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Review

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Bioremediation of Arsenic metal from water and soil by *Bacillus species* - A review

Sutapa Bhowmik[1], Shiv Charan Prajapati[2], Shravan Kumar[3], Kumari Priyanka[4], Reena Saxena[1*]

[1]Suresh Gyan Vihar University, Gyan Vihar Marg, Jaipur, India. [2]Department of Paint Technology, Government Polytechnic Bindki Fatehpur, Uttar Pradesh, India. [3]Department of Biochemical Engineering, Harcourt Butler Technology University, Kanpur, Uttar Pradesh, India. [4]S S Jain Subodh PG College, Rambagh Circle, Jaipur, India.

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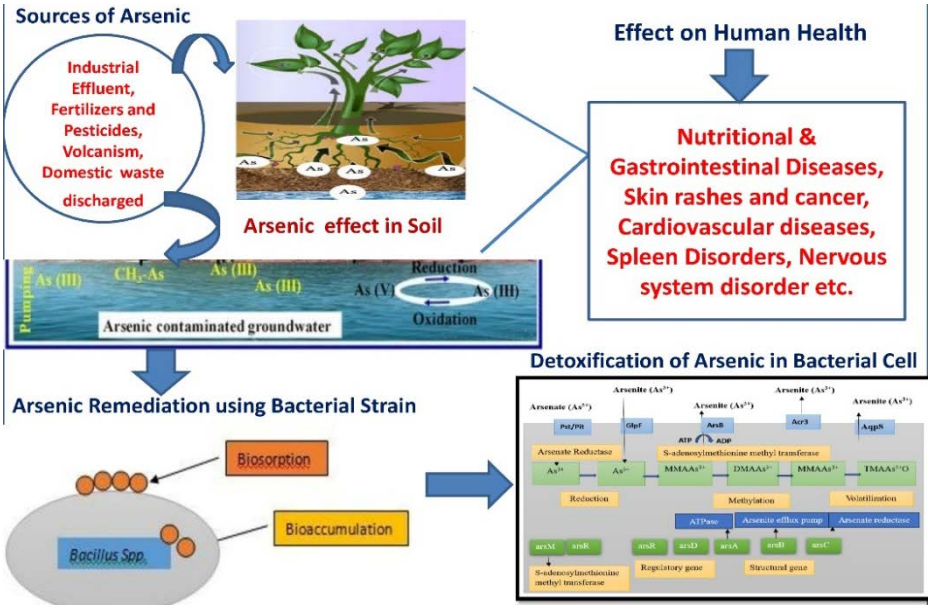
ABSTRACT

Water pollution due to the presence of arsenic (As) is a serious problem worldwide since it can cause several diseases such as cancer, gastrointestinal disorders, enlarged liver, spleen disorders and cardiovascular diseases etc. The current techniques for cleaning waters have limitations, such as producing toxic sludge, requiring a lot of labour, and being costly. Therefore, new techniques that are cost-effective and efficient are required. Arsenic (As) bioremediation using arsenic-resistant microorganisms through volatilization, phytoextraction, biosorption and bioaccumulation has proven to be a very effective method. Bioremediation is an environmentally safe and effective procedure. Numerous investigations have been carried out about the molecular mechanism behind microorganism mediated arsenic (As) bioremediation. This has helped us better understand how microorganisms and arsenic (As) interact and work to reduce and remove environmental arsenic (As) pollution. Among the different bacterial genera actively participating in bioremediation of arsenic in the environment, the genus Bacillus has demonstrated exceptional capabilities due to its diverse biochemical and genetic routes. Model Gram-positive bacteria called Bacillus species have been the subject of lots of research due to their capacity for biosorption, the molecular mechanisms underlying their survival, and their capacity to eliminate and detoxify heavy metals. This review article has focus on the importance of Bacillus species for the removal of arsenic (As) from contaminated soil and water.



Keywords: Bioremediation, Heavy metals, Arsenic (As), Elimination, Microorganism

INTRODUCTION

Naturally occurring arsenic (As) is a hazardous metalloid[1] that has been found in rock (1-2 mg/kg), soil (5-10 mg/kg), and seawater (1-3 µg/L)[2,3] in addition to air and volcanic ash (0.02 µg/m^3) which allows methylated arsine species and arsine gas (AsH_3) to reach the environment.[4] Few of its man-made origins are pesticides and herbicides[5] fossil fuel combustion, mining, smelting, wood preservation, sludge, manure[6] paint pigments, ceramic, glass industry, and food additives.[7,8,9] It is hazardous to living systems at concentrations above 0.5 ppm, which causes cutaneous cancer symptoms.10 Anaemia, chronic respiratory problems, gastrointestinal disorders, enlarged liver, spleen disorders, weakness, weight loss, lethargy, and loss of appetite[11] and cardiovascular disease.[12] Serious worries about arsenic is, that could arise from the arsenic contamination of groundwater and the food chain.[13] The well-known carcinogen arsenic (As) is a common

*Corresponding Author: Dr. Reena Saxena, Suesh Gyan Vihar University, Jaipur
Tel: +91 7976162255 Email: reena.saxena@mygyanvihar.com

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food, drink and soil contaminant that is harmful to human health everywhere. Adeloju et al. reported Arsenic (As) in rocks, soil, water, biota and the atmosphere.[14] Rock-water interactions are the main cause of arsenic release and the deterioration in groundwater quality in aquifer systems. Groundwater pollution with arsenic (As) is a major worldwide health risk. This is because drinking water is the primary means by which Arsenic (As) contaminated groundwater exposes millions of people worldwide.[15] As the main known source of arsenic ingestion is believed to be drinking water, the World Health Organisation (WHO) established the arsenic(As) drinking water guideline value in 2022 at 10 μg L^{-1},[16] and the majority of nations have since adopted this as the maximum content in drinking water while there are very few recommended values for the concentrations of arsenic(As) in food have been reported. Excess arsenic(As) exposure, can result in a variety of illnesses, whether acute or chronic including cancer.[17] The metalloid arsenic (As) is widely utilised in the global glass, herbicide, pesticide, and wood preservation industries.There are many ways that humans can come into contact with arsenic, but the most important one is through eating and drinking food and water that have been tainted. The risk of exposure and serious sickness is increased by high use of arsenic contaminated water.[18] Agricultural soils that are irrigated with water that is rich in arsenic (As) may accumulate arsenic in the soil, crops and enter the food web.[19] Soil arsenic pollution is frequently caused by human activities such as the use of pesticides, fertilizers, and wood preservatives; glassworks; mining; smelting; electronics; batteries; paints; adhesives; weapons; and the dumping of industrial effluents.20 Inorganic forms of arsenic that can be found in nature include arsenite (As^{3+}), arsenate (As^{5+}), arsenic (As^{0}), and arsenide (As^{3-}). These include arsenobetaine, methylarsonic acid, arsanilic acid, dimethylarsinic acid (cacodylic acid), and other organic forms of arsenic. Because of its high solubility, mobility, and bioavailability, arsenite (As^{3+}) is one of the more dangerous Arsenic forms and it's toxicity is 100 times more than arsenate (As^{5+}). The most hazardous type of arsenic is arsine (AsH_3). Because of its structural similarities to phosphate, arsenate (As^{5+}), can enter cells through phosphate transporters. Nevertheless, the enzymes in charge of significant metabolic pathways are inhibited by arsenite (As^{3+}).[21]

