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Harnessing Microbial Potential for the Bioremediation of Arsenic-Contaminated Environments

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ABSTRACT

Environmental toxicity is an issue of great concern worldwide and poses a major health threat to mankind. In last decade, the number of research activities concerning the removal of heavy metal contamination has been increasing. Arsenic is an extremely toxic metalloid that severely threatens the biota. Microbial bioremediation process for the removal of arsenic from the contaminants is gaining considerable attention globally due to its environmental compatibility and possible cost-effectiveness. A wide range of microorganisms such as bacteria, fungi, yeasts and algae can act as biologically active methylators, which are able to detoxify arsenic. Although microorganisms cannot destroy metals, they can alter their chemical properties via a surprising array of mechanisms such as biosorption, redox reaction and most importantly volatilization. The ubiquity of arsenic in the environment has led to the evolution in microorganisms of arsenic defence mechanisms based on the presence of the arsenic resistance operon (ars), which codes for a regulatory protein (ArsR), an arsenite permease (ArsB) and an enzyme involved in arsenate reduction (ArsC). The main theme of this chapter is to provide an update on the recent literature concerning the strategies for the remediation of arsenic using microorganisms and to alleviate arsenic pollution.

Key words: Arsenic toxicity, Microorganisms, Detoxification, Biotransformation.

INTRODUCTION

Unabated increments in population and industrialization have caused environmental toxicity and threat not only to the human life but also to the ecosystem. Industrialization and various anthropogenic activities add a significant number of contaminants in the soils. Current researches have enabled to exploit the inherent traits of natural resources to overcome the environmental contamination (Singh et al., 2021).

'Bioremediation' is one of the most effective processes for pollution abatement and this process exploits biological agents to eliminate toxic waste from the environment. At present, bioremediation is an alternative tool that offers the possibility to destroy various

toxicants including heavy metals using microorganisms such as bacteria and fungi. Compared to other methods (encapsulation, vapour extraction, stabilization and electrokinetics), bioremediation (a biological process) is a more promising and less expensive way for cleaning up contaminated soil and water on site under aerobic conditions (Ahemad and Khan, 2011; Kamaludeen *et al.*, 2003). The nature of pollutants, soil structure, temperature, pH, moisture content, hydrogeology, nutritional state, redox-potential and microbial diversity of the site are the most essential parameters required for the bioremediation process (Dua *et al.*, 2002; Zaidi *et al.*, 2009; Xing *et al.*, 2024). Bioremediation activity through microorganisms is accelerated by several factors such as supplementing nutrients (nitrogen and phosphorus), electron acceptors (oxygen), and substrates such as methane, phenol and toluene (Ma *et al.*, 2007; Baldwin *et al.*, 2008). Microorganisms use the contaminants as a source of energy in the degradation mechanism. The majority of rock minerals are rich in vital elements (e.g., P, Fe, S, Mg and Mo), but some may also contain toxic heavy metals or metalloids. At present heavy metal contamination is one of the most important environmental concerns.

Table 1. List of major arsenic minerals occurring in nature (Kushwaha *et al.*, 2025).

Minerals	Composition	Occurrence
Niccolite	NiAs	Vein deposits
Cobaltite	CoAsS	Metamorphic rocks
Arsenopyrite	FeAsS	Mineral veins
Orpiment	As ₂ S ₃	Hot springs
Realgar	AsS	Hot springs, clays and lime stones
Arsenolite	As ₂ O ₃	Secondary mineral formed by oxidation of arsenopyrite, native arsenic and other arsenic minerals
Scorodite	FeAsO ₄ .2H ₂ O	Secondary mineral
Claudetite	As ₂ O ₃	Secondary mineral formed by oxidation of realgar, arsenopyrite and other arsenic minerals
Conichalcite	CaCu(AsO ₄)(OH)	Secondary mineral

Heavy metals are elements that are characterized by relatively high density and high relative atomic weight with an atomic number greater than 20 (Raskin *et al.*, 1994) and exhibit metallic properties such as ductility, malleability, conductivity, cation stability, and ligand specificity.

Some heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, V and Zn are beneficial for the organisms at low quantity but excessive amounts of these elements have detrimental effect. Heavy metals such as Pb, Cd, Hg and As are toxic to organisms and are thus the “main threats” to humankind. Heavy metals may modify soil biological properties and also affect the number, diversity and activities of soil microorganisms. The toxicity of the heavy metals on microorganisms depends on soil temperature, pH, clay minerals, organic matter, inorganic anions and cations, and chemical forms of the metal (Friedlova, 2010; Giller *et al.*, 1998).

Microorganisms have developed unique ways to deal with toxic heavy metals by oxidizing or reducing them to non-toxic or relatively less toxic forms.

ARSENIC: AN OMNIPRESENT ENVIRONMENTAL TOXIC METALLOID

The main reasons for environment contamination are toxic organic and inorganic chemicals. These chemicals pose a major health threat to the human population. Arsenic (As), the 20th most abundant element in the earth crust (National Research Council 1977), is a naturally occurring toxic metalloid and belongs to group V of the periodic table of elements and is thus classified as a heavy metal. More than 300 arsenic containing minerals are found in nature with approximately 60% being arsenates, 20% being sulphides and sulphosalts, 10% being oxides and the rest being arsenites, arsenides and metal alloys. Arsenopyrite (FeAsS) is the most abundant arsenic containing mineral. Table 1 shows the major arsenic mineral occurring in nature (Smedley and Kinniburgh, 2002).



