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Whole Cell Arsenic Biosensor- A Cheap Technology for Bioavailable Arsenic (As) Determination

Rahul Soangra[1], CB Majumder[1] and Partha Roy[2]

[1]*Department of Chemical Engineering, Indian Institute of Technology, Roorkee, India*
[2]*Department of Biotechnology, Indian Institute of Technology, Roorkee, India*
srahul@vt.edu

ABSTRACT

The threat of arsenic pollution to public health and wild life has led to an increased interest in developing systems that can monitor bioavailable arsenic in potable water. This paper reviews the principal milestones in the development of arsenic biosensor combining a selective biological recognition element (microorganism) and a sensitive transducer (microilluminator), are environmental tools applied for environmental bioavailable arsenic pollution monitoring. We have discussed here the mechanism of Arsenic intake and expression of reporter gene in the bioreporter. The bioluminescent bioreporter integrated circuit (BBIC) can be developed in which a bioreporter is engineered to luminescence when target substrate (arsenite) is encountered, while the circuit detects the luminescence and after processing the signal, the results are communicated by remote sensing. BBIC provides a low power, inexpensive, selective and highly sensitive Arsenic biosensors.

Key words: Bioreporter, Reporter genes, arsenic pollution, bioreporter integrated circuit (BBIC)

INTRODUCTION

Life arose in an alien and hostile environment of this earth. Before the atmosphere became oxidizing, the concentration of dissolved metal ions in primordial oceans were undoubtedly much higher than today. One of the earliest challenges of the first cells would have been the ability to detoxify heavy metals, transition metals and metalloids, including As (III). The presence of arsenic resistance (ars) genes in the genome of every living organism sequenced to date illustrates that ars genes must be ancient [1]. The minimum set of genes needed for arsenic resistance is arsRBC [3]. The ubiquity of environmental arsenic provides the selective pressure that maintains resistance genes in present day organisms from E.coli to humans [1].

Arsenic is the 20th most abundant element in the earth's crust, and found ubiquitously in nature. About 155,000 metric tonnes of arsenic are released into the environment each year [2]. Arsenic enters the biosphere primarily by leaching from the geological formations. Anthropomorphic sources include mining and arsenical containing fungicides, pesticides and herbicides [1]. Current estimates are that 35-50 million people in the West Bengal and Bangladesh area, over 10 million in Vietnam, and over 2 million in China are exposed to unacceptable arsenic intake through potable water consumption [8]. The safety limit for arsenic in drinking water for most European countries and United states, 10 µg/L; elsewhere, 50 µg/L [8].

Systemic and chronic exposure to arsenic is known to lead to serious disorders, such as vascular diseases (Blackfoot disease and hypertension) and irritations of the skin and mucous membranes as well as dermatitis, keratosis, and melanosis. Inorganic arsenic is a human carcinogen, and ingestion of inorganic arsenic increases the risk of developing cancer of the bladder, liver, kidney, and skin. The clinical manifestations of arsenic intoxication are referred to as arsenicosis. Currently, the largest case of arsenic poisoning takes place in Bangladesh. It is estimated that out of 4 million tubewells, 1.12 million are affected by arsenic contamination and that between 20 and 30 million people (15-25% of the population of the Bangladesh) are exposed to arsenic levels above 50µg/L.

Arsenic is a toxic metalloid which belongs to group XV of the periodic system together with nitrogen, phosphorous, antimony and bismuth and it has two biologically important states, As(V) and As(III), as the oxyacids arsenic acid (H_3AsO_4) or arsenous acid, also called arsenic trioxide (As_2O_3)[1,3]. In solution at neutral pH, arsenic acid exists as the arsenate oxyanion. The pKa of arsenous acid is 9.2, so that, at neutral pH, it would be

primarily present in solution as neutral As(OH)3. As(III) as a soft metal ion, forms strong bonds with functional groups such as thiolates of cysteine residues and the imidazolium nitrogens of histidine residues[1]. Arsenite is uncharged at neutral pH and appears to gain access to the cytoplasm by less specific mechanisms, possibly including diffusion across the membrane. It crosslinks sulfhydryl (thiol) groups of enzymes, forming stable adducts that permanently disable the enzyme. This mechanism is even more destructive to the cell than that of arsenate [6]. Arsenite has a high affinity for thiol groups and affects respiration by binding to the vicinal thiols in pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. It also affects the function of the glucocorticoid receptor [7].

Arsenate is a chemical analog of phosphate and can uncouple mitochondrial oxidative phosphorylation. Arsenate enters the microbial cells readily through phosphate uptake proteins. Its primary mode of toxicity is then to displace phosphate in the production of adenosine triphosphate(ATP), the primary energy currency of the cell. The resulting molecules hydrolyze spontaneously, causing the cell to deplete its energy sources rapidly [6]. Organisms take up As(V) via phosphate transporters and As(III) by aquaglyceroporins. As(V) is reduced to As(III) which is either extruded from the cells or sequestered in intracellular compartments, either as free arsenite or as conjugates with GSH or other thiols[1]. Arsenic is an essential toxin; it is required in trace amounts for growth and metabolism but is toxic at elevated concentrations. Arsenic is used as an osmolite in some marine organisms and its use for energy is widespread in prokaryotes with representative organisms from Crenarchaeota, thermophilic bacteria, low and high G + C gram-positive bacteria, and Proteobacteria[6].

In higher eukaryotes, glutathione reduces arsenate to arsenite, which then accepts a methyl group from S-adenosyl-methionine producing MMA or DMA with the help of methyltransferase. The methylated forms of arsenic, such as monomethylarsonic (MMA) and dimethylarsonic acid (DMA) are less toxic and are main route of detoxification in some mammals [7].

TOXICITY OF ARSENIC COMPOUNDS

Arsenic occurs in four oxidation states: As+5, As+3, As0 and As-3.

