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### A REVIEW ON BIOREMEDIATION OF ARSENIC FROM CONTAMINATED GROUND WATER

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**Abstract:** Arsenic is most commonly occurring toxic heavy metal in the environment and causes major risk to human health. It is often responsible for the contamination of soil and ground water which is widespread throughout the world. Depending upon the nature of carcinogenicity and toxicity of arsenic to human and animal health, remediation of arsenic-contaminated ground water has become a high priority. There are various physicochemical-based conventional technologies available for removal of arsenic from water. But these technologies are high cost and cause the production of toxic by-products. Therefore, research on new sustainable and cost-effective arsenic removal technologies for water has recently become an area of intense research activity. Bioremediation technology provides great potential for future application due to its environmental compatibility and possible cost-effective in removal of contamination of pollutants from natural environment. It depends on microbial activity to remove, mobilize arsenic from water. And various removal techniques like biosorption, biomethylation-demethylation and oxidation-reduction processes were also used for removing arsenic from water. This review mainly represents an overview on the contamination of arsenic in ground water and bioremediation of arsenic in contaminated ground water by using various biological methods.

**Keywords:** Arsenic; Arsenate; Arsenite; Biosorption; Ground water contamination.

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#### INTRODUCTION

The contamination of heavy metals in water is recognized as severe environmental problem. Toxic metals contaminate water which is harmful for the human consumption. Arsenic (As), is a toxic metalloid widely distributed in aquatic environments and originated from human activities such as industrial production of pesticides and wood preservatives, coal combustion, mining and smelting operations (Wang and Mulligan 2006). The reports have been showed that the elevated levels of As in soils and ground water through worldwide. The regions where the ground water contamination by As mainly occurs in parts of Bangladesh, Argentina, China, India(West Bengal), Chile, Hungary, Taiwan, Mexico, Romania, Vietnam and many parts of USA. The World Health

Organization recommended the concentration of As in drinking water is 10µg/L where as this level is raised to 50µg/L in Bangladesh (Smith *et al.*, 2000). The contamination of drinking water and food become a major problem now-a-days. The high concentration of As in diet which is generated from drinking and irrigation water, from crops (through soil), meat products and vegetables causes so many health problems in human in various countries (Mayorga *et al.*, 2013). It has been reported that the intake of As from drinking water can increase the risk of cancer in the lungs, liver, skin, bladder and kidneys (Wang *et al.*, 2002).

To overcome the poisonous effects of As in ground water contamination there are various methods which have been developed such as physico-chemical methods, conventional

methods for removal of As from contaminated water. Although traditional methods have also been reported which have many limitations like production of toxic by-products, high cost and difficulties in operations (Litter *et al.*, 2010). But there are biological methods which solve these drawbacks because they are easy to operate and do not produce secondary pollution. Microorganisms like bacteria, fungi and yeast are used to clean up these heavy metal contaminated water by a process called bioremediation (Strong and Burgess 2008). Bioremediation is a process in which microorganism uses the contaminants as nutrient and energy sources (Tang *et al.*, 2007). This method is an alternative tool to remove or destroy the poisonous effects of metal pollution through biological activity and this method is also cost effective (Kamaludeen *et al.*, 2003). The precipitation and redox processes governed by microorganisms are the potential applications in bioremediation of metal pollution (Gadd 2004). Thus bioremediation is the best method which involves the use of naturally occurring living organisms and used as best method to overcome all the above limitations in alleviating the arsenic effect on ground water contamination. This review mainly represents an overview of effect of arsenic on ground water contamination and its bioremediation.

### **Sources of Arsenic**

The most abundant element in the earth's crust is As where it has the levels between 1 and 2mg/kg. The sources of As are both natural and anthropogenic. The natural sources for the release of As into groundwater include volcanic eruption, marine sedimentary rocks, weathering of rocks and minerals, hydrothermal ore deposits and biological activities which contribute to the release of As in complex soil-water interface (Mahimairaja *et al.*, 2005; Niazi *et al.*, 2012). Generally As is found in inorganic forms but due to the microbial activity it is occurring in the organic form i.e., in methylated arsenic form. It has been widely used in agricultural herbicides, insecticides, and feed additives for cultivation of crops and production of poultry. As is also combined with various ores of metals like lead, copper, gold and

