensitive plasmids pTPT21and pTPT31 harboring the luciferase gene *lucGR* under the control of the *mer* operator/promoter (O/P) of R100 and the *ars* operator/promoter of p1258 and R773 were generated. The study revealed that high substrate (luciferin) concentration inhibits light production for *Pseudomonas fluorescence* after prolonged incubation. Further, pTPT31 evidenced better detection limits for arsenite than pTPT21 because of the Gram-negative origin of the *ars* part of the former. Later, Petanen and Romantschuk (2002) demonstrated the same luminescent bacterial strain *P. florescence* OS8 (pTPT31) as an efficient sensor for the determination of total and bioavailable As(III) in different soil samples. With the motive of lower background noise, a recombinant plasmid pJAMA–*arsR*–ABS was engineered by Stocker et al. (2003). The strain had a second *arsR* binding site in front of the *luxAB* gene. This adjustment resulted in slightly lower background luciferase activity and reduced the response time by 3–5-fold to the same arsenite concentration. In another effort, the bioreporter strain *E. coli* MC1061 (pT0031) was utilized in suspension to measure bioavailable As(III) in addition to other heavy metals (Hakkila et al., 2004).

Harms et al. (2005) employed the luminescent bacterium *E. coli* DH5α (pJAMA–*arsR*) harboring the luciferase gene *luxAB* from *Vibrio harveyi* to study arsenic in groundwater and reported the interference of phosphate, silicate and iron (Fe) on luminescence production. Addition of EDTA was proposed as a remedy to ion interference that resulted in dissociation of arsenic from all complexes, subsequently leading to accurate estimation of arsenic in tap water. A similar study by Trang et al. (2005) reported the capability of the bioreporter *E. coli* DH5*α* (pJAMA–arsR) containing a bacterial luciferase gene (*luxAB)* for estimation of arsenic in water to a detection limit of 7 μg/L. Baumann and Van der Meer (2007) described a method for As(III) analysis in rice using the bioreporter *E. coli* DH5*α* (pJAMA–*ars*R). A multiwell plate bioassay was utilized for high sample-throughput analysis, and a detection limit of 6 ng/g of dry rice was achieved. In an effort to make system more field applicable, Ivask et al. (2007) immobilized the recombinant bioreporter *E. coli* MC1061 (parslux CDABE) on the tip of an optical fiber. It was demonstrated that the storage of a microbes-bearing tip in $CaCl_2$ at −80 °C maintained microbial luminescence activity for a longer period. The biosensor developed had a detection limit of 141 μg/L As(V) and 18 μg/L As(III). Arsenate is efficient in inducing certain gene promoters such as the UFO1 gene of *Saccharomyces cerevisiae*, which is responsible for producing an ubiquitin ligase complex. Captivating the advantage of the fact, a yeast luminescent strain (*S. cerevisiae pdr5Δ–luxAB* strain) was constructed (Bakhrat et al., 2011). A low detection limit was achieved by subtracting the *arsABC* gene unit from the construct that enabled the accumulation of arsenate inside the cell. The recombinant was insensitive to other metal ions such as cadmium and mercury, but was inducible with UV irradiation. A chip-based analysis system was reported by Elad et al. (2011) and Elad and Belkin (2012) for continuous 10-day online monitoring of toxicants in water. A bacterial bioreporter harboring the *luxCDABE* luciferase gene was immobilized on the biochip to facilitate luminescent signals in a 0.5–2.5 h exposure time. Two more reporter strains for other contaminants were also used in the device. The biochip demonstrated a corresponding increase in bioluminescence with the addition of arsenic and other toxic compounds in the water flow. Ahmed et al. (2012) reported a study pertaining to the effects of arsenic toxicity on the growth and nutrient uptake of *Lens culinaris* L. The bioreporter strain *E. coli* HB101 pUCD607 harboring the lux gene was employed to evaluate bioavailable arsenic level. Siegfried et al. (2012) undertook a comparative study of bioreporter-based test kit with two commercially available chemical test

Table 2
Characteristics of arsenic biosensors with their detection limits and induction periods/response time.

Biosensor specifications	Detection limit for As (III) (μg/L)	Induction period/ response time (h)	Limitations	Reference
Luciferase-based biosensors				
S. aureus RN4220(pT0021) harboring *arsR–lucFF* gene construct	7.7	2	Low sensitivity and induction coefficient, need of substrate addition, O_2 dependent measurement	Tauriainen et al. (1997)
E. coli LF20012 harboring *arsB–luxAB* gene construct	10	1	Phosphate interference, non linear detection range	Cai and DuBow (1997)
E. coli MC 1067 (pT0031) harboring *arsR–lucFF* gene construct	7.7	1.5	Fast luminescence decay in *in vitro* assay, high background fluorescence, variable optimal pH for bioreporter growth and luminescence production	Tauriainen et al. (1999)
P. fluorescens OS8 (pTPT31) harboring *arsR–lucGR* fusion	0.77	2	Low induction coefficient, semi-quantitative analysis, non-specificity	Petanen (2001)
Luciferase gene containing *E. coli* DH5α (pJAMA–*arsR*–ABS) gene construct	4	1	Non-linear response	Stocker et al. (2003)
E. coli DH5α (pJAMA–*arsR*) harboring *arsR–luxAB* gene construct	7.5	1	Effect of groundwater constituents on luminescence production, possibility of false positive and negative results	Trang et al. (2005)
S. cerevisiae pdr5Δ luxAB gene construct	0.0007	1	Low genetic stability	Bakhrat et al. (2011)
Luminescent *Photobacterium leiognathi*	4000	0.5	Poor detection limit, complex media composition, non-specificity	Ranjan et al. (2012)
E. coli DH5α (pASPW2–*arsR–luxCDABE*)	0.74	2	Interference by Antimony, maintenance of bacterial culture	Sharma et al. (2013)
E.coli arsRp: luc	3.75	2	Need of cell lysis for luminescence signal	Hou et al. (2014)
***lacZ*based biosensors-**				
E. coli JM109(R773) harboring *arsR–lacZ* fusion	7.7	17	High background noise, low sensitivity, Non-specificity	Scott et al. (1997)
E. coli DH5α (pMV–*arsR*–ABS) harboring *arsR–lacZ* fusion with second binding site for *arsR* protein	8	1	Semi-quantitative analysis, long growth period	Stocker et al. (2003)
B. subtilis (*ars*23) spores harboring *arsR–lacZ* fusion	12	12	Poor detection limit, long spore germination period	Date et al. (2007)
E. coli JM109/pSB1A2–BBa–J33201(BioBrick) harboring *arsR–urease* and *arsR–lacZ* fusions	5	5	Semi-quantitative analysis, interference by lactose and urea degrading microbes	Aleksic et al. (2007)
E. coli DH5α strain 2245 harboring *arsR–lacZ* gene construct	0.2	22	Qualitative measurement, tedious gene construct, long incubation period	Wackwitz et al. (2008)
E. coli 1971,1981,1982 harboring *arsR–ccp* gene construct	4	4	Qualitative measurement	Wackwitz et al. (2008)
E. coli BBa_J33203 harboring *arsR–lacZ* fusion	5	2	Interference by bicarbonate ions, prolonged response time with freeze dried cells	Joshi et al. (2009)
B. subtilis (*ars*23) spores harboring *arsR–lacZ* fusion	7.7	2.5	Sporing media optimization required	Date et al. (2010b)
B. subtilis (*ars*23) spores captured on microfluidic platform CD	7.7	3	Unsuitable for onsite measurement	Date et al. (2010a)
E. coli DH5αf′ harboring plasmid pMV arsR:: ABS	10	2	Effect of phosphate and other industrial waste on luminescence signal	Ivanina and Shuvaeva (2009)
E. coli JN109–pSB1A2–BBa_J15501 harboring *arsR–lacZ* and *xylE* gene construct	50	24	Long response period, interference by bicarbonate ions, possibility of false positive results due to lactose	De Mora et al. (2011)
E. coli harboring *arsD–lacZ* fusion	100	0.5	Poor detection limit, effect of other metal ions not mentioned	Chiou et al. (2011)
E. coli DH5α (pPROBE′*arsR*–ABS–RBS–*lacZ* strain 2245) harboring *arsR–lacZ* fusion	0.8	4.23	Risk of electrode contamination and perturbation with repeated use	Cortes-Salazar et al. (2013)
Green fluorescent protein (*gfp*)-based biosensors				
E. coli pIRC140 harboring *arsR–gfp* fusion	1	12	Long induction period, non-linear response, high background signal	Roberto et al. (2002)
E. coli DH5α (pPR–*arsR*) harboring *arsR–gfp* gene construct	7.7	1	Low sensitivity than luciferase sensor, delayed fluorescence production	Stocker et al. (2003)
E. coli DH5α (pVLASI) harboring *arsR–gfp* fusion	30 7.7	2 8	High salt, phosphate, silicate and pH conditions decreases *gfp*, background fluorescence, effect of other metal ions on *gfp* expression	Liao and Ou (2005)
E. coli harboring 3 copies of *arsR–gfp* gene	7.5	5	High background noise	Tani et al. (2009)
E. coli DH5α (ars–puc18–yfp) harboring yellow fluorescent protein	615	2	Delayed fluorescence production, poor detection limit, require sensitive instrumentation	Hu et al. (2010)
ArsRCis–gfp trans based biosensor	5	0.66	Tedious bioassay protocol, effect of high Ca and lyoprotectant on fluorescence signal	Kawakami et al. (2010)
E. coli harboring *arsR–egfp* gene construct	10	3.33	Longer storage cause decline in fluorescence signal, short storage stability	Buffi et al. (2011a)
ArsRCis–gfp trans based solid phase biosensor	5	0.5	Non-specificity, variable results in hard water conditions	Siddiki et al. (2011)
Magnetic nanoparticle based thermo-responsive biosensor	1	0.08		Siddiki et al. (2012a)

