CHAPTER 2

Review of Literature
REVIEW OF LITERATURE

Arsenic (As) toxicity is termed as the worst environmental catastrophe in human history by World Health Organization. It is widely distributed in nature and its presence in both drinking and groundwater has roused great concern. Transport of toxic As from water and soil to the plant increases As content in different parts of the edible food crops which eventually causes serious health hazards among human and ruminants. Thus remediation of As is necessary for human welfare.

2.1 Arsenic in the environment

2.1.1 Arsenic in rocks and minerals

Arsenic is enlisted as the most hazardous pollutants by the Environmental Protection Agency and consider as a potential threat to the human health (Brown and Ross, 2002). It is a toxic trace element for soil, water, plant, animal, and human. Arsenic input to the environment either through natural processes of weathering of As-bearing rocks or use of As-contaminated groundwater for irrigation and anthropogenic activities, such as mining operations, smelting of base metal ores, combustion of coal, and application of agricultural pesticides, are widely reported (Sanyal and Nasar, 2002).

Arsenic makes entry into terrestrial and aquatic environments through natural geological processes (geogenic) and human (anthropogenic) activities. The natural pool of As in surface soils is developed through net geological, hydrological and soil-forming bio-geo-chemical processes. Generally, the nature of soil As is controlled by the lithology of the parent rock materials, volcanic activity, weathering history, transport, sorption, biological activity and precipitation (Kabata-Pendias and Adriano, 1995).

Contamination of ground water with As influence on soil-plant-animal-human continuum have been reported from various parts of the world at different points of time (Dhillon and Dhillon, 2003; Mukhopadhyay and Sanyal, 2004).

A high As level in groundwater are not necessarily related to areas of high As concentrations in the source rocks or sediments. Distinctive groundwater As problems occur in both reducing and oxidising conditions (Smedley and Kinniburgh, 2002). A number of As
species exist in the environment with different valence states, which play an important role for their behaviour and toxicity in the aqueous system (Jain and Ali, 2000).

According to Dermatas et al. (2004), inorganic As (AsIII &AsV) is highly toxic to plants because it uncouples phosphorylation and inhibits phosphate uptake. At higher concentrations, As interferes with plant metabolic processes and inhibits growth. Under severe conditions it may lead to plant death.

The input of As to soil from various sources becomes detrimental to plant growth through its uptake to the toxic limit, thereby facilitating its entry into the food chain. There is also possibility of biomagnification of toxin as it travels up in the food web (Sanyal and Nasar, 2002; Ghosh et al., 2004).

![Arsenic dynamics in contaminated soil and aquatic ecosystems](image)

**Fig 2.1 Arsenic dynamics in contaminated soil and aquatic ecosystems**

**2.1.2 Arsenic in drinking and ground water**

Arsenic contamination of surface and ground water occurs worldwide and several million people are at risk from As contaminated drinking water in West Bengal (Chakraborti et al., 2002). Arsenic is a known carcinogenic metalloid and as per World Health Organization (WHO) it is highly toxic for humans when its concentration in drinking water exceeds 10 µg L⁻¹; however, for India and Bangladesh the permissible limit has been fixed as 50 µg L⁻¹ (Huq et al., 2006).
The origin of As contaminated groundwater in West Bengal, India and Bangladesh is geological (Smedley and Kinniburgh, 2002). Arsenate (AsV) is the dominant form of As in drinking water and its concentration in surface and ground water depend on daily and seasonal variations in raw water input (McNeill et al., 2002). Worldwide, the four major incidents of groundwater As contamination were in Asia: Bangladesh; West Bengal (India); Inner Mongolia (China); and Taiwan (Chowdhury et al., 2000; Ghosh et al., 2004).

Arsenic may accumulate in groundwater through oxidation of arsenopyrites in aquifer sediments as atmospheric oxygen invades the aquifer due to lowering of groundwater level by its large-scale lifting for agricultural irrigation, especially for cultivation of summer (boro) paddy when the groundwater recharge is at its minimum (Sanyal and Dhillon, 2005).

Drinking of As contaminated tube well water has become a serious health threat in Bangladesh (Paul and De, 2000). Arsenic contaminated tube wells are believed to be responsible for poisoning nearly two third of the country’s population (Feltz et al., 1991) observed an elevated concentration of As in irrigation water and bottom sediments in western United States.

Groundwater As contamination is particularly serious in Bangladesh and West Bengal, India (Chowdhury et al., 2001). About a third of the land area of West Bengal, more than 90% of the tested shallow tube wells exceed the WHO guideline value (Chakraborti et al., 2001). The aquifer water of the Lower Gangetic Plain (LGP) of West Bengal used for irrigation purpose has the total As content in the range of 0.1 to 0.35 mg L⁻¹, which is 23 times higher than the WHO permissible limit (Sarkar et al., 2012).

Till date limited attention has been paid to the risk of using contaminated groundwater for irrigation. Irrigation water with high levels of As may result in land degradation and food safety (Duxbery and Zavala, 2005; Brammer and Ravenscroft, 2009). Such findings are important and a fixed source of As contamination for drinking water may become a diffuse and uncertain source of contamination when As finds its way into the food web (Sanyal and Nasar, 2002). This contamination exposes at least 100 million people to the risk of cancer and other As related diseases (Guha-Mazumder, 2008).

The contamination of drinking water aquifers in Bangladesh and West Bengal, India, has now become the world’s largest As health problem as it potentially affects millions of people (Anawar et al., 2002; Roychowdhury et al., 2002; Chakraborti et al., 2001; Smith et al., 2001; Chowdhury et al., 2000).