Arsenic (As) is removed from water using a variety of chemical-based treatment methods, such as coagulation, ion-exchange, adsorption and reverse osmosis.[22] The majority of large-scale practical applications of these systems have a number of drawbacks, including the production of sludge, high energy needs, high material costs, and additional environmental effects. In an effort to overcome these constraints, numerous researchers examined a range of biological agents as practical, economical, and ecologically acceptable biosorbents for treating water contaminated with arsenic (As). As a result, biosorption has become a popular and affordable method for arsenic cleanup[23], due to intricate structures of microbes that can absorb metal in a variety of ways depending on the microbial cell. The high efficiency, purity and economy of microorganism-mediated bioremediation make it an innovative and promising method for reducing arsenic (As) contamination.[24] The use of Bacillus species (sp.) is one of the numerous biological therapeutic choices due to its peptidoglycan layer on its gram-positive wall, which is its specific characteristic. Additionally Teichoic acids and cell wall-associated acids are features of gram-positive cells; their phosphate groups are essential for the uptake of metals.[25] Previously some researchers have documented bioremediation methods thoroughly as a general remedy for the cleanup of arsenic (As).[26,27,18] This work provides the basis for the current analysis, which evaluates the most recent bioremediation methods in use and their applications in detail.

Microorganisms based bioremediation is considered as to be the outstanding creature for the detoxification of pollutants as it is cheap, simple, and eco-friendly clean-up method.[28] Some Bacillus bacterial species have demonstrated potential in bioremediation processes, such as the elimination of heavy metals from contaminated areas. These microbes have the ability to absorb, sequester, or even convert heavy metals to reduce their harmful effects on the environment.Because of their resilience and plasticity, Bacillus species are good choices for bioremediation applications. Bacillus species have the ability to reduce heavy metal contamination in soil, water, and other ecosystems through mechanisms such as biosorption, bioaccumulation, and biotransformation.It's crucial to remember that different species and strains of Bacillus, as well as environmental factors, might have varying effects on the efficiency of heavy metal remediation. Furthermore, even while bioremediation methods might be sustainable and kind to the environment, they frequently need close monitoring and tuning to get the desired results.This review paper presents a thorough and methodical explanation of the various mechanisms and functional microorganisms like Bacillus Sp. associated with arsenic (As) remediation, which may open up intriguing new possibilities for the use of arsenic bioremediation in the future. Because of their dynamic metabolism, bacteria are able to live and grow in areas with high levels of arsenic contamination by using their genetic makeup to target and eliminate arsenic. It has been found that the genetic makeup and kind of bacteria, which are strongly influenced by the environmental conditions in which they live, have a significant impact on the arsenic resistance mechanisms that bacteria exhibit.[29]

This review's novelty comes from its focussed investigation of various Bacillus species and theircapacities for arsenic bioremediation from water and soil both. In addition, the article examined the current research data to identify gaps in the understanding of arsenic treatment. It is concluded in this review paper that Bacillus sp. could prove to be a viable option for heavy metal bioremediation in the future. The paper also emphasizes the novel strategy of using indigenous microbiota primarily for bioremediation, which lowers expenses and environmental effect in comparison to non-native species. Review's components under this study mark notable developments in the field, offering fresh avenues for investigation and useful applications in heavy metal bioremediation.

MICROORGANISMS USEFUL FOR ARSENIC (AS) BIOREMEDIATION

Microorganisms are present everywhere and are crucial to both human health and ecological balance. Microorganisms have the ability to modify the valence states of As, so mitigating its toxicity

and potentially influencing its movement and transformation in various environments.[30] Unlike organic pollutants, which biodegrade over time, arsenic (As) is not biodegradable; instead, it can be removed and detoxified by changing its solubility and status while being impacted by microorganisms.[31] Through the intricate reduction-related metabolic processes,[32] decomposition,[33,34] bio adsorption [26,35,] Volatilization, methylation,[36] bacteria are essential to the mobilisation, transformation, and bioremediation of Arsenic.According to the earlier research, bacteria may precipitate their own hydroxides, which could cause immobilization, and they may use their metabolites to solidify and stabilize As.[37] Another study by Hare et al. states that microorganisms should be able to create enough stabilisers or metabolites to immobilise arsenic.[28] Microorganisms are under pressure to maintain their arsenic detoxification systems in order to survive, due to the naturally occurring arsenate and arsenite in water and soil environments that could enter the cells through the phosphate-transport system. According to Musingarimi et al. detoxification operons, which are encoded on plasmids or genomes, are one of the most prevalent ways that microorganisms resist arsenic.[38]

Molecular Processes Associated with Microbial Remediation

Microbes bind heavy metals and clean them through enzymatic reduction processes with oxidoreductases for minimizing their influence on the environment.[39] Arsenic resistant microorganisms isolated from variety of environmental samples mostly act on As^{3+} and As^{5+} through redox processes and transforming inorganic to organic forms.[34] Furthermore, the bacteria possess vast gene reserves that enable them to flourish in a variety of challenging environments, and that is how they occupy every ecological niche. This sheds light on the possible advantages of applying biotechnological methods that employ genomes for arsenic cleanup.[40]

Oxidative Phosphorylation's significance

One cannot undervalue the importance of oxidative phosphorylation in living things [9].As^{5+} has the ability to block oxidative phosphorylation, because of its structural resemblance to phosphate [6] .As^{5+} obstructs phosphorylation metabolic processes and prevents adenosine triphosphate from being synthesized [1]. The aquaglyceroporinproteins act as the entry point for As^{3+}.[6,41,42] Instantaneously upon internalization, it binds itself to the respiratory enzymes via their sulphur residue.[7,43]

Energy for cellular processes is produced by mitochondrial oxidative phosphorylation.[44] Energy in the form of ATP, the universal energy unit of cells, is necessary for all forms of life. In eukaryotes, Oxidative phosphorylation (OXPHOS) primarily produces ATP in the mitochondria.[45] Primarily occurring in mitochondria, oxidative phosphorylation is a series of redox reactions that phosphorylate adenosine diphosphate (ADP) to make ATP. These reactions use reduced nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide ($FADH_2$) as a substrate.[46]

As^{5+} imitates and replaces phosphates in glycolysis and cellular respiration; it causes disruptions in phosphorylation processes. As^{5+} binds to adenosine diphosphate (ADP) in the reaction medium to form ADP-arsenate, which denotes the absence of high-energy ATP phosphate connections. The absence of typical high-energy phosphate connections causes the uncoupling of oxidative phosphorylation.