Figure 1. Global concern of arsenic contamination in drinking water (Xing et al., 2024).

Arsenic occurs in nature in four oxidation states (+5, +3, 0, and -3), with pentavalent arsenate [+5, As (V)], and trivalent arsenite [+3, As (III)] being the most common forms. Arsenate is toxic, but adsorbs strongly to many common minerals, limiting its mobility.

On the other hand, Arsenite is relatively mobile in the environment, and is more toxic than arsenate. Arsenic in the atmosphere comes from volcanoes (gas fumes), microorganisms, and by burning fossil fuels. Arsenite can be reduced by microorganisms in soil to the volatile compounds arsine (AsH_3) and methylarsines under anoxic conditions.

Inorganic arsenic compounds are more toxic than organic arsenic compounds. At present contamination of drinking water by arsenic is a global concern that enters into the drinking water from natural and anthropogenic sources (Fig. 1). The permissible limit of arsenic in drinking water is 10 $\mu\text{g/L}$. Exposure to >10-50 $\mu\text{g/L}$ arsenic in drinking water can results into skin, cardiovascular, renal, hematological and respiratory disorders (Smedley and Kinniburgh, 2002).

Unlike other heavy metals, arsenic cannot be destroyed once it has entered the environment (Valls and de Lorenzo, 2002) and cause harmful health effects to humans, animals and other organisms by entering the food chain as organoarsenic compounds (arsenobetaine). Arsenic enters into the body through consumption of food/water and inhalation. Absorption of arsenic through the skin is minimal, and thus, hand washing and bathing with water containing arsenic does not pose threat to humans.

ARSENIC: TOXICITY TOWARDS HUMAN HEALTH

Humans are exposed to arsenic through soil, food, water, and air and can cause irritation of the stomach and intestines, decreased production of red and white blood cells, lung irritation and tumor development. Exposures to high concentrations of inorganic arsenic can also cause several disorders such as brain damage, hypertension, coronary artery disease, peripheral vascular disease, and atherosclerosis (Fig. 2). Exposure to arsenic is also related to type II diabetes (Walton *et al.*, 2004). The formation of strong bonds with functional groups, such as the thiols of cysteine residues and the imidazolium nitrogens of histidine residues from cellular proteins is the key factor for arsenite toxicity. Arsenate toxicity is the result of the mimetic effect of arsenate (AsO_4^{3-}) and phosphate (PO_4^{3-}) (Tseng, 2004).

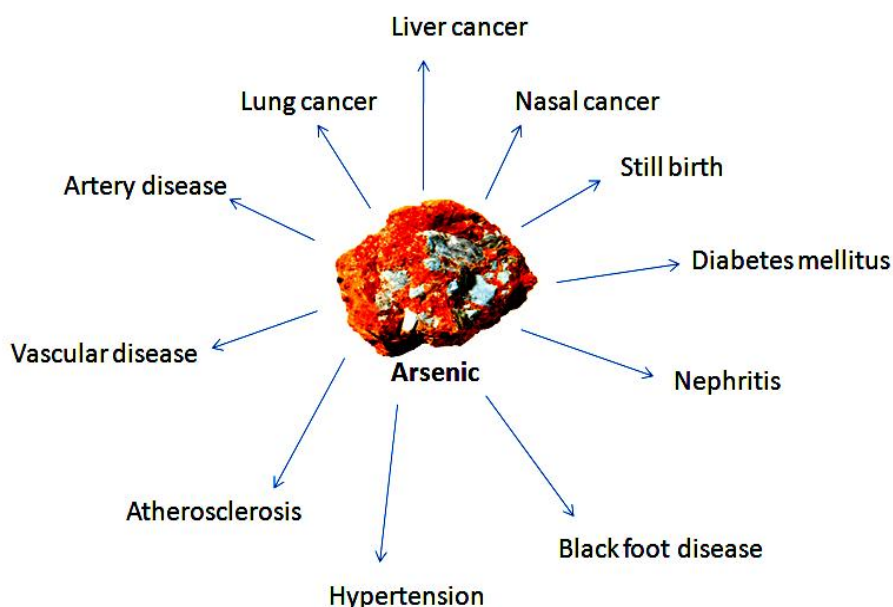


Figure 2. Arsenic toxicity towards human health (Kushwaha *et al.*, 2025).

A significant exposure to arsenic results into adverse effects on human health and are totally dependent on the duration and dose of metalloid exposure. Based upon the toxicity it can be classified as acute toxicity and chronic toxicity. Acute toxicity involves short-term exposure of arsenic and results into nausea, vomiting, profuse watery diarrhoea and diffuse skin rash etc. (Ratnaike, 2013). Continuous exposure to small quantity of arsenic leads to chronic toxicity and causes skin lesions, respiratory symptoms and reproductive disorders (Smith *et al.*, 2000; Milton and Rahman, 2002, Sen and Chaudhuri, 2008). Arsenic shows mutagenic and genotoxic effects on humans, and it has been associated with increased risk of lung, liver and other cancers and other developmental problems (Bodwell *et al.*, 2006; Navas-Acien *et al.*, 2006; Liu and Waalkes, 2008; Prozialeck *et al.*, 2008). There is an increased risk of arsenic toxicity, even at the low dose of 10 ppb (permissible limit of arsenic

set by WHO), leading to several physiological damages to human beings (Prozialeck *et al.*, 2008).