Table 2 (*continued*)

Biosensor specifications	Detection limit for As (III) (μg/L)	Induction period/ response time (h)	Limitations	Reference
A. niger harboring *arsA–egfp* fusion protein	1.8	12	Possibility of flocculation in reaction mixture, regeneration require additional incubation, generation of weak attractive forces to the magnet Effect of storage conditions and media requirement uncertain, long exposure time, unsuitable for onsite monitoring	Choe et al. (2012)
E. coli AW3110 with two plasmids	10	5	Assembly of two plasmids could be integrated in one, effect of other metal ions not detected	Chen et al. (2012)
E. coli strain 1598 harboring plasmid pPROBE–ArsR–ABS	10	1.20	Semi-quantitative analysis	Truffer et al. (2014)

kits for analyzing As(III) and As(V) in groundwater samples from Bangladesh. The bioreporter-based test kit comprised of *E. coli* DH5α–2697 harboring the luxCDABE gene under the expression control of *arsR* and its operator/promoter (O/P). The bioreporter-based test kit outstandingly achieved a testing rate of 160 samples/day as compared to 60 samples/day with the commercial kits. An online biosensor for multi-analysis of As(III), Cd(II), Hg(II) and Cu (III) was developed by Jouanneau et al. (2012), employing two bacterial biosensors, namely Lumisens III and Lumisens IV. In the first assembly, bioreporters were immobilized in agarose hydrogel in a multiwall card and in the second one freeze-dried bacteria were utilized. *E. coli* K12 MG1655 pBArslux was used particularly for arsenic detection. It was concluded that the Lumisens IV system was more successful in terms of stability, sensitivity, reproducibility and time consumption (90 min against 360 min). It also provided longer online analysis of environmental samples. The only limitation was the lack of specificity of the promoter towards reporter genes. In addition to recombinant *E. coli*, the marine luminescent bacterium *Photobacterium leiognathi* in immobilized form has also been exploited for As(V) analysis (Ranjan et al., 2012). Different experimental variables and media composition were optimized to obtain maximum luminescence. An inhibition effect of As(V) on the microbe was observed upon encapsulation in sodium alginate beads. Another study has reported the development of a bioreporter bacterium for effective low-level arsenic monitoring (Sharma et al., 2013). The gene construct has been developed exploiting pASPW2 as the expression vector in *E. coli* DH5α bearing *asrR* and the operator/promoter region of plasmid R773 along with *luxCDABE* from *Vibrio fishcheri*. Recently, a bioreporter *E.coli* arsRp:: luc deduced from pUC 18 with the luminescence-producing luc gene was employed to examine the impact of different long-term fertilization regimes on the bioavailability of arsenic in soil. It was concluded that the developed biosensor is more efficient in assessing the bioavailability of arsenic in soil as compared to chemical extractions (Hou et al., 2014). The various characteristics of luciferase gene-based arsenic biosensors have been summarized in Table 2.

2.1.2. *lacZ-based biosensors*

The *β*-galactosidase (*lacZ*) gene has also been reported as a candidate for arsenic biosensing as it provides reproducible and quantitative detection by different means. *β*-Gal-based signal transduction could take place through the electrochemical polarization of *p*-aminophenol (PAP) produced from *p*-aminophenyl *β*-D-galactopyranoside (PAPG) enzymatically or through an enzyme-catalyzed reaction on X-gal, producing a colorimetric response. Both these techniques provide faster analysis than luciferase. For the first time, Scott et al. (1997) reported an *arsR–lacZ* fusion-based strain harboring the plasmid R773 regulatory region and *E. coli* JM 109 as host. The parameters for cell lysis and the induction period were optimized to obtain a readable electrochemical signal. However, the system had the constraint of sensitivity; the *lac*Z gene being a natural inhabitant of bacterial cells caused high background activity and lead to misinterpretation. The recombinant was also inducible with other oxoanions like bismuthate, nitrate, sulfate, phosphate and carbonate to some extent. Stocker et al. (2003) also constructed a recombinant pMV–*arsR*–ABS for *β*-galactosidase-based biosensor. It was developed in a more raw and robust form as a qualitative paper strip test. The developed strip was stable for 2 months at −20 °C. Later, Date et al. (2007) reported the construction of a spore-forming bacterial bioreporter *B. subtilis* (*ars* 23), containing the *lacZ* gene under the expression control of the *ars*R regulatory protein. The cell suspension grown from spores made the analysis method simple, economical and applicable to harsh environmental conditions. They also facilitate preservation, storage and transport of the system for onsite

estimation. A bienzymatic system harboring urease and *lacZ*, named BioBrick (BBa–J33201), was proposed with a low detection limit of 5 μg/L (Aleksic et al., 2007). Both the enzymes were modeled as arsenate-responsive pH indicators to either increase or decrease pH in the absence or presence of arsenate, respectively. *arsR* and *arsD* repressor function was utilized to control the expression of *lacZ* and dual repressors Lamda and Lac I were thought to repress urease production and hence pH increase in the absence of arsenic. In the presence of arsenic, pH was decreased due to *lacZ* activity. A simple pH electrode/indicator or ISFET was enough for analysis. Unfortunately, the proposed gene construct was not achieved completely. Owing to the non-uniform signals from the bioreporter gene inserts and the need of the standard calibration system for samples of interest, a new calibration bioassay using a multiple bioreporter cell line was put forward (Wackwitz et al., 2008). The workers developed four recombinant *E. coli* cell lines indicative of effective expression of *β*-galactosidase on arsenite induction. Among them, *E. coli* DH5*α* (pPROBE′*arsR*–ABS–RBS–*lacZ* strain 2245) was found to be most sensitive. Six cell lines, based on the variable catalytic activity of cytochrome C peroxidase (CCP), were also constructed. The bioreporter strains 1971, 1981 and 1982 had a detection limit of 4 μg/L. Joshi et al. (2009) constructed two whole cell-based arsenic biosensors. The first construct exploited the fermentative property of the *E. coli* recombinant BBa–J33203 ("BioBrick" developed in a previous study) and the second was based on the endospores of recombinant *B. subtilis* harboring catechol-2,3-dioxygenase (*xylE*) as the reporter gene. The applicability of the spore-forming bioreporter strain *B. subtilis* (*ars*-23) to serum and fresh water samples was demonstrated by Date et al. (2010b). Further, the ability of spores to germinate in a complex sample matrix improved their potential as high-throughput field-portable bioreporters, which was proved by their further work (Date et al., 2010a). In this study, an engineered 12 cm diameter polymethyl methacrylate (PMMA) centrifugal compact disk (CD) with microfluidic channels and reservoirs for reagents was employed. The platform had three different reservoirs for spores, As(III) solution and chemiluminescent substrate. The reagents were mixed with each other by rotating the disk at defined spun velocities. The whole system produced a good agreement with spiked and fresh water samples in a 3 h response time. Factors such as faster mass transfer, reaction kinetics and improved surface-to-volume ratio enhanced the efficiency of the system. Exclusion of the cell-lysis step for the *β-gal* reaction with substrate was recommended through the use of X-gal against o-nitrophenyl-*β*-D-galactopyranoside (ONPG) as substrate (Ivanina and Shuvaeva, 2009). The study was carried out on the *E. coli* DH5α f′-harboring plasmid pMV arsR:: ABS. It was concluded that X-gal reduces As(V) to As(III) and provides better results. The developed biosensor was successfully applied to real snowmelt water samples. To increase the field applicability of the *β*-gal system, a new recombinant was constructed, namely *E. coli* JM 109–pSB1A2–BBa–J15501 (De Mora et al., 2011). This strain had an additional reporter gene *xylE* encoding catechol-2,3-dioxygenase in addition to *β*-galactosidase to a produce pH-dependent color change in arsenic-contaminated samples. Both the reporter genes worked together to establish a relation between arsenic concentration and pH change. A very unique combination of the whole-cell system with microfluidics and electrochemical detection was reported by Chiou et al. (2011). An *arsD*-*β*-galactosidase fusion protein was engineered in an *E. coli* bioreporter. The workers integrated the arsenite-induced expression of *β*-galactosidase with electrochemical measurement of *p*-aminophenol (PAP) which was formed as a result of *β*-galactosidase catalytic activity on *p*-aminophenyl-*β*-D-galactopyranoside (PAPG). The novelty of the study was the coupling of a micro-concentrator with a fabricated microfluidic chip containing micro-channels to concentrate the cells in a minute zone of electrochemical analysis, which significantly increased the sensitivity and reduced the response time of analysis. The developed microchip could be easily modulated from an electrochemical to an optical detection platform, which has broadened its application for other toxicants as well. The immense potential of miniaturization technology was proved in another report by Cortes-Salazar et al. (2013), where the same electrochemical reaction was performed in micro-wells of a 16-well microchip with a gold working electrode and a silver reference electrode in each well. The bioreporter *E. coli* DH5α (pPROBE′*arsR*–ABS–RBS–*lacZ* strain 2245) harboring the *arsR*–*lacZ* fusion gene reported in an earlier study was employed as the biocomponent. Since the bioreporter was an auxotroph for thiamine, it provided non-variable readouts in replicate assays and an improved *lacZ* ribosome-binding site resulted in a sensitive estimation of 0.8 μg/L. The microchip-facilitated detection was extremely fast as it avoided the cell lysis and PAP separation procedure required in other electrochemical methods. The developed system recorded a response time of 25–50 min for a sample of water containing 7.5 μg/L arsenite.