Out of 20 countries where groundwater As concentration and human suffering there from have been reported so far, the magnitude is considered to be the highest in Bangladesh,
followed by West Bengal, India (Chowdhury et al., 2000; Chakraborti et al., 2001; Roychowdhury et al., 2002). An estimated population of 25 million of Bangladesh was exposed to As concentrations of more than 0.05 mg L⁻¹ and the number would be approximately doubled if WHO limit of 0.01 mg L⁻¹ were considered (School of Environmental Studies and Dhaka Community, 2000).

Fig 2.2 Groundwater arsenic contamination status in West Bengal
(http://www.soesju.org)

The widespread As contamination in many parts of the groundwater aquifers of nine districts (Malda, Murshidabad, Nadia, North and South 24 Parganas, Burdwan, Hoogly,
Howrah and Kolkata) are reported to contain As exceeding the WHO earmarked safe limit of 0.05 mg L\(^{-1}\) (Ghosh, 2005). Mandal et al. (1999) reported that a large number of people of West Bengal located pre-dominantly in seven districts adjoining the river Bhagirathi, as well as the contiguous districts in Bangladesh are affected by widespread As contamination in ground water and the problem seems to be triggered off by large scale withdrawal of ground water for agricultural irrigation.

Sanyal and Nasar (2002) expressed concern over the wide spread As contamination in ground water in different parts of West Bengal as well as the contiguous districts in Bangladesh. Presently the contamination in West Bengal is found spreading over 111 blocks of 12 districts, (http://www.soesju.org/arsenic/wb.htm) of which nine districts are severely affected (area 38,861sq. km. and population 50.4 million). Arsenic loading of the ground water which is used as irrigation source varied from 0.06 to 0.53 mg L\(^{-1}\) in Nonaghata mouza of Haringhata block of Nadia district in West Bengal (ICAR, 2003) where the present study has been carried out.

A high degree of such contamination was also found in the affected area of Gotera and Ghentugachi mouzas of Chakdah block of Nadia district (ICAR, 2003) (ranging from trace to 0.89 mg L\(^{-1}\)).

2.1.3 Arsenic in soil environment

As stated earlier, presence of As in environmental materials may be of both natural and anthropogenic origin. In soils, the native As content varies widely, and is often governed by the geological history of the parent materials. In case of alluvial soils, the nature of the sediment load, deposited by flowing rivers, is also of particular importance in this regard. In addition, the use of arsenicals as pesticides in agriculture, and a number of human activities (such as use of fertilizers, emissions from non-ferrous smelters, coal-fired and geothermal plants and the disposal of industrial and municipal wastes, etc.) contribute towards As input to soils (Sanyal, 1999). Huang (1994) identified, other secondary factors such as climate (e.g. rainfall, relative humidity), organic and inorganic components of soils and redox potential status as factors affecting As level in soils. Soils derived from shales and granites showed elevated As concentration of upto 250 mg kg\(^{-1}\) (Colbourn et al., 1975), while the Australian soils, originating from quartzite, contained 100 to 200 mg of As kg\(^{-1}\) (Fergus, 1955). Typical amounts of As in natural uncontaminated soils varied from 5.00 to 6.00 mg kg\(^{-1}\) in Austria.
(Aichberger and Hofer, 1989), while that in the Belgian soils was 10.0 mg kg⁻¹ (Cottenie and Verloo, 1984).

Arsenic has four oxidation states: −III, 0, +III, and +V, the last two being the most common in the terrestrial environment. AsV is the predominant species in aerobic soils, whereas AsIII predominates in anaerobic environments such as submerged soils. Interconversion between these two As species is driven by both biotic and abiotic processes and strongly influenced by the redox potential and pH. Both AsV-reducing and AsIII-oxidising bacteria are present in soil. The bioavailability of As is more enhanced to rice plants grown under submerged conditions than to those grown under aerobic conditions (Xu et al., 2008; Li et al., 2009). AsIII concentrations as high as 20 μM have been reported in the soil solutions from paddy fields contaminated by irrigation of As-laden groundwater, causing As toxicity and yield losses in rice (Panaullah et al., 2009). Methylated As compounds, such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (TMAO) are found in some soils, sometimes as a minor component (Huang et al., 2006), but can reach high concentrations (Abedin et al., 2002).

Research studies of West Bengal conducted at the selected affected areas, revealed that the total and Olsen extractable (i.e., 0.5M NaHCO₃, pH 8.5– extractable As which constitutes the soil As pool amenable to plant uptake) As varied from 8.4 mg kg⁻¹ to 24.3 mg kg⁻¹ and from 2.90 mg kg⁻¹ to 15.8 mg kg⁻¹, respectively (ICAR, 2001; ICAR, 2003; SWID, 2003). The soil of Nonaghata village, in particular, under Haringhata block was heavily infested. It contained 11.3 – 14.7 mg kg⁻¹ of total As and 3.84 – 3.79 mg kg⁻¹ extractable As (Ghosh et al. 2004) and 4.7 – 16.6 mg kg⁻¹ total As, 0.74 – 2.98 mg kg⁻¹ extractable As in low land rice field (Biswaas and Kole, 2012). The soil As contents of these areas were often higher than those reported for the soils of several other countries like Argentina, China, Italy, Mexico, France, Australia, (0.01-62.6 mg kg⁻¹) (Sanyal and Nasar, 2002).

2.2 Arsenic in food chain

Accumulation of As in rice is recognized as a new disaster for South-East Asia where rice is the major cultivated crop and used as a staple food (Meharg, 2004). The entry of As into human body through contaminated drinking water has received much attention in India and abroad. The food crops, irrigated with contaminated water are also a vital source of As toxicity. But, this issue has only been recognised in recent years (Sanyal and Nasar, 2002; Meharg and Rahaman, 2003; William et al., 2005; Meharg et al., 2009).
Application of contaminated water in agricultural purpose made the soil a secondary source of As pollution. Arsenic availability in soil solution depends on redox potential, pH and microbial activities (Mahimairaja et al., 2005). In case of arable crops, As is adsorbed by iron hydroxides and becomes largely unavailable to plants. In contrast, because of its specific physiological behaviour clubbed with reduced soil environment make rice higher accumulator of As (Williams et al., 2007).