sulphurous minerals of sulphur and iron (Nriagu *et al.*, 2007). It is detected in more than 200 different minerals; the most important ores are arsenopyrite (FeAsS), realgar (AsS) and orpiment (As<sub>2</sub>S<sub>3</sub>). It has been reported that the natural occurrence of arsenic in ground water in many parts of the world is from mine wastes, weathering and leaching of geological formations. The concentration of arsenic in groundwater may vary significantly which depends on the redox characteristics of the bedrocks and aquifer sediments. The release of As into environment from anthropogenic activities include combustion of fossil fuel, mining operations, wood treatment, electrolyte processes, sewage sludge, municipal and industrial waste, pigments, fertilizers, alloys, electronics, pesticides, cosmetics and As trioxide stockpiles, that are highly soluble in water (Mohan and Pittman 2007; Naidu *et al.*, 2006b). The As-rich fossil fuels will produce arsenic oxide (As<sub>2</sub>O<sub>3</sub>) which is highly toxic and it is the main anthropogenic source of atmospheric contamination (Cullen and Reimer 1989). The steel industry (13%), mining industry (16%), coal ash (22%) and commercial wastes (40%) are the major contributors of As in ground water (Basu *et al.*, 2014; Eisler 2004). Higher levels of As have also been found in the northern hemisphere because of the presence of many industrial sites when compared to the southern hemisphere (Cullen and Reimer 1989).

### **Speciation of Arsenic**

Arsenic is a metalloid found in the environment in four oxidation states. The most forms of arsenic occurring in aquatic environment is arsenite (As(III)) and arsenate (As(V)) species which have shown in Figure 1. The species As(V) is found mainly in the toxic conditions whereas As(III) is predominant in reducing conditions (Tamaki and Frankenberger 1992). The important factors responsible for the identification of the relative proportions of these oxidation states is depend on the biological and local physico-chemical conditions which include the redox potential (Eh) and the hydrogen potential (pH). The pKa values for As(V) are pKa1=2.2, pKa2 =6.9, and pKa3 = 11.5, and those for As(III) are pKa1=9.2 and pKa2 = 12.1.

The solubilisation of arsenic is depends upon its speciation and this solubilisation of As element is due to the reduction of arsenate into arsenite (Smedley and Kinniburgh 2002; Oremland and Stolz 2003). In soil and natural waters arsenic is found in methylated forms such as monomethylarsonous acid [MMA(III)], monomethylarsonic acid [MMA(V)], dimethylarsinous acid [DMA(III)], dimethylarsinic acid [DMA(V)], trimethylarsine [TMA(III)] and trimethylarsenic oxide (TMAO) (Cullen and Reimer 1989) are shown in Figure 2. And in marine animals arsenobetaine which is found as a common organoarsenic species where as in plants like algae arsenosugars are the major arsenic species. Some reports suggested that the methylated organoarsenic species are less mobile than inorganic arsenic species (Xu *et al.*, 1991). Moreover organoarsenic species is considered as less toxic when compared to inorganic species and some methylated arsenic

species like MMA(III) and MMA(V) are found to be more toxic than inorganic arsenic because they are more efficient in causing DNA breakdown (Styblo *et al.*, 2000).

