(IJRST) 2016, Vol. No. 6, Issue No. II, Apr-Jun **e-ISSN: 2249-0604; p-ISSN: 2454-180X**

MICROORGANISM– AS BIOSENSOR FOR ARSENIC DETECTION

Dr. Seema Dwivedi
Gautam Buddha University

ABSTRACT

Arsenic is the 20th most abundant element found universally on earth and it is consumed by the human through various means including drinking water, and food, etc. Arsenic also has a permissible limit within which it is safe but above that it is toxic and may harm human health. So it is very necessary to monitor the arsenic concentration in different stuffs of human use. Although there are many traditional techniques available that are being used from a long time to detect and estimate the arsenic level in drinking water and soil but they are costly, less efficient and also have toxic effect to environment since different chemicals are used in them. Hence developing efficient and eco-friendly detection and estimation tools is of great concern of today's situation where arsenic toxicity is increasing day by day. Use of microbes as detection and estimation tool is showing great potential. In this review paper we have tried to discuss different methods of arsenic detection using engineered microorganism. Microbes can be engineered by inserting a number of different bioreporter genes including luxCBDAE, luxAB, and lacZ genes which makes microorganisms fluorescent that gives signal when they exposed to arsenic and that fluorescence signal is amplified and detected by a device called- bioluminescent bioreporter integrated circuits (BBICs). Such bio-based techniques have advantages over conventional methods in terms of more sensitive, more specific and less worthy.

Keywords- Arsenic, bioreporter, bioluminescence, luxCBDA, luxAB, lacZ.

1. INTRODUCTION

A biosensor such as Microbial biosensor is an analytical device that consists of a biological recognition component with a signal transducer to convert the response (28-29)(**Figure 1**). The living cells have a number of enzymes and produce a response to different analytes precisely and selectively hence it is a lees time consuming, cheap process and also have no negative effects to working environment (30-31). The immobilization between bio elements and transducer should be intimate and stable for the transfer of response from the recognition elements to the transducers. For a good microbial biosensor it is basic requirement to integrate the corresponding microbes into the transducer (32). The signal transferred from the microorganisms to the transducer and the reusability of such biosensor determined by the immobilization. So in the development of a biosensor immobilization plays a very crucial role. Different old and conventional methods used for immobilization (such as encapsulation, adsorption, entrapment, covalent binding and cross linking) in the adverse condition suffer from poor, long term or negative effects. To providing higher reliability and stability of the bio-elements nanotechnology

provide an alternative for better immobilization by using nanomaterial such as nanoparticles, nanotubes, and fiber optics (33). A new technique for the microbial biosensor known as microbial fuel cell (MFC) proposed in past decade is based on optical transducer as a main transducer. The MFC provides high sensitivity and selective sensing power with the additional benefit to produce sustainable electricity from biodegradable organic compounds by microbial metabolism (34-35).

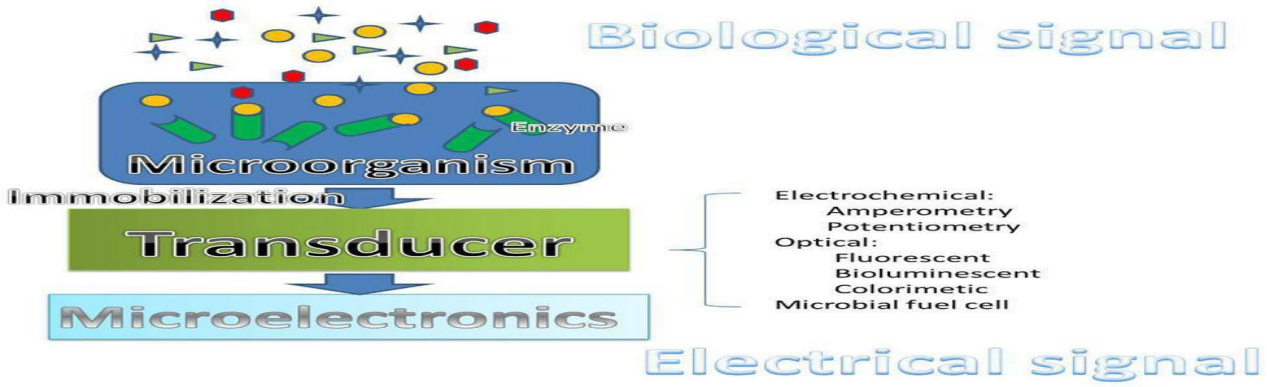

Figure1.Aschematicrepresentationofmicrobialbiosensor

Sources: OpenJournalofAppliedBiosensor Vol.2 No.3(2013), Article ID:35377,11 pages