Arsenate
This oxyanions is analog of phosphate, and as such it is potent inhibitor of oxidative phosphorylation, the key reaction of energy metabolism in metazoans, including humans.

Arsenite
It is the most toxic of arsenic oxyanions. It readily binds to reactive sulphur atoms (SH groups) of many enzymes, including those involved in respiration.

Arsenic Trioxide (As_2O_3)
It is the most common form of arsenic used for a variety of agricultural, manufacturing and medical purposes. It is highly toxic.

Methylated Forms of Arsenate and Arsenite
Compounds such as methylarsonic acid (MMAV), monomethylarsonous acid (MMAIII) and dimethylarsenic acid (DMAV) are produced by algae and as excretory products of animals.

Arsines
Arsines in the -3 oxidation state, occurring as highly toxic gases, such as H_3As and $(CH_3)_3As$.

Organoarsenic Compounds
Arsenobetaine is molecular analog of osmotic regulating compound, betaine, where arsenic substitutes for the original nitrogen atom. They occur in marine animals but are not toxic to animals that eat these organisms, including humans.

Synthetic Organoarsenic Compounds
Substances, such as roxarsone (4-hydroxy-3-nitrophenylarsonic acid) are used as feed of poultry. They do not accumulate in these organisms, and are ultimately excreted. And their subsequent breakdown by bacteria in soils will release As(V) into the environment.

Arsenate anion is the prevalent chemical species under oxic conditions and tends to be strongly adsorbed onto several common inorganic mineral surfaces, especially amorphous iron minerals like ferrihydrite, and aluminium oxides in clays. In oxic sub-surface systems with a high Eh (oxidation-reduction potential) and an abundance of adsorptive minerals, As(V) tends to be immobilized by remaining sorbed onto the solid phase. Also, As(III) is the prevalent chemical species under anoxic conditions (lowEh), and because it sorbs to fewer such minerals, it partitions into aqueous phase and is thus more mobile and off course more toxic than As(V)[4].

The Ecology of Arsenic
There are two kinds of prokaryotes which reduce As(V) to As(III). These are Dissimilatory arsenate-respiring prokaryotes (DARPs) and Arsenate –resistant microbes (ARMs). DARPs make use of As(V) as an electron

acceptor in anaerobic respiration. These prokaryotes oxidize a variety of organic (hydrogen and sulphide) electron donors resulting in production of As(III) using Arr as reductase. Whereas ARMs do not gain energy from the process, but use it as a means of coping with high arsenic in the environment. Similarly, there are two kinds of organisms which oxidize As(III) to As(V). These are Chemolithoautotrophic arsenite oxidizers (CAOs) and Heterotrophic arsenite oxidizers (HAOs). Although the arsenite oxidases of CAOs and HAOs have notable similarities, the arsenate reductases of DARPs and ARMs are very different [4]. These prokaryotes use arsenic oxyanions for energy generation either by oxidizing arsenite or by respiring arsenate. The predominant form of inorganic arsenic in aqueous, aerobic environments is arsenate whereas arsenite is more prevalent in anoxic environments. Arsenate is strongly adsorbed to ferrihydrite and alumina, a property that constrains its hydrologic mobility. Whereas arsenite adsorbs less strongly, which makes it the more mobile oxyanions[5].

Dissimilatory Arsenate-Reducing Prokaryotes

It has been discovered that As(V) serves as a nutrient to certain anaerobes by functioning as their respiratory oxidant. The reaction is energetically favorable when coupled with the oxidation of organic matter because As(V)/As(III) oxidation/reduction potential is +135mV. Two of the ε-Proteobacteria, Sulfurospirillum arsenophilum and *Sulfurospirillum barnesii*, conserve energy by linking the oxidation of lactate to the reduction of As(V) to As(III) [Gibbs free energy (ΔG^o) = -295kJ/mol lactate]. DARPs use a variety of electron donors including hydrogen, acetate, formate, pyruvate, butyrate, citrate, succinate, fumarate, malate, and glucose.

A haloalkaliphile *Bacillus selenitireducens* grows well at 10 mM As(V), possibly because the product As(III) is charged at high pH and cannot enter the cell, Sulfurospirillum species grow best at 5mM. To date, no obligate DARPs have been found, because all the strains examined can use other electron acceptors for growth. For example, *Desulfotomaculum auripigmentum* and *Desulfomicrobium* strain Ben-RB also respire sulphate. *S. Barnesii* also respires selenate, nitrate, fumarate, Fe(III), thiosulphate, elemental sulphur, dimethylsulphoxide, and trimethylamine oxide[5].

Arsenite-Oxidizing Prokaryotes

Arsenite-Oxidizing Prokaryotes include both heterotrophic arsenite oxidizers (HAOs) and Chemolithoautotrophic arsenite oxidizers (CAOs). Heterotrophic oxidation of As(III) is viewed primarily as a detoxification reaction that converts As(III) encountered on the cell's outer membrane into the less toxic form, As(V), perhaps making it less likely to enter the cell. CAOs couple the oxidation of arsenite (e.g., electron donor) to the reduction of either oxygen or nitrate and use the energy derived to fix CO2 into organic cellular material and achieve growth. In HAOs the oxidation of As(III) is catalyzed by a periplasmic enzyme that is distinct from the dissimilatory arsenate reductase[5].

Environment Depletion

One of the theory of subsurface mobilization of arsenic includes (i) the oxidation of As-containing pyrites, (ii) the release of As(V) from reduction of iron oxides by autochthonous organic matter(e.g., peat), (iii) the reduction of iron oxides by allochthonous organic matter (from dissolved organics in recharging waters), (iv) the exchange of adsorbed As(V) with fertilizer phosphates. Microorganisms play a crucial role in the direct reduction and oxidation of arsenic species, as well as the iron minerals contained in these aquifers [5]. Fig. 1 shows Arsenic Cycling.