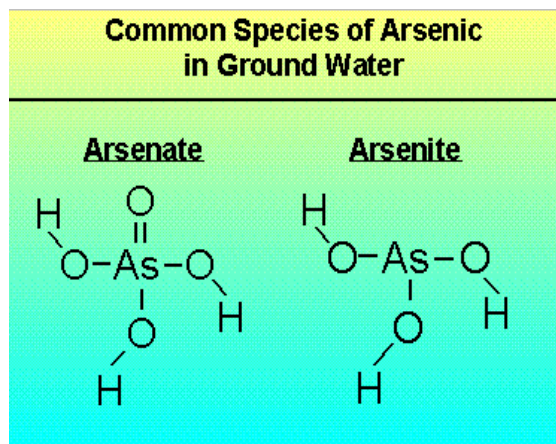


Figure 1. Difference in Molecular configuration of Arsenate and Arsenite

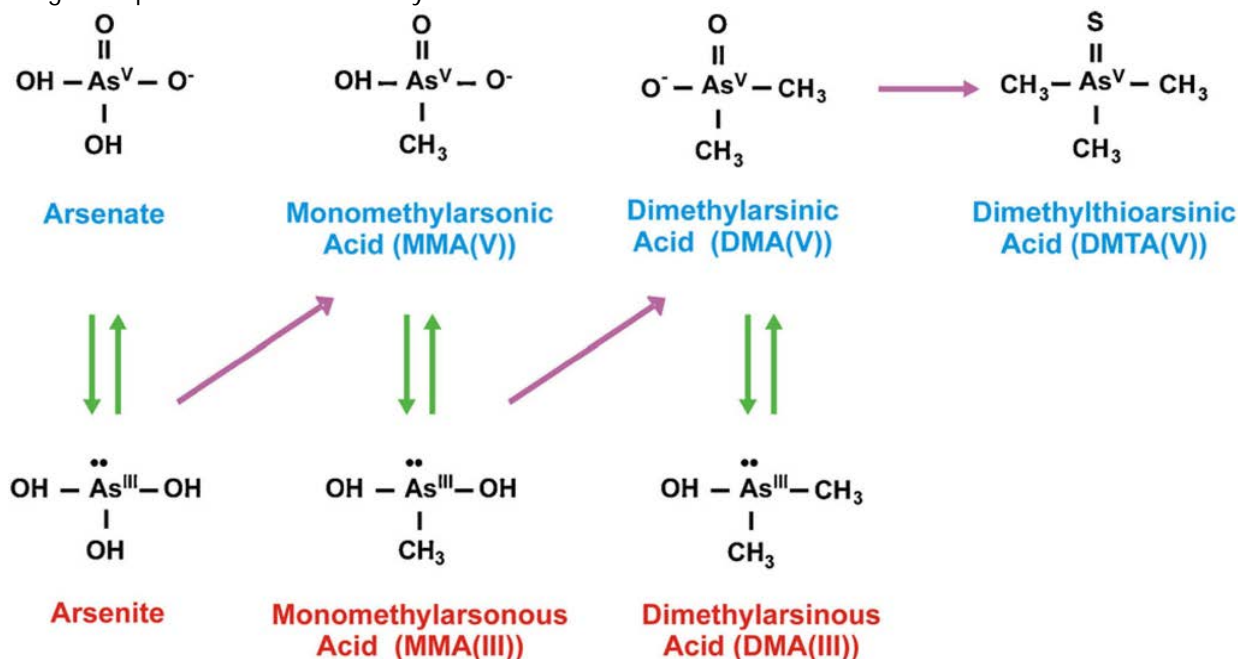


Figure 2. Methylated forms of Arsenic

### Toxicity of Arsenic

Arsenic is highly toxic to both plants and humans in their ionic forms. The arsenic toxicity in plant species such as wheat, rice, or lettuce may show relative differences in their responses (Stolz *et al.*, 2007; Shri *et al.*, 2009). For example, in maize plants it has been found that arsenate is more toxic than arsenite but there is an opposite response in lettuce, wheat and rice plants (Abbas and Meharg 2008). The

arsenic exposure to drinking water may cause various health problems which leads to the development of various types of cancers that affecting lungs, skin and digestive system (Centeno *et al.*, 2002). It has been reported in many areas like Argentina, West Bengal (India) Taiwan and Bangladesh (Mazumder *et al.*, 1992) that the acute toxicity of arsenic may include anemia, gastrointestinal tracts and diarrhea. The chronic arsenic exposure may

cause a number of adverse health effects which include effects on skin, respiratory and gastrointestinal tracts, hematopoietic system, cardiovascular system, nervous system, endocrine system etc. The toxicity of arsenic mainly depends upon its speciation. Arsenite inhibiting more than 200 enzymes in human cells by binding to the sulphhydryl groups of the enzymes and thus induces the functional impairments. Arsenic causes mutagenic, teratogenic and carcinogenic effects by replacing the phosphate in the DNA double helix (Mandal and Suzuki 2002). Various studies have suggested that the arsenic causes intracellular reduction from As (V) into As (III) that can lead to the production of free radicals which is responsible for the chromosomal and cellular damage (Del Razo *et al.*, 2001). The toxicity of various As species is in the following order: As(III) > As(V) > MMA > DMA (Basu *et al.*, 2014). Ground water is the major source of drinking water which has the elevated levels of As and has been associated with various negative health effects in humans (Bhattacharya *et al.*, 2002). Ground water contamination is one of the major pathways of human exposure to inorganic arsenic and ground water contamination is more with arsenic when compared to surface water (Argos *et al.*, 2012). As is mainly used in industrial process for the production of antifungal food preservatives which can lead to the contamination of soil. The source for environmental arsenic contamination is caused by the incineration of preserved wood products, pressure treated with chromate copper arsenate. Another source of chronic arsenic exposure is the consumption of food grown in arsenic-contaminated areas and it has been confirmed that contaminated ground water used to cultivate the agricultural products like vegetables and rice for human consumption which may be an important pathway of arsenic ingestion (Chakraborti *et al.* 2004). It has been implicated that the exposure of As results in a range of diseases, including various types of cancer and cardiovascular disease (Jomova *et al.*, 2011).