1.1. Environment Depletion of Arsenic:

Arsenic immobilization on the subsurface includes-1) As containing pyrites oxidation, 2) By the autochthonous organic matter reduction of iron oxides (e.g.- peat), 3) By allochthonous organic matter (from dissolved organics in recharging water) reduction of iron oxides, 4) Adsorbed As(v) exchange with fertilizer phosphates. In the direct reduction and the oxidation of arsenic species microorganisms play an important role cause uninterrupted reduction of arsenic species.

1.2. Mechanism of arsenic resistance in *Escherichia coli*:

1.2.1. Uptake of Arsenic in E. coli:

GlpF- a member of aquaporin superfamily facilitate the transport Sb (III) and As (III)to the cells. As(V) is taken up through the phosphate transport system . High Vmax and lees specific Pit system fulfills the cells phosphates need and also leads to arsenate accumulation (under phosphate rich condition). Differentiation between phosphate and arsenate is done by Pst 100-fold better than Pit. The Pst system is induced by phosphate starvation condition. Inactivation of the Pit system through mutation is the only way for the cell to adept arsenate stress. By the bacterial Ars C enzyme Arsenate is reduced in to arsenite and glutathione using glutaredoxin as the source of reducing potential. Inducible ion efflux system that reduces the concentration of intracellular arsenic is the basic process of the arsenic detoxification in the bacteria. Anion export is driven by the bacteria depending upon chemiosmotic gradient. The transport of the

As(V) can not be done by ion efflux system. The problem of As(V) efflux can be solved by the enzyme Arsenate reductase (ArsC in the case of E.coli) that reduce the As (V) to As(III). So this enzyme spread the range of resistance for both As (III) and As (V).

Figure-3: 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..

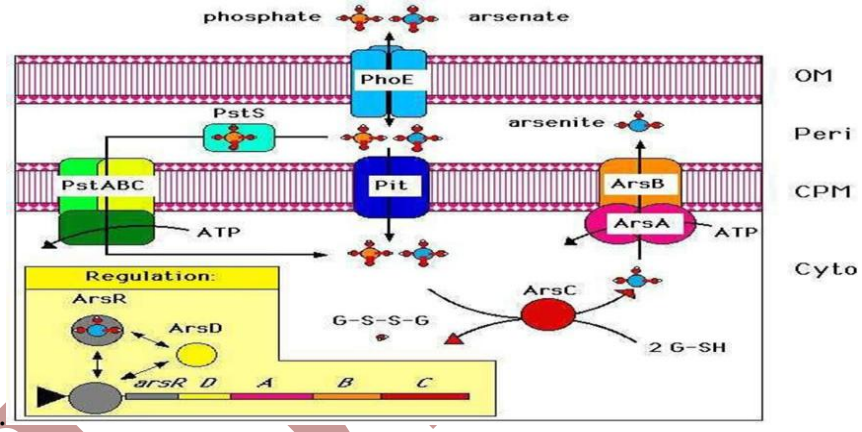

Figure-4: Transport and resistance to arsenate in E.Coli,

1.3. Reporter Gene System of Biosensor:

To construct the bioreporter microorganisms there are several types of genes available to use as reporter, and the signal which generated can be categorized as either colorometric, fluorescent, chemoluminescent, electrochemical or luminescent . In some cases of bio reporter, the signal only arises after the secondary substrate is added to the bioassay such as LuxAB, Luc, and aequorin, some bioreporter uses external light source such as GFT and UMT to activate signal and in few bioreporters the signals are self-induced. In last types of reporters no exogenous substrate or external activation being required such as luxCDABE gene (4).

