

## REVIEW ARTICLE

# Bio-remediation Techniques of Ground water Arsenic Contamination: A Review

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### ABSTRACT

Groundwater is a major source of drinking water world-wide. Contaminants of groundwater may be organic compounds, microorganisms or inorganic pollutants. Major groundwater contaminants known are electron donors. These ions of heavy metal make the drinking water toxic. One of the most ubiquitous, toxic and highly prevalent ion present in groundwater of India and Bangladesh are the Arsenite  $As(III)$  ion and Arsenate  $As(V)$  ion. Studies show that more than 50 million inhabitants of India and Bangladesh have been affected by Arsenic contamination. It's well-known that  $As(III)$  is more toxic and highly soluble in water while  $As(V)$  is relatively less toxic and less soluble in water. Therefore it's a prominent practice in Arsenic decontamination from groundwater to first oxidize  $As(III)$  to  $As(V)$  followed by easy filtration of less soluble  $As(V)$  by ion exchange, coagulation, reverse osmosis or adsorption. This purification process can be carried out both in-situ and ex-situ. Instead of using chemical oxidizing reagents to oxidize  $As(III)$  to  $As(V)$ , it's seen that certain oxidize  $As(III)$  much more efficiently. Microbes like engineered *Escherichia coli* with *ArsR* are known to show enhanced bioaccumulation of  $As(III)$  ions. Even phytoremediation and remediation by fungal biomass is observed to occur naturally where plants like *Pteris vittata* and fungi like *Aspergillus fumigatus* absorb  $As(III)$  ions and in turn decontaminate ground water. The present review opens the insights of the ground water contamination and subsequent bioremediation techniques to decontaminate Arsenic from the ground water, with a detailed comparison on physico-chemical methods, microbial remediation, phytoremediation, fungal remediation and bioremediation using various transgenic organisms of the ground water Arsenic contamination

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### INTRODUCTION

Ground water is the most common form of drinking water used across the world [1]. There are three major types of groundwater pollutants known- organic compounds, inorganic pollutants and certain microorganisms. These pollutants are majorly acquired through natural geochemical causes, pipeline leakages, infiltration of polluted surface water, landfills and many other causes [1]. The purification of these compounds may be carried out both in-situ (commonly using permeable reactive barriers- PRBs) and ex-situ conditions (commonly using pump and treat methods) [2]. In recent times, biological treatment of groundwater to remove metal ions and make the drinking water stable is gaining much attention. Unlike conventional methods of purification, biological treatment requires no reagents; it may be followed as a sole protocol or done along with other physico-chemical protocols. The most common mechanism of approach for biological treatment includes potential charges undergoing redox reactions [1]. Studies show that certain microbes reduce metal ions and hence make it less mobile and less harmful [3], while other microbes oxidize the metal ions and hence make it less soluble in water and easier to filter out [4]. Some common groundwater metal ion contaminants are ions of iron (Fe), manganese(Mn) and most prevalently arsenic(As) [5]. Arsenic contamination is prevalent in countries like USA, China, Chile, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, Japan, India, Vietnam and Bangladesh [6-8]. Maximum contamination of Arsenic is found in Bangladesh followed by West Bengal in India [9-14]. More than 50 million people of West Bengal (India) and Bangladesh alone have been affected by Arsenic intake via drinking water containing more than 0.05 mg per liter of Arsenic, among these more than 3 million people have been severely affected due to Arsenic poisoning [15-19]. Studies show that Arsenic contamination of groundwater of Bangladesh is due to neighboring contaminated bedrock of the

Brahmaputra[20]. Arsenic poisoning due to contaminated drinking problem has hence become a nationwide crisis in India [21], In fact World health organization(WHO) has called Arsenic as “the largest poisoning of the population in the human history” [22]. Arsenic is known to discharge into groundwater by industrial release, percolation of contaminated surface water, due to natural Arsenic rich aquifer sediments and due to irrigation [23-25]. Arsenic is a known human carcinogen, it is found to be present in atmosphere, pedosphere, hydrosphere and biosphere [26]. It is ubiquitous and has either natural or anthropogenic origin. According to WHO, acceptable and tolerable Arsenic concentration that is, the maximum contaminant level (MCL) of Arsenic in drinking water is 10µg per liter [10], but the acceptable concentration of Arsenic in drinking water is 7µg per liter in Australia and New Zealand, 10µg per liter in most of the European countries(according to European commission 2003) [27] and its over 50µg per liter in India and Bangladesh [28].