### **Arsenic concentration in Aquifers**

The most important sources of arsenic are geothermal activities, chemical conditions and reductive dissolution in certain areas of Northern part (Aiuppa *et al.*, 2003; Angelone *et al.*, 2009). Moderate concentration of arsenic is usually present in surface water i.e., 0.2 to 2 µg/l (Smedley and Kinniburgh 2002). However, the higher levels of arsenic, up to 1000 µg/l are found in some local conditions, mine drainage system and geothermal inputs. Water present in the underground contains the highest concentration of arsenic where it favours the physico-chemical conditions for the solubilisation of the metalloid, especially when arsenic is present in the form of As (III) (Smedley and Kinniburgh 2002).

### **Arsenic Remediation by Conventional Technologies**

There are various technologies that are available for the treatment of As removal from contaminated water. The conventional technologies include the methods like simple coagulation, adsorption, flocculation, ion exchange and membrane processes (Mondal *et al.*, 2013). These technologies also includes *in situ* remediation approaches like combined coagulation/flocculation, use of zero-valent iron, adsorption methods, low cost natural adsorbent materials and photochemical technologies. But these *in situ* remediation processes have some limitations such as high impact of geochemical and microbial processes and interference from oxides, carbonates, sulphides and hydroxides. Moreover, zero-valent iron produces toxic solid wastes (Litter *et al.*, 2010). Even though the conventional technologies are extensively being used, they possess a number of limitations which includes the release of harmful by-products and sludge from redox processes, regeneration of adsorbents in adsorption techniques, requirement of pH in adjustment in coagulation, dissolved solids and other inorganic ions in the ion exchange process (Litter *et al.*, 2010).

### **Bioremediation**

To overcome all the limitations during the application of conventional and traditional methods for the removal of arsenic from

contaminated water, bioremediation which is a best alternative method and has received significant role in recent times because of its cost effectiveness and environmental compatibility. Bioremediation is a process which uses the living organisms mainly microorganisms for the detoxification of toxic pollutants and cause the degradation of organic compounds into simple and harmless compounds like CO<sub>2</sub> and H<sub>2</sub>O. Now-a-days it is also becoming attractive for As-rich drinking water systems, especially representing an economically sounding solution for low income countries (Malik *et al.*, 2009). It converts the highly toxic and mobile As(III) into less toxic and immobilized As(V) form. Bioremediation of heavy metals is achieved through various methods like bioaccumulation, biosorption and biovolatilization.

The arsenic bioremediation mainly depends on microbial activity to detoxify, mobilize or immobilize arsenic through oxidation-reduction, sorption, biomethylation processes (Wang and Zhao 2009).

**i. Oxidation:** For the oxidation of As(III) enzymatically, a wide range of bacteria isolated from various contaminated water which has an ability to synthesize arsenite oxidases. The studies have been reported that this reaction is performed by microorganisms (Santini *et al.*, 2001) that mainly include heterotrophic bacteria and chemoautotrophic bacteria. The heterotrophic bacteria is involved in the detoxification mechanisms which converts As(III) less toxic As(V) form. The autotrophic As(III) oxidisers utilize As(III) as electron donor where as oxygen is used as electron acceptor and carbon dioxide as the carbon source. But an exception that a facultative chemoautotroph under  $\gamma$ -proteobacteria which oxidizes As(III) utilize nitrate as the terminal electron acceptor under anoxic conditions (Hoeft *et al.*, 2007). Chemoautotrophic bacteria produces organic substances which are utilized by the heterotrophic As(III)-oxidizing bacteria for their growth (Battaglia-Brunet *et al.*, 2002). Arsenite oxidation in some chemolithotrophic enzymes is combined with oxygen or nitrate reduction processes and produces energy which can be used to fix CO<sub>2</sub> (Xu *et al.*, 1991;