Arsenite binds to sulfhydryl groups and dithiols groups of proteins, whereas arsenate can inhibit oxidative phosphorylation (Ordon *et al.*, 2005). Arsenic causes gene amplification and chromosomal damage at lower doses and can enhance mutagenesis by inhibiting DNA repair (Li and Rossman, 1989). Arsenite may act as carcinogen by causing DNA hypomethylation leading to aberrant gene expression (Zhao *et al.*, 1997). A progressive increase in CpG methylation within the p53 promoter was observed by chronic exposure to arsenite (Mass and Wang, 1997).

ARSENIC: A STEP TOWARDS BIOREMEDIATION

There have been increasing demands of removal of arsenic from contaminated soils and groundwater in order to minimize its detrimental effect on living beings. A plenty of remediation methods have been developed that may achieve complete or partial reduction of arsenic from contaminated area in order to limit the metalloid toxicity towards human health.

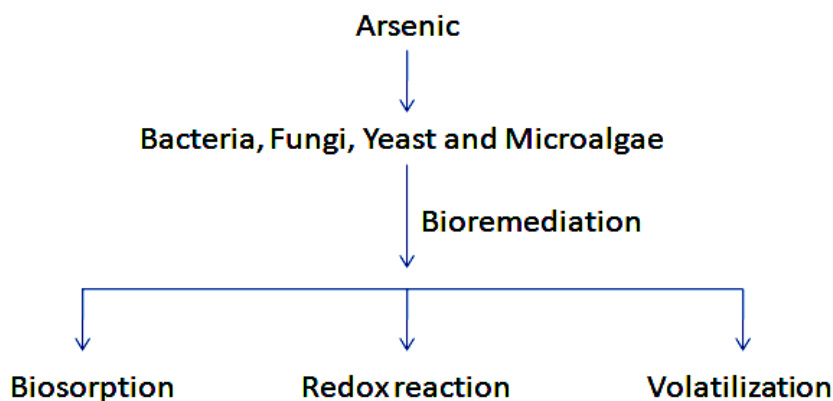


Figure 3. Mechanisms associated with bioremediation of Arsenic by microorganisms (Xing et al., 2024).

Coagulation, filtration, adsorption, activated alumina, ion exchange, reverse osmosis and electro dialysis techniques for arsenic removal are avoidable due to expensive and inappropriate process involved into it. Currently, bioremediation of arsenic using microorganisms has substituted the previous techniques due to the costly and time-consuming processes. Microorganisms have a prominent role in biogeochemical cycle of arsenic and can convert it into less toxic forms by oxidising or reducing it. Microorganisms exposed to the higher concentrations of toxic arsenic may develop various strategies to resist themselves against metalloid stress and can be used as successful bioremediation agents.

At present, bioremediation of arsenic using microorganisms has received immense interest due to its potential, cost effectiveness, and environmentally friendly property. Several microbial strains of bacteria and fungus have been employed in the field of bioremediation of arsenic for last few years (Kushwaha et al., 2025; Xing et al., 2024). Microorganisms have evolved several strategies in order to tolerate the uptake of arsenic which could be utilized as attractive approaches for removal of arsenic from contaminated sites.

The adaptive mechanism involves efflux of heavy metal ions outside the cell, accumulation of the metal ions inside the microbial cell and oxidation-reduction of the metal ions to a less toxic state (Nies, 1999). Arsenic-resistant microbes utilize arsenic either as a means of generating energy (Xing et al., 2024; Santini *et al.*, 2000) or using arsenate as a terminal electron acceptor in an aerobic respiration (Stolz and Oremland, 1999). Microbes transform inorganic arsenic compounds by a detoxification process, biomethylation into monomethylarsonic acid (MMA), dimethylarsenic acid (DMMA), trimethylarsine (TMA) and trimethylarsine-oxide (TMAO) (Ridley *et al.*, 1977; Woolson, 1977, Cullen and Reimer, 1989; Gadd, 1993). The bioremediation of arsenic includes three major approaches as discussed below (Fig. 3).

Biosorption

Microorganisms have potential to accumulate (bioaccumulation) arsenic from arsenic containing substrate by biosorption process. The process of bioaccumulation of arsenic in microbial cells (Mahimairaja *et al.*, 2005) is influenced by soil pH, moisture, aeration, temperature, concentration and speciation of arsenic, soil amendments, and rhizosphere. Non-genetically engineered arsenic accumulating bacteria *Marinomonas communis* was isolated from marine and non-marine environment in Japan with high removal efficiency of this metalloid (Takeuchi *et al.*, 2001). The cellular accumulation of arsenic could be due to higher uptake and lower efflux by a regulatory protein of the *ars* operon that has a specific binding site for arsenite (Ghodsi *et al.*, 2011).

Biosorption is an alternative method for the complete or partial removal of pentavalent arsenic from the contaminants using Gram-negative bacteria (Loukidou *et al.*, 2001). Visoottiviseth and Panviroj (2001) also aimed to remove toxic arsenic compounds by introducing fungal strains isolated from arsenic polluted areas in Ron Phibum District, Nakhonsi Thammarat Province, Thailand and found that the fungal isolate PRMT2-401 was proved to be the most effective arsenic removing agent. Arsenic removal is also influenced by the age of the culture and cell viability. Dead cells are unable to remove arsenic (Visoottiviseth and Panviroj, 2001). Hossain and Anantharaman (2006) reported that *Bacillus subtilis* had potential to absorb arsenic (III) from aqueous solution.