(IJRST) 2016, Vol. No. 6, Issue No. II, Apr-Jun e-**ISSN: 2249-0604; p-ISSN: 2454-180X**

1.4. **LuxAB Bioreporter:**

The gene which is together responsible for generating the light signal is known as luxAB bioreporter and it contains only luxA and luxB gene. In this bioreporter for light emission a substrate is required to the cells.

1.5. **LuxCDABE Bio-reporter:**

LuxCDABE bioreporters contain all five genes of lux cassette instead of having only the luxA and luxB genes, so permitting for a totally independent light generating system which required neither recruitment of minor addition of substrate nor any excitation by an external source of light. So in this biosensor bioluminescence results approximately within one hour by bioreporter just exposed to a target analyte with quantitatively increase of bioluminescence .LuxCDABE bioreporter system is highly attractive due to their less time consuming and easy to use with the ability to perform the experiment repetitively in real time and on-line.

2. WHOLE-CELL BACTERIAL BIOSENSORS

Toxicity of variety of environmental media including soil, sediment and water can be demonstrated by the use of bacteria as biosensor in which bacteria is connected with transducer which convert a cellular response into detectable signals. These engineered bacterial biosensors which have a reporter gene which generate a signal upon exposer to contaminating analytes (e.g. As). In the cell the sensing component stimulates the reporter gene by a biochemical pathway when biosensor is exposed to chemical or physical changes. Visible light is emitted by the reporter gene in the cell which is the measurable response of the reporter gene and indicator of the degree of physical or chemical changes. A lot of biosensors for the detection of toxicity by physical and chemical changes in the analyte have been developed. But now researchers are trying to develop such biosensors that respond to a particular analyte. Such types of biosensors have been developed for the heavy metals and metalloids including Arsenic, mercury, and lead (6).

(IJRST) 2016, Vol. No. 6, Issue No. II, Apr-Jun **e-ISSN: 2249-0604; p-ISSN: 2454-180X**

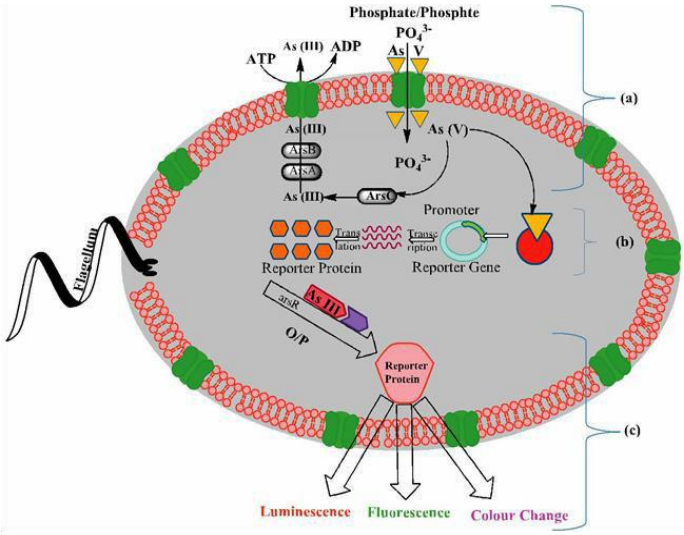

Figure-5: Schematic representation of whole-cell-based biosensor for (a) Arsenic [As(V) and As(III)] transport by phosphate channel (b) Working mechanism of signal transducer for arsenic biosensor, and (c) Detection of As(III) by luminescence, fluorescence and color change(7).

2.1 Luciferase- based Biosensors:

On the basis of luminescent activity of the luciferase gene a several number of recombinant strains have been developed. Luciferase provides a simple and sensitive measurement of the gene expression and regulation. Researchers have reported the initial gene constructs containing luciferase (luxAB) in fusion with arsenic resistance operon and their regulation(9). After that another scientist reported a luminescent recombinant bacterial strain for arsenic analysis (10). The luciferase gene (lucFF) of the firefly Photinus pyralis was expressed under the regulatory control of the ars-operon of the *S. aureus* plasmid P1258 in a shuttle vector plasmid pT002 in three different host strains, namely *S. aureus* RN 4220, *B. subtilis* BR15 and *E. coli* MC1061. Among the above three differen strains *S. aureus* was found to be most sensitive and showed the detection limit of 7.5mg/L ,4.03 mg/L , and 37mg/L for arsenic. At -20^{O}C in 25% (v/v) glycerol. The recombinant strain was stable for 6 months and this shows that this bioreporter is stable and simple arsenic bioreporter.