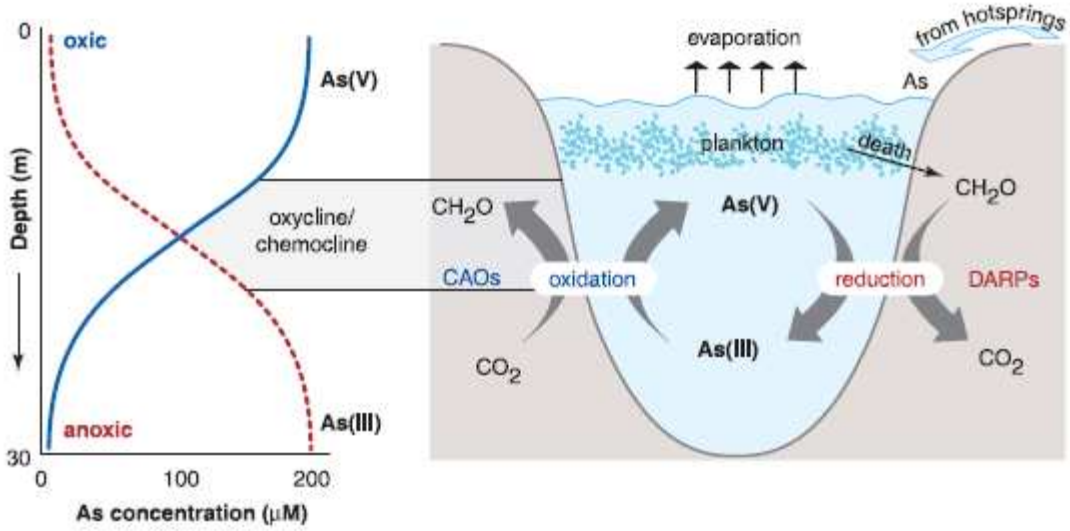


Fig. 1 Arsenic cycling occurs in the region of the chemocline. Arsenate reduction is mediated by DARPs that use released organic matter from dying plankton to fuel their respiration. Arsenite oxidation (aerobic and anaerobic) is mediated by CAOs that also contribute to secondary production by "fixing" CO_2 into organic matter [5]

In Bangladesh, perhaps the initial process is the oxidation of the original As(III)- containing minerals (e.g., arsenopyrite) during transport and sedimentation by the pioneering CAOs and HAOs. This results in accumulation of As(V) onto surfaces of oxidized minerals like ferrihydrite. Human activities such as intensive irrigated agriculture, digging of wells, and lowering of ground water tables would provide oxidants (e.g., oxygen, nitrate) that would further stimulate As(III) oxidation. There is buildup of microbial biomass (and its associated organic matter) and the creation of anoxic conditions. This organic matter, in conjunction with other sources either from decomposing buried peat deposits or from that dissolved in seasonal recharge from agricultural surface waters, would in turn promote the dissimilatory reduction of adsorbed As(V) by DARPs and the eventual dissolution of adsorbent minerals like ferrihydrite [4].

Reporter Gene Technology

Reporter genes code for proteins that produce a signal that allows a protein to be determined in a complex mixture of other proteins and enzymes. Reporter genes are now being used as part of a signal transduction event in many biosensing systems and have been used to develop assays for such diverse compounds as metal ions, toxic organic species, viruses and antibodies. A molecular recognition event is usually coupled to a reporter event to provide the required sensitivity and selectivity to detect the analyte. The molecular recognition event recognizes the selectivity of the system and reporter event generates a signal which controls the sensitivity of the bioreporter. Reporter proteins can be monitored by a variety of detection systems such as electrochemical, fluorescence, bioluminescence and chemiluminescence .Table 1 illustrates several reporter proteins, and their reactions to produce biological signal. Many organisms having reporter proteins with different emission wavelength have been found e.g., single-celled algae, sea walnuts, jellyfish, fireflies, worms and even some mushrooms are bioluminescent because certain photoproteins or enzymes are present[9].

Several types of reporter genes are available for use in the construction of bioreporter organisms, and the signals they generate can usually be categorized as colorimetric, fluorescent, luminescent, chemiluminescent or electrochemical. Although each functions differently, their end product always remains the same – a measurable signal that is proportional to the concentration of the arsenite to which they have been exposed. In some instances, the signal only occurs when a secondary substrate is added to the bioassay (luxAB, Luc, and aequorin). For other bioreporters, the signal must be activated by an external light source (GFP and UMT), and for a select few bioreporters, the signal is completely self-induced, with no exogenous substrate or external activation being required (luxCDABE). The following sections outline in brief some of the reporter gene systems available and their existing applications.

Bacterial Luciferase (Lux)

Bacterial luciferases are flavin dependent monooxygenases that catalyze the oxidation of reduced flavin mononucleotide (FMN) and a long chain aldehyde to FMN and the corresponding long chain carboxylic acid with light emission at 490nm. The genes *lux*A and *lux*B code for the α and β subunits of bacterial luciferase, and genes such as *lux*C, *lux*D, and *lux*E code for enzymes responsible for the synthesis of the long chain aldehyde substrate. Although tetradecanal is the natural substrate synthesized by luciferase-containing bacteria, shorter chain aldehydes (e.g., decanal) elicit a much higher luminescence response than does tetradecanal. It is possible to use the *lux*A and *lux*B genes in reporter systems without the other *lux* genes. The luciferase activity is then determined by adding decanal to the system exogenously [9]. Fig. 2 shows bioluminescence by bioreporters containing *lux* genes.