Besides drinking water, food and inhaled particulates share notable amount of As intake in human (Mondal et al., 2010). In West Bengal, rice is a staple food and it uptakes 10 folds higher As over that in other cereals. Therefore, this crop becomes a principal source regarding the entry of As into human system (Zhao et al., 2010). Rice is a dominant source of As intake even to the population not exposed to high concentrations of As in drinking water (Kile et al., 2007; Meharg et al., 2009; Halder et al., 2012). Arsenic intake from rice is significant, accounting for~ 50% even for populations exposed to elevated As in drinking water (Ohno et al., 2007; Mondal and Polya, 2008). In West Bengal, rural people on average consume 400 g rice per day (Carbonell-Barrachina et al., 2009), and are thus exposed to the threat of As-related health hazards (Guha-Mazumder, 2008; Guha-Mazumder et al., 2012).

2.3 Arsenic toxicity

2.3.1 Arsenic toxicity in human

Arsenic is a widely occurring toxic metal in natural ecosystems. As small as 0.1 g of As trioxide can prove lethal to humans (Jarup, 1992). Toxicity of As to humans depends on the concentration and the length of exposure. Arsenical toxicity develops insidiously after six months to two years or more depending on the amount of intake of As-laden groundwater (in case of contaminated groundwater serving as the drinking water source as in West Bengal and Bangladesh), and As concentration in it.

Early symptoms of As poisoning include skin disorders, weakness, languor, anorexia, nausea and vomiting with diarrhoea or constipation. With the progress of poisoning, the
symptoms attain more characteristic features, which include acute diarrhoea, oedema (especially of the eyelids and ankles), skin pigmentation, ars nical melanosis and hyperkeratosis, enlargement of liver, respiratory diseases and skin cancer. In severe cases, gangrene in the limbs and malignant neoplasm are also observed (Mandal et al., 1996). “Bell Ville Disease” (typical As induced cutaneous manifestations among the people of Bell Ville) in Argentina, “Black Foot Disease” in Taiwan and “Kai Dam” disease in Thailand are well established as health disorders due to As poisoning (Sanyal and Dhillon, 2005).

Arsenic can be toxic through its interaction with sulphydryl groups of proteins and enzymes by denaturing the proteins and enzymes within the cells (Gebel, 2000) and through an increase of reactive oxygen species in the cells, consequently causing cell damage (Ahmad et al., 2000). Oxidative stress induced by trivalent arsenicals inhibits glutathione (GSH) reductase (Styblo and Thomas, 1997). AsIII is known to inhibit more than 200 enzymes in the body (Abernathy et al., 1999) and, because AsV has a similar structure as phosphate, it can substitute for phosphorus in the body, which can lead to replacement of phosphorus in the bone for many years (Arena and Drew, 1986). Because AsV is hydrolyzed easily (in the cell), it prevents subsequent transfer of phosphate to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP; the energy currency of the cell) and thus depletes the cell of its energy (Winship and Mare, 1992). Arsine, the most toxic of the arsenicals (Leonard and Gerber, 1994), is known to cause hemolysis of red blood cells, leading to hemolytic anaemia, which is primarily responsible for the development of oliguria renal failure (Fowler and Weissberg, 1974). Besides, exposure to As causes cardiovascular and peripheral vascular diseases, neurological disorders and various forms of cancer (Abernathy et al., 2003; Freeman et al., 2004).

2.3.2 Arsenic toxicity in Plant

Plants take up As mainly as AsV. Plants exhibit a certain degree of As tolerance at lower concentration, but above critical limit, growth will be reduced (Miteva, 2002). Exposure to AsV causes considerable stress in plants, including inhibition of growth and physiological disorders (Stoeva et al., 2005). Cytoplasmic AsV interferes with metabolic processes involving phosphate, giving it the potential to be toxic to plants, but it is probably reduced in the cytoplasm to As (Stoeva and Bineva, 2003). AsIII reacts with sulphydryl group (SH) of enzymes and tissue proteins, inhibiting cellular function and causing death (Ullrich-Eberius et al., 1989). Arsenic induced oxidative stress in plants resulted oxidative
damage to biomolecules and enzymatic irregularities and eventually cause cell death (Gunes et al., 2009).

AsV is an analogue of phosphate and thus enters the plant system through phosphate transporters, which has been experimentally proven through various physiological and electrophysiological studies demonstrating inhibition of AsV uptake in the presence of phosphate (Abedin et al., 2002; Srivastava et al., 2007). In contrast, AsIII exists as a neutral molecule [As(OH)_3] at the prevailing pH and redox conditions of the environment, and hence, its uptake occurs via aquaglyceroporin channels of nodulin 26-like intrinsic protein family, which transport various neutral molecules including salicylic acid and boric acid (Isayenkov and Maathuis, 2008; Zhao et al., 2009). Rice an efficient accumulator of As, under submerged condition, uptake AsIII through transporters of NIP (nodulin intrinsic protein) family (Ma et al., 2008). Pteris possesses efficient uptake systems for AsV / AsIII for their transport to shoots, and for their final sequestration in the vacuoles in addition to the fact that it is hypertolerant to As (Su et al., 2008; Xie et al., 2009; Indriolo et al., 2010).