2.1.3. Green fluorescent protein (gfp)-based biosensors

Green fluorescent protein has been the choice of reporter gene due to the foremost advantage of no substrate requirement in contrary to luciferase- and *lacZ*-based approaches. It also holds the credit of providing continuous rather than end-point measurement. Roberto et al. (2002) reported a *gfp* bioreporter construct *E. coli* (p1 RC1140) containing operator/promoter, *arsR* gene and some part of *arsD* from p1RC120 fused with *gfp* from *Aequorea victoria*. The bacterial biosensors demonstrated stable fluorescence signal after a 12-h induction period with a detection limit of 1 μg/L and a nonlinear response between 1 μg/L and 10 mg/L. However, the obstruction due to background signals from recombinant strains remained a dilemma. Stocker et al. (2003) proposed a second binding site of the *arsR* protein before the reporter gene to avoid background expression of the *arsR* promoter. Another *gfp* bioreporter was developed for As(III), As(V) and Sb(III) monitoring in contaminated groundwater samples (Liao and Ou, 2005). An *E. coli* DH5*α* (pVLAS1) strain harboring the *S. aureus* plasmid fused with red-shift *gfp* was employed in the study. The bacterial biosensor had a detection limit of 30 μg/L, 75 μg/L and 91 μg/L for As(III), As(V) and Sb(III), respectively. An induction period of 2 h was required, but 8 h of incubation decreased the detection limit to 7.5 μg/L. To overcome intrapopulation response variability and carry out sensitive and precise detection, Busam et al. (2007) suggested use of flow cytometry and interpretation of its data by means of an artificial neural network-based adaptive approach. The fluorescence response of *E. coli* DH5α pPROBE–ArsR–ABS against As(III) was utilized to build the algorithm, which was applicable to other bioreporter responses too.

A new reporter system harboring the *arsP/O* and the *arsR* gene in trans placement and three copies of the reporter unit (*ars* promoter/operator–*arsR*–*gfp*) was demonstrated to double the signal-to-noise ratio without reducing background noise (Tani et al. 2009). This improvement was held responsible for the lower detection limit of 7.5 μg/L. Theytaz et al. (2009) proposed the integration of microfluidics and miniaturization as a contemporary practice in present biosensors. Attempting the same goal, a biochip was developed with channels for free flow of samples and filters to avoid microbial mobility. The bioreporter *E. coli* DH5α probe–*gfp* (tagless)–*arsR*–ABS trapped in the biochip was employed for fluorescence production after one hour. The biochip was demonstrated to respond substantially well at ≥ 50 μg/L As(III). The drawbacks of the study were a high detection limit and requirement of a fluorescence microscope for result retrieval. Since *gfp* suffers from a high background signal and delayed response, Hu

et al. (2010) studied the idea of a yellow fluorescent protein-based biosensor for arsenic. The construct *E. coli* DH5α (ars–puc18–yfp), also called WCB–11, displayed a time- and dose-dependent response against As(III) and As(V) with all-time low background fluorescence. Buffi et al. (2011a) developed a miniaturized bacterial biosensor in which *E. coli* recombinant cells bearing arsenic-induced *egfp* bioreporters were entrapped in agarose beads. The agarose beads frozen at −20 °C were assembled on a polydimethylsiloxane (PDMS) chip and integrated with microfluidic technology, to furnish a cheap, arsenic biosensor for on-site monitoring. The chip was limited by a decrease in fluorescence with a longer storage period. To overcome the limitation, Buffi et al. (2011b) suggested the conditioning of a PDMS chip frozen at −20 °C with rich LB medium for 30 min as an alternate to replenishing and reactivating the cells for better signal development in lesser response time.

To avoid various practical anomalies with whole-cell bacterial bioreporters, an advanced protein–DNA complex as a sensing component was reported (Kawakami et al., 2010). In this study, the *gfp*-tagged *arsR* protein acted as a trans element and the *ars* promoter/operator (P *ars*/O *ars*) provided the cis counterpart to form an arsenic-sensitive complex. An improvement in the same assay protocol was reported by Siddiki et al. (2011) in which the trans–cis element complex was immobilized on the microwell surface and dissociation of the complex in the presence of arsenic was monitored for quantitative analysis. The fluorescence readout of the *gfp*-tagged trans element after dissociation from cis was undertaken separately. This step avoided the background noise problem that was the main concern in whole-cell-based biosensors. The developed systems provided a suitable onsite monitoring tool for arsenic monitoring through a lyophilized solid phase hand-held flourometer. Later, the same sensor was employed by the group to measure As(III) moieties in milk, and other dairy products (Siddiki et al., 2012a). Another study by Siddiki et al. (2012b) demonstrated the capability of magnetic nanoparticles in developing a highly advanced thermoresponsive solid-phase arsenic biosensor. Experimentally, *arsR* DNA was tagged with *gfp* and the complementary operator–promoter DNA was attached to magnetic nanoparticles through streptavidin–biotin conjugation. This assembly enabled magnet-induced aggregation of nanoparticles and subsequent fluorescence-based arsenic analysis. Since the microbial degradation of arsenic-containing herbicides leads to more toxic products, an initiative to selectively measure organic arsenic was carried out by Chen et al. (2012). For this purpose, a dual plasmid assembly was engineered in host *E. coli* AW3110 in which the first plasmid pBADarsR had *arsR* from *Acidithiobacillus ferrooxidans* (AfArsR) under the control of the arabinose promoter and the second plasmid pACYC184–*parsO*–*gfp* had the *gfp* reporter gene fused to the *A. ferrooxidans arsO* promoter. This genetic engineering provided selective analyte response through tunable *gfp* expression and shift in selectivity order with arabinose concentration. In continuation to this work, Chen et al. (2014) further diminished the sensitivity of the repressor AfArsR towards inorganic As(III) by replacing the Cys102 with a serine residue (C102S). To combat limitations of bacterial biosensors, a more robust and stable fungal-based biosensor was reported (Choe et al., 2012). The fungal *arsA* gene of the isolated strain *A. niger* was hypersensitive to low levels of arsenic. So, a gene construct harboring *acrA*–*gfp* was transcribed into the fungal model system. The pre-grown hyphae produced better dose-dependent detectable florescence than the fungal conidia, but suffered from significant background autoflorescence. Truffer et al. (2014) recently developed an automated electronic system to illuminate, assemble and detect fluorescence produced from the *E. coli* strain 1598 harboring the plasmid pPROBE–ArsR–ABS. The bioreporter was encapsulated in agarose minibeads and placed on a microfluidics PDMS platform with two parallel channels for As(III) analysis. Each measurement in two parallel channels was carried out at an interval of 20 min. The device responded to the 10 μg/L concentration at 80 min and 50 μg/L concentration at 120 min. The results of the various studies on *gfp*-based arsenic biosensors are summarized in Table 2.