On the other hand, Pokhrel and Viraraghavan (2006) exploited *Aspergillus niger* (coated with iron oxide) for 95% removal of As (V) and 75% removal of As (III) from aqueous solution. Cernansky *et al.* (2007) used filamentous fungus *Aspergillus clavatus* DESM for biosorption of arsenic (ranging from 0.25 to 100 mg/L) from aqueous solutions by Freundlich equilibrium sorption model. *Stenotrophomonas maltophilia* SAAnt 15 and *Serratia marcescens* SAAnt 16 were able to grow in the presence of arsenite and arsenate. *Stenotrophomonas maltophilia* SAAnt15 was resistant to 10 mmol/L arsenite and 20 mmol/L arsenate, whereas *Serratia marcescens* SAAnt 16 grew in 15 mmol/L arsenite and upto 500 mmol/L arsenate (Botes *et al.*, 2007). The higher arsenate tolerance of bacteria may be due to the significant reduction of arsenate to arsenite (Kushwaha et al., 2025). According to Cernasky *et al.* (2007), *Neosartorya fischeri* was the potential heat-resistant fungus for the biosorption of arsenic. In another study, *Streptococcus* and *Xanthomonas* genus isolated from Ronphibun District, appeared to be novel arsenic absorbing bacteria (Aksornchu *et al.*, 2008). Littera *et al.* (2011) reported two fungal strains namely: *Aspergillus niger* and *Neosartorya fischeri* for their biosorption capacity of As (V) ranging from 0.2 to 5.0 mg/L at two different pH values (pH 5 and pH 7). The biosorption of arsenic increased linearly with increasing arsenic concentration. Treatment with FeCl₃ and HCl did not show any significant

enhancement in arsenic biosorption, but treatment with ferric oxyhydroxide was found to be the most potent (Littera *et al.* 2011). Kamsonlian *et al.* (2012) reported efficient biosorption of Arsenic (III) using palm bark (PB) biomass under various experimental parameters.

Despite of bioaccumulation ability of arsenic by bacterial and fungal species, some algal species (*Fucus gardneri* and *Chlorella vulgaris*) are also known to bioaccumulate (Granchinho *et al.*, 2001; Maeda *et al.*, 1985) this metalloid.

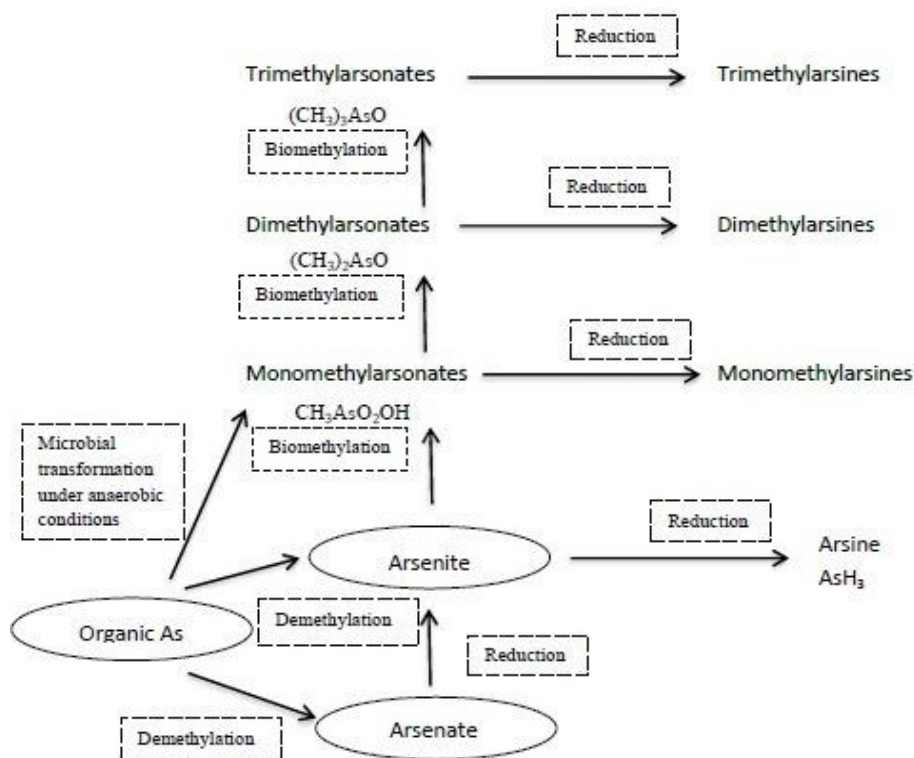


Figure 4. Arsenic volatilization pathway (Singh *et al.*, 2021).

Microbial-mediated redox reaction

Arsenic-resistant bacteria have the potential to grow chemolithotrophically with oxygen as an electron acceptor and As (III) as an electron donor (Santini *et al.*, 2000; Duquesne *et al.*, 2001). Bacteria obtain metabolic energy from As (III) oxidation (Ilyaldinov and Abdrashitova, 1981). *Bacillus* sp., *Pseudomonas* sp. (Frankenberger and Losi, 1995), *Alcaligenes faecalis* (Phillips and Taylor, 1976) and *Alcaligenes* sp. (Osborne and Ehrlich, 1976) were capable of oxidizing As (III) to As (V). Gihring *et al.* (2001) reported that *Thermus aquaticus* and *Thermus thermophilus* had ability to oxidize As (III) to As (V). *Sulfurospirillum barnesii*, *S. arsenophilum*, *Desulfotomaculum auripigmentum*, *Bacillus asoselenatis*, *B. selenitireducens*, *Crysiogenes arsenatis*, *Sphingomonas* sp., *Pseudomonas* sp. and *Wolinella* sp. (Ahmann *et al.*, 1994; Lovley and Coates, 1997; Newman *et al.*, 1998; Stolz and Oremland, 1999) use As (V) as a terminal electron acceptor in an aerobic respiration. Microorganisms possess As (V) reduction mechanisms that impart into resistant towards arsenic toxicity. In addition, *arsC* enzyme present in the cytoplasm of *Escherichia coli* and *Staphylococcus aureus* reduces As (V) to As (III) for its rapid release from the cell, that results into development of resistance towards arsenic (Xing *et al.*, 2024; Diorio *et al.*, 1995).