2.3.3 Arsenic toxicity to microorganisms and their resistance

Arsenic contamination of soil and water has a direct impact on microbial community and structure. Microbial ability to grow at high metal concentration is found coupled with a variety of specific mechanism of resistance and environmental factor. Smith et al. (1998) observed that many bacterial communities adapt to As contaminated environment by developing resistance and tolerance mechanism. Exposure to As might exert a selective pressure on plasmid harbouring bacteria to trigger resistant gene on plasmid to encode and express under such stressed condition to combat As toxicity (Smith et al., 1998). Resistance mechanisms of microorganism include microbial surface sorption, enzymatic transformation, and precipitation by oxidation/reduction reaction and biosynthesis of metal binding proteins or extracellular polymers, whereas environmental factors may include the surrounding pH and redox potential metal speciation, soil particulates, and soluble organic matters (Srinath et al., 2002; Zoubilis et al., 2004). Arsenic resistant bacteria have also been isolated worldwide by several groups (Anderson and Cook, 2004; Mateos et al., 2006; Chang et al., 2007; Duquesne et al., 2008; Valenzuela et al., 2009).

Aksornchu et al. (2008) isolated six bacterial strains which might have potential in bioremediation of As and other contaminants. Microbial resistance to toxic As is widespread in natural environment (Venezuela et al., 2009). Achour et al. (2007) confirmed the wide
distribution of phylogenetically diverse As resistant bacteria in the environment. Anderson and Cook (2004) isolated 17 morphologically distinct As-resistant bacteria that belonged to genera *Exiguobacterium*, *Aeromonas*, *Bacillus*, *Pseudomonas*, *Escherichia*, *Acinetobacter* and all the bacteria showed high tolerance capacity for As (0–100 mM AsV or 0–20 mM AsIII).

Oliveira et al. (2009) enumerated and characterized As-tolerant diazotrophic bacteria in a long-term heavy metal contaminated soil and he found that most genera recovered from the contaminated soil were also found in the uncontaminated soil. Krumova et al. (2008) isolated and identified some bacterial strains which could grow in the presence of AsIII (19mM) and AsV (100mM).

Microorganisms play an important role in the geobiocycling of elemental As. Chen and Shao (2009) isolated 54 AsIII resistant bacteria from deep-sea sediments from the Southwest Indian Ridge, which showed varied tolerance to AsIII of 2–80 mM and the bacteria mainly belonged to *Proteobacteria* and *Actinobacteria*.

Twenty one As resistant bacteria belonging to different genera of Gram-positive and Gram-negative bacteria were isolated from agricultural soils of Bangladesh and showing varying resistant pattern to different species of As (Bachate et al., 2009).

Pepi et al. (2007) reported four highly resistant isolates (ORAs1, ORAs2, ORAs5 and ORAs6), showed minimum inhibitory concentration values equal or superior to 16.68 mmol L\(^{-1}\) and 133.47 mmol L\(^{-1}\) in the presence of AsIII and AsV and they grouped in the three genera *Aeromonas*, *Bacillus* and *Pseudomonas*.

Pepi et al. (2007) suggested the presence of As resistant *Aeromonas*, *Bacillus* and *Pseudomonas* isolated from contaminated sediments of the Orbetello Lagoon, Italy.

*Corynebacterium glutamicum*, which is used for the industrial production of amino acids and nucleotides, is one of the most As -resistant microorganisms described to date (up to 12 mM AsIII and >400 mM arsenate) (Mateos et al., 2006).

Bahar et al. (2012) reported a new AsIII oxidising bacterium, found to tolerate as high as 60 mM AsIII in culture media and was closely related to *Stenotrophomonas panacihumi*. Arsenic resisting capacity exceeding 100mM of AsV is considered as hyper tolerant As resistant microbes (Jackson et al., 2005).

Several As resistant bacteria have been isolated from the As prone zones of West Bengal and Bangladesh. *Bacillus indicus* and *Denococcus indicus* are two novel As resistant bacteria isolated from As contaminated aquifers located in the Chakdah block of Nadia district of West Bengal, India (Suresh et al., 2004).
Islam et al. (2004) isolated *Aeromonas media*, *Aeromonas popoffii* and *Bacteroides* sp., *Pseudomonas* sp., *Cytophaga* sp., *Nitrosolubus multiformis*, *Clostridium aminobutyricum*, *Sulfuricurum kuijense* from As rich Bengal delta sediments.

In 2005, Gault et al. isolated *Desulfovibrio desulfuricans* (delta proteobacteria) and *Clostridia* sp. from a shallow aquifer located in Chakdah block, Nadia district, West Bengal.

Arsenic resistant bacteria *Pseudomonas aeruginosa*, *Acinetobacter*, *Pseudomonas resinovorans*, *Bacillus circulans*, *Acinetobacter calcoaceticus*, *Pseudomonas alcaligenes*, *Vogesella indigofera* were isolated from As contaminated soil of North 24 Parganas, West Bengal and resistance varied widely in the range of 50–125 mM for AsV and 10–100 mM for AsIII (Banerjee et al., 2011).

Bachet et al. (2009) conceived that phylogenetically diverse As resistant bacteria present in agricultural soils of Bangladesh could be capable of reducing AsV to AsIII under aerobic conditions apparently for detoxification purpose.

Jackson et al. (2005) isolated bacteria that belonged to bacterial groups *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*. Some of these bacteria were capable of growing at high levels of AsV (up to 275 mM), although AsIII tolerance was much lower (10 mM). Chowdhury et al. (2009) isolated *Planococcus* KRPC10YT from As infested bore-well of West Bengal, India and the bacterium was resistant to higher concentrations of AsV (30 mM) and AsIII (20 mM). Shivaji et al. (2005) obtained a novel As resistant bacterium *Bacillus arsenicus* from As sediments in Chakdah district of West Bengal, India.

### 2.4 Microbial transformation of arsenic

Microbial transformation of As has great implications on its geochemical cycling in the environment because different forms of As vary in solubility, mobility, bioavailability, and toxicity (Smedley and Kinniburgh, 2002). Arsenic is toxic to microorganisms, but certain bacteria survive its exposure and, transform its species through oxidation, reduction and methylation/volatilization (Osborne and Ehrlich, 1976).