2.1.4.. Biosensors based on alternative approaches

In addition to *E. coli*, certain other bacteria such as the photosynthetic bacterium *Rhodovulum sulfidophilum* have also been exploited as hosts for arsenic biosensors (Fujimoto et al., 2006). A novel carotenoid-based arsenite biosensor plasmid pSENSE–As was constructed by inserting carotenoid photosynthesis responsible *crtA* gene from *R. sulfidophilum* and operator/promoter (*O/Pars*) region and *arsR* gene from *E. coli* into the broad-host-range plasmid vector, pRK415. When pSENSE–As was transferred to the *R. sulfidophilum* strain CDM2 (*crtA*-deleted mutant), it showed a change in color from yellow to red in the presence of arsenic due to increased production of the carotenoid, spheroidenone. The detection limit of the biosensor strain was 3 μg/L, visible to the naked eye on 24-h incubation. The study demonstrated the commercial potential of the developed method as a red spot paper test. Another whole-cell carotenoid-based biosensor was reported harboring the *arsR* gene of *E. coli* and the *crtI* gene of *Rhodopseudomonas palustris* under the expression control of the *ars* operon (Yoshida et al., 2008). The biosensor was reported to produce a red-colored pigment lycopene from the colorless carotenoid phytone in response to As(III). The novelty of the investigation was reflected in the choice of *crtI* as the reporter gene and use of blue-green non-sulfur photosynthetic bacteria instead of luciferase- or *gfp*-containing *E. coli* mutants. Liu et al. (2012) developed an SPR-based biosensor comprising of highly sensitive AuNPs-modified 3D hydrogel matrix. The study included ArsA–ATPase binding to ArsD and response to As(III). It was concluded that the ArsA–ArsD complex is involved in channelizing As(III) from ArsD to ArsA, and this transfer is influenced by Mg^{2+} ions and ATP.

A very unique kind of label-free cytotoxicity assessment approach was reported by Xing et al. (2005). The study used a real-time cell electronic sensing (RT–CES) platform in which the cells were grown onto the surfaces of microelectronic sensors. The microelectronic sensors were comprised of circle-on-line electrode arrays integrated into the microtiter plate. The system conferred online monitoring of changes in cell viability, morphology and adherence properties based on change in electrical impedence. Although the concentration of As(III) studied was very high, the automated approach provided a very effective and dynamic cytotoxicity assessment system. Few efforts have been made to convert microbes as communicators. In this respect, an interesting study was reported by Prindle et al. (2012) based on intercellular synchronization. A microfluidic array was developed that allows many separate colonies of sensing bacteria to grow and communicate rapidly by gas exchange. They wired thousands of small oscillating colonies, or 'biopixels', in a microfluidic array to develop a sensor. Coupling between biopixels involves redox signaling by hydrogen peroxide (H_2O_2) and the native redox-sensing machineries of *E. coli*. The two coupling mechanisms act synergistically and yield extremely consistent oscillations, with a temporal accuracy of about 2 min, compared to 5–10 min for a signal. Another study integrated the cells with logic gates (Wang et al., 2013). The genetic logic gates can function as a biological filter and an amplifier to enhance the selectivity and sensitivity of cell-based biosensors. An engineered modular genetic AND logic gate in a genetically manipulated *E. coli* cell was developed for the detection of three environmental signals, As(III), Hg(II) and Cu(II) (Wang et al., 2013). A special kind of plasmid was constructed for specific metal ions, by eliminating some restriction sites. The sensory modules were then wired to a genetic logic AND gate to

Table 3
Characteristics of cell free arsenic biosensors with detection limits and induction period/response time.

Biosensor specifications	Detection limit (μg/L)	Induction period/Response time (min)	Limitations	Reference
DNA-based biosensors				
CT-DNA based electrochemical biosensor	385	2	Increase in guanine signal above 0.5 mm As(III), non-specificity	Ozsoz et al. (2003)
CT-DNA based electrochemical biosensor with $[Co(phen)_3]^{3+}$ and $[Ru(bipy)_3]^{2+}$ as DNA markers	77,000	10	Poor detection limit, Inefficient electrode regeneration, non-specificity	Labuda et al. (2005)
Layer by layer assembled CT- DNA based electrochemical biosensor	77,000	10	Poor detection limit, non-specificity	Ferancová et al. (2007)
DNA functionalized SWCNT hybrid biosensor	0.05	3	Non-specificity, Electrode regeneration problem	Liu and Wei (2008)
Calf thymus DNA based SPR biosensor	10	0.5	Sophisticated electrode preparation, non-specificity	Solanki et al. (2009)
Aptamer-based biosensors				
Aptamer–cationic polymer complex based Gold nanoparticle biosensor	5.3	2	AuNPs susceptibility to surroundings	Wu et al. (2012b)
Aptamer–CTAB complex based Gold nanoparticle biosensor	0.66	2	Instable practical application	Wu et al. (2012a)
Aptamer–CV nanoparticle based biosensor	0.2	2		Wu et al. (2012c)
Aptamer–hemin system based biosensor	6	15	Hemin instability in aqueous solution	Wu et al. (2013)
Protein-based biosensors				
Acetylcholine based electrochemical biosensor	0.015	0.5	Non-specificity	Stoytcheva et al. (1998)
Acid phosphatase and polyphenol oxidase based electrochemical biosensor	0.15	20	Preconcentration step losses reusability, high PPO amount needed	Cosnier et al. (2006)
Arsenite oxidase based electrochemical biosensor	1	0.16	Interference from ppm level humic aid	Male et al. (2007)
L–cysteine based electrochemical biosensor	1.2	5	Uncontrollable porosity of hydrogel polymer, effect of oxidizing agents on performance	Sarkar et al. (2010)
Acetylcholine based electrochemical biosensor	0.825	70	Interference by other metal ions, low storage stability	Sanllorente-Méndez et al. (2010)
Acid phosphotase based electrochemical biosensor	0.825	70	Non-specificity, fluctuating background currents	Sanllorente-Mendez et al. (2012)
Cyctochrome C based electrochemical biosensor	692	0.5	Poor detection limit	Fuku et al. (2012)

distinguish As(III), Hg(II), Cu(II), Zn(II) and the 3-oxo-C6 homoserine lactone (3OC6HSL) quorum sensing molecule and combinations thereof with quantitative fluorescent output. A reporter-free inhibition-based whole-cell detection system has been developed by Zhai et al. (2013). The study involved ferricyanide-mediated bioreaction to produce prussian blue (PB) for simple colorimetric analysis of As(III). *E. coli* DH5α was engaged to donate electrons for ferrocyanide formation from ferricyanide and subsequent PB formation with addition of ferric ions. As(III) and other toxicants cause inhibition of respiratory activity and reduction in PB production and blue color formation. The developed method had a detection limit of 25 mg/L with a response time of 30 min.