Battaglia-Brunet *et al.*, 2002). The microbial oxidation of As(III) occurs over a wide range of pH which depends upon the type of the species. Some species like *Sulfolobus acidocaldarius* (Sehlin and Lindström 1992) and *Thiomonas arsenivorans* (Dastidar and Wang 2009) can able to oxidize As(III) at low pH ( $\leq 4$ ). But most of the oxidizing species have demonstrated the optimum oxidation at near neutral range of pH. It has been reported that the optimum pH range for *T. arsenivorans* strain b6 has 6 (Dastidar and Wang 2009). Therefore these differences in optimum pH value suggest that pH modification is necessary for different cultures so with this we can achieve the maximum As(III) oxidation for bioremediation.

**ii. Reduction:** Reduction process can be accomplished by several bacteria which involve two mechanisms; the first one is related to detoxification mechanism. In this mechanism the cells are mediated by arsenate-reductase enzyme which is present in the cytoplasm (Rosen 2002) and has shown in the following Fig 3. This enzyme has been identified as a membrane bound heterodimer protein and consists of 2 subunits ArrA and ArrB (Macy *et al.*, 2000). Dissimilatory anaerobic respiration is another reduction process, which has been described in bacteria belonging to various phylogenetic groups including obligate or facultative anaerobic micro-organisms (Pérez-Espino *et al.*, 2009). These micro-organisms utilize As(V) as terminal electron acceptor (Lièvreumont *et al.*, 2009). The bacterial strain named MIT-13 was reported as the first arsenate-respiring strain and was later named *Geospirillum arsenophilus*. The recent studies reported that the other arsenate reducing bacteria with respiratory arsenate reductase activity are *Desulfuorososinus* species strain Y5 (Pérez-Jiménez *et al.*, 2005), *Shewanella* sp. strain ANA-3 (Malasam *et al.*, 2008), *Clostridium* sp. strain OhISAs (Stolz *et al.*, 2006) and *Alkaliphylus oremlandii* strain OhILAs (Fisher *et al.*, 2008). Several studies have been established that these two mechanisms play significant attention in the solubilisation of arsenic leading to major

contamination of groundwater (Islam *et al.*, 2004; Challenger 1945).

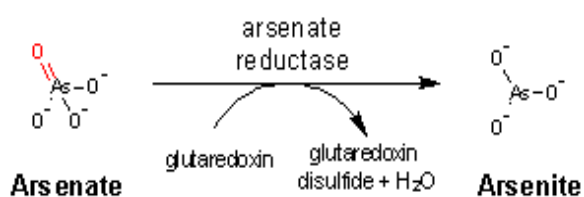


Figure 3. Reduction of Arsenic by Arsenate Reductase

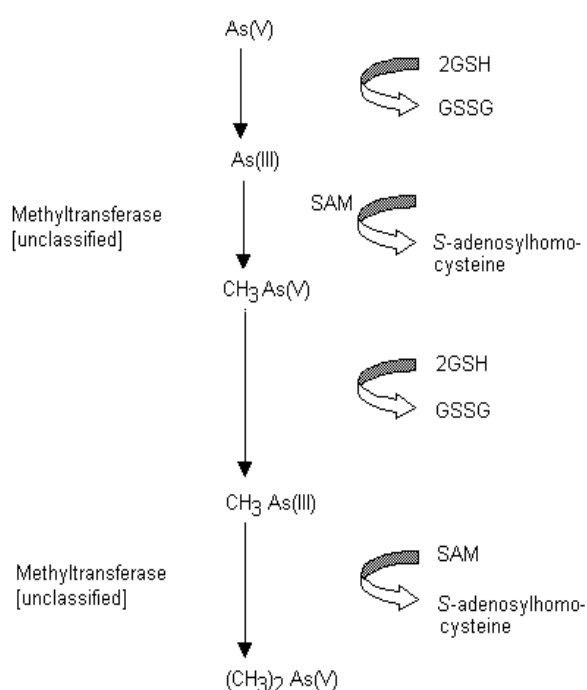


Figure 4. Arsenic Methylation in Mammals

**iii. Methylation:** Arsenic methylation is considered as a detoxifying mechanism in living organisms carried out by bacteria, fungi and other eukaryotes (Stolz *et al.*, 2006). The enzyme used in this methylation process is methyltransferase and S-adenosylmethionine is the source for methyl groups, the thiol is the reducing agent and glutathione is involved in reduction process are shown in following Fig 4. It has been proposed that the mechanism of As in fungus named *Scopulariopsis brevicaulis* which is involved in the repeating sequence of pentavalent arsenic species to trivalent arsenic species followed by the oxidative addition of a methyl group (Challenger 1945). This methylation produces the arsenic methylated products such as monomethylarsenic acid, monomethylarsonous acid, dimethylarsinic acid(DMA(V)), dimethylarsinous acid, trimethylarsenine oxide, and lastly