For the study of the ground water a scientist used a luminescent bacterium E.coli DH5α (pJAMA-arsR) entertaining luciferase gene luxAB from *Vibrio harveyi* and reported the phosphate, silicate, and iron (Fe) interference in the luminescence production (11). For the solution of ion interference addition of EDTA has been proposed that results in separation of arsenic from all other complexes and accurate estimation of arsenic in the tap water. A scientist reported a similar study in which they reported the bacterial luciferase gene (luxAB)

E.coli DH5α (pJAMA-arsR) bioreporter capability for the detection of As in water to a detection limit of 7 mg/L (12). Luminescent signal in a 0.5 – 2.5 hrs. exposure time facilitated by a bacterial bioreporter containing the luxCDABE luciferase gene immobilized on biochip has been reported. For the other contaminants two more strain can also be used in the device. On the addition of the arsenic and other toxic compounds in the water flow biochips demonstrated a corresponding increase in the bioluminescence. Effects of the arsenic toxicity on the growth and nutrient uptake (Lens culinarisL study) is reported by a scientist, for the estimation of the arsenic level by using E.coli strain HB101 pUCD607 containing the lux gene as a bioreporter. For the analysis of As(III) and As(V) in the ground water samples of Bangladesh, a scientist assumed a comparative study of bioreporter based test kit with two commercially available chemical kit (14).

2.2. LacZ-Based Biosensors:

For the arsenic bio sensing the β-galactosidase (lacZ) gene has been reported as a factor that provides a reproducible and quantitative detection by different way. Through the electrochemical polarization of p-aminophenol (PAP) produced from p-aminophenyl β-D- galactopyranoside (PAPG) the transduction of the β-Gal-based signal take place enzymatically on X-gal, producing a coloromtric response. In comparison to luciferase these both techniques provide a quick analysis. An arsR-lacZ fusion based strain containing plasmid R773 regulatory region and E.coli as a host was reported first time in 1997 (15) . To find the reliable electrochemical signal the parameters for cell lysis and induction period were optimized .The lac-Z gene as a natural inhabitant of bacterial cells produced high background activity and to lead misinterpretation, however the system had the constraint sensitivity. A mechanism of construction of spore forming bacterial bireporter *B. subtilis* (ars23) having lacZ gene under the expression control of arsR regulatory protein has been reported (16). Cells having spores is economically beneficial and applicable to harsh environment conditions, make the analysis method simple. This type of system also facilitates the storage preservation and transport of the system for in-situ analysis. A bioenzymatic system containing urease and lacZ with low detection limit capacity of 5 mg/lit was proposed and named BioBrick (J33201) (17). For the control the expression and function of lacZ, arsR and arsD gene is used and to repress the production of urease which is responsible for increase in the pH in the absence of arsenic, dual repressors Lamda and Lac I are used. The pH will be decreased due to lacZ activity in the presence of arsenic. In this system a simple pH electrode /indicator is enough for analysis. The microorganism E. coli DH5α (strain 2245) harboring arsR–lacZ gene construct is most sensitive for arsenic detection and have the limit of 0.02µg/lit, but the incubation period is 22 hours which is very time taking. Microorganism *B. subtilis* (ars23) spores harboring arsR– lacZ fusion have the detection limit of 7.7µg/lit with the incubation for 2.5 hour.