2.2. Cell-free arsenic biosensors

2.2.1. DNA-based biosensors

The non-reproducible and practically non-feasible nature of whole-cell biosensors indicated the need for other biorecognition components. DNA provided the required platform for the purpose. It provides three modes of interactions, namely electrostatic interaction with the negatively charged phosphate, binding interaction with the minor and major grooves of the DNA double helix and intercalation between the stacked base pairs of native DNA (Arora et al., 2007). DNA-based arsenic biosensors have been reported as listed in Table 3. These DNA-based sensors, in the majority of cases, signal by oxidative damage in the presence of arsenic. Thus, Ozsoz et al. (2003) investigated calf thymus DNA and 17-mer oligonucleotides as the biocomponent for electrochemical detection of As(III) based on the guanine oxidation signal. The special feature of the study was investigation of the effect of As(III) on dsDNA, ssDNA and 17-mer oligonucleotides. Carbon paste or pencil graphite working electrode was employed for differential pulse voltametry (DPV) and potentiometric stripping analysis. Labuda et al. (2005) reported an advanced form of the DNA biosensor with addition of a certain electrochemical DNA marker and a DNA oxidation catalyst to increase the sensitivity. A Co(III) complex of 1,10-phenanthroline $[Co(phen)_3]^{3+}$ and Ru(II) with bipyridine $[Ru(bipy)_3]^{2+}$ were employed for the purpose. Calf thymus DNA (CT-DNA), immobilized on the surface of a screen-printed electrode (SPE), was subjected to different concentrations of aqueous As(III), As(V), dimethyl arsenic acid, phenyl arsenic and *p*-arsanilic acid. Although the system was reported to have a poor detection limit (75 mg/L), it presented the successful correlation of the DNA marker signal with the As(III) level. Another biosensor based on the same DNA marker $[Co(phen)_3]^{3+}$ as the indicator of undamaged DNA after As(III) exposure was reported (Ferancová et al., 2007). The basic advancement from the previous study was the use of carbon multiwalled nanotubes (MWNT) as the signal enhancer over the screen-printed electrode. The advantage of high electric conductivity of carbon nanotubes (CNTs) was utilized by Liu and Wei (2008) to construct an electrochemical biosensor. The concept of direct oxidation of As(0) to As(III) on DNA-functionalized single-walled CNT-modified glassy carbon electrode was explored. The developed biosensor had an operational stability over a broad pH range and showed 16 times reusability. Advancement in DNA-based arsenic biosensors was seen with the advent of a self-assembled monolayer (SAM) and surface plasmon resonance (SPR) technology (Solanki et al., 2009). SAM enabled thin film formation of biomolecules on a detection platform and it furnishes fast current flow within a polymer matrix as compared to the thick film approach utilized in previous studies. The study, for the first time, proposed possible interaction between As_2O_3 with dsCT-DNA. Although the study achieved a very low detection limit in minimum response time, it suffered two main drawbacks. Firstly, it was very difficult to fabricate large quantities of such sophisticated dsCT-DNA/MCE/Au bioelectrodes, for field investigation of As(III). Secondly, as with all DNA-based biosensors, it lacked specificity towards As(III).

2.2.2. Aptamers-based biosensors

Recently, a new category of a biorecognition tool called aptamers has become available. Aptamers are artificial, single-standard RNA or DNA oligonucleotides, *in vitro* preorganized for selective binding to a number of target analytes. They have the advantage of specificity, real-time and onsite monitoring that provide a commercial potential for use as biosensors. Aptamers provide multiple

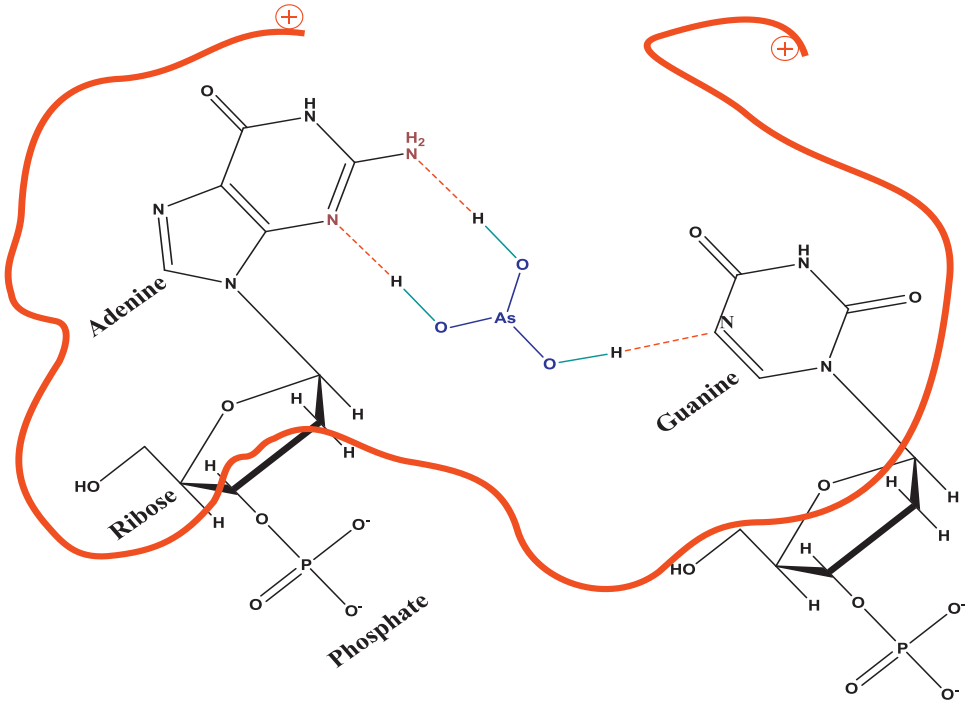
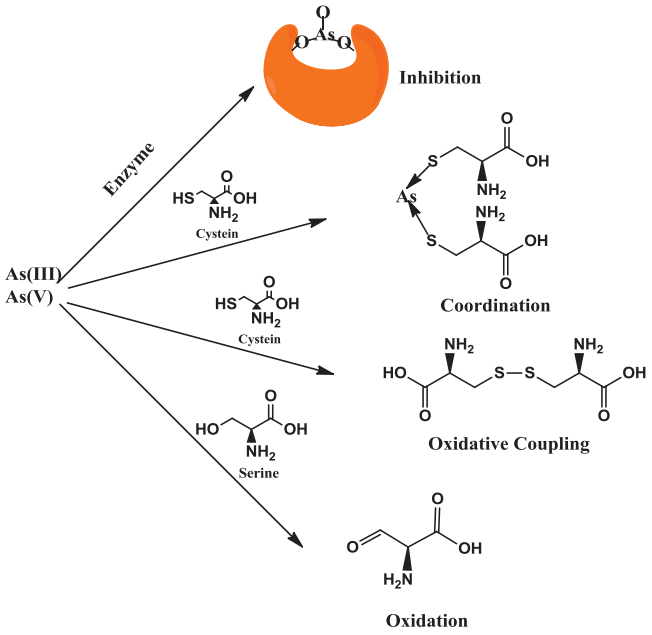


Fig. 1. Possible structure of arsenic species binding site with aptamer.

complementary binding options via directed unsaturated hydrogen bonding with the nucleotide bases and their self-assembly-induced recognition behavior has been utilized in various sensing techniques. The possible mode of interaction of arsenic with an aptamer is depicted in Fig. 1. These features make the apatmers quite different from the DNA in anion-sensing affinity for As(III) species. The basic bioassay principle for As(III) detection explores the affinity of the aptamer Ars-3 towards arsenic and some cationic polymers or surfactants that induces gold nanoparticle (AuNP) aggregation. This change in particle property is subsequently relayed as the change in the absorption or resonance scattering assay of AuNPs. In a preliminary study, a water-soluble cationic polymer polydiallyldimethylammonium (PDDA) salt was employed to aggregate AuNPs and hybridize the Ars-3 aptamer through electrostatic interactions (Wu et al., 2012b). The introduction of As(III) induced aptamer–As(III) complex formation and hence PPDA was set free to aggregate AuNPs and change their color from wine red to blue. In the absence of As(III), PDDA gets hybridized to Ars-3, that made it unavailable for AuNP aggregation; thus, the red wine color persists. To achieve better sensitivity, a cationic surfactant cetyltrimethylammonium bromide (CTAB) was employed in the same strategy and it showed significant advancement with a detection limit of 40 μg/L for the naked eye, 0.66 μg/L for colorimetric and 0.77 μg/L for resonance scattering assay (Wu et al., 2012a). The low detection limit was attributed to the formation of a CTAB-induced nanostructured supramolecule with aptamers and low background signals as compared to cationic polymers. In a study by Wu et al. (2012c), As(III)-induced size alteration of Ars-3–crystal violet (aptamer–CV) nanoparticles was employed to conduct Rayleigh scattering spectral (RRS) assay-based detection. The unique structure of CV with one positive charge and three phenyl rings promotes the formation of lipid-like aptamer–CV micelles with a hydrophobic phenyl ring inner core and a hydrophilic positively charged surface. The concentration of the aptamer decided the size of nanoparticles, as 200 nM (low aptamer) concentration led to large nanoparticles and 600 nM (high aptamer) concentration formed small nanoparticles. The addition of As(III) caused alteration in nanoparticle size and hence change in RSS intensity. The association of As(III) with the aptamer–CV complex through its three hydroxy groups (OH^-) in aqueous solution resulted in inter- or intra-molecular hydrogen bonds that led to significant changes in average particle size. The detection limit of the biosensor with 600 and 200 nM aptamer concentration was 0.2 and 0.3 μg/L, respectively. On addition of As (III), the average particle size increased from 28.7 to 73 nm in the former case and decreased from 273 to 168 nm in the latter one. The most recent report of Wu et al. (2013) employed the hemin-peroxidase system for sensitive and selective colorimetric detection of As(III). The bioassay characterization utilized the fact that the Ars-3 aptamer provisionally inhibits the catalytic activity of hemin on its substrate, 3,5,3′,5′-tetramethylbenzidine (TMB), that yields the cationic radical of blue color. Addition of As(III) inverted this effect owing to formation of the aptamer–As(III) complex that resulted in a yellow diamine product due to complete oxidation of TMB. To avoid aggregation of hemin molecules into inactive dimmers in an aqueous system, G-quadraplex structures were produced that maintained their catalytic active conformation. These aptamer-based biosensors proved to be an efficient tool for As(III) detection without many limitations and are the representative of the most recent and advanced technology. They could also be combined with microfluidics to develop miniaturized field biosensors. They provided the advantage of repetitive and reliable results over microbial biosensors and showcased effective field application.