The production of ATP from ADP is propelled by the energy produced from these oxidation/reduction events, which occur during oxidative phosphorylation when electrons from $FADH_2$ and NADH mix with O_2.

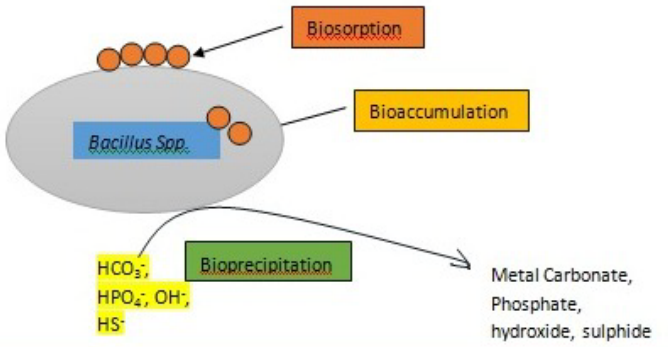

Figure 1. Bacterial interactions with heavy metals accumulated in soils and water

Oxidation Step: electron transport chain

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightarrow NAD^+ + H_2O$$
$$FADH_2 + \tfrac{1}{2}O_2 \rightarrow FAD + H_2O$$

Phosphorylation step

$$ADP + P_i \rightarrow ATP$$

P_i = Inorganic Phosphate

Biosorption and Bioaccumulation of As

Microbe's ability to biologically absorb and accumulate metals, including metalloids, is thought to be a useful technique for bioremediation. Functional microorganism can interact with As in various forms including redox, biomethylation, biosorption, and bioaccumulation, thereby playing an important role in As bioremediation and ecological balance. The surface group of microbial cells such as carboxylic, thiol, hydroxyl, amine and phosphate are capable of binding arsenic and facilitates the process of biosorption.[30] By attaching to proteins or peptides, bioaccumulation is a technique for reducing the toxicity of free As carried by glycerol and phosphate transporters.[24]

Based on cell membranes, biosorption is a physicochemical heavy metal uptake process that is independent of metabolism. It works by means of substances that are negatively charged and found in cell membranes.[47] Different physiochemical circumstances, including pH, temperature and the presence of other ions, might cause biosorption to occur. There are various steps involved in the biosorption process, including ion exchange, chelation/complexation, adsorption, and precipitation.[48]

In Ion exchange polysaccharides found in the cell walls of microorganisms serve as metal ion exchange sites because of their opposing charges.[49] While in chelation/complexation, electrostatic interaction causes complexation or coordination between a metallic ion chelating agent and a polymer produced by bacteria. Metals are removed from the solution by reacting with active groups on the cell wall, such as carboxyl, hydroxyl, thiol and amino group to create complexes on the cell surface.[48] During adsorption process heavy metals are eliminated by microorganisms through surface adsorption. Metals build accumulated on the cell surface and interact with different functional groups during this process. Driven by non-specific forces such as van der waal interactions or columbic attractions between charged solute species and the adsorbing phase, this phenomenon primarily occurs on the surface of materials.[50] Precipitation carried out when metal ions precipitate; they can either stay unchanged or attach themselves to the bacterium by interacting with the bio sorbent's surface functional groups.[51]

However, bioaccumulation and the initial step of the biosorption process are almost the same; there are several key differences, most notably in the process's route. Bioaccumulation starts quickly and progresses to a moderate level depending on metabolic activity that requires ion exchange or physical adsorption for metal attachment and intracellular transit.[52]

Bacillus species as possible heavy metal elimination agents

Bacillus species (Bacillus sp.) are rod-shaped, gram-positive bacteria that are members of the phylum Firmicutes. They naturally produce spores. Bacillus sp. are distinguished by their ability to generate spores under harsh settings. This process is typically initiated by a shortage of nutrients.[53] Research has made it possible to employ the distinctive qualities of Bacillus sp. to the best advantage of humankind, as a result of the beneficial applications of these species in various sectors. Numerous Bacillus sp., including Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Bacillus pumilus and Bacillus coagulans are used widely worldwide for different applications.[54] Numerous research findings have demonstrated the antibacterial and anti-cancerous benefits of B. subtilis, indicating that it is safe to use as a probiotic.[55] Given that gram-positive bacteria frequently predominate contaminated locations because of their greater metabolic capabilities and increased ability for biosorption. Bacillus sp. is being investigated widely for their potential part in mitigating heavy metals from polluted sites through bio adsorption and bioaccumulation.[56,57]

Heavy metals in the environment can be eliminated by microorganisms using a variety of techniques (Figure.1) The genus Bacillus uses biosorption, bioaccumulation, and bioprecipitation as their primary heavy metal removal techniques[47].

However, As^{3+} has a greater biological significance than the other because of its capacity to impede normal protein folding, reduce cysteines, and bind to sulfhydryl groups with such strength. Although As^{5+} likewise inhibits phosphate anion transporters to cause toxic cell function, it is dangerous because it can be converted to the more toxic As^{3+}.[58]

Numerous Bacillus species and Pseudomonas species are among the extremely effective microbes because they produce a variety of enzymes and secondary metabolites that improve the bioremediation process. Wide range of secondary metabolites produced from Bacillus species, including isocoumarins, fatty acids, polyketides, lipopeptides, and macrolactones. These metabolites are capable of eliminating organic contaminants and heavy metals and metalloids.[59]

The ars operon uses the following genes to mediate the bioremediation of As in Bacillus-containing bacteria: arsA, arsB, arsC, arsD, and arsR. Where, arsA and arsB have ATPase activity, and arsC encodes an arsenate reductase that changes As^{3+} to As^{5+}.[60] The most widely used the aio gene cluster, sometimes referred to as aso, aox, or aro, controls the As^{3+} oxidation pathway. arr Gene code for dissimilatory As^{5+} reduction sometimes it is referred to as respiratory As^{5+} reduction and work as a later evolutionary response to ambient arsenic stress. Two distinct methods exist for microorganisms to reduce As^{5+}, one is dissimilatory As^{5+} reduction, which is encoded in the arr gene system, and other is activation of cytoplasmic As^{5+} reductases via the ars operons.[58] The intracellular uptake of As^{5+} via phosphate membrane systems initiates the metabolic cascade of cytoplasmic As^{5+} reduction. Since As^{5+} is a molecular analogue of phosphorus, As^{5+} ions can enter bacterial cells via phosphate-specific transport (Pst), phosphate inorganic transport (Pit), or periplasmic binding proteins. The gene that codes for the As^{5+} reductase, arsC, is increased once it enters the cytoplasmic milieu.[58]