***lux*AB Bioreporters**

*lux*AB bioreporters contain only the *lux*A and *lux*B genes, which together are responsible for generating the light signal. However, to fully complete the light-emitting reaction, a substrate must be supplied to the cell. Typically, this occurs through the addition of the chemical decanal at some point during the bioassay procedure. Numerous *lux*AB bioreporters have been constructed within bacterial, yeast, insect, nematode, plant, and mammalian cell systems [10].

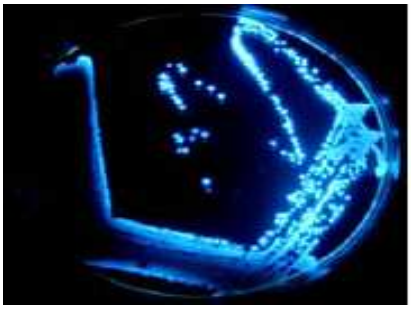


Fig. 2 Bioluminescence emitted from individual colonies of microbial cells containing the genes for bacterial luciferase [10]

ß-galactosidase

Lactose ----------> Galactose + Glucose

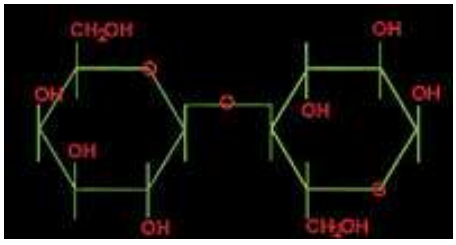


Fig. 3 Structure of lactose Sugar

_lux_CDABE Bioreporters

Instead of containing only the *lux*A and *lux*B genes, bioreporters can contain all five genes of the *lux* cassette, thereby allowing for a completely independent light generating system that requires no extraneous additions of substrate or any excitation by an external light source. So in this bioassay, the bioreporter is simply exposed to a target analytic and a quantitative increase in bioluminescence results, often within less than one hour. Due to their rapidity and ease of use, along with the ability to perform the bioassay repetitively in real-time and on-line, makes *lux*CDABE bioreporters extremely attractive. Consequently, they have been incorporated into a diverse array of detection methodologies ranging from the sensing of environmental contaminants to the real-time monitoring of pathogen infections in living mice [10].

Firefly Luciferase (Luc)

Firefly luciferase was isolated from *Photinus pyralis* and differs from the bacterial luciferases in its structure and light-emitting reaction. Firefly luciferase is encoded by the *luc* gene. In the presence of adenosine triphosphate (ATP) and molecular oxygen, firefly luciferase catalyzes the oxidation of its substrate luciferin to oxyluciferin yielding CO_2 and light (λ_{max} = 560nm, quantum yield is approx. 0.88). The bioluminescence signal is linear over 8 orders of magnitude of luciferase concentration, and the enzyme can be detected at subattomole levels, making this luciferase an attractive choice for quantitative analytical applications [9].

β – Galactosidase (Lac Z)

Beta galactosidase is an enzyme that indicates the breakdown of the sugar lactose. Lactose is a type of sugar found in milk. It is composed of two rings. The rings contain 5 carbon atoms, one at each corner and an oxygen atom. Attached to the carbon atoms area a hydrogen atom and an OH group or another carbon. Fig. 3 shows the structure of lactose. The rings are bound together by an oxygen bridge. This bridge can be broken when the enzyme beta galactosidase binds to lactose and a water molecule reacts with the oxygen atom in the bridge (Hydrolysis reaction). Association of lactose with beta galactosidase facilitates the reaction between water and lactose. This breaks the oxygen bridge and results in the production of two simple sugars (glucose and galactose). Like lactose ONPG (o-nitrophenyl-β-D-galactopyranose) is a molecule composed of two rings held together by an oxygen bridge. Beta galactosidase also forms a complex with ONPG molecules and is able to hydrolyze them. Fig. 4 shows the hydrolysis of ONPG.

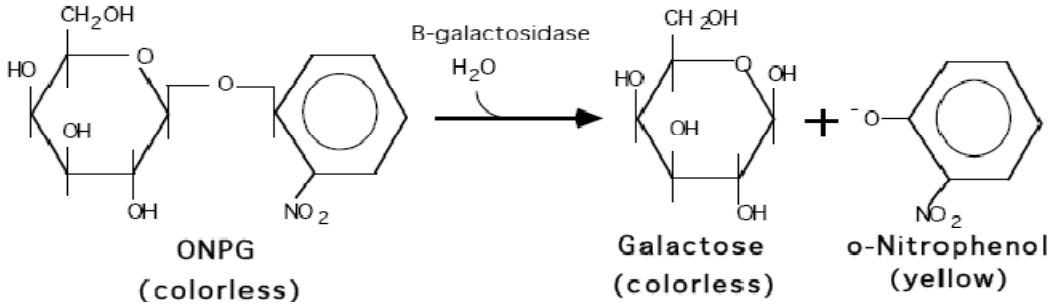


Fig. 4 Hydrolysis of ONPG

The amount of o-nitrophenol formed can be measured by determining the absorbance at 420 nm. If excess ONPG is added, the amount of o-nitrophenol produced is proportional to the amount of ß-galactosidase and the time of the reaction. The reaction is stopped by adding Na2CO3 which shifts the reaction mixture to pH 11. At this pH most of the o-nitrophenol is converted to the yellow colored anionic form and beta galactosidase is inactivated. The reaction can be run using whole cells that have been permeabilized to allow ONPG to enter the cytoplasm. However, since whole cells are present, the absorbance at 420 nm is the sum of the absorbance due to o-nitrophenol and light scattering due to the cells. The contribution of light scattering can be determined by measuring the absorbance at 550 nm where o-nitrophenol doesn't absorb. The light scattering at 420 nm is 1.75x the light scattering at 550 nm, so the absorbance of o-nitrophenol is determined by subtracting 1.75 x OD550 nm. The corrected absorbance is then used to calculate the activity of ß-galactosidase.