Volatilization

Volatilization is the process in which certain species of microorganism such as fungi and bacteria methylate inorganic arsenic species to form methylarsenicals.

Microorganisms are able to biomethylate inorganic arsenic species to monomethylarsine (MMA) and dimethylarsine (DMA) (Ridley *et al.*, 1977; Woolson, 1977; Gadd, 1993) and these compounds are susceptible for volatilization due to their low boiling point and/or high vapour pressure. Methylarsenicals are released from the microorganisms as a gaseous product. *Methanobacterium bryantii* was exploited first time for the conversion of As (V) to small amounts of volatile methylarsines (McBride *et al.*, 1971).

Clostridium collagenovorans, *Desulfovibrio vulgaris* and *D. gigas* were also utilized in the formation of methylarsines (Michalke *et al.*, 2000). *Achromobacter* sp. and *Enterobacter* sp. form monomethylarsine and dimethylarsine as by-products during the conversion process of As (V). On the other hand, *Aeromonas* sp. and *Nocardia* sp. form monomethylarsine, dimethylarsine and trimethylarsine (TMA) by the conversion of As (V) (Woolson, 1977). *Escherichia coli*, which is common in the intestines of humans and other animals, can form volatile trimethylarsine (Frankenberger and Arshad, 2002). Species of *Penicillium*, *Aspergillus* and *Pseudomonas* are also capable of volatilizing inorganic arsenic. This should be noted that the substrates acted upon by the microorganisms, as well as the volatile products released, typically depends upon the nature of the microorganisms and vary from organism to organism. Fig. 4 shows arsenic volatilization pathway modified from Frankenberger and Arshad (2002).

Thus, arsenic volatilizing organisms offer the possibility to stimulate bioremediation of arsenic contaminants. Studies have reported the rate of arsenic volatilization to be only 10% of total arsenic content (Hassler, 1984). The large discrepancy in the rate of volatilization is most attributable to varying soil conditions and differing microbial communities.

Factors affecting volatilization mechanism

Soil pH, moisture, temperature, organic matter and heavy metals have significant influence on the rate of volatilization by promoting or inhibiting microbial growth.

Temperature has a large impact on volatilization. The optimal temperature for volatilization in *Penicillium* was 20°C (Frankenberger and Arshad, 2002) and it established that the optimal temperature for volatilization will correspond to the incubation temperature that favours the growth of organism, as enzymatic rates are maximum under these conditions.

Environmental pH is also an important parameter for the rate of volatilization. Acidic pH favours the volatilization process (Baker *et al.*, 1983). Arsenic species are generally more mobile under acidic conditions, making them more readily available to volatilizing organisms (Cox and Alexander, 1973).

The presence of heavy metals in soil can have a drastic effect on the rate of volatilization. For example, chromium concentrations of ~100 µM inhibited the volatilization process, whereas similar iron concentrations have been shown to stimulate it (Frankenberger and Arshad, 2002).

The organic material promotes volatilization process because it could recycle organic wastes and improves the quality of soil. Soils with ~11% organic matter favoured the highest rates of arsenic volatilization (Akins and Lewis, 1976). Additions of carbohydrates always lowered volatilization rates, while the supplementation of amino acids had positive or negative

effects on volatilization depending on the specific amino acid supplied (Huysmans and Frankenberger, 1990).

Soil moisture level has a distinct impact on volatilization process as well. Soil samples with moisture levels more than 250g H₂O kg⁻¹ soil led to a decrease in volatilization rates. Dry soils do not promote the volatilization mechanism (Gao and Burau, 1997).

Apart from above mentioned factors, Phosphorus has a more complex effect on volatilization process. Phosphorous, an important constituent for microbial growth when added to colonies of volatilizing bacteria, inhibits the formation of trimethylarsine (Xing et al., 2024, Cox and Alexander, 1973) and was explained by the fact that arsenic and phosphorous are chemical analogs. It is probable that arsenic species enter cells through the phosphate transport system, leading to competition between the uptake of phosphorus and arsenic (Kertulis et al., 2004).

Arsenic substrate also contributes in promoting the rates of volatilization. Each volatilizing organism has its specific substrate it is capable of transforming. In general, organic arsenicals favour the higher rate of volatilization (Frankenberger and Arshad, 2002).

Volatilization can occur under both aerobic and anaerobic environments. *Penicillium* exhibits higher rate of volatilization under anaerobic conditions (Frankenberger and Arshad, 2002). This is due to the reason that As (III) is more plentiful under reduced conditions.

ARSENIC DETOXIFICATION: A MOLECULAR APPROACH

As (V) reduction to As (III) by arsenate reductase occurs in many organisms, including Archaea and Bacteria. Dissimilatory arsenate reductases from bacteria are heterodimeric proteins. They are located either at the periplasm or membrane-associated in bacteria such as *Chrysiogenes arsenatis*, *Bacillus selenitireducens*, and *Shewanella* sp. (Silver and Phung, 2005).