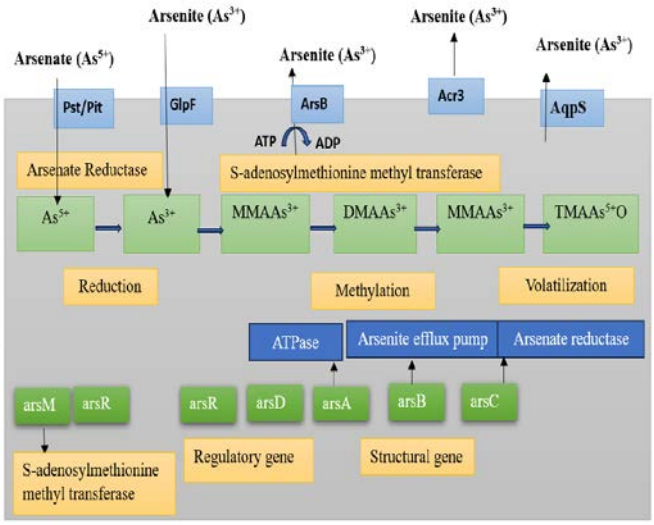

Figure 2. A Schematic representation of the pathways connected to the detoxification of arsenic within a microbial cell for the aim of bioremediation of As.

The figure demonstrates that the genes arsB, acr3, and aqpS, along with Glycerol uptake facilitator protein (GlpF) and phosphate transporters, are in charge of As uptake and efflux. Reduction, methylation, and volatilization are the three processes that underpin the conversion of As into its many forms. Arsenite S-adenosylmethionine methyltransferase is encoded by the arsM gene and is responsible for the methylation-based conversion of arsenite to different methylated forms. Here, arsC and arsM control the

bioconversion process.Together with arsA, which codes for an ATPase, functions arsB,which codes for an arsenite efflux pump.[69]

MMAAs^{3+} - Monomethyl arsenite
DMAAs^{3+} - Dimethyl arsenite
MMAAs^{3+} - Trimethyl arsenite
TMAAs^{5+}O - Trimethyl Arsine Oxide

Global reach for bioremediation of Arsenic by *Bacillus* spp.

Arsenic is soluble in water hence it is challenging to remove it from the environment.[61] The enzyme known as arsenic oxidase is present in the protoplasm of bacteria that oxidize arsenic[1], converts the poisonous As^{3+} into the less dangerous form As^{5+} because microbes utilizearsenicin their metabolic processes as a source of energy.[62,63] According to Liao et al., Bacillus is a significant arsenic-reducing bacterium[64] Arsenic can be removed by Bacillus sp. like Bacillus megateriu,[65,66] B. aryabhattai.[67] An analysis was conducted to determine the arsenic-removing capacity of Bacillus cereus strain W2, which was isolated from soil in Miyazaki Prefecture, Japan.[68] Ghosh et al. discovered that B. cereus anaerobically respired As^{5+}.[13] The process used for absorption between B. subtilis bacteria and arsenic at concentrations of (0.5, 0.2, 0.1 mg/ml) under pH 7 conditions at 35 °C and for a period of (24, 48, 72 hours). The optimal elimination duration was found to be 24 hours, with the highest removal of arsenic being 86% at 0.1 mg/ml and the lowest removal being 56% at 0.5 mg/ml.[13] Different strain of Bacillus spp. has been utilized globally to carry out bioremediation of Arsenic. Some of them are as follows:

Bacillus megaterium strain UM-123, which was isolated from soil in Miyazaki, shows promise as a new arsenic-removing bacteria.[65] Arsenic is efficiently eliminated from culture media containing As^{3+} and As^{5+} by Bacillus megaterium strain UM-123, 0.386 mg As/g (dry weight) removed in 24 hours from a medium containing 5.0 mg As/l (As^{3+}), it exhibits remarkable removal efficiency. Dried cells of strain UM-123 exhibited a preference for As^{3+} over As^{5+} when tested for arsenic adsorption. While As^{5+} adsorption was negligible, As^{3+} adsorption were substantial. Having an adsorption capability of up to 0.127 mg As/g (dry weight), the As^{3+} adsorption equilibrium data followed the Langmuir adsorption isotherm.[65] In arsenic solutions up to 1.0 mg As/l, strain UM-123 demonstrated a high degree of selectivity for As^{3+} adsorption, with a ratio of As^{3+}adsorption exceeding 93%. The ability to successfully remove arsenic from tainted water was made possible by this selectivity.According to the findings; strain UM-123 may be used to bioremediate water sources contaminated with arsenic. Even at starting values of up to 0.2 mg As/l, it can lower arsenic concentrations in solution to 0.01 mg As/l or below. This suggests that it could be used to treat water bodies that have been poisoned by arsenic.The distinct qualities of Bacillus megaterium strain UM-123 render it a propitious contender for bioremediation tactics aimed at addressing arsenic pollution in water supplies. Its potential importance in environmental rehabilitation efforts is highlighted by its high arsenic removal efficiency, selectivity for As^{3+}, and effectiveness at low concentrations.

Another research carried out using groundwater contaminated by arsenic in Burdwan, West Bengal, Dey et al. isolated Bacillus sp. KM02[1]. Both high concentrations of As^{3+} and As^{5+} were resistant to the isolate. Furthermore, 100 ppm of arsenate and arsenite containing nutritional broth media has potential for reducing the quantity of arsenic1.51.45% of arsenite was considered to be the non-significant elimination. At the 5% level of significance, however, a substantial clearance of 53.29% arsenate was noted from the medium following a 72-hour incubation period. The isolate was unable to decrease arsenate, although it possesses the unusual ability to oxidize arsenite to less poisonous arsenate[1]. It found possible that Bacillus sp. KM02 have special traits or adaptations that allow it to endure and perhaps flourish in arsenic-contaminated surroundings. Understanding the relationships that organisms have with their environments and possibly finding solutions to problems with environmental contamination depend on this kind of research.