Various studies have shown that Arsenic exposure on humans especially via food and water causes cancer, gangrene, diabetes, cardiovascular diseases, Bowen’s disease, hyperkeratosis, spotted keratosis, diffused keratosis, spotted melanosis, diffused melanosis, black foot disease, non-pitting oedema, skin carcinoma and many other such diseases [29-33][21,12]. In a cytological scale, Arsenic exposure on humans causes mitochondrial damage, altered DNA methylation, altered DNA repair, cell proliferation, oxidative stress and tumor promotion due to co-carcinogenesis [34]. It is generally found to be deposited in the human body within kidney, lungs and skin along with traces of Arsenic deposited in bones and muscles. However, chronic Arsenic exposure leads to its deposition on hair and nails [35].

### BIOREMEDIATION OF ARSENIC

In groundwater, Arsenic exists in two forms namely, the trivalent Arsenite As(III) and the pentavalent Arsenate As(V). As(III) is the most common form, especially found in anaerobic groundwater. It is more toxic, thermodynamically stable and more soluble in water than As(V), As(III) also interacts lesser with solid surface and hence more difficult to remove [1,18][36-39]. As(III) in reducing condition exists as  $H_3AsO_3$  with a pKa of 9.22 while As(V) in oxidizing condition exists as  $H_2AsO_4$  with a pKa of 2.19 or as  $HAsO_4$  with a pKa of 6.94 [40]. The most prominent factor affecting the relative distribution of As(III) and As(V) ions is the pH and redox potential of the water [41].

Therefore a general conventional protocol for treatment of Arsenic contaminated groundwater is to oxidize As(III) to As(V) using chemical oxidizing agents like  $H_2O_2$ ,  $KMnO_4$ ,  $O_3$ ,  $Cl_2$  and  $MnO$  [42-44]. Oxidation of arsenite to arsenate is a prerequisite for any type of Arsenic treatment [45]. This is followed by physiochemical removal of As(V) by methods like coagulation, filtration, adsorption (usually on iron oxides, activated alumina, calcite, organic matter, clay or hydroxides), reverse osmosis, lime softening, filtration, ion exchange, electro dialysis reversal and other electrochemical treatments, evaporation, nanofiltration, precipitation with iron or sulphur ions, and flocculation[46-57]

### COMMON PHYSICO-CHEMICAL TECHNIQUES OF ARSENIC REMEDIATION FROM GROUND WATER

#### Coagulation –

A common and efficient coagulating agent used in case of Arsenic is ferric chloride or alum. Studies have shown that pH plays a key role in differentiating and selective coagulation of As (III) and As(V) ions and the relative efficiency of ferric chloride and alum in coagulation of Arsenic. Alum coagulates As(V) under a highly restricted pH range unlike ferric chloride. Another important factor determining the coagulation is the source water composition and the relative abundance of As(III) and As(V). For instance, ferric chloride coagulates As(III) less efficiently than As(V), the coagulation of As(III) is also sensitive to source water composition. While Alum cannot coagulate As(III) at all, even the efficiency of coagulation of As(III) by ferric chloride is quite low and drastically decreases when the source water has other organic or sulphate contaminants [58].

Another widely used approach is electro-coagulation of aqueous Arsenic ions using various metal electrodes with iron electrodes showing the most efficiency in coagulation. Also it was noted that with increasing current, the rate of coagulation increases and hence the concentration of aqueous Arsenic decreases. During electro coagulation of As(III), it first gets oxidized to As(V) and then forms complex with metal hydroxides, while As(V) directly gets coagulated. As a result the electro coagulation of As(III) is more efficient than direct coagulation of As(III), whereas electro coagulation and direct coagulation shows no difference in efficiency for As(V) [59].