Scheme 3. Schematic representation of mode of interaction between protein and arsenic species.

2.2.3. *Protein-based biosensors*

Apart from DNA and aptamers, certain proteins have been employed as the sensing material for arsenic. The mechanism for sensing arsenic relies on the HSAB principle whereby the soft acid arsenic has affinity for binding soft sulfur in protein structures. Apart from this, the sulfur of cysteine is susceptible to oxidation in the presence of arsenic and can be utilized for sensing. The probable mechanisms of arsenic interaction with proteins are depicted in Scheme 3. Most protein-based biosensors developed for As(III) or As(V) are based on the inhibition phenomenon. The same strategy was employed by Stoytcheva et al. (1998) to develop an acetylcholinesterase-based As(III) amperometric biosensor. The bioassay principle involved the catalytic action of acetylcholinesterase on acetylcholine iodide, resulting in the formation of the redox active product thiocholine that provided the signal for As (III) estimation. In the presence of As(III), thiocholine oxidation current decreased to levels proportional to As(III) concentration. A method was also developed for As(III) and As(V) differentiation in wastewater samples. A very interesting study on fluorescent arsenite analysis through napthyl-labeled phytochelatin-like peptide was reported by Parker et al. (2005). Since arsenite is neutral at physiological pH and has affinity for thiol group, cysteine preorganized peptides were designed and synthesized for quenching-based florescence analysis of arsenite. However, to reduce the interference of positively charged ions, amino acids carrying positively charged substituents at neutral pH, were introduced in the peptide designs. Peptides Napth-Cys-Lys-Cys-Lys-Gly and Napth-Cys-Pro-Gly-Cys-Lys-Lys with no distinct secondary structure and a tighter hairpin turn in secondary structure, respectively, were found to sense arsenite. The study showed a path intended for protein designing for biosensing but has to deal with the specificity aspect. Another study for As(V) analysis was reported through the application of acid phosphatase (AcP) and polyphenol oxidase (PPO) (Cosnier et al., 2006). The bi-enzymatic system was employed to catalyze the hydrolysis of phenylphosphate to phenol by AcP and subsequent phenol polarization using PPO. The inhibitory effect of As(V) on AcP and the anion accumulation potential of layered double hydroxides was utilized to perform the analysis. For the first time, exploitation of a molybdenum-containing arsenite oxidase from the chemolithoautotroph NT 26 was reported for mediator-free oxidation of arsenite to arsenate and

subsequent analysis of the former ion (Male et al., 2007). Arsenite specificity of the enzyme and sensitivity of a multiwall carbon nanotube (MWCNT) on glassy carbon (GC) electrode enabled fast investigation with excellent reproducibility. The developed biosensor was employed to estimate arsenite in tap, river and commercial mineral water samples. L-Cysteine immobilized in an acrylamide matrix on the working electrode has also been demonstrated to act as a reducing agent for the conversion of arsenate into arsenite (Sarkar et al., 2010). In this reaction, L-cysteine itself was oxidized to L-cystine accompanied by electron transfer. This system enabled the amperometric detection of arsenic on three different kinds of transducer assemblies. The S1 transducer assembly had carbon as the working electrode, S2 had a glassy carbon working electrode and in the S3 system a platinum electrode was employed. The sensitivity order of the three electrodes towards arsenic was S3 > S1 > S2. The developed biosensor displayed comparable results with other conventional techniques and was applicable to field testing. The only drawback described by the authors was the need for freshly prepared electrodes because of uncontrolled porosity of the acrylamide polymer matrix. This defect constrained the use of the biosensor for large-scale on-line water monitoring. Another electrochemical biosensor based on acetylcholine inhibition by As(III) was reported by Sanllorente-Méndez et al. (2010). The study was based on the well-known fact of enzyme inhibition by toxic heavy metals. Amperometric determination was accomplished on an screen-printed electrode (SPE) through covalent immobilization of the enzyme on the electrode surface. The study suffered the limitations of short storage stability (15 day) of the bioelectrodes at 4 °C and non-specificity. Later, Sanllorente-Mendez et al. (2012) reported the first acid phosphatase-based As(V) biosensor with use of the novel substrate 2-phospho-L-ascorbic acid. The optimization of experimental variables by means of central composite design (CCD) was unique about the study. Cytochrome-C (Cyt-C), being an important component of the electron transport chain in mitochondria and sensitive to all toxic compounds, has also been exploited as a biorecognition element (Fuku et al., 2012). An electrochemical biosensor was constructed with Cyt-C, immobilized on a boron-doped diamond electrode. Square-wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) were performed to study the Cyt-C interaction with arsenic and cyanide. Subtractive normalized Fourier transform infrared spectroscopy (SNFTIR) was carried out to confirm efficient protein adsorption onto the electrode. An UV–vis study of the Cyt-C with analyte confirmed proper binding. It was revealed that their interaction was through amino acid of the basic protein structure rather than through the heme moiety of Cyt-C. The comparison of techniques employed, detection limits and response time achieved in developed biosensors have been summarized in Table 3.

3. Conclusion

During the last two decades, a number of arsenic biosensors have been developed based on whole-cell biosensor to biomolecules (protein, DNA and aptamer)-based biosensors. Whole-cell-based biosensors have showed successful utilization in the analysis of arsenic in environmental samples including groundwater and soil. However, their use is limited by the incubation period and the lower detection limits. This disadvantage of the whole-cell-based biosensors have been overcome with the advent of biomolecular biosensors, whereby the unique interaction characteristics of arsenic are taken into consideration for biosensing. Protein-based biosensors in majority are based on the dosimetric strategies; in a similar manner, DNA-based biosensors are based on oxidative strategies. Quick response and better detection limits have extended the capabilities of the biomolecular biosensors. The advent of aptamer-targeted technology has further provided the essential tool for further lowering the detection limit to sub-ppb levels that has opened up a futuristic pathway for target-specific biosensor development. The integration of microfluidics and miniaturization technology with biosensors has made analysis possible with very small sample volume (50–500 μl) and has increased their target niche from environmental to clinical samples. Synthesis of target-specific protein motifs through docking studies could further take the biosensing approach to new levels. However, few of these biosensors have been tested for their robustness to various environmental matrices. Further challenges exist in the development of biosensors that could furnish arsenic sensing in complex matrices including health related matrices such as blood, urine, etc. and water samples with high TDS and salinity including seawater.

Acknowledgment

Dr. J.N. Babu is thankful to DST-SERB, New Delhi for providing financial assistance under DST Fast track Young Scientist Programme (Ref. No. 240/2010). We also acknowledge the contribution of Dr. Neetu Purohit, Centre for Comparative Literature, Central University of Punjab, Bathinda, Punjab, India, for linguistic and grammatical editing of this manuscript. Further, due to limited focus of the article, some references could not be included, for which we apologize.