Another study was performed in Jorhat, Assam where arsenic contaminated groundwater was treated with Bacillus sp. IIIJ3-1 13. B. cereus strain IIIJ3-1 has the ability to convert and accumulate As^{3+} with efficiency, making it a possible As^{3+} accumulator in areas contaminated by Arsenic. The strain shows promise for developing bioremediation techniques for contaminated groundwater and other ecosystems due to its resistance to transformation and its capacity to sequester arsenic inside the cells.In-depth genetic, chemotaxonomic, biochemical, and Eco physiological characterizations of a novel B. cereus strain IIIJ3-1 member that is capable of effectively converting and accumulating As^{3+} were provided in their work. Based on its genomic, metabolic, and chemotaxonomic characteristics, strain IIIJ3-1 appears to be a unique, non-clonal member of the B. cereus group that has the capacity to accumulate As^{3+} in areas which are contaminated with arsenic. The bacterial strain IIIJ3-1 offers a novel approach to remediate As^{3+} in heavily contaminated irrigational fields, like those in West Bengal, and arsenic-affected ground water because of its capacity to withstand and accumulate the most toxic form of inorganic As (As^{3+}) within its system.[70]

In the hypersaline, alkaline, arsenic-rich anoxic muds of Mono Lake, California, two gram-positive anaerobic bacteria, Bacillus arsenicoselenatis and Bacillus selenitireducens, were isolated[71].Both Bacillus selenitireducens and Bacillus arsenicoselenatis are haloalkaliphiles, which means they do well in conditions with high salinity and alkaline pH values. These bacteria's capacity to respire selenium and arsenic oxyanions makes them remarkable. These bacteria are able to substitute arsenic and selenium compounds for oxygen in their respiratory chains by acting as terminal electron acceptors in them in anoxic conditions. Because of their metabolic adaptability, they can flourish in settings with low or no oxygen or other conventional electron acceptors. Both expanded by lactate oxidation to acetate plus CO_2 and dissimulators reduction of As^{5+} to As^{3+}. However, As^{3+} is typically more poisonous and mobile than As^{5+}. However, As^{3+} can be precipitated using digenetic iron or sulphur to remove it from the solution[72,73] to decontaminate As^{5+}contaminated soils, dissimilatory reduction may therefore still be an option. In a groundwater sample that contained arsenic and was regularly used for irrigation in Taif City, Saudi Arabia, Mohamed & Farag discovered several bacterial strains.[66] Upon molecular identification, the isolates were found to belong to two distinct taxa, Bacillus and Lysinibacillus, based on

the results of 16S replacement DNA sequencing. Arsenic up to 15 mg/L was resisted by B. cereus strains EA4, EA5, and EA6.The 16S rRNA gene sequences of 6 isolates were submitted to GenBank, where they were assigned accession numbers as L. sphaericus EA1, B. fusiformis EA2, Lysinibacillus sp. EA3, B. cereus strains EA4, EA5, and EA6. A mixed culture of L. sphaericus EA1, B. fusiformis EA2, and Lysinibacillus sp. EA3 and B. cereus strain EA5 was found to be effective in bioremediating arsenic oxychloride up to 99.7% and 94.9%, respectively[66].The capacity of the novel Bacillus strains to extract arsenic from aqueous solution was tested at fixed temperature (300ºC), pH level (7), agitation speeds (100 rpm), cell weight (0.1 g wet weight), and contact duration (12 h). The starting arsenic concentration in the 20 ml/100 ml conical flask was 15 mg/L. The percentage of removed arsenic for each individual strain was greater than 83%. B. cereus EA5 (94.9%) and B. fusiformis EA2 (94.7%) exhibited the highest level of arsenic adsorption, with L. sphaericus EA1 (93%) and B. cereus EA4 (92.8%) following closely behind. However, Lysinibacillus sp. EA3 and B. cereus EA6 showed the lowest percentage of arsenic bioremediation. There were also two distinct uses of mixed cultures. L. sphaericus EA1, B. fusiformis EA2, and Lysinibacillus sp. EA3 are included in one mixed culture; B. cereus EA4, B. cereus EA5, and B. cereus EA6 are included in the other. The former has the maximum activity of 99.7% arsenic elimination.

In batch studies,Podder &Majumder,examined the capacity of a Bacillus arsenicus MTCC (Microbial Type Culture Collection, Chandigarh, India) 4380 biofilm supported on sawdust(SD)/$MnFe_2O_4$ composite to biosorb/bioaccumulate As^{3+} and As^{5+}.[74] The sawdust that has been acid-treated has a reduced ability to absorb As^{3+} and As^{5+}. Its primary purpose was to providing a template with a high specific area for $MnFe_2O_4$ loading. They discovered that effective increment in the adsorption capacity can be achieved by adding a sawdust template that has been acid-treated. They noted that the template might be able to increase the effective adsorption area and stop $MnFe_2O_4$ particles from aggregating during the adsorption process, which would greatly increase the adsorption capacity.[74] According to their research, As^{3+} and As^{5+} from artificially produced wastewater could be successfully bioabsorbed or bioaccumulated using Bacillus arsenicus, which immobilized on the SD/$MnFe_2O_4$ composite surface. For both As^{3+} and As^{5+}, the ideal contact duration and temperature were found to be 220 minutes and 30°C, respectively, duringbiosorption/bioaccumulation.While practically unaffected by rising temperatures, the ideal contact time for reaching equilibrium increased with concentration. The rate at which immobilized bacterial cells bioabsorbed or accumulated $As^{3+}$and As^{5+}declined as concentration increased.[74] Overall, Bacillus sp. has the ability to reduce heavy metal contamination in soil, water, and other ecosystems through mechanisms such as biosorption, bioaccumulation, and biotransformation. It's crucial to remember that different species and strains of Bacillus, as well as environmental factors, might have varying effects on the efficiency of heavy metal remediation. Table.1 summarizing the comparisons between different Bacillus sp. in terms of their efficiency and conditions required for arsenic bioremediation.

Expression of Operon in Resistance to Arsenic

The bacteria's plasmids or chromosomes contain the operon for the arsenic resistance system (ars).The genes for ars serve as inorganic arsenic compound detoxifying agents. The arsenite efflux permease (Ars B) which is encoded by the ars B gene of the ars operon, catalyzes the extrusion of As^{3+} during the detoxification process of As^{3+}.[75] Three genes, known as arsC (reduction of arsenate to arsenite), arsR (transcriptional repressor), and arsB (which may potentially serve as a component of Ars AB-As^{3+} translocating ATPase, the ATP-driven efflux pump), comprise most detoxifying operons[81,60,38] .Furthermore, two extra genes (arsD-metallochaperone and arsA-ATPase) are also present in some detoxifying operons.[81,38] The ArsA subunit of the efflux pump receives cytosolic As^{3+} once it is bound by the ArsD metallochaperone.[60] Bacillus sp. utilize several removal mechanisms to regulate arsenic biotransformation, consisting of exopolysaccharide complexation, cell membrane binding, reduction (detoxification), efflux, and adsorption on the cell surface. Normally, ArsC converts As^{5+}, a less harmful version that transforms the cell into As^{3+}, a more toxic form, which is subsequently taken out of the cell by ArsB[82,38]; (Figure 3). According to Villegas-Torres et al. , Bacillus sphaericus's capacity to withstand arsenic may be attributed to the arsC gene; this could be horizontally transmitted between bacteria obtained from Columbian oil-contaminated soil that has high arsenic levels.[83] According to another study by Flores et al. the draft genome sequences of two indigenous strains of Bacillus (ZAP17 and ZAP62) from Araro microbial mat hot springs, Mexico were identified.