trimethylarsine (Bentley *et al.*, 2002 ; Thomas *et al.*, 2004). These trivalent methylated intermediates such as MMA(III) and DMA(III) are found to be readily oxidized chemically and biologically (Oremland and Stolz 2003). Bacteria causes the formation of gaseous product from arsenic in methylation (Bentley and Chasteen 2002; Stolz *et al.*, 2006). Methylcobalamine is considered to be the most efficient methyl donor in anaerobic bacteria (Bentley and Chasteen 2002). Anaerobic bacteria such as *Clostridium collagenovorans* (fermentative bacterium) and sulphate reducing bacteria, *Desulfovibrio gigas* and *Desulfovibrio vulgaris* produce small amounts of trimethylarsine (Michalke *et al.*, 2000; Stolz *et al.*, 2006) where as *Methanobacterium formicum* which is a methanogen, found to be very efficient at producing methylated arsines which are given in Table 1. Methylation process plays an important role in the biogeochemical cycle of arsenic because methylated compounds are often volatile but may not necessarily contribute to the detoxification mechanisms.

Table 1. Reported microorganisms in arsenic biomethylation

| Microorganisms                   | Mechanism   |
|----------------------------------|---|
| <i>Methanobacterium formicum</i> | As methylation and demethylation under favourable conditions                    |
| <i>Penicillium species</i>       | Methylarsonic and dimethylarsinic acid methylation to trimethylarsine           |
| <i>Chlorella vulgaris</i>        | Biosorption and accumulation of As and converted into (CH <sub>3</sub> )AsO(OH) |
| <i>Polyohysa peniculus</i>       | As(V) methylation into dimethylarsine   |
| <i>Fusarium oxysporum meloni</i> | As(V) accumulation and methylation into dimethylarsine                          |
| <i>Closterium aciculare</i>      | As(V) methylation into methylarsinic (III) species                              |

**iv. Bioaccumulation:** Bioaccumulation of As is a process which is mainly involved in the biosorption of arsenic by microbial biomass and its byproducts. This process is mediated by the physiological uptake of arsenic by microorganisms through metabolically active and passive processes. The uptake of As(V) by living organisms is via phosphate transporter, whereas As(III) in water is an inorganic equivalent of nonionized glycerol and can be transported across cell membranes by

glyceroporin membrane channel proteins (Rosen 2002). Microbes like bacteria, fungi and algae results in the accumulation of high concentrations of arsenic. The plants even from substrates, having low concentrations also causes the accumulation of arsenic (Kaise *et al.*, 1997; Waring and Maher 2005). The reports showed that marine *Polychaete* species causes the accumulation of arsenic concentrations up to 2739 mg/kg dry weight (Waring and Maher 2005). It has been reported that the hyper accumulation of arsenic in fern *Pteris vittata* was observed up to 22,630 mg arsenic/kg. (Ma *et al.*, 2001). Plants may remove the accumulated arsenic from soils and groundwater by transporting it into the plant tissue.

**v. Biosorption:** Biosorption is a remediation process which is emerged as a cost-effective and eco-friendly alternative method for removal of As and heavy metal-contaminated water (Cheraghi *et al.*, 2014). It is the process of removing heavy metals such as As by using biosorbents is called biosorption. Arsenic removal by biosorption involves the non-living biomass to bind and remove As from water by physico-chemical (e.g., adsorption, chelation and precipitation) reactions. Biosorbents are composed of different functional groups including carboxyl, hydroxyl, phenolic, sulfydryl, alcoholic, phenolic and ester, which have great potential to remove As from water through complexation, ion exchange, co-precipitation, diffusion reactions (Demirbas 2008; Sud *et al.*, 2008). Biosorption to biomass is an attractive technology to remove heavy metals and arsenic from aqueous solutions (Loukidou *et al.*, 2003; Say *et al.*, 2003). It helps in the removal of arsenic from groundwater by resulting in the transfer of soluble arsenic into solid phases and used for the drinking water treatment process. It has been reported that immobilized algae biomass, *Scytonema*, was able to remove As(III) from water effectively (Prasad *et al.*, 2006). It is demonstrated that effectiveness of sorghum biomass is also having high adsorbing capacity of arsenic from aqueous solutions (Haque *et al.*, 2007). To improve the capacity of biosorption process of biomass, physical or chemical pretreatments