Nies (1999) stated that for survival under metal-stressed conditions, bacteria have evolved several mechanisms to tolerate heavy metal ions. It mainly involves efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell and oxidation-reduction of the heavy metal ions to a less toxic state.

Many bacteria are known for their ability to transform inorganic species by oxidation or reduction (Macy et al., 2000; Oremland and Stolz, 2005). Some bacteria metabolize As in
a way that transforms water-soluble AsIII into the safer, insoluble AsV (Oremland et al., 2004), suggesting it might someday be possible to use the bacteria to clean up or reduce As contamination of aquifers. Such transformations occur incidentally or, through specific detoxification mechanisms (Mukhopadhyay et al., 2002; Rosen, 2002; Oremland and Stolz, 2003).

Biomethylation of As is another way of transformation where AsV or AsIII are being methylated. In some bacteria, AsIII is first oxidized to AsV by a specific enzyme and subsequently methylated (Gihring and Banfield, 2001). Biomethylation may not necessarily contribute to the detoxification mechanisms but plays an important role in the biogeochemical cycle of As because methylated compounds are often volatile.

Conversion of metal(loid)s to their volatile derivatives by organisms is a well-known phenomenon in nature (Challenger, 1945). Arsenic biovolatilization starts with a reduction of AsV to AsIII and through a series of methylation reaction forms less toxic volatile organo-arsenicals (Turpeinen et al., 1999; Bentley and Chasteen, 2002). During As volatilization, some species of fungi and bacteria methylate inorganic As species to relatively less toxic volatile methylarsenicals (Rodriguez et al., 1999; Cernansky et al., 2009).

These microbes are widely distributed in nature. Successful exploration of these strain with advanced research could be explored in bioremediation to detoxify As contaminated environments.

2.4.1 Bioaccumulation of Arsenic

Bioaccumulation of As mainly involves the biosorption of As by microbial biomass and its by-products and physiological uptake of As by microorganisms through metabolically active and passive processes. AsV uptake by living organisms is via phosphate transporter and then reduced to AsIII, which may be sequestered in the intracellular environment in its free form or conjugated form with glutathione, while AsIII in water is an inorganic equivalent of nonionized glycerol and can be transported across cell membranes by glyceroporin membrane channel proteins (Rosen, 2002).

Metal-accumulating bacteria are often found among metal-resistant bacteria (Srinath et al., 2002). Takeuchi et al., 2007 reported that As accumulation in Marinomonas communis cells amounted to 2290 mg As kg⁻¹ (dry weight) when incubated in a medium containing 5mg L⁻¹ of AsV. Bacterial strain Pseudomonas is capable of accumulation of 4 mg of As g⁻¹ of dry weight under laboratory condition (Joshi et al., 2008).
Ghodsi et al. (2011) reported bacterial strain *B. macerans* which had the ability to accumulate 36% of AsIII after 48 h under *in-vitro* culture study. It has been reported that a number of bacterial species namely *Proteus* sp., *Bacillus* sp., *E. Coli*, *Flavobacterium* sp., *Corynebacterium* sp., *Pseudomonas* sp., posses varying degrees of As accumulating abilities (Qin et al., 2006). However, except for some genetically engineered microorganisms (Kostal et al., 2005) no bacteria that could be applied for this purpose have been found to date.

Other than bacteria, fungi also act as a good accumulator of toxic arsenicals. *Phaeolus schweinitzii*, *Scopulariopsis breviculis*, *Neosartorya fischeri* have been shown to be capable of As bioaccumulation (Cernansky et al., 2007). *Penicillin janthinellum, Fusarium oxysporum* were able to accumulate AsV up to 39.54 μg and 304.06 μg under laboratory experimentation (Su et al., 2010).

One emerging technology that has received more attention in recent years is the development of biosorbents with high affinity and specificity. Kostal et al. (2005) presented a new method for the selective removal of As by using engineered *E. Coli* cells. The metalloregulatory protein ArsR, which offers high affinity and selectivity toward AsIII, was overexpressed in *E.coli* in an attempt to increase the bioaccumulation of As and accumulation was 5 and 60 fold higher levels of AsV and AsIII than control cells without ArsR over expression. An As-chelating metallothionein (fMT) from the As -tolerant marine alga *Fucus vesiculosus* was expressed in *Escherichia coli*, resulting in 30 and 26 fold higher AsIII and AsV binding, respectively (Singh et al., 2008).

Biosorption of As by microbial biomass may be helpful to remove As from groundwater. However, it might not assist As removal from contaminated soils since usually it is not easy to separate the microbes from soil (Wang and Zhao, 2009).

### 2.4.2 Oxidation of Arsenite (AsIII)

Green (1918) first time reported As oxidising bacteria in cattle-dipping fluids in South Africa and identified as *Bacillus arsenoxydans*. Later on, different species of As oxidising bacteria have been identified and isolated from contaminated mines (Santini et al., 2000), hot creeks (Salmassi et al., 2002), ground water (Fan et al., 2008), sediments (Valenzuela et al., 2009) and soil (Bahar et al., 2012) worldwide.

Most of the other precedence of reporting represented diverse groups of bacteria like *Alkaligenes* (Osborne and Ehrlich, 1976), *Bordetella* (Bachate et al., 2012), *Agrobacterium* (Salmassi et al., 2002), *Achromobacter* (Cai et al., 2009), *Thermus* (Gihring and Banfield
2001), *Stenotrophomonas* (Bahar et al., 2012), while the genus *Bacillus* is also one of the most widely reported groups contributing to the family of As oxidising bacteria (Shivaji et al., 2005; Pepi et al., 2007).