These cultures were shown to be capable of growing on arsenate As^{5+} at concentrations up to 64 mM and arsenite As^{3+}, at concentrations up to 32 Mm [84] .Two functioning ars operons are carried by Bacillus paralicheniformis ZAP17 and Bacillus altitudinis ZAP62, enabling them to withstand high As^{5+} or As^{3+} concentrations. Additionally, two arsenic resistance operons, ars RBC and ars RBCDA, in both strains were found to be potential As resistance determinants by genome mining. ArsA (arsenial pump-driving ATPase), ArsB (arsenical efflux pump protein), ArsC (arsenate reductase), ArsD (arsenical efflux pump protein), and ArsR (metalloregulator/ars operon repressor) proteins were predicted to be distinctly grouped with their respective clades corresponding to other characterized bacterial species. Researchers's findings demonstrated that the expression of the arsRBC and arsRBCDA genes occurred in the presence of As^{3+}, which allowed us to further assess the functionality of the ars operons in the ZAP17 and ZAP62 strains.[84]

Table 1. *Bacillus* species' capacity to absorb Arsenic

Bacterial Strains	Initial Metal Concentration	Arsenic Uptake Ability	Conditions required	Reference
***Bacillus*sp.KM02**	100ppm	51.45%(As^{3+}) 53.29% (As^{5+})	Temperature – 30^0C, Incubation Period – 72 h	[1]

***Bacillus megaterium* strain UM-123**	1 mg As/ l	93% As^{3+}	Optimum pH – 7. Temperature – 35^{0}C,	[65]
***Bacillus sp.* KUJM2**	0.5 mg/l	89.87% As^{3+} 91.22%As^{5+}	Optimum pH – 7.0±0.2 Temperature – 35^{0}C	[77]
***Bacillus*sp.IIIJ3–1**	350smM(As^{5+}) 10mM(As^{3+})	350mM(As^{5+}) 10mM(As^{3+})	Temperature – 30^{0}C, Incubation Period – 24 h	[13]
B. selenatarsenatis	250 mg-As/kg 2400 mg-As/kg	65%	anthraquinone-2,6-disulfonate(AQDS), Incubation time -7 days	[77]
Bacillus licheniformis	3 mM of As^{5+} 2 mMAs^{3+}	100% As^{5+} 100% As^{3+}	Optimum pH – 7	[78]
***B.cereus*W2**	50mg/L	1.870mg/L(As^{3+})	Optimum pH – 7.5. Temperature – 30±2 ^{0}C, Contact time -30 mins.	[68]
Bacillus macerans	128 mM arsenite	92%	Incubation Time – 144 h	[79]
Bacillus megaterim		73%	Incubation Time – 120 h	
***B.cereus*EA5**	15mg/L	94.9%	Optimum pH – 7.	[66]
***B.fusiformis*EA2**		99.7%	Temperature – 30^{0}C, Contact time -12 h	
***B.arsenicus*MTCC 4380**	2000mg/L 1800mg/L	89.462%(As^{5+}) 83.043%(As^{3+})	Ideal Contact time -220 mins, Temperature-30 ^{0}C	[74]
Bacillus safensis	0.027 mM (2 ppm)	37.54%	Incubation period – 24 h, Temperature – 37 ^{0}C	[21]
Lysinibacillus sp.		32.33%		
Bacillus sp. EIKU23	100 mg /l As^{3+} 100 mg /l As^{5+}	38%As^{3+} 22.6%As^{5+}	Temperature -30 ^{0}C, Incubation time – 60 mins.	[80]

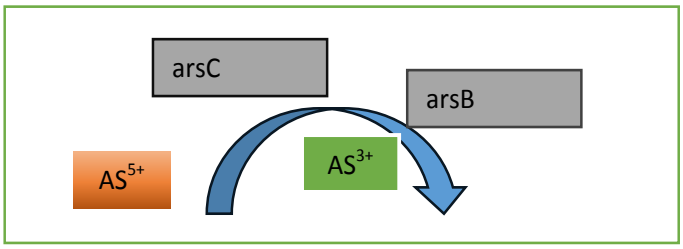

Figure 3. *Bacillus* species have a mechanism for bio regulating arsenic.

The biomass dosage, temperature, contact time, pH of the solution, and operating parameters all have an impact on how well As^{3+}ions are bio absorbed. At pH 7.5, it was discovered that the biomass of B. cereus has a biosorption capacity of 32.24 mg/g for As^{3+} [68].The ideal conditions were 30 minutes of contact time, 6 g/L of biomass, and 30±2 °C of temperature. As^{3+} biosorption data conform to a Langmuir isotherm that has been linearly transformed when the coefficient of correlation (R^2) is greater than 0.9968. The rate constant of biosorption is effectively predicted using explanation of As^{3+} dynamics using a pseudo-second-order model. The sorption process of As^{3+} onto B. cereus biomass is endothermic, spontaneous, and practicable, as indicated by thermodynamic characteristics. B. cereus is used to desorbed the As^{3+}ions using both 1M Hydrochloric acid (HCl) and 1M Nitric acid (HNO3).The Fourier Transform Infrared Spectroscopy (FTIR) study explains, how the surface functional groups of B. cereus cells, which may contain amide, amino, and hydroxyl groups, are involved in the chelating features of As^{3+} ion coordination. B. cereus is a cheap biomass that has a sizable capability for biosorption[85] .B. cereus strain W2 was able to remove dry cells up to 0.18 mg As/g and retain As^{3+} and As^{5+} up to 1.87 mg As/g of dry cell weight[68], following its first isolation, Techno Suruga Laboratory Co., Ltd. (Japan) identified this bacterium. It was then cultivated using Nutrient Broth (NB) from Nissui Pharmaceutical Co., Ltd. (Japan) and incubated at 30°C for all studies. During the stationary growth phase, Bacillus sp. strain DJ-1 has shown a 9.8 mg g-1 As^{5+} biosorption capability.[86] After being cultured for 72 hours at 35 °C, Bacillus sp. KUJM2 eliminated 89.87% and 91.22% of As^{3+} and As^{5+}, respectively, at pH 7.[76]