#### Filtration –

Zerovalent iron has been extensively studied and proven effective in Arsenic ions removal from ground water. When As (III) is allowed to flow through a glass column filled with iron fillings and quartz sand, with sufficient aeration, it was seen that along with oxidation of Fe(II), As(III) gets partially oxidized to

As(V) which in turn gets adsorbed by the newly formed hydrous ferric oxides. These hydrous ferric oxides after subsequent filtrations are removed physically there by reducing the Arsenic content substantially [60]. This process of filtration is affected by the presence of phosphates and silicates in the source water. The presence of these contaminant ions decreases the oxidation of As(III) to As(V) and hence is subsequent adsorption and filtration.

Recent studies have shown that the process of Arsenic ions filtration may be carried out in a house hold level by bringing about the co-precipitation of Arsenic ions with ferric and hypochlorite salts. Followed by manual filtration of the precipitate by passing the source water through a sand filter [61].

#### Reverse osmosis -

Reverse osmosis can remove As(V) ions more efficiently than As(III) ions. The removal of As(III) by reverse osmosis is facilitated by high pH as As(III) is weakly acidic [62]. The reverse osmosis process of Arsenic ions removal mainly depends on temperature, pressure, pH and Arsenic ions concentration. An experimental setup of RO membrane with spiral wound module predicted the optimal conditions as a concentration of 0.2 to 0.3 mg/l, a pressure of 190 to 210 psi and a temperature of 23 to 30 °C within a pH of 6 to 8 respectively [63]. It has been noted that this physiochemical protocol of chemical oxidation followed by separation is inefficient and difficult especially at low Arsenic concentrations, costly, requires highly specific operating conditions like high pressure and temperature which are difficult to achieve and maintain in-situ, low recovery of water and it also leaves behind harmful residues and unwanted toxic byproducts, sludge and brine [1][18][64-70]. In order to make the treatment more efficient, bioremediation techniques are adapted. As(III) is partially oxidized to As(V) and then it's removed by biosorption [54].

#### BIOTRANSFORMATION OF As(III) to As(V)

Microbes show ability to transform As(III) to less toxic and less soluble forms by changing the oxidation number by bio-transformation such as oxidation, reduction, methylation [71]. Microbes like *Microbacterium lacticum* [64], *Pseudomonas spp.*, [72] *Azoarcus spp.*, [73] *Thermus spp.*, [74-75] *Bosea spp.*, [76] *Alcaligenes spp.*, [77] are known to oxidise As(III) to As(V), but they are not isolated from the ground water. Hence for treating As(III) ions, these microbes must either be introduced to the ground water artificially by ecological interference or the contaminated water must be treated ex-situ. Microbes like *Proteobacterium spp.*, [78-79] *Firmicutes spp.*, [80] *Kocuria spp.*, [81] *Stenotrophomonas alcaligenes*, *Herminiimonas spp.*, [82] *Rhodococcus spp.*, [83] *Lactobacillus spp.*, [82] *Achromobacter spp.*, *Aliihoeflea spp.*, [84] *Bacillus spp.*, [85] *Lysinibacillus spp.*, [86] *Pseudomonas spp.*, [87] can oxidize As(III) to As(V) and are isolated from habits such as soil, industrial sewage, discharged water, evaporated ponds or can be purchased from culture collection centers [23]. As a result these microbes can treat As(III) contaminated groundwater in-situ naturally. Some microbes like *E.coli* in prokaryotes and *saccharomyces cerevisiae*, *Arabidopsis thaliana* in eukaryotes are also known to reduce As(V) to As(III) and hence increasing its toxicity [88-93]. Reduction of As(V) in bacteria is carried out by ars operons such as arsC (arsenate reductase), arsA, and arsB in arsAB-ATPase efflux pump [94].