Arsenate reductase genes (*arr*) have been well-characterized in *Shewanella*; the *arr* genes conform an operon that is chromosomally joined to the *ars* operon (detoxification) (Saltikov and Newman, 2003). Organisms take up As (V) via phosphate transporters and As (III) by glyceroporin membrane proteins (Mukhopadhyay et al., 2002) or hexose transporters (Liu et al., 2004). As (V) is then reduced to As (III), which is either extruded from cells or sequestered in intracellular compartments, either as free arsenite or as conjugates with glutathione (GSH) or other thiols. Detoxification-based As (V) reduction has been exploited in *E. coli*, and has also been documented for other group of bacteria (Silver and Phung, 1996; Silver and Phung, 2005). As (III) is the inducer that triggers up-regulation of the *ars* operon and it is also a specific substrate for an efflux pump (Fig. 5). Gram-positive and Gram-negative bacteria are found to be resistant to both arsenite and arsenate (Kushwaha et al., 2025). This is in the form of an *ars* operon consisting of *arsR* (determining the regulatory repressor), *arsB* (encoding the membrane arsenite permease pump), and *arsC* (encoding an intracellular arsenate reductase) gene in the order *arsRBC*. The *ars* operon occurs more widely in those bacterial genomes with over 1000 or 2000 genes. The ArsR regulatory protein and ArsB efflux pump protein provide resistance to arsenite, and the *ars* operon confers resistance to arsenite and arsenate. ArsA is an intracellular ATPase protein that binds as a dimer to the membrane ArsB protein.

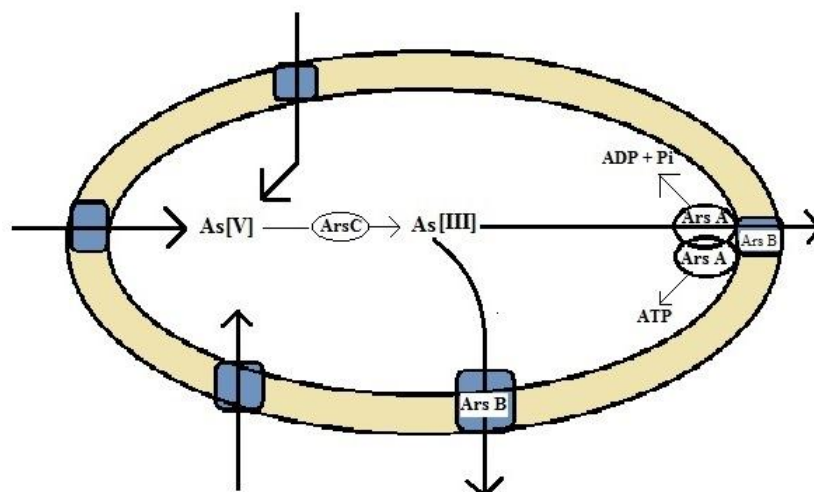


Figure 5. Detoxification mechanism of Arsenic in prokaryotic organisms (Yin et al., 2022).

The arsenite membrane efflux pump functions either chemiosmotically with ArsB alone or as an ATPase (with the ArsAB complex). ArsD is thought to be a *trans*-acting corepressor of the *arsRDABC* operon, in addition to ArsR (Silver and Phung, 1996). In *E. coli* plasmid R733, the *ars* operon comprises five genes, but in the *Staphylococcus* plasmid pl258, the *ars* operon contains only three genes (Silver and Phung, 1996).

Members of the genera *Corynebacterium* include both pathogens (such as *C. diphtheriae*) and many non-pathogenic members that have vast biotechnological applications such as production of amino acids. Members of the coryneform group were much more resistant to arsenic.

The genes involved in arsenic resistance in *C. glutamicum* form two operons, *ars1* and *ars2*, distant from each other on the chromosome (Ordonez *et al.*, 2005). *ars1* and *ars2* have a similar genetic structure that is based on the three-component system (*arsRBC*). The first genes of the *ars1* and *ars2* operons are *arsR1* and *arsR2*, which encode putative arsenite regulatory proteins. The second genes, *arsB1* and *arsB2* encode the arsenite efflux pump (arsenite permease). The rest of the genes encode putative thioredoxin-dependent arsenate reductases with predicted protein tyrosine phosphatase activity. In addition to this, arsenite permease (*arsB3*) gene and arsenate reductase (*arsC4*) gene are responsible for arsenic resistance in *C. glutamicum* genome. The involvement of the arsenite permease genes *arsB1*, *arsB2*, and *arsB3* in arsenic resistance in *C. glutamicum* was confirmed using gene-disruption techniques. The resistance levels suggested that resistance to arsenite in *C. glutamicum* is due to the activity of operons *ars1* and *ars2*, operon *ars1* being more crucial than *ars2*. *C. glutamicum* grown in a medium supplemented with a low level of phosphate increases As (V) uptake (Mateos *et al.*, 2006). Fig. 6 describes the gene organization of the *ars* operons from different bacterial species.