An excellent method for cleaning up As-contaminated soils is the coexistence of anthraquinone-2,6-disulfonate(AQDS) and Bacillus selenatarsenatis SF-1, a respiratory reducing bacterium that reduces As^{5+}. This co-presence enhanced the removal efficiency of arsenic from contaminated soil[77].Researchers discovered that during 7 days of incubation with both AQDS and B. selenatarsenatis, the accumulated aqueous arsenic (As) accounted for 65% of the original solid-phase, which is almost twice as compare with B. selenatarsenatis alone (35%).[77] In Hokko Prefecture, Japan, two types of polluted soils (soils layer L and layer H as described by the researchers) were gathered from a former factory site.When AQDS was added to As-contaminated soils (soil L, 250 mg-As/kg; soil H, 2400 mg-As/kg), the arsenic removal efficiencieswhich are determined by dividing the total quantity of dissolved arsenic by the original amount in the soils increased to 56% and 40% in soil layer L and layer H, respectively. In contrast, the removal efficiencies were 32% and 19%, respectively, in the absence of AQDS.[77]

In another study, arsenite-resistant bacteria were isolated and the minimum inhibitory concentration (MIC) was determined by Ghodsi et al., applying the spread plate method and the agar dilution method with PHG-II agar plates(1 g yeast extract, 2 g glucose, 4 g pepton, and 15 g agar per liter) that have been exposed to sodium arsenite (pH 7).[79] The results showed that 69 and 25 % of the isolates resistant to arsenite were bacilli that were classified as gram positive and negative, respectively.[79] It is associated with bacteria including Corynebacterium vitarumen, Bacillus macerans, and

Bacillus megaterim. Its maximal MIC was 128 mM/L.Following 48 hours of growth, the removal efficiencies of arsenite for Bacillus macerans and Bacillus megaterim were 60% and 38%, respectively.[79] When Bacillus macerans was grown for 144 hours and Bacillus megaterim was grown for 120 hours, the removal efficiency of arsenite was 73% and 92%, respectively.[79]

Gram-negative, As-tolerant Bacillus aryabhattai strain NBRI014 accession no. (KT238896) was isolated by Singh et al. from the rice rhizosphere of Uttar Pradesh. The bacterial strain NBRI014 was assessed for its potential to be utilized in arsenic bioremediation by estimating its biovolatilization and bioaccumulation capabilities. Their research also shows that bacteria have the ars operon, which may have been important element reducing arsenic's toxicity. The outcomes show that after 36 hours of incubation, there is an increase in the amount of arsenate in the bacterial biomass.[67] With longer incubation times, there was a rise in bioaccumulation and biovolatilization; the highest accumulation was seen after 12 hours. By expressing ars genes and seven novel up regulated proteins, the strain NBRI014 was able to remove a significant amount of arsenic in their investigation. Additionally, the elemental composition and relative distribution of As in bacterial cells were demonstrated by Singh et al.'s investigation, and this was further supported by FTIR analysis, which identified functional groups involved in arsenic binding.

Tripti et al. extracted soil from the rhizosphere of Amaranthusviridis (green amaranth), which was planted in an area contaminated with arsenic.[78] Using 16S rRNA gene sequencing, arsenic-tolerant bacteria from the rhizosphere of Amaranthusviridis were discovered and identified as Bacillus licheniformis. Subsequently, the capacity of Bacillus licheniformis to reduce the above quantities of As^{5+} to $As^{3+}$and to absorb and eliminate arsenic at 3, 6, and 9 mM As^{5+} and 2, 4, and 6 mM As^{3+} was evaluated. As^{5+} and As^{3+}were found to have minimal inhibitory concentrations (MICs) of 10 and 7 mM, respectively. In contrast, at 6 mM As^{5+}, 76% As^{5+} was eliminated from the media and 56% was converted to As^{3+}. At 3 mM, 100% of As^{5+} was absorbed by the bacteria, releasing 42% As^{3+} into the medium. $As^{3+}$was absorbed by the bacteria 100% of the time at 2 mM, but only 40% of the time at 6 mM. The difference in growth, absorption, and protein content of cells was used to quantify the toxicity, availability, and speciation of arsenic, and these factors were all significantly impacted by pH. While both acidic and basic pH promoted development, but at varying pH values, As^{5+} and As^{3+}were most hazardous at about a neutral pH.Bacillus licheniformis was seen to have first uptaken As^{5+} and then reduced to As^{3+}, which was extruded in the growth media, while the concentration of As^{5+} was progressively decreased and of As^{3+} was gradually raised in the remaining media. Tripti et al.offered a potential explanation in which they described how the phosphate transporter system helps As^{5+} enter bacterial cells initially, and then it reduced to As^{3+} inside the cell with the help of (arsC) gene product, arsenate reductase[87]. After As^{3+} accumulated in the cell, an antiporter protein channel, or the product of the arsB gene, ejected it.[88]

After being isolated from the rhizosphere of A. viridis, Bacillus licheniformis DAS-2 was chosen for additional research[89].The isolate's 16S rRNA nucleotide sequences have been placed in GenBank under the accession number KF664028 and the species name B. licheniformis DAS-289. The bacterium's capacity to withstand As^{5+}[MIC 8 mM] and As^{3+}[MIC 6 mM] is exceptional. Additionally, the bacteria had sufficiently eliminated and absorbed 100% of the As^{5+} and $As^{3+}$from the growing medium, especially at the lower concentration of arsenic enrichment. The same medium that had previously been exclusively enriched by As^{5+} was used to measure As^{3+} and it was also discovered that As^{5+}concentration were dropping out of the medium. This occurrence represented the conversion or decrease of absorbed or eliminated As^{5+} into As^{3+}, which may be a survival tactic used by Bacillus licheniformis DAS-2 to withstand arsenic(As) poisoning. Different arsenic(As) concentrations and pH markedly changed the efficiency of Bacillus licheniformis DAS-2's arsenic tolerance (maturing, removal, transformation As^{5+} to As^{3+}. The findings corroborate the theory that Bacillus licheniformis DAS-2's arsenic(As) toxicity is modulated by pH, which may be a more effective way to reduce arsenic pollution. In growth media enriched with As^{5+} at concentrations of 3, 5, and 7 mM, the potential for arsenic removal/uptake was 100%, 60%, and 35%, respectively. At 3 mM As^{5+}enrichment, 80% of the absorption As^{5+} was converted to As^{3+}, and at enrichment of 7mM As^{5+} and at a neutral pH, this percentage progressively dropped to just 17%[89]. It was discovered that pH affected the amount of arsenic that was poisonous to Bacillus licheniformis DAS-2. This was investigated with changing the growth, uptake/removal, reduction, and chemical toxicity measurements.[89]