*Microbacterium lacticum* oxidizes As(III) under neutral pH at ambient temperature and in the presence of methanol as a substrate. This oxidation was found to be highly efficient and the As(V) was removed by zero valent iron in the form of activated charcoal or FeCl<sub>2</sub> [64]. In fact it has been noted that the bioremediation of Arsenic is much more efficient when As ions are a contaminant along with Fe ions [1]. The reason for this enhanced efficiency of simultaneous bioremediation of Fe and As ions has been postulated to be because the efficiency of bio-oxidation of As(III) to As(V) is enhanced by simultaneous bio-oxidation of Fe(II) ions and the As(V) formed is adsorbed by the **biogenic** Fe oxides formed by the preceding process [54]. This is postulated by ligand exchange mechanism where Fe(III) adsorbs As(V) and gets itself adsorbed by cation exchange mechanism [95].

As7325 strain of *Pseudomonas spp.* is a facultative anaerobic Gram negative rod that flourishes in groundwater containing acetate, lactate or succinate at a temperature of 30°C, pH of 7 and aerobic condition. It was found to be efficient in oxidizing As(III) to As(V). It is found in aquifers of endemic area of Blackfoot disease in Taiwan. Due to its high tolerance of Arsenic, it's a potential Arsenic bioremediator. In fact even pellets of cells of this strain are used as a bio-adsorbant for As(V) ions formed [62].

#### BIO SORPTION OF As(V)

Many organisms show high tolerance to adsorb and accumulate As(V) ions from ground water. Microbial biomass is one of the best sorbents for Arsenic and other metal pollutants [96]. It is possible to selectively allow the growth of Arsenic tolerant strains of certain microbes and hence perform artificially directed evolution of such efficient Arsenic tolerant bio accumulators [23]. This tolerance towards

bioaccumulation is mainly achieved by mechanisms like intracellular bioaccumulation, periplasmic biosorption or biotransformation to less toxic forms of As(V) [23][18]. The uptake of As(V) ions by an organism is generally facilitated by cellular mechanisms such as phosphate transporter proteins PST and PII, while the uptake of As(III) is facilitated by glycerol transporter GLPF [97]. The uptake As(V) and As(III) ions must then be remediated and released out [98]. This efflux of As(III) is facilitated by ArsAB-ATPase pump [99]. Certain proteins such as aqua glyceroporins have the ability to simultaneously uptake and release Arsenic ions bi directionally [98].

*Lysinibacillus phaericus B1 CDA* strain is a circular convex rod shaped gram positive colony which shows optimum growth at a temperature of 37°C and a pH of 7. It is isolated from the soil and seen to accumulate and remove upto 5mg of As(III) ions per gram of its cell (dry biomass). It can hence reduce 50% of groundwater As(III) content showing a MIC values of 500nM Arsenic. But it cannot accumulate As(V) ions, hence As(V) enters the cell through the phosphate transport system and gets reduced to As(III) in the presence of the cytoplasmic enzyme arsenate reductase. This type of reduction is also beneficiary as it acts as a source of energy in stress conditions for the bacteria and various plant cells can sequester As(III) in vacuoles. This is done to avoid the toxicity of As(III) [23][99-104].

*Lactobacillus acidophilus* is commonly found in Arsenic contaminated ground water. It shows high efficiency biosorption of As(III) ions form the source water. Studies have shown that this biosorption can be efficiently carried out at a pH of 7. Moreover increasing the biomass of *L.acidophilus* increases its As(III) ions sorption capacity[105]. Other microbial strains that has the ability to adsorb As(III) ions are *Lactobacillus crispatus*, *Lactobacillus casei*, *Acetobacter spp.*, and some of the Fungi capable to adsorb As(III) ions are *Zygosaccharomyces spp.*, *Rhizopus oryzae*, *Penicillium chrysogenum*, *Penicillium purpurogenum*, *Penicillium canescens*, *Aspergillus niger*, and some of the algae possessing this ability are *Scytonema* and *Lessoniani greescens*[106].

Dead fungal biomass which is a common byproduct of fermentation does not only produce less sludge but is also an efficient sorbent for As(V) [107]. *Aspergillus fumigates* a filamentous fungi is used to treat Arsenic polluted groundwater [108]. Since *Aspergillus fumigates* is a common waste from fermentation industries, its utilisation as a biosorbent is novel way of waste management. Moreover *Aspergillus fumigates* is a cultivatable and economic biosorbent. In recent times, pellets of *Aspergillus fumigates* are made for biosorption. The adsorption of As(V) on these pellets follows freundlich isotherm model [24] These pellets are disposable, biodegradable and can be easily composted.