2.3. Green Fluorescent Protein (GFP) – Based Biosensors:

In the GFP-based biosensors genes coding for a photo-protein and green fluorescent protein (GFP) have been used from the jellyfish *Aequorea victoria* (18). The emission of a green fluorescence due to the production of GFP in the jellyfish is measured in order to estimate analyte concentration. GFP system serves as a reporter, but compared to the luc operon it has less sensitivity. GFP system without disrupting the cell metabolism and adding any substrate gives a real time detection(19). GFP also does not depends on interior reducing equivalents being produced by the cells, which means may be that the reporter gene not as equal sensitive to the growth or nutritional position of the microbes used in biosensor (18). The use of GFP in the biosensor attracting researcher due to the ease in the detection and the lesser metabolic cost to the host cells (20). GFP have the ability to alter its stability and spectral properties by the structural alteration and produce mutants to improve the fluorescence intensit, and thermostability of biosensor (20). Since GFP is a very stable, its accumulation in the cells with time results in the background fluorescence which cause in false positive results. To minimize the background fluorescence mutants can be developed with less stable GFP protein (19). Rather than measuring fluorescence activity of protein, measuring number of proteins provides more stable results. Fusion of E.coli plasmid- p1RC1140 containing operator/promoter, arsR gene and some part of arsD from p1RC120 with the GFP of *Aequorea victoria* sourse is made to construct a GFP bioreporter. With GFP-based biosensor a stable fluorescence signal is detected after 12 hour induction period and the detection limit of 1 mg/lit and a nonlinear response between 1 mg/lit to 10 mg/lit has been reported. However the limitation due to background signals from recombinant strains continued a problem. To avoid the background signals expression an arsR promoter-a new second binding site of arsR protein is inserted before the reporter gene (21). Another GFP bioreporter has been developed for monitoring and detection of As (III), As (V) and Sb (III) in ground water samples (22). The 2 hour incubation period is sufficient for this bioreporter, but 8 hour induction decreases the detection limit to7.5mg/lit.

3. USE OF BACTERIAL DEFENSE MECHANISM AGAINST ARSENIC:

To achieve the significant bioluminescence in the response to specific contaminants (arsenic here) a bacteria can be genetically engineered. Microbes used as biosensor have two essential genetic elements- a promoter gene and a reporter gene. A promoter less reporter gene which lack regulatory signals of its own is attached to the regulatory signals of ars promoter using reporter gene fusion technology. When the contaminant (arsenic) is presents in the cell's environments then the promoter gene (ars promoter) is turned on (transcribed). For resistance to E.coli from arsenic oxyanions the ars operon containing arsA , arsB , arsC , arsD and arsR gene is present in the normal cell. The promoter of ars operon is linked to the other genes that transcribed and translated into protein which help the cells in combining/ adapting to the contaminants. Arsenite sensing protein is arsR, and we can take the biochemical capacities of arsR as an advantage. It binds to DNA in sequence

specific manner in the absence of arsenite and prevents the defence gene of arsenic from becoming transcribed by RNA polymerase. Repression is not complete hence less amount of ArsR is always expressing. The ArsR changes its habits when arsenite enters in the cell and binds the arsenic compound and in turn loos its affinity for the DNA so the protein "fall off" the DNA. For the defense mechanism cell produces arsenic pump and arsenate reductase in large amount because of the ArsR no longer represses the defense mechanism (23). These types of genes or the parts of the gene have been removed and replaced with a reporter gene in case of a bioreporter. The production of reporter protein that finally develops into a detectable signal (light) requires only activation of reporter gene (lux gene cassette). Bacterial lux system containing five genes lux-C, -D, -A, -B and -E is utilized in many bioluminescence analysis system. The enzyme luciferase is responsible for generating bioluminescence response encoded by the luxA and luxB together. The long aldehydes are converted into fatty acid by the enzyme luciferase with a simultaneous production of photons which is visible at 490nm as blue-green light. By using a multienzyme-fatty acid complex involving three protein known as reductase, transferase, and synthase encoded by the lux-C, -D, and geneE respectively recycling back of fatty acid into the aldehyde substrate is done . The complex of luxAB or the complex luxCDABE cassette can only be used for making lux fusions. If we use only luxAB genes the cells must be supplied with an aldehyde substrate before the light production. Whenever we use entire luxCDABE cassette, it allows for a completely self-contained bioluminescent response, no requirement of any exogenouse substrate additions. Then the bioreporter senses a particular analyte (arsenite here) in the environment through the presence of a signal (4).