Cell- Based Sensing Systems or Bioreporters

Whole cells as the sensing element offers some unique advantages compared with isolated proteins, whole cells are often less susceptile to changes in environmental conditions such as pH and temperature and the presence of other solutes. Cell based sensing systems provide a measure of the bioavailability of a given analyte, because the analyte must be transported inside the cells or bound to cellular receptors to produce a response. The bioavailability of certain analytes, such as toxins and pollutants, is an important consideration in decisions regarding remediation of contaminated environment because it is better measure of toxicity than the total analyte concentration. Cell-based sensing systems can either be specific for a certain analyte or can respond broadly to a group of analytes. Fig. 5 shows anatomy of bioreporter organism.

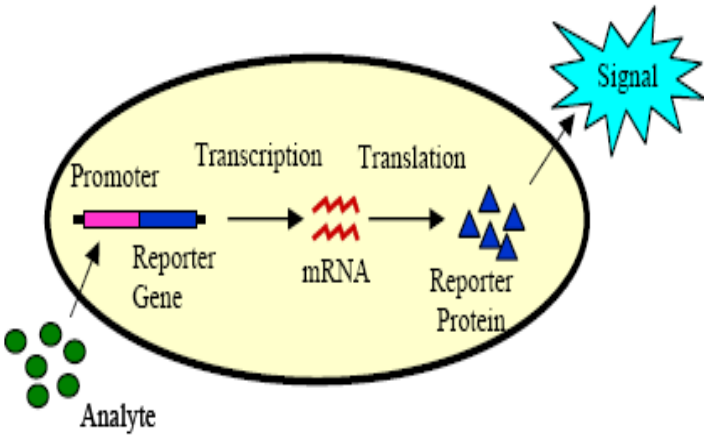


Fig. 5 Anatomy of a bioreporter organism. Upon exposure to a specific analyte, the promoter/reporter gene complex is transcribed into messenger RNA (mRNA) and then translated into a reporter protein that is ultimately responsible for generating a signal [10]

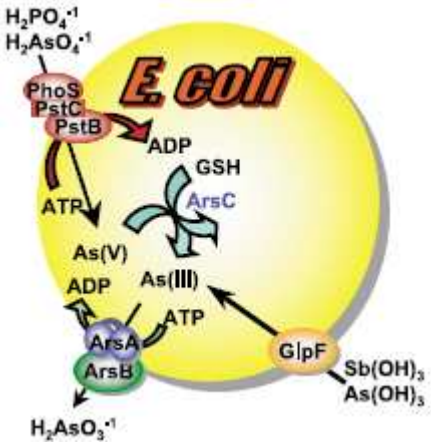


Fig. 6 Arsenate (As(V)) is taken up by phosphate transporters, and As(III) is taken up by aquaglyceroporins (GlpF in *E. coli*, and arsenate is reduced to arsenite by the bacterial ArsC enzyme. Glutathione and glutaredoxin serve as the source of reducing potential. In *E. coli*, arsenite is extruded from the cells by ArsB alone or by the ArsAB ATPase [1]

One type of sensing system of broad specificity uses a reporter gene as a marker for bacterial cell metabolism. This reporter gene codes for a protein, typically a luciferase,that gives rise to a signal under normal metabolic conditions. When the sensor is exposed to an environment containing toxic ions, the cell metabolism is inhibited, decreasing the reporter enzyme activity. This gives a qualitative estimate of ecotoxicity. The light measured in the cells is related to the toxicity of the environment. In these whole cell sensors, any compound that affects either the cellular metabolism or causes stress in the cell induces a response. This system can be applied to a wide variety of potentially hostile environments to provide initial warning of adverse effects and trigger subsequent analysis and remediation actions [13].

The resistance mechanism of microorganisms to metals and organic compounds is the result of several enzymes and proteins acting in concert to neutralize the toxicity of these compounds. The production of proteins and enzymes that confer this resistance is regulated by a control system made up of regulatory proteins and specific promoter regions of chromosomal or plasmid DNA. The proteins and /or enzymes are expressed only when the specific compound is present. By inserting a gene for a reporter protein into the plasmid, a signal is produced in response to a specific compound. Certain strains of bacteria can survive in environments contaminated with arsenite, arsenate, and antimonite because they possess the necessary genetic configuration to make them resistant to these species. Resistance is conferred by the ars operon, which consists of five genes that code for three structural proteins, ArsA, ArsB, ArsC, and two regulatory proteins, ArsR and ArsD. The proteins ArsA, ArsB, and ArsC form a pump system that extrudes antimonite, arsenite and arsenate from the cell once the anions reach the cytoplasm of the bacterium. Arsenite and antimonite trigger the transcription and translation of the genes that constitute the pump proteins. Although all organisms have their own defense mechanism against arsenic but here we are interested in exploiting arsenic resistance mechanism in *Escherichia coli*.

Arsenic Resistance Mechanism in *Escherichia coli*

Arsenic uptake in *E.coli*: GlpF is an aquaglyceroporin, a member of the aquaporin superfamily. Aquaglyceroporins are multifunctional channels that transport neutral organic solutes such as glycerol and urea. Chemical properties of Sb(III) and As(III) are very close and GlpF plays its role in their transport into the cell. Uptake of As(V) is via phosphate transport system. Under abundant phosphate conditions, the high V_{max} but less specific Pit system fulfills the phosphate need of the cell and leads also to arsenate accumulation. Under conditions of phosphate starvation, the more specific Pst-system is induced. Pst discriminates between phosphate and arsenate 100-fold better than Pit. Thus one way for the cell to adapt to arsenate stress is to inactivate the Pit system by mutation, which leads to moderate arsenate tolerance due to the discrimination between arsenate and phosphate by the Pst system [11]. Arsenate is reduced to arsenite by the bacterial ArsC enzyme and glutathione and glutaredoxin serves as the source of reducing potential. The bacterial detoxification of arsenic is often based on inducible ion efflux systems that reduce the intracellular concentration of arsenic by active export. Since anion export from the bacterial cells is driven by the chemiosmotic gradient, simple arsenic As(III) efflux systems are composed of just on efflux protein. As(V) cannot, however be transported with this system [23]. The solution to the problem of As(V) efflux is the enzyme arsenate reductase (ArsC in case of E.coli), which catalyzes the reduction of As(V) to As(III), the substrate of the efflux system. Thus this enzyme extends the spectrum of resistance to include both As(III) and As(V)[12]. Fig. 6 and Fig. 7 shows arsenic uptake and extrusion mechanism of *E.coli*.

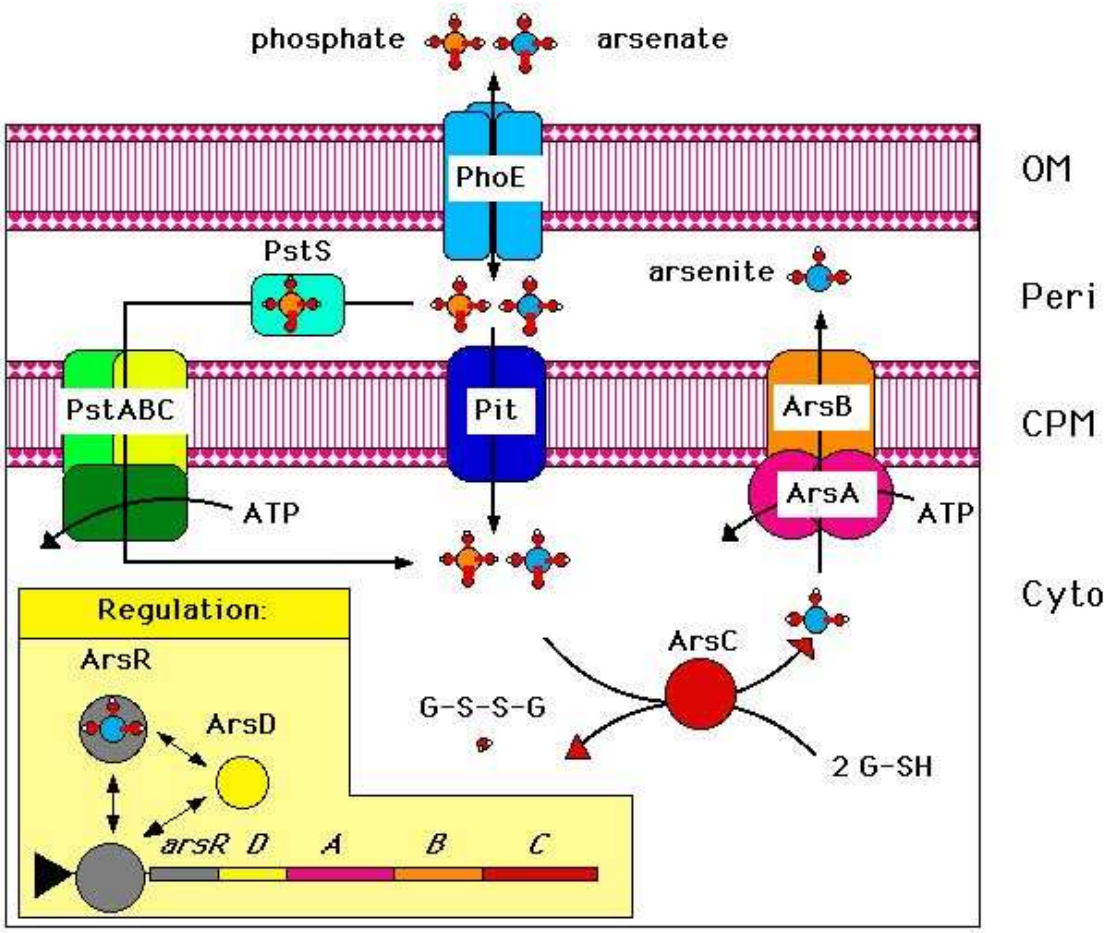


Fig. 7 Transport and resistance to arsenate in E. Coli, Arsenate and phosphate enter the periplasmic space through the outer membrane porin, the PhoE protein. Both anions are transported into the cytoplasm by the Pit protein or the Pst system (which is more specific for phosphate). Within the cell, arsenate is reduced to arsenite by the ArsC protein (dependent on glutaredoxin and glutathione) and arsenite is pumped out of the cell by the ArsAB efflux ATPase. The arsRDABC operon is regulated by the ArsR repressor protein and the ArsD co-regulator protein. OM, outer membrane; Peri, periplasmic space; CPM, cytoplasmic membrane; Cyto, cytoplasmic space; G-SH, reduced glutathione; G-S-S-G, oxidized glutathione. Phosphate, arsenate and arsenite are shown as tetragonal or trigonal oxyanions [11]

Exploitation of Bacterial Defense Mechanism against Arsenic

Bioluminescent Bioreporters are bacteria that can be genetically engineered to achieve bioluminescence in response to specific contaminants (arsenic here). They contain two essential genetic elements: a promoter gene and a reporter gene. With reporter gene fusion technology a promoterless reporter gene that lacks regulatory signals of its own, is coupled to the regulatory signals of ars promoter. The promoter gene (ars promoter) is turned on (transcribed) when the contaminant (arsenite), is present in the cell's environment. In a normal cell, the promoter gene is linked to other genes that are likewise transcribed and then translated into proteins that help the cell in either combating or adapting to the contaminant e.g., ars operon containing *arsA*, *arsB*, *arsC*, *arsD*, and *arsR* is present in E.coli to provide resistance to arsenic oxyanions. ArsR is an arsenite sensing protein. We take advantage of the biochemical capacities of ArsR. It has two binding capacities: In the absence of arsenite, it binds to a specific element on the DNA and thereby prevents the arsenic defence genes from becoming transcribed by RNA polymerase. Repression however is not complete and small amounts of ArsR, the arsenate reductase and the arsenite pump are always present. When arsenite enters the cell, ArsR changes its habits; it will immediately bind to the arsenic compound and lose affinity for the DNA binding site with the result that the protein "falls off" the DNA. As a consequence, ArsR no longer represses the defence mechanism so that the arsenic pump, and the arsenate reductase are produced by the cell in larger amounts [9, 10, 13].

In case of a bioreporter, these genes, or portions of the genes, have been removed and replaced with a reporter gene. Consequently, turning on the promoter gene now causes the reporter gene to be turned on. Activation of reporter genes (*lux* gene cassette) leads to production of reporter proteins that ultimately develops sometime of detectable signal (light). Many of the bioluminescence assays utilize the bacterial *lux* system, consisting of five genes *lux*C,D,A,B, and E. *lux*A and B together encode for the enzyme luciferase which is responsible for generating the bioluminescent response. Luciferase converts long-chain aldehydes into fatty acids with a concomitant production of photons, visible as blue-green light at 490nm. The fatty acids are recycled back into the

aldehyde substrate by a multienzyme fatty acid complex consisting of three proteins, a reductase, transferase, and synthetase encoded by the *lux*C, D, and E genes, respectively. *lux* fusions can consist of just the *lux*AB complex or the complete *lux*CDABE cassette. If only the *lux*AB genes are used, the cells must be supplied with an aldehyde substrate, typically decanal, before light production is possible. Utilization of the entire *lux*CDABE cassette, however, allows for a completely self-contained bioluminescent response, negating the requirement for any exogenous substrate additions. Therefore, the presence of a signal indicates that the bioreporter has sensed a particular target agent (Arsenite) in the environment. Table -2. Lists some of the arsenic biosensors developed. Fig. 8 shows exploitation of arsenic resistance mechanism to make arsenite biosensor. Fig. 9 shows some gene fusions to make arsenite biosensors.

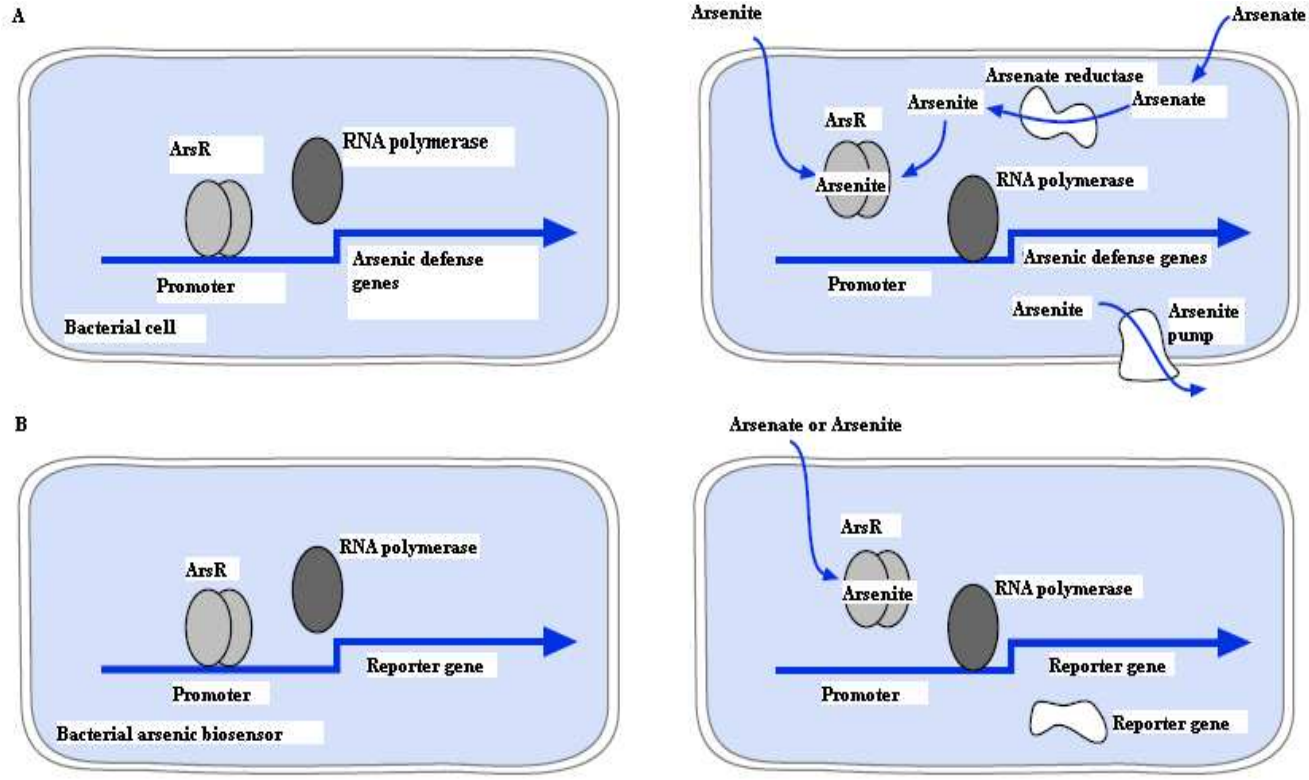
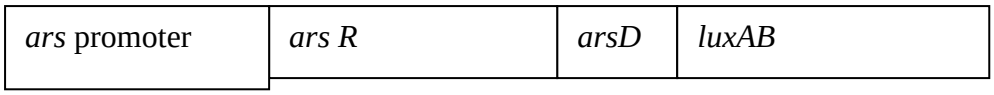


Fig. 8 Principle of the arsenic biosensors. A) The ArsR regulatory protein binds to a specific DNA element and thus inhibits the expression of the genes coding for the arsenic defense mechanism. When arsenite enters the cell, it binds to ArsR. ArsR dissociates from the DNA and RNA polymerase can access. The arsenic defense genes (the ArsR protein itself, the arsenite pump and the arsenite reductase) are expressed in high amounts. B) In the biosensor cells, the ArsR control DNA element is coupled to a reporter gene. Expression of the reporter gene is inhibited by the ArsR, but when arsenite enters the cell, it again dissociates and the reporter gene product is synthesized. These products are either luciferase, green fluorescent protein or β-galactosidase[13]

pRLUX plasmid



pT0031 plasmid

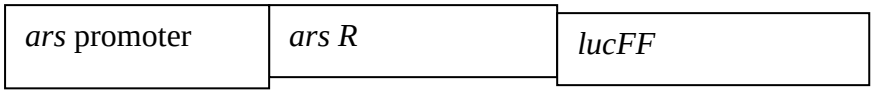


Fig. 9 pRLUX plasmid [15] and pT0031 plasmid [19]

Bioluminescent Bioreporter Integrated Circuit (BBIC)

The bioreporters are placed on a CMOS integrated circuit (IC) that detects bioluminescence and performs signal processing to get the sensor data digitally. The basic building blocks of the integrated circuit are the microluminometer and the transmitter. The microluminometer includes integrated photodetector and signal processor and the transmitter perform wireless data transmission. The concept of System-on-Chip is applicable in BBIC. Bioreporters are joined to microluminometer, which are chips designed to measure the emitted light. The amount of light generated by bioluminescence is proportional to the concentration of the analyte of interest

(arsenic). The light is absorbed by a photodiode array on the device resulting in the generation of photocurrent, which is proportional to the intensity of the light. The signal-processing portion of the microluminometer should filter the luminescent signal from photodetection noise, digitize this signal, and prepare it for transmission. It can easily detect very small optical signal, which is proportional to the concentration of the targeted substance. Incident light on the photodetector produces a current that is collected (integrated) on the feedback capacitor of the integrator. The photocurrent is converted to a digital signal by an analog-to-frequency converter circuit. Remote frequency (RF) transmitters can be incorporated into the overall integrated circuit design for wireless data relay. BBIC mass, size, and power requirements are minimal, and, since they are completely self contained units, operational capabilities are realized by simply exposing the BBIC to the desired test sample. The chip can be as tiny as 2.25sq mm (1.5mm X 1.5mm) [10, 24]. Fig. 10 shows assembly of BBIC.

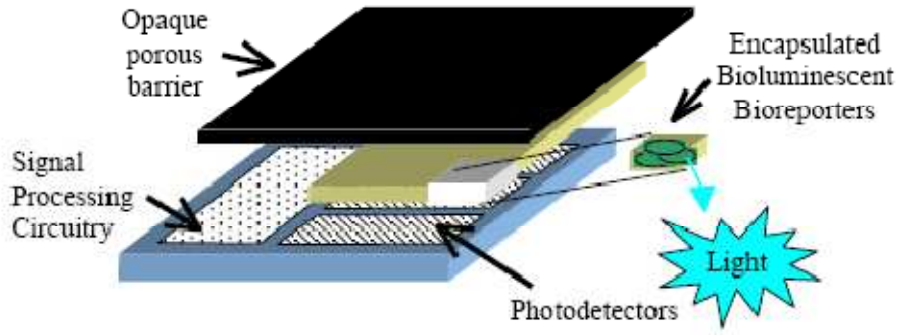


Fig. 10 Assembly of a bioluminescent bioreporter integrated circuit (BBIC) sensor [10]

CONCLUSION

Worldwide, arsenic along with fluoride is now recognized by the WHO as the most serious inorganic contaminant in drinking water. The regional distribution of the high-arsenic waters in Bangladesh is extremely patchy and shows seasonal variation. Because of this high degree of variability, all wells must be tested separately and repeatedly for arsenic. The identification of safe tube wells is an important mitigation strategy. As people are unaware of the water quenching their thirst is hygienic or toxic. In Bangladesh, wells are tested and painted green if waters contain arsenic concentrations below 50 µg/L and painted red if arsenic concentration are above 50 µg/L. In order to test each one of the roughly 9 million private drinking water wells, BBIC looks as an inexpensive, reliable and sensitive field method. BBICs can be crafted to sense many contaminants such as ammonia, arsenic, cadmium, chromate, cobalt, copper, lead, mercury, PCBs, and zinc. It is possible to design BBICs that can survive in extreme or highly contaminated surroundings. In Bangladesh and West Bengal, water containing arsenic has to be pumped and sent to an off-site laboratory for arsenic analysis using Hydride generation AAS/ICP-MS techniques. HG-AAS/ICP-MS analysis is extremely sensitive and accurate, and is by far the best method available for detecting arsenic in environmental sources. However, it also requires expensive and bulky instrumentation, a trained technician, the use of hazardous chemicals, and a significant allotment of time. As an alternative, bioreporters may be used as sensors for the ground water contaminants. Analysis can occur on-site, where bioreporters can be simply combined with ground water samples and be allowed to incubate for a set of time. Resulting bioluminescence can be measured using a field portable photomultiplier unit interfaced to a laptop computer. Duplicate samples can be sent to an off-site lab for HG-AAS and ICP-MS determination of arsenic concentration. bioreporter technology will provide a robust, cost-effective, quantitative method for rapid and selective detection and monitoring of bioavailable Arsenic in Potable water. Environmental monitoring by rapid and remote monitoring using BBIC devices can strategically pinpoint areas of Arsenic hazard.

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