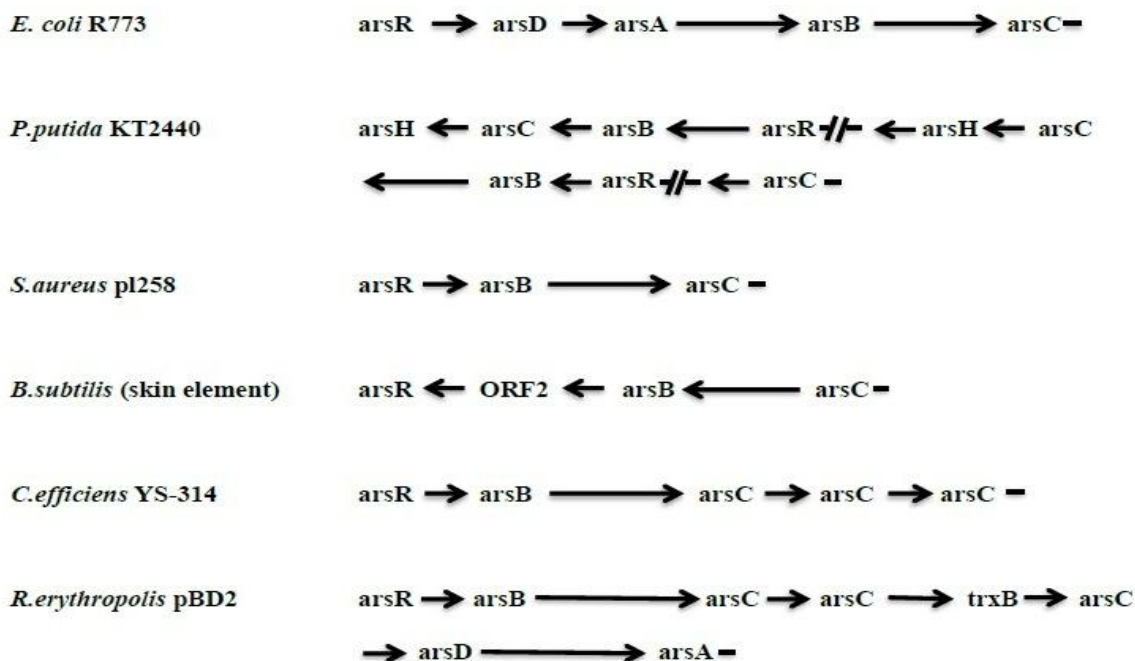


Figure 6. Genes involved in arsenic resistance in different bacteria. Arrows represent Open Reading Frames (ORFs) found in the sequenced DNAs (modified from Mateos *et al.*, 2006; (Yin *et al.*, 2022).

Arsenic methylation is also associated with detoxification. Interestingly, microbial methylation of arsenic compounds is very similar to the process carried out in animal tissues. S-adenosylmethionine is the methyl group donor in eukaryotic cells and the methylation product is dimethylarsinic acid (cacodylic acid).

On the other hand, S-adenosylmethionine (Qin *et al.*, 2006) and methyl-cobalamin (Bentley and Chasteen, 2002) are the methyl donors in the prokaryotic cells. Methyltransferase acts as a catalyst during methylation process, Methylated forms of arsenic are more toxic than the unmethylated form for some microbes (Stolz *et al.*, 2006).

ROLE OF MICROORGANISMS IN ARSENIC REMOVAL

Currently, the frequent occurrence of arsenic as an impurity in many metal-rich sulphide deposits and drinking water source is problematic. It results into a number of difficulties such as metallurgical problems and environmental issues. Microorganisms play a tremendous role in arsenic removal from two major arsenic contaminated sites as discussed below (Yin *et al.*, 2022).

Bio-oxidation of arsenic-bearing minerals

Bio-oxidation is a process where the desired metal remains in the rock, but the mineral structure is converted to allow better penetration of chemicals that can solubilise it. Bio-oxidation is a time consuming and less expensive process but it also generates potentially toxic components. *Acidithiobacillus ferrooxidans* were used for the partial removal of arsenic (occurring in the form of FeAsS) from galena (PbS) concentrate (Yin *et al.*, 2022; Makita *et al.*, 2004) and formed an anglesite (PbSO₄) concentrate. The rest of the bio-oxidized arsenic was precipitated as amorphous compounds.

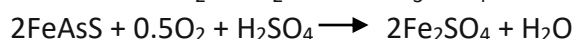
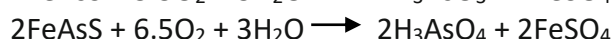
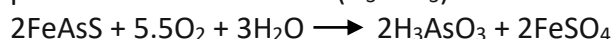
Treatment of Arsenic-contaminated water

Drinking (ground and surface) water sources are becoming polluted due to the release of arsenic from minerals during mining operations that result into major environmental issue. Microbiological methods for arsenite oxidation in arsenic-contaminated wastewaters are alternative economically viable process. Microorganisms that promote the reduction of arsenate, play a significant role in precipitation processes. *Desulfomicrobium* Ben-RB and *Desulfotomaculum auripigmentum* can concomitantly reduce As (V) to As (III) which leads to the formation of both intracellular and extracellular arsenic trisulfide precipitates (Newman *et al.*, 1997). The sulphate-reducing bacteria (SRB) are also potential agents for the bioremediation of arsenic by oxidizing simple organic compounds and utilizing sulphate as an electron acceptor, resulting into the sequestration of arsenic. Similar results were observed by Teclu *et al.* (2008) who also showed that SRB cells can remove significant quantities of As (III) and As (V) by biosorption process. Luo *et al.* (2008) used an ethanol-fed sulphate reducing bioreactor system to remove arsenic from neutral to alkaline mine wastewater. Battaglia-Brunet *et al.* (2006) reported the process of arsenic removal from mine drainage water by utilizing indigenous bacteria under both aerobic and anaerobic conditions and established a treatment process for the discharge effluent of mine waters.