4. BIOREPORTER DEVICE SYSTEM (BBIC)

The bioluminescent bio reporter integrated circuits (BBICs) is consist of two main components- a photo detector for capturing the on chip luminescent signal and a signal processor for managing and storing information derived from bioluminescence. For the wireless data relay remote frequency transmitters are also be incorporated into the overall integrated circuit design. Within a BBIC the bioreporter/bio sensing elements are completely self-contained. A chip designed called microluminometer joined with bioreporters to measure the emitted light. The generation of the photocurrent resulted from the light absorbed by a photodiode array on the device is proportional to the intensity of the light. The filtration of the luminescent signal from photo detection noise, digitize this signal and get ready for transmission is done by the signal processing portion of the microluminometer .Small optical signals can be easily detected by this which is proportional to the concentration of the targeted substance. Photodetector after detecting light produces a current which further collected on the feedback capacitor of the integrator. Through an analog-to-frequency converter the photocurrent is converted into a digital signal.

For the wireless data relay a remote frequency transmitters can be incorporated into overall integrated circuit design. The operational capabilities of the BBIC are realized by simply exposing the BBIC to the desired test sample. BBIC mass, size and power requirements are

minimal. The size of the chip is as tiny as 2.25sq mm(1.55mm X 1.5mm)(8).

5. RESULT INTERPRETATION METHOD

A dose-response curve must be established in order to correlate the induction coefficient with the concentration of analyte (here arsenic). After that a plot of the results from the sample and comparison between the dose- response curves can be made. This comparison reveals the bioavailable concentration of the arsenic in the unknown samples. Running of the experiment with unknown concentration a dose-response curve can be produced. The luminescence measurement or induction coefficient calculation against the known concentration of the arsenic is represented by dose-response curve. For the determination of the unknown concentration the induction coefficient is calculated and compared with the dose-response curve to determine the concentration of the arsenic associated with that induction coefficient (6). Until the threshold level of concentration is reached the response of the biosensor is nonlinear after which the response is linear.When the concentration is so high the response peak rapidly decreases and cell cannot expel the arsenic and begins to die. Usually as compared to the As (V) response the response of As (III) is higher which must be reduced to the As (III) before the activating the system.

6. CONCLUSION

Arsenic detection by different types of biosensor using specific microorganism is an advance method which do not involve any chemical. The purpose of the using microorganisms as biosensor is making cheap and natural device that is useful for arsenic detection in the water as well as soil. In this paper we have discussed the advance methods and mechanisms of the arsenic detection by using microorganism as biosensor. when arsenic is attached to the microorganisms the protein responsible for the fluorescence is activated and device which is attached to the microorganisms measure the amount of fluorescent light and by comparing the emitted light with amount of arsenic we can calculate the total amount of arsenic present in the water or soil sample. In this short review paper we have tried to find out the best method of arsenic detection by using microorganism and we found different microorganisms that have good efficiency for arsenic detection. Luciferase gene based bioreporter (*S.aureus* RN4220 strain) for arsenic detection gives the best detection limit 4.03mg/lit. LacZ gene based bioreporter, *E. coli* DH5α (pPROBE′arsR–ABS–RBS–lacZ strain 2245), has detection limit of 0.8µg/lit in the water. The bioreporter based on green fluorescence protein (GFP) also provide an efficient detection limit for the arsenic, in this method the most frequently used and efficient microorganism is E. coli pIRC140 harboring arsR–gfp fusion which has the detection limit of 1µg/lit. It has a limitation of 12 hour exposure time. The different detection limit observed by the use of different bioreporter gene and different microorganisms in different experiment we can conclude that this technique is a powerful and efficient to detect very less amount of arsenic in

the arsenic contaminated water or soil. This technique also provides a very cheap method of arsenic detection compared to chemical or conventional techniques. For the detection of the arsenic with the use of microorganism, we should develop an efficient device which can detect the amount of the fluorescent light. To solve this problem we use device known as **bioluminescent bioreporter integrated circuit (BBIC) sensor** which efficiently measures the light emitted by microorganisms when exposed to arsenic in the contaminated water or soil.