Oxidation of AsIII represents a potential detoxification process that allows microorganism to tolerate higher levels of AsIII and has an immense importance in bioremediation (Bahar et al., 2012). Microbial oxidation of AsIII is a critical link in the global As cycle by converting more toxic AsIII into less mobile and less toxic AsV species (Ehrlich, 2001). Oxidation of AsIII generate driving energy for the growth of autotrophic bacteria, here AsIII acts as the electron donor, whereas oxygen is used as the electron acceptor and carbon dioxide as the carbon source (Garcia-Dominguez et al., 2009) while for heterotrophic bacteria AsIII oxidation is a detoxification mechanism catalysed by a periplasmic enzyme called AsIII oxidase (Muller et al., 2003).

An AsIII oxidising bacterium, *Agrobacterium alberimagni* AOL15 oxidising 585 µM of AsIII in 24 h was isolated by Salmassi et al. (2002) and they found that AsIII does not support chemolithotrophic growth of AOL 15. Complete oxidation of AsIII in 40 h at a lower concentration (1mM) by *Alcaligenus* sp RS-19 was reported by Yoon et al. (2009).

Valenzuela et al. (2009) isolated an AsIII oxidising bacteria *Pseudomonas putida* from As enriched sediments of Northern Chile which was capable of completely oxidize 0.5 mM of AsIII under laboratory incubation study. Nagvenkar and Ramaiah (2010) reported that percent of AsIII biotransformed from the growth medium was quite rapid by a strain of *Enterobacteriaceae* (as much as 92% of the As in the growth medium by 120 h).

Bahar et al. (2012) isolated a new AsIII oxidising bacterium *Stenotrophomonas* sp. MM-7 from soil. This strain was capable of completely oxidizing 500 µM of AsIII to AsV within 12 h of incubation in a batch experiment. Arsenic oxidation rate of the strains UPB-24 and UPB-31 was 416 µM of AsIII h⁻¹ at concentration of 5 mM reported by Bachet et al. (2012).

Microorganisms have specific enzymes or respiratory chains to mediate the redox transformations of As. This may be correlated to the presence of As respiratory reduction genes (*arr*), or AsIII oxidation genes (*aox/aro/aso*) (Chang and Kim, 2010).

In general As is toxic to living system, but microorganisms developed some mechanism to combat with toxic As (Bhattacharjee and Rosen, 2007). Bacterial oxidation of
AsIII to AsV represents a way of As detoxification and has an immense importance in bioremediation.

Table 2.1: Some important microorganisms that can oxidize arsenite (AsIII)

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<th>Microorganisms</th>
<th>Mechanism</th>
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<tr>
<td><em>Pseudomonas arsenitoxidans</em> NT-26</td>
<td>Chemolithoautotrophic AsIII oxidation under oxic conditions</td>
<td>Santini et al. (2000)</td>
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<tr>
<td><em>Sulforospiillum barnesii</em></td>
<td>Dissimilatory AsV reduction under anaerobic conditions</td>
<td>Zobrist et al. (2000)</td>
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<tr>
<td><em>Thermus</em> HR13</td>
<td>Heterotrophic AsIII oxidation under aerobic conditions and dissimilatory AsV reduction under anaerobic conditions coupled with lactate oxidation</td>
<td>Gihring and Banfield (2001)</td>
</tr>
<tr>
<td><em>Thermus aquaticus</em></td>
<td>Heterotrophic AsIII oxidation to AsV</td>
<td>Gihring and Banfield (2001)</td>
</tr>
<tr>
<td><em>Sphingomonas</em></td>
<td>AsV reduction under aerobic conditions via a detoxification process</td>
<td>Macur et al. 2001</td>
</tr>
<tr>
<td><em>Desulfitobacterium</em></td>
<td>Dissimilatory AsV reduction using formate as the sole carbon source and electron donor</td>
<td>Niggemyer et al. (2001)</td>
</tr>
<tr>
<td><em>Bacillus strain</em> JMM-4</td>
<td>AsV reduction while the lactate is oxidized to CO2 via the intermediate, acetate</td>
<td>Santini et al. (2002)</td>
</tr>
<tr>
<td><em>Azoarcus strain</em> DAO1</td>
<td>Anaerobic AsIII oxidation with inorganic C as the carbon source and nitrate as the electron acceptor</td>
<td>Rhine et al. (2006)</td>
</tr>
</tbody>
</table>

Conventional removal processes of As from contaminated environments are generally requires an oxidation step to transform AsIII to AsV. However, oxidation via chemical oxidants such as chlorine, hydrogen peroxide and ozone are widely used as part of As removal processes (Simeonova and Luster, 2005). These result in high costs and may produce many harmful by-products. The use of biological treatment, either in situ or ex situ, has gained significant attention during the last two decades due to its advantages over conventional treatment methods. Thus, microbial transformation of AsIII to AsV could be an eco-friendly and cost effective alternative to conventional methods.
2.4.3 Reduction of arsenate (AsV)

Arsenate reduction contributes to the detoxification and resistance. Arsenate reduction can be achieved by various bacteria possessing cytoplasmic AsV reductase (ArsC). Despite its toxicity, a number of microorganisms are capable of using either the oxidized form of inorganic As, AsV or the reduced form AsIII in their metabolism (Silver and Phung, 2005). Some bacteria use AsIII as the electron donor for chemoautotrophic growth while other bacteria use AsV as the terminal electron acceptor in anaerobic respiration, thermodynamic considerations suggesting that dissimilatory reduction of AsV could provide enough energy for microbial growth (Laverman et al., 1995). Dissimilatory AsV reducing bacteria (DARB) may be involved in the solubilization, fate and transport of As by reducing AsV to AsIII (Ahmann et al., 1997).

Bacteria, fungi and algae are able to reduce AsV to AsIII and subsequently to arsine (Frankenberger and Losi, 1995). However, the effect of microbial activity on the transformation and movement of As in soil is difficult to quantify (Smith et al., 1998).