PATHWAY TOWARDS THE OXIDATION OF REDUCED MINERALS

Chemolithoautotrophic microorganisms have evolved a metabolic pathway for the oxidation of reduced minerals such as arsenopyrite in order to produce ATP. These microbes promote the dissolution of arsenic-bearing minerals by oxidation of associated iron or sulphur (or both) and release a range of compounds of varying arsenic oxidation state (Yin *et al.*, 2022). *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* are the most effective mesophilic microorganisms responsible for the bio-oxidation of arsenic mineral (Singh *et al.*, 2021; Hackl and Jones, 1997).

The oxidation of arsenopyrite (FeAsS) occurs in the presence of oxygen, which results in the production of arseneous (H₃AsO₃) and arsenic acids (H₃AsO₄).



Strain WAO (closely related to *Bosea thiooxidans*) could mediate oxidation of sulphide in the pyrite lattice at circumneutral pH under aerobic environment, leading to the release of pyrite-bound arsenic. Arsenopyrite (FeAsS) oxidation by strain WAO results in complete oxidation of arsenic to arsenate (Rhine *et al.*, 2008). For most chemolithoautotrophic bacteria, the oxidation of arsenite is coupled to the respiratory electron transport chain and the reduction of oxygen (Battaglia-Brunet *et al.*, 2006; Drewniak *et al.*, 2008). Chlorate compounds may represent an alternative electron acceptor supporting microbial oxidation of arsenite. *Dechloromonas* sp. strain ECC1-pb1 and *Azospira* sp. strain ECC1-pb2, could grow by linking the oxidation of As (III) to As (V) with the reduction of chlorate compound (Sun *et al.*, 2010). Transformation of As (III) to the less toxic form As (V) by microorganisms is also mediated by heterotrophic arsenite oxidizers. The process utilizes an organic carbon source for cell growth, not to acquire energy (despite arsenite oxidation is an exothermic reaction).

Table 2. Bioremediation of Arsenic from microorganisms of diverse sources.

S. No.	Microorganisms	References
1.	<i>Bacillus</i> sp. and <i>Aneurinibacillus aneurinilyticus</i>	Deya <i>et al.</i> , 2016
2.	<i>Brevibacillus reuszeri</i> and <i>Rhodococcus</i> sp.	Neeratanaphan <i>et al.</i> , 2015
3.	<i>Rhodopseudomonas palustris</i>	Zhao <i>et al.</i> , 2015
4.	<i>Enterobacter</i> sp., <i>Klebsiella pneumonia</i>	Abbas <i>et al.</i> , 2014
5.	<i>Enterobacter asburiae</i> and <i>Enterobacter cloacae</i>	Selvi <i>et al.</i> , 2014
6.	<i>Acinetobacter brisouii</i> , <i>Pseudomonas abietaniphila</i> , <i>Exiguobacterium aestuarii</i> , and <i>Planococcus rifietoensis</i>	Bhakta <i>et al.</i> , 2014
7.	<i>Actinobacteria</i> , <i>Microbacterium</i> , <i>Pseudomonas</i> and <i>Rhizobium</i>	Paul <i>et al.</i> , 2014
8.	<i>Brevibacillus</i> sp.	Mallick <i>et al.</i> , 2014
9.	<i>Gammaproteobacteria</i>	Pous <i>et al.</i> , 2015
10.	<i>Actinobacteria</i> , <i>Firmicutes</i> , <i>Nitrospirae</i> , <i>Tenericutes</i> and <i>Gemmatimonadetes</i>	Luo <i>et al.</i> , 2014

RECENT ADVANCES IN THE BIOREMEDIATION OF ARSENIC

In spite of toxicity of arsenic, certain microorganisms can use these metalloids for their respiration and different oxidation reactions. Several microorganisms especially bacteria have been employed in the field of bioremediation of arsenic in last decade (Kushwaha *et al.*, 2025; Yin *et al.*, 2022). The genetic capacity of isolates can be exploited for the analysis of heavy metal remediation (Khusro *et al.*, 2014). Table 2 shows current trends of arsenic removal using microorganisms.

CONCLUSION

Arsenic (As) is a naturally occurring ubiquitous toxic metalloid which is introduced into the environment through natural geochemical and anthropogenic activities. Many studies have been investigated in last few years in order to reduce arsenic toxicity from the surrounding environment. Considerable effort has also been made to develop efficient and cost-effective technologies in order to remove arsenic from contaminants.

The potential removal of arsenic from solutions through bioremediation process has been shown to be an interesting approach towards the metalloid detoxification. In addition, the bioremediation and detoxification of metalloid is dependent on various factors. The bioremediation and biodeterioration of arsenic using microorganisms have been taken into account in last few years due to easy availability and large cultivation of microorganism of interest. Microorganisms have potential ability to bind and detoxify arsenic through various mechanisms resulting changes in solubility and toxicity of various arsenic species.

A better understanding of arsenic detoxification pathway will help researchers to deal with the biotransformation of arsenic into less toxic forms with maximum efficiency. Thus,

further investigations need to be carried out in order to remove arsenic from the contaminants by focussing on the development of clean arsenic-free environment.

The isolation of arsenic resistant microorganisms from diverse sources and the understanding of the mechanism behind its detoxification may contribute to the development of improved bioremediation processes.

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