References

Ahmed, F., Alexander, I.J., Mwinyihija, M., Killham, K., 2012. J. Environ. Sci. 24 (6), 1106–1116.
Aleksic, J., Bizzari, F., Cai, Y., Davidson, B., De Mora, K., Ivakhno, S., Seshasayee, S., Nicholson, J., Wilson, J., Elfick, A., 2007. IET Synth. Biol. 1 (1), 87–90.
Anawar, H.M., 2012. Talanta 88, 30–42.
Arora, K., Prabhakar, N., Chand, S., Malhotra, B., 2007. Sens. Actuators B—Chem. 126 (2), 655–663.
Baeumner, A.J., 2003. Anal. Bioanal. Chem. 377 (3), 434–445.
Bakhrat, A., Eltzov, E., Finkelstein, Y., Marks, R.S., Raveh, D., 2011. Cell Biol. Toxicol. 27 (3), 227–236.
Baumann, B., Van der Meer, J.R., 2007. J. Agric. Food Chem. 55 (6), 2115–2120.
Beer, P.D., Gale, P.A., 2001. Angew. Chem. Int. Ed. 40 (3), 486–516.
Buffi, N., Merulla, D., Beutier, J., Barbaud, F., Beggah, S., van Lintel, H., Renaud, P., van der Meer, J.R., 2011a. Lab Chip 11 (14), 2369–2377.
Buffi, N., Merulla, D., Beutier, J., Barbaud, F., Beggah, S., van Lintel, H., Renaud, P., van der Meer, J.R., 2011b. Bioeng. Bugs 2 (5), 296–298.
Busam, S., McNabb, M., Wackwitz, A., Senevirathna, W., Beggah, S., van der Meer, J. R., Harms, H., 2007. Anal. Chem. 79 (23), 9107–9114.
Cai, J., DuBow, M.S., 1997. Biodegradation 8 (2), 105–111.
Cervantes, C., Ji, G., Ramirez, J.L., Silver, S., 1994. FEMS Microbiol. Rev. 15 (4), 355–367.
Chen, C.M., Misra, T.K., Silver, S., Rosen, B.P., 1986. J. Biol. Chem. 261, 15030–15038.
Chen, D., Huang, C., He, M., Hu, B., 2009. J. Hazard. Mater. 164 (2–3), 1146–1151.
Chen, J., Sun, S., Li, C., Zhu, Y., Rosen, B.P., 2014. Environ. Sci. Technol. 48 (2), 1141–1147.
Chen, J., Zhu, Y.-G., Rosen, B.P., 2012. Appl. Environ. Microbiol. 78 (19), 7145–7147.
Chiou, C.H., Chien, L.J., Chou, T.C., Lin, J.L., Tseng, J.T., 2011. Biosens. Bioelectron. 26 (5), 2484–2488.
Choe, S.-I., Gravelat, F.N., Al Abdallah, Q., Lee, M.J., Gibbs, B.F., Sheppard, D.C., 2012. Appl. Environ. Microbiol. 78 (11), 3855–3863.
Chowdhury, U.K., Biswas, B.K., Chowdhury, T.R., Samanta, G., Mandal, B.K., Basu, G. C., Chanda, C.R., Lodh, D., Saha, K.C., Mukherjee, S.K., 2000. Environ. Health Perspect. 108 (5), 393.
Corbisier, P., Ji, G., Nuyts, G., Mergeay, M., Silver, S., 1993. FEMS Microbiol. Lett. 110 (2), 231–238.
Cortes-Salazar, F., Beggah, S., van der Meer, J.R., Girault, H.H., 2013. Biosens. Bioelectron. 47, 237–242.
Cosnier, S., Mousty, C., Cui, X., Yang, X., Dong, S., 2006. Anal. Chem. 78 (14), 4985–4989.
Cullen, W.R., Reimer, K.J., 1989. Chem. Rev. 89 (4), 713–764.
Date, A., Pasini, P., Daunert, S., 2007. Anal. Chem. 79 (24), 9391–9397.
Date, A., Pasini, P., Daunert, S., 2010a. Anal. Bioanal. Chem. 398 (1), 349–356.
Date, A., Pasini, P., Sangal, A., Daunert, S., 2010b. Anal. Chem. 82 (14), 6098–6103.