Oremland and Stolz (2003) reported that some specialist “dissimilatory AsV-reducing prokaryotes” are able to respirate using AsV as the electron acceptor, in place of oxygen, under anaerobic conditions. Although pH and concentration dependent, the oxidation/reduction potential of AsV/AsIII is in the region of +60 to +135 mV, which is sufficient to support growth when organic matter is supplied as the electron donor.

Lim et al. (2008) from the batch and column tests suggested that in natural groundwater system, *Shewanella sp.* the iron-reducing bacteria, participates in the reduction of AsV and sulfate, with concurrent oxidation of lactate to acetate and the decrease of Eh. The sulfides produced by the microbial activity precipitate AsV and AsIII.

Niggemyer et al. (2001) revealed that the dissimilatory AsV reducing bacterial Strain GBFH couples the oxidation of formate to the reduction of AsV when formate is supplied as the sole carbon source and electron donor.

Researchers have reported on isolating AsV reducing bacteria from As rich soils and sediments using anaerobic media where AsV serves as the sole terminal acceptor (Nicholas et al., 2003). Stolz and Oremland (1999) provided the reduction reactions that select bacteria can perform on these oxyanions of As. The following equations show the reduction of AsV and AsIII by the organism *C. arsenatis*, *S. barnesii*, *D. auripigmentum*, *B. selenitireducens*, and *B. arsenicoselenatis*. 
\[
\text{Lactate}^- + 2\text{HAsO}_4^{2-} + 2\text{H}^+ \rightarrow 2\text{H}_2\text{AsO}_2^- + \text{HCO}_3^-
\]

\[
\text{Acetate}^- + 2\text{HAsO}_4^{2-} + 2\text{H}_2\text{AsO}_4^+ + 5\text{H}^+ \rightarrow 4\text{H}_3\text{AsO}_3 + \text{HCO}_3^-
\]

The first “respiratory AsV reductase” characterised was from \textit{Chrysiogenes arsenatis}, isolated from gold mine wastewater. The protein was found in one of the outer compartments, called the periplasm, of this Gram-negative bacterium and consisted of 87 and 29 kDa subunits (Kraft and Macy, 1998). It is related to the dimethylsulfoxide (DMSO) family of mononuclear molybdenum-containing enzymes.

\textbf{Table 2.2: Some important microorganisms that can reduce arsenate (AsV)}

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Shewanella} sp. Strain ANA-3</td>
<td>AsV respiration and detoxification</td>
<td>Saltikov et al. (2005)</td>
</tr>
<tr>
<td>\textit{Sulfurospirillum barnesii} SES-3</td>
<td>AsV respiration and detoxification</td>
<td>Laverman et al. (1995)</td>
</tr>
<tr>
<td>\textit{Desulfotomaculum auripigmentum} OREX-4</td>
<td>AsV respiration and detoxification</td>
<td>Newman et al. (1997)</td>
</tr>
<tr>
<td>\textit{Bacillus selenitireducens} strain MLS 10</td>
<td>Respiratory AsV reduction</td>
<td>Silver and Phung (2005)</td>
</tr>
<tr>
<td>\textit{Chrysiogenes} AsVs</td>
<td>Respiratory AsV reduction</td>
<td>Kraft and Macy (1998)</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus} plasmid PI 258</td>
<td>Arsenic resistance</td>
<td>Ji and Silver (1992)</td>
</tr>
</tbody>
</table>

Zobrist et al. (2000) reported that the cell suspension of \textit{Sulfurospirillum barnesii} was able to reduce AsV to AsIII. Liao et al. (2011) cultured and identified 10 facultative anaerobic AsV reducing bacteria and one strictly aerobic AsIII oxidising bacterium from As rich groundwater in Taiwan.

Several bacteria of β or γ-proteobacter groups have been reported to reduce AsV (Simeonova et al., 2004). Although the precise mechanism of As mobilization remains to be characterized in detail, respiration of adsorbed AsV by dissimilatory AsV reducing prokaryotes may play a role, resulting in the formation of potentially more mobile AsIII (Oremland and Stolz, 2005). These organisms were stimulated under anaerobic conditions in laboratory microcosms by the addition of acetate as a proxy for organic matter enhanced rates of As mobilization in analogous sediments from West Bengal (Islam et al., 2004).
2.4.4 Biomethylation of arsenic

Bacterial methylation of As has great environmental significance for biological removal of As. Inorganic As is biomethylated to both volatile species such as monomethylarsine (MMA), dimethylarsine (DMA), and trimethylarsine (TMA) and non-volatile species such as methylarsonic acid, dimethylarsinic acid, and trimethylarsenic oxide. Arsenic biomethylation can be considered as a detoxification of As in the environment because the toxicity of organic As is much less than that of inorganic As (Kaise et al., 1985). Production of volatile methylated species has further importance in the removal of As from environmental samples (soil/sediment) and biosolids (sludge). Biomethylation of inorganic As to volatile methylarsine is conducted by microorganisms referred to as As Methylating Bacteria (AsMB) (Islam et al., 2005).

Microorganisms including bacteria, fungi, yeasts, and algae can act as biologically active methylators that are able to methylate As to gaseous arsines. Biomethylation of inorganic As has been extensively studied in methanogenic bacteria under anaerobic conditions. Anaerobic biomethylation of As only proceeds to dimethylarsine, which is stable in the absence of oxygen but can be rapidly oxidized under oxygenated conditions. Depending on environmental conditions, arsines may be slowly oxidized to organic forms, MMA, DMA or TMAO (Takamatsu et al., 1982). The trivalent methylated intermediates such as MMAIII and DMAIII are found to be readily oxidized chemically and biologically (Oremland and Stolz, 2003).

Biomethylation could be proved previously for various bacteria e.g. Proteus sp., E. coli, Flavobacterium sp., Pseudomonas sp., Corynebacterium sp. (Shariatpanahi et al., 1981) as well as for species of the genera Nocardia, Achromobacter, Aeromonas, Enterobacter, and Alcaligenes (Shariatpanahi et al., 1983).