7. FUTURE PROSPECT

The use of microorganisms as a biosensor provides a very efficient and powerful techniques for the arsenic detection in this time when all countries are suffering from a serious arsenic contamination like Bangladesh which is the largest arsenic contaminated country in the world. Different techniques developed by the researchers with the use of biosensor for arsenic detection provide a powerful method for arsenic detection in water and soil but they need some improvement in the biosensor such as improvement in the detection limit which should measure very less amount of arsenic. The incubation period of the engineered microorganism is also an important limiting factor for the quick estimation of the arsenic contamination. It will also be beneficial to develop such techniques which can provide better results with very low detection limit in the minimum time. Another factor of an accurate arsenic detection in the water or soil is to have an efficient detection device which can be used for the detection of the emitted light by the bioreporter microorganism. It is also a matter of need in the improvement of the arsenic detection device to minimize the detection of background noise which most of the time provide false positive results. Developing such bioreporter based detection device which can be easily operated by everyone to check the arsenic contamination in their daily use drinking water will significantly help in fighting against arsenic hazard especially for rural area population it will be very healthful.

REFERENCES

1- Aaltonen, Emil KJ, and Maria Silow. "Transmembrane topology of the Acr3 family arsenite transporter from Bacillus subtilis." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1778.4 (2008): 963-973.

2- Rosen, Barry P. "Biochemistry of arsenic detoxification." *FEBS letters* 529.1 (2002): 86-92.

3- Alexander, D. C., et al. "Blazing towards the next millennium: Luciferase fusions to identify genes responsive to environmental stress." *Water, air, and soil pollution* 123.1-4 (2000): 81-94.

4- Soangra, Rahul, C. B. Majumder, and Partha Roy. "Whole Cell Arsenic Biosensor-A Cheap Technology for Bioavailable Arsenic (As) Determination."*European Journal of Advances in*

Engineering and Technology 2.5 (2015): 52-61.

5-Chunhui Dai, Seokheun Choi Technology and Applications of Microbial biosensor, , 2013, 2, 83-93

6- Strosnider, Heather. "Whole-cell bacterial biosensors and the detection of bioavailable arsenic." *US Environmental Protection Agency* (2003).

7- Kaur, Hardeep, et al. "Advances in arsenic biosensor development–A comprehensive review." *Biosensors and Bioelectronics* 63 (2015): 533-545.

8- Hug, S., et al. "Arsenic contamination of ground water: disastrous consequences in Bangladesh." *EAWAG News* 49 (2000): 18-20.

9- Corbisier, P., et al. "luxAB gene fusions with the arsenic and cadmium resistance operons of Staphylococcus aureus plasmid pI258." *FEMS microbiology letters* 110.2 (1993): 231-238.

10- Tauriainen, Sisko, et al. "Measurement of firefly luciferase reporter gene activity from cells and lysates using Escherichia coli arsenite and mercury sensors." *Analytical biochemistry* 272.2 (1999): 191-198.

11- Harms, Hauke, et al. "Effect of groundwater composition on arsenic detection by bacterial biosensors." *Microchimica Acta* 151.3-4 (2005): 217-222.

12-. Trang, Pham Thi Kim, et al. "Bacterial bioassay for rapid and accurate analysis of arsenic in highly variable groundwater samples." *Environmental science & technology* 39.19 (2005): 7625-7630.

13- Ahmed, FR Sadeque, et al. "Effect of arsenic contaminated irrigation water on Lens culinaris L. and toxicity assessment using lux marked biosensor."*Journal of Environmental Sciences* 24.6 (2012): 1106-1116.

14- Siegfried, Konrad, et al. "Field testing of arsenic in groundwater samples of Bangladesh using a test kit based on lyophilized bioreporter bacteria."*Environmental science & technology* 46.6 (2012): 3281-3287.

15- Scott, Donna L., et al. "Genetically engineered bacteria: electrochemical sensing systems for antimonite and arsenite." *Analytical chemistry* 69.1 (1997): 16-20.