Similarly in case of phytoremediation, plants such as *Pteris vittata*, *Pityrogrammacalo melanos* and *Mimosa pudica* can effectively adsorb As(V) from the ground water [69][109]. General methods of phytoremediation seen is phyto-extraction, rhizofiltration (both in situ and ex situ), phytostabilization, phytovolatilization and phytodegradation[110]. These techniques of phytoremediation are well explored and widely used in remediation of metal pollutants of the ground water, soil, surface water, municipal and industrial waste waters. *Pteris vittata* is a plant that can hyper accumulate upto 23,000 µg of As(V) ions per gram of its biomass by rhizo-filtration [111]. The accumulation is mostly in the shoots region above ground tissue and this is carried out by transportation of As(V) from the roots to the shoots [112]. Once the As(V) is accumulated, its then reduced within the plant body to As(III) [113].

## TRANSGENIC ARSENIC BIOREMEDIATORS

Most of the naturally bioremediation methodologies discussed so far are inefficient and fix the polluted ground water in extremely small quantities. One approach to overcome this drawback is by performing these techniques ex-situ by artificially introducing the bioremediators in laboratory conditions. But another approach that is gaining much attention in recent times with humongous scope of research is genetically modified organisms as bioremediators. One of the first genetically modified phytoremediator for ground water Arsenic was proposed by Richard B Meagher's team. A genetically modified *Arabidopsis thaliana* plant containing arsC gene (coding for arsenic reductase) and ECS gene (coding for γ-glutamylcysteinesynthetase) of the *E.coli* was created [114]. This transgenic *Arabidopsis thaliana* was observed to accumulate 2 to 3 folds more Arsenic than the normal *Arabidopsis thaliana* expressing its own arsC and ECS genes [114]. In contrast, studies have also been conducted involving transgenic *E.coli* expressing genes that code for phytochelatin synthase (AtPCS) obtained from *Arabidopsis thaliana*. These phytochelatins are metal binding **cysteine** rich peptides that reportedly increases the ability of *E.coli* to accumulate As(V) ions by 50 folds [115].

A highly efficient transgenic *Brassica napus* (canola) can accumulate upto 4 times more Arsenic than its non-transgenic counterpart. They are genetically modified to express 1 aminocyclopropane-1-carboxylate deaminase gene obtained from *Enterobacter cloacae* (P7 CV westar). *Brassicca juncea* is known to

bioaccumulate As(V) effectively and store these ions in its shoot as As(III) trithiolate complex. This is done in the presence of Arsenic contaminated soil containing dithiol Arsenic chelator dimers.

### FUTURE SCOPE OF RESEARCH AND PERSPECTIVES

If the molecular mechanism of Arsenic ions remediation and accumulation is known for other Arsenic bioremediators, one can work on designing a transgenic form of that bioremediator with higher efficiency, better growth, adaptability and greater biomass for increased bioaccumulation capacity [114]. Hence future studies must be led to identify these mechanisms and analyze the genes involved in Arsenic fixation in various other organisms. Along with this, studies must also be dedicated to identify Arsenic ion remediation capacity and ability in other organisms including microorganisms, plants and fungi. Various stress induced adaptation of these organisms must be carried out to enhance their Arsenic fixation capacity in-situ. Research must also be conducted to enhance the efficiency and make ex-situ remediation techniques cost effective for Arsenic from ground water. Finally, other heavy metal pollutants of the groundwater such as chromium, lead, and iron must be identified and appropriately addressed. Their physico-chemical remediation techniques must be replaced with the newly explored bioremediation techniques.

### CONCLUSION

Arsenic contamination in ground water, its cause and effect was studied. The classical physico-chemical approach to remediate Arsenic contaminated groundwater was compared with Bioremediation approach. Various bioremediators of Arsenic were studied and reported, some of their biochemical and molecular Arsenic fixation mechanisms were also analyzed and recent developments along with future scope for this field was reported.

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