Shariatpanahi et al. (1983) reported that the rates of methylation and demethylation of monosodium methylarsonate at 10 and 100 mg L\(^{-1}\) of normal culture media by Aeromonas, Nocardia, Enterobacter, Flavobacterium, Achromobacter, Pseudomonas, and Alcaligenes species followed first-order composite kinetics.

Mechanism of methylation starts with either AsV or AsIII. In higher eukaryotes, glutathione reduces AsV to AsIII, which then accepts a methyl group from S-adenosylmethionine producing MMA or DMA. An 85 kDa methyltransferase has been identified as mediating the last step (NRC, 1999). In some bacteria, AsIII is first oxidized to
AsV by a specific enzyme, arsenite oxidase, with the AsV subsequently methylated (Gihring and Banfield, 2001).

**Table 2.3: Some reported microorganisms in arsenic biomethylation**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Polyohysa peniculus</em></td>
<td>AsV methylation into dimethylarsine</td>
<td>Cullen et al. (1994)</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>Biosorption and accumulation of As and converted into (CH3)AsO(OH)</td>
<td>Kaise et al. (1997)</td>
</tr>
<tr>
<td><em>Scopulariopsis breviculae</em></td>
<td>AsV methylation to (CH3)3As species</td>
<td>Andrews et al. (2000)</td>
</tr>
<tr>
<td><em>Methanobacterium formicicum</em></td>
<td>As methylation and demethylation under favorable conditions</td>
<td>Michalke et al. (2000)</td>
</tr>
<tr>
<td><em>Closterium aciculare</em></td>
<td>AsV methylation into methylarsenic(III) species</td>
<td>Hasegawa et al. (2001)</td>
</tr>
<tr>
<td><em>Fusarium oxysporum meloni</em></td>
<td>AsV accumulation and methylation into dimethylarsine</td>
<td>Granchinho et al. (2002)</td>
</tr>
<tr>
<td><em>Fucus gardneri</em></td>
<td>AsV methylation into dimethylarsine</td>
<td>Granchinho et al. (2002)</td>
</tr>
<tr>
<td><em>Penicillium</em> sp. <em>Ulocladium</em> sp.</td>
<td>AsV methylation to arsine</td>
<td>Edvantoro et al. (2004)</td>
</tr>
</tbody>
</table>

Biotransformation of As in As enriched soils, involving As oxidation and methylation as As detoxifying mechanisms (Osborne and Ehrlich, 1976; Woolson, 1977; Tamaki and Frankenberger, 1992), plays an important role in the distribution and mobilisation of As in soil (Yan-Chu, 1994). Microbial methylation of As to volatile As effectively reduces its toxicity. In soils, the amount of As volatilised by microorganisms is governed by nutrient levels and microbial growth (Sanford and Klein, 1988).

Cox and Alexender (1973) isolated microorganism from soil and sewage in culture containing various As compounds. The organisms were able to produce trimethylarsine from AsV and AsIII at acidic pH suggested acidic condition may promote trimethylarsine production.
Fig 2.3: Microbial formation of trimethylarsenic from inorganic arsenic

A culture of *Pseudomonas*, occurring in marine environment, showed the highest ever measured production rate of TMA (under aerobe conditions) with 585 nmol min$^{-1}$ g of cells$^{-1}$ (wet weight) (Bentley and Chasteen, 2002). Qin et al. (2006) reported that heterologous expression of *arsM* from *Rhodopseudomonas palustris* conferred AsIII resistance to an As sensitive strain of *E. coli*, ArsM catalyzes the formation of a number of methylated intermediates from AsIII, with trimethylarsine as the end product. The net result is loss of As, from both the medium and the cells, which is a description of a mechanism of AsIII resistance through methylation and subsequent volatilization.

Biomethylation may not necessarily contribute to the detoxification mechanisms but plays an important role in the biogeochemical cycle of As because methylated compounds are often volatile. Further, the addition of microbes with As methylating ability to As polluted soil could be a potential strategy in order to enhance the rate of As volatilisation from soils (Sanford and Klein, 1988; Huysmans and Frankenberger, 1991; Thomas and Rhue, 1997).
2.4.5 Biovolatilization of arsenic

Biomethylation of As has been widely studied, but its application in As remediation has not been fully exploited. It produces volatile As species. Therefore, biovolatilization might be developed as an ex-situ method for As removal under controlled conditions. As summarized by WHO (WHO, 2001), volatilization can substantially contribute to As removal from top soils, up to 12–35% year⁻¹. It is a natural process that occurs globally, and species capable of As volatilization have been isolated from many diverse environments, including soils, rivers, hot-springs (Turpeinen et al., 1999). Several species of soil dwelling microorganisms have shown As volatizing potential (Frankenberger and Arshad, 2002; Islam et al., 2007; Mohapatra et al., 2008).

Over a century ago, As biovolatilisation by a fungal strain Scopulariopsis brevicaulis was first obtained (Challenger, 1945). Recently, more microorganisms capable of conducting these processes have also been found. Following different transformation process methylarsenicals are released from the microbe as a gaseous product (Fig 2.4). Arsenic biovolatilization starts with a reduction of AsV to AsIII and through a series of methylation reactions, it forms less toxic volatile organo-arsenicals (Turpeinen et al., 1999; Abedin et al., 2002; Bentley and Chasteen, 2002).

Some anaerobic methanogenic bacteria are capable of reducing inorganic AsV to AsIII. They can then convert either AsIII or methanearsonic acid to gaseous dimethylarsine through volatilization pathways (McBride et al., 1978). Escherichia coli, which is common in the intestines of humans and other animals, can form volatile trimethylarsine (Frankenberger and Arshad, 2002).

More importantly, from a bioremediation perspective