De Mora, K., Joshi, N., Balint, B., Ward, F., Elfick, A., French, C., 2011. Anal. Bioanal. Chem. 400 (4), 1031–1039.
Diesel, E., Schreiber, M., van der Meer, J.R., 2009. Anal. Bioanal. Chem. 394 (3), 687–693.
Dietrich, B., 1993. Pure Appl. Chem. 65 (7), 1457–1464.
Diorio, C., Cai, J., Marmor, J., Shinder, R., DuBow, M.S., 1995. J. Bacteriol. 177, 2050–2056.
Du, J., Cui, J., Jing, C., 2014. Chem. Commun. 50 (3), 347–349.
Elad, T., Almog, R., Yagur-Kroll, S., Levkov, K., Melamed, S., Shacham-Diamand, Y., Belkin, S., 2011. Environ. Sci. Technol. 45 (19), 8536–8544.
Elad, T., Belkin, S., 2012. Bioeng. Bugs 3, 124–128.
Ezeh, V.C., Harrop, T.C., 2012. Inorg. Chem. 51 (3), 1213–1215.
Ezeh, V.C., Harrop, T.C., 2013. Inorg. Chem. 52 (5), 2323–2334.
Ferancová, A., Adamovski, M., Gründler, P., Zima, J., Barek, J., Mattusch, J., Wennrich, R., Labuda, J., 2007. Bioelectrochemistry 71 (1), 33–37.
Forzani, E.S., Foley, K., Westerhoff, P., Tao, N., 2007. Sens. Actuators B—Chem. 123, 82–88.
Fujimoto, H., Wakabayashi, M., Yamashiro, H., Maeda, I., Isoda, K., Kondoh, M., Kawase, M., Miyasaka, H., Yagi, K., 2006. Appl. Microbiol. Biotechnol. 73 (2), 332–338.
Fuku, X., Iftikar, F., Hess, E., Iwuoha, E., Baker, P., 2012. Anal. Chim. Acta 730, 49–59.
Gao, C., Yu, X.Y., Xiong, S.Q., Liu, J.H., Huang, X.J., 2013. Anal. Chem. 85 (5), 2673–2680.
Gibbon-Walsh, K., Salaün, P., van den Berg, CM., 2010. Anal. Chim. Acta 662 (1), 1–8.
Hakkila, K., Green, T., Leskinen, P., Ivask, A., Marks, R., Virta, M., 2004. J. Appl. Toxicol. 24 (5), 333–342.
Harms, H., Rime, J., Leupin, O., Hug, S.J., van der Meer, J.R., 2005. Microchim. Acta 151 (3–4), 217–222.
Hou, Q.H., Ma, A.Z., Lv, D., Bai, Z.H., Zhuang, X.L., Zhuang, G.Q., 2014. Appl. Microbiol. Biotechnol. 98 (13), 6137–6146.
Hu, Q., Li, L., Wang, Y., Zhao, W., Qi, H., Zhuang, G., 2010. J. Environ. Sci. 22 (9), 1469–1474.
IARC, 2004. Arsenic in Drinking-Water. International Agency for Research on Cancer (IARC), Lyon, France, 84, pp. 39–267.
Ivanina, A.V., Shuvaeva, O.V., 2009. J. Anal. Chem. 64 (3), 310–315.
Ivask, A., Green, T., Polyak, B., Mor, A., Kahru, A., Virta, M., Marks, R., 2007. Biosens. Bioelectron. 22 (7), 1396–1402.
Ji, G., Silver, S., 1992. J. Bacteriol. 174 (11), 3684–3694.
Joshi, N., Wang, X., Montgomery, L., Elfick, A., French, C., 2009. Desalination 248 (1), 517–523.
Jouanneau, S., Durand, M.J., Thouand, G., 2012. Environ. Sci. Technol. 46 (21), 11979–11987.
Kawakami, Y.., Siddiki, M.S.R., Inoue, K., Otabayashi, H., Yoshida, K., Ueda, S., Miyasaka, H., Maeda, I., 2010. Biosens. Bioelectron. 26 (4), 1466–1473.
Labuda, J.,.K, Bubnicova, K., Kovalova, L.., Vanickova, M., Mattusch, J.., Wennrich, R., 2005. Sensors 5 (6), 411–423.
Liao, V.H.C., Ou, K.L., 2005. Environ. Toxicol. Chem. 24 (7), 1624–1631.
Liu, C.,, Balsamo, V., Sun, D., Naja, M., Wang, X., Rosen, B., Li, C.Z., 2012. Biosens. Bioelectron. 38 (1), 19–26.
Liu, Y., Wei, W., 2008. Electrochem. Commun. 10 (6), 872–875.
Lohar, S., Sahana, A., Banerjee, A., Banik, A., Mukhopadhyay, S.K., Sanmartín Matalobos, J.S., Das, D., 2013. Anal. Chem. 85 (3), 1778–1783.
Ma, J., Sengupta, M.K., Yuan, D., Dasgupta, P.K., 2014. Anal. Chim. Acta 831, 1–23.
Male, K.B., Hrapovic, S., Santini, J.M., Luong, J.H., 2007. Anal. Chem. 79 (20), 7831–7837.
Mandal, B.K., Suzuki, K.T., 2002. Talanta 58 (1), 201–235.
Mello, L.D.., Kubota, L.T., 2002. Food Chem. 77 (2), 237–256.
Merulla, D., Buffi, N., Beggah, S., Truffer, F., Geiser, M., Renaud, P., van der Meer, J.R., 2013. Curr. Opin. Biotechnol. 24 (3), 534–541.
Owolabi, J.B., Rosen, B.P., 1990. J. Bacteriol. 172, 2367–2371.
Ozsoz, M., Erdem, A., Kara, P., Kerman, K., Ozkan, D., 2003. Electroanalysis 15 (7), 613–619.
Parker, K.J., Kumar, S, Pearce, D.A., Sutherland, A.J., 2005. Tetrahedron Lett. 46 (41), 7043–7045.
Petanen, T., 2001. Assessment of Bioavailable Concentrations and Toxicity of Arsenite and Mercury in Contaminated Soils and Sediments by Bacterial Biosensors (doctoral dissertation). University of Helsinki, Helsinki, Finland.
Petanen, T., Romantschuk, M., 2002. Anal. Chim. Acta 456 (1), 55–61.
Petursdottir, A.H., Gunnlaugsdottir, H., Jorundsdottir, H., Mestrot, A., Krupp, E.M., Feldmann, J., 2012. Anal. Bioanal. Chem. 404 (8), 2185–2191.
Prindle, A., Samayoa, P., Razinkov, I., Danino, T., Tsimring, L.S., Hasty, J., 2012. Nature 481 (7379), 39–44.
Ranjan, R., Rastogi, N.K.., Thakur, M..S., 2012. J. Hazard. Mater 225, 114–123.
Richter, J.., Lischka, S., Piechotta, C., 2012. Talanta 101, 524–529.
Roberto, F.F, Barnes, J.M., Bruhn, D.F., 2002. Talanta 58 (1), 181–188.
Rosen, B.P., 1999. Essays Biochem. 34, 1–15.
Sahana, A., Banerjee, A., Lohar, S., Panja, S., Mukhopadhyay, S.K., Matalobos, J.S., Das, D., 2013. Chem. Commun. 49 (65), 7231–7233.
Salaun, P., Gibbon-Walsh, K.B., Alves, G.M., Soares, H.M., van den Berg, C.M., 2012. Anal. Chim. Acta 746, 53-–62.
Saleem M., 2013. IOP Conference Series: Material Science and Engineering 51, Bristol, UK, p. 012012.
Sanllorente-Mendez, S., Dominguez-Renedo, O., Arcos-Martinez, M., 2012. Talanta 93, 301–306.
Sanllorente-Méndez, S., Domínguez-Renedo, O., Arcos-Martinez, M.J., 2010. Sensors 10 (3), 2119–2128.
Sarkar, P, Banerjee, S., Bhattacharyay, D., Turner, A.P., 2010. Ecotoxicol. Environ. Saf. 73 (6), 1495–1501.
Scott, D.L., Ramanathan, S., Shi, W., Rosen, B.P., Daunert, S., 1997. Anal. Chem. 69 (1), 16–20.
Sharma, P., Asad, S., Ali, A., 2013. J. Biosci. 38 (2), 251–258.
Siddiki, M.S.R., Kawakami, Y., Ueda, S., Maeda, I., 2011. Sensors 11 (11), 10063–10073.
Siddiki, M.S.R., Shimoaoki, S., Ueda, S., Maeda, I., 2012a. Sensors 12 (10), 14041–14052.
Siddiki, M.S.R., Ueda, S., Maeda, I., 2012b. Br. Med. Clin. Biotechnol 12 (1), 76.
Siegfried, K., Endes, C., Bhuiyan, A.F.M.K., Kuppardt, A., Mattusch, J., van der Meer, J. R., Chatzinotas, A., Harms, H., 2012. Environ. Sci. Technol. 46 (6), 3281–3287.
Singh, M., Verma, N., Garg, A.K., Redhu, N., 2008. Sens. Actuators, B 134 (1), 345–351.
Solanki, P.R., Prabhakar, N., Pandey, M.K., Malhotra, B.D., 2009. Int. J. Environ. Anal. Chem. 89 (1), 49–57.
Stocker, J., Balluch, D., Gsell, M., Harms, H., Feliciano, J., Daunert, S., Malik, K.A., van der Meer, J.R., 2003. Environ. Sci. Technol. 37 (20), 4743–4750.
Stone, R., 2008. Science 321 (5886), 184–185.
Stoytcheva, M., Sharkova, V., Panayotova, M., 1998. Anal. Chim. Acta 364 (1), 195–201.
Tahir, M.A., Rasheed, H., Malana, A., 2008. Drink. Water Eng. Sci. Discuss. 1, 135–154.
Tani, C,, Inoue, K., Tani, Y., Harun-ur-Rashid, M., Azuma, N., Ueda, S., Yoshida, K., Maeda, I., 2009. J. Biosci. Bioeng. 108 (5), 414–420.
Tauriainen, S., Karp, M., Chang, W., Virta, M., 1997. Appl. Environ. Microbiol. 63 (11), 4456–4461.
Tauriainen, S., Virta, M., Chang, W., Karp, M., 1999. Anal. Biochem. 272 (2), 191–198.
Tauriainen, S.M., Virta, M.P.J., Karp, M.T., 2000. Water Res. 34 (10), 2661–2666.
Theytaz, J., Braschler, T., Van Lintel, H., Renaud, P., Diesel, E., Merulla, D., Van der Meer, J., 2009. Proc. Chem. 1 (1), 1003–1006.
Trang, P.T.K., Berg, M., Viet, P.H., Mui, N.V., van der Meer, J.R., 2005. Environ. Sci. Technol. 39 (19), 7625–7630.
Truffer, F, Buffi, N., Merulla, D., Beggah, S., van Lintel, H., Renaud, P., Geiser, M., 2014. Rev. Sci. Instrum. 85 (1), 015120.
Wackwitz, A., Harms, H., Chatzinotas, A., Breuer, U., Vogne, C., Der Meer, V., Roelof, J., 2008. Microb. Technol. 1 (2), 149–157.
Wang, B., Barahona, M., Buck, M., 2013. Biosens. Bioelectron. 40 (1), 368–376.
Wang, J., 1999. J. Pharm. Biomed. Anal. 19 (1), 47–53.
Williams, P., Islam, M., Adomako, E., Raab, A., Hossain, S., Zhu, Y., Feldmann, J., Meharg, A., 2006. Environ. Sci. Technol. 40 (16), 4903–4908.
Williams, P.N., Lei, M., Sun, G., Huang, Q., Lu, Y., Deacon, C., Meharg, A.A., Zhu, Y.-G., 2009. Environ. Sci. Technol. 43 (3), 637–642.
Wu, Y., Liu, L., Zhan, S., Wang, F., Zhou, P., 2012a. Analyst 137 (18), 4171–4178.
Wu, Y., Zhan, S., Wang, F., He, L., Zhi, W., Zhou, P., 2012b. Chem. Commun. 48 (37), 4459–4461.
Wu, Y., Zhan, S., Xing, H., He, L., Xu, L., Zhou, P., 2012c. Nanoscale 4 (21), 6841–6849.
Wu, Y., Wang, F., Zhan, S., Liu, L., Luo, Y., Zhou, P., 2013. RSC Adv. 3, 25614–25619.
Xing, J.Z., Zhu, L., Jackson, J.A., Gabos, S., Sun, X.J., Wang, X.B., Xu, X., 2005. Chem. Res. Toxicol. 18 (2), 154–161.
Yoshida, K., Inoue, K., Takahashi, Y., Ueda, S., Isoda, K., Yagi, K., Maeda, I., 2008. Appl. Environ. Microbiol. 74 (21), 6730–6738.
Zhai, J., Yong, D., Li, J., Dong, S., 2013. Analyst 138 (2), 702–707.
Zhu, Y.G., Williams, P.N., Meharg, A.A., 2008. Environ. Pollut. 154 (2), 169–171.