In the Murshidabad district's Asanpara village (Bhagobangola I block), arsenic-contaminated soil yielded arsenic-resistant bacteria (ARB), which were then biochemically identified [21]. By using 16S rRNA sequencing and phylogenetic analysis, Bacillus safensis and Lysinibacillus sp. were recognized. Isolated bacteria were able to grow in arsenite concentrations of around 77 mM and 89 mM and arsenate concentrations of around 561 mM and 721 mM, respectively[21]. Its unchecked development at extremely high arsenic concentrations can be explained by these bacterial possibility that these bacteria bio transform arsenite into arsenate. Scanning electron microscopy (SEM) shows changed in size as a defence against arsenic stress. Bacillus safensis shows around 38%, 35%, and 35% bioremediation in the presence of 2 ppm, 10 ppm and 50 ppm arsenic, respectively. Lysinibacillus sp. shows around 32%, 31%, and 31% bioremediation[21] .Lysinibacillus sp. and Bacillus safensisare hyper tolerant towards a variety of hazardous heavy metals, including arsenic (As^{3+} and As^{5+}).

Native bacteria that are resistant to arsenic (As) and possess distinct Arsenate (As^{5+}) and arsenite (As^{3+}) MIC levels were measured in paddy fields throughout various locations of Chhattisgarh, India.[90] Bacillus nealsonii strain ARP2 and Bacillus tequilensis strain ART2, respectively, were identified as these isolates using 16S rRNA gene sequencing. With isolate ARP2 displaying arsenate reductase activity, As^{5+} was quickly reduced to As^{3+} with rate of reduction of 37.5 µM/min. Similar to Bacillus tequilensis strain ART1, it could oxidize As^{3+} into As^{5+}using the arsenite oxidase enzyme, with a rate of oxidation of 21.8 $\mu Mmin^{-1}$. The isolates Bacillus tequilensis ARP2 and Bacillus tequilensis ART2 eliminated from the culture media containing arsenic,

93±0.2% and 77±0.14% of As^{5+} and As^{3+}, respectively, according to quantitative assessment of arsenic (As) using an atomic absorption spectrophotometer. The interaction between arsenic and the cell membrane was revealed by the FTIR analysis, and this finding was confirmed by SEM and Transmission Electron Microscopic (TEM) techniques, which indicated that arsenic accumulation led to a subsequent increase in cell volume. As^{5+} had an observed MIC value of 350 mM for ARP2, whilst As^{3+} resistance of 18 mM was demonstrated by ART2. Their metabolic processes to use arsenic in the redox reactions occurring inside the cell produced their remarkable resistance to high arsenic-concentrations. These two isolates' ability to withstand arsenic showed that they could be used for long-term arsenic (As) bioremediation.[90] Members of the Bacillus species are typically found in habitats polluted by arsenic.[91] The capability of Bacillus species for adaption of a wide range of settings, including those tainted with arsenic, is well recognized. Because of their ability to withstand arsenic, certain species in the Bacillus genus are able to survive in these contaminated environments. Certain bacteria have developed defence mechanisms against arsenic, such as sequestering arsenic within cells or converting arsenate to arsenite, in order to detoxify the metal or survive its harmful effects[91]. This resistance to arsenic contamination can have important ramifications for our understanding of the microbial ecology in polluted habitats and for environmental remediation efforts. Species that have evolved to survive in arsenic concentrations hazardous to other living forms have a high potential for arsenic bioremediation. For the purpose of metabolizing arsenic, they have undergone a number of evolutionary modifications. Since they have been found to possess chromosomal or plasmid-borne arsC genes, which may be able to remove arsenic from contaminated environments and due to this reasonthey have garnered a lot of attention recently in arsenic bioremediation. Overall it has been noticed that arsenic oxidation and degradation are significantly influenced by the prevalence of Bacillus isolates carrying the arsC and aioA genes.[92] These genes are linked to the metabolism of specific bacteria and resistance to arsenic. Arsenate reductase, which is produced by the arsC gene, is an enzyme that converts arsenate (As^{5+}) to arsenite(As^{3+}) which is less harmful and can be taken out of cells more readily. This system aids in the bacteria's ability to withstand high ambient arsenic levels. Corresponding to this, the aioA gene codes for arsenite oxidase, an enzyme that helps detoxify arsenic in aerobic environments by oxidizing arsenite(As^{3+}) to arsenate(As^{5+}). It is well known that some Bacillus species are capable of metabolizing arsenic. These gene-carrying Bacillus isolates have a major impact on the detoxification and cycling of arsenic in a variety of settings, including soil and water. The general destiny and mobility of arsenic in ecosystems can be impacted by their existence and activities. It also indicates their potential for use in upcoming bioremediation procedures.[93-95]

CONCLUSIONS

The removal of arsenic from soil and water can be accomplished through bioremediation, particularly when Bacillus sp. are involved. Bacillus sp. is known for their ability to grow in a range of environments and for possessing characteristics that make them suitable for use in bioremediation procedures. These processes includes oxidation, reduction, and methylation, Bacillus species can change arsenic into less mobile or hazardous forms. As^{3+}can be oxidized by certain Bacillus strains to form arsenate As^{5+}, which is less poisonous and more readily precipitates or immobilizes in soil. Bacillus sp. is capable of absorbing arsenic from their surroundings and can store arsenic inside of their cells, which lowers the arsenic amount in the nearby water or soil. The high efficiency, purity and economy of microorganism-mediated bioremediation make it an innovative and promising method for reducing arsenic (As) contamination. Different enzymes produced by Bacillus sp. can take part in the activities involved in the transformation of arsenic. Arsenate reductase enzymes, for instance, have the ability to change arsenate into arsenite, which makes it easier to remove from the environment. Bacillus sp. may work in concert with other environmental microbes to remove arsenic more effectively. Overall, because of their broad metabolic capacities, environmental adaptability, and genetic modification amenability, Bacillus sp. show tremendous potential for bioremediation of arsenic-contaminated water and soil. The use of biological techniques, in particular bacterial-mediated bioremediation, is an efficient, practical, and green way to clean contaminated soil and water. As their molecular mechanisms are suggestive of the resistance, transport, efflux, and detoxification systems that function in high and low concentrations of heavy metals to maintain and regulate metal homeostasis at the cellular level. More investigation is needed to identify and analyze to unidentified molecular mechanisms of resistance, which can help clarify the function of Bacillus sp. in bioremediation at a far deeper level and enable the effective removal of contaminants, including Arsenic (As).

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest for publication of this work in public domain.

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