can also be used. So it is reported that the pretreatment of *Penicillium chrysogenum* (a waste byproduct from antibiotic production) with surfactants (hexadecyl-tri-methyl ammonium bromide and dodecylamine) and a cationic polyelectrolyte was able to remove more amounts of As(V) from waters (Loukidou *et al.*, 2003).

**vi. Bio-volatilization:** Biovolatilization is a process which is developed as an *ex-situ* method for As removal. The reports showed that the fungal species *Penicillium sp.* were capable of volatilizing 25.8–43.9 mg of arsenic during cultivation period of 5-days (Visoottiviseth and Panviroj 2001). It has been showed that the arsenic volatilization rates significantly increased by the augmenting contaminated soils (1390 mg arsenic/kg soil) with methylating fungi (*Penicillium sp.* and *Ulpladium sp.*) (Edvantoro *et al.*, 2004). The order of ability of arsenic biovolatilization is *Neosartorya fischeri* > *Aspergillus clavatus* > *A. niger*. The studies reported that the use of pure cultures of different fungal strains leads to the production of volatile derivatives of arsenic under laboratory conditions (Čerňanský *et al.*, 2009). The estimated range of average amount of volatilized As for all fungal strains is from 0.026 to 0.257 mg of As(III) where as for As(V) is 0.024 to 0.191 mg.

**viii. Phytoremediation:** Phytoremediation is a plant-based green technology which utilizes the plants for the *in-situ* treatment of the contaminated soil and water (Salt *et al.*, 1995). It is a low-cost *in-situ* technology which produces low amount of waste and causes minimal disturbances. To remediate groundwater, the plants with deep roots can be used (Wang *et al.*, 2006). The application of phytoremediation is based on the As hyperaccumulating capacity of plants. It has been reported that the plant *Pteris vittata L.*, which is the hyperaccumulator of As and can accumulate high concentrations up to 23,000 mg/kg of As in above-ground biomass from the soil (Ma *et al.*, 2001). To modify the plants for arsenic uptake, transport and sequestration, several genetic engineering strategies may be used which increase the arsenic-hyper accumulating capacity of plants. In plants of

*Arabidopsis thaliana*, the engineering tolerance and hyper accumulating capacity of arsenic was enhanced by combining As(V) reductase (ArsC) and g-glutamylcysteine synthetase (ECS) expression (Dhankher *et al.*, 2002).

## CONCLUSION

Arsenic contamination of ground water is a major problem and has an expensive impact on human health which can impair the quality of life. To overcome this, several conventional and physico-chemical technologies have been employed for arsenic removal. Various biological methods using microorganisms can also be used to treat arsenic contaminated groundwater and soil. Bioremediation is such a process involves the use of naturally occurring microorganisms alone or in association with dead biomass/biomass residues, could be the best alternative and cost-effective arsenic removal technology. A number of microbial transformations of arsenic and their mechanisms have been reviewed in this paper. These mechanisms of arsenic mobilization are distinct and it is now widely accepted by researchers that micro-organisms have a major role in these processes. Bioaccumulation, biosorption and biovolatilization are considered as a part of arsenic bioremediation that have been accomplished by microorganisms as detoxification mechanisms. In this review, phytoremediation method have also been discussed which represents an effective *in-situ* technology for the remediation of arsenic contaminated soils and groundwater. Biovolatilization shows a potential for future development to remove arsenic from soils and waters. Currently, bioremediation methods have been mainly investigated in laboratory studies under well defined conditions. Further full-scale demonstrations are required and cost-effectiveness analysis should be conducted to assess the applicability. Despite there being a number of methods to minimize arsenic contamination little is known about the most cost-effective way of dealing with groundwater arsenic contamination. Behavioral research needs to be conducted to ascertain effective methods to mitigate arsenic exposure. Further research should be focused on

biosorption technique because these biosorbents could also offer commercial purpose in the future.

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