16- Date, Amol, Patrizia Pasini, and Sylvia Daunert. "Construction of spores for portable bacterial whole-cell biosensing systems." *Analytical Chemistry*79.24 (2007): 9391-9397.

17- Aleksic, J., et al. "Development of a novel biosensor for the detection of arsenic in drinking water." *IET Synthetic biology* 1.1 (2007): 87-90.

18-Roberto, Francisco F., Joni M. Barnes, and Debby F. Bruhn. "Evaluation of a GFP reporter gene cons for environmental arsenic detection." *Talanta*58.1 (2002): 181-188.

19- Tauriainen, Sisko, et al. "Recombinant luminescent bacteria for measuring bioavailable arsenite and antimonite." *Applied and environmental microbiology* 63.11 (1997): 4456-4461.

20-. Date, Amol, Patrizia Pasini, and Sylvia Daunert. "Integration of spore-based genetically engineered whole-cell sensing systems into portable centrifugal microfluidic platforms." *Analytical and bioanalytical chemistry* 398.1 (2010): 349-356.

21-. Stocker, Judith, et al. "Development of a set of simple bacterial biosensors for quantitative and rapid measurements of arsenite and arsenate in potable water." *Environmental science & technology* 37.20 (2003): 4743-4750.

22- Liao, Vivian Hsiu-Chuan, and Kun-Lin Ou. "Development and testing of a green fluorescent protein-based bacterial biosensor for measuring bioavailable arsenic in contaminated groundwater samples." *Environmental toxicology and chemistry* 24.7 (2005): 1624-1631..

23-S Ripp and GS Sayler, Advanced Bioreporter Technologies for Targeted Sensing of Chemical and Biological Agents www.ceb.utk.edu/bioprimer.pdf ,2015.

24-Roberto, F., J. Barnes, and D. Bruhn. 2002. .Evaluation of a GFP Reporter Gene Construct for Environmental ArsenicDetectionTalanta. 58:1, pp. 181-188.

25- Tauriainen, S. M., M. P. J. Virta, and M. T. Karp. "Detecting bioavailable toxic metals and metalloids from natural water samples using luminescent sensor bacteria." *Water Research* 34.10 (2000): 2661-2666.

26-Maryam Daneshpour 1, Nooshin Shabab1, Amir Roointan2 ,Alireza Rahmani3 ,Gholam Abbas Chehardoli4 , Massoud Saidijam1 Designing A Bacterial Biosensor For Arsenic Detection In Water Solutions.

27MisoPark,ShenLongTsaiandWilfredChen,MicrobialBiosensors:EngineeredMicroorganismsasthe Sens ingMachinery

28- Mohanty, Saraju P., and Elias Kougianos. "Biosensors: a tutorial review."*Potentials, IEEE* 25.2 (2006): 35-40.

29-. Chauhan, S., Vibhuti Rai, and H. B. Singh. "Biosensors." *Resonance* 9.12 (2004): 33-44.

30- Lei, Yu, Wilfred Chen, and Ashok Mulchandani. "Microbial biosensors."*Analytica chimica acta* 568.1 (2006): 200-210.

31- Su, Liang, et al. "Microbial biosensors: a review." *Biosensors and Bioelectronics* 26.5 (2011): 1788-1799.

32- D'souza, S. F. "Immobilization and stabilization of biomaterials for biosensor applications." *Applied biochemistry and biotechnology* 96.1-3 (2001): 225-238.

33- Tuncagil, Sevinc, et al. "Gold nanoparticle modified conducting polymer of 4-(2, 5-di (thiophen-2-yl)-1H-pyrrole-1-l) benzenamine for potential use as a biosensing material." *Food chemistry* 127.3 (2011): 1317-1322.

34- Choi, Seokheun, and Junseok Chae. "Optimal biofilm formation and power generation in a micro-sized microbial fuel cell (MFC)." *Sensors and Actuators A: Physical* 195 (2013): 206-212.

35- Du, Zhuwei, Haoran Li, and Tingyue Gu. "A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy." *Biotechnology advances* 25.5 (2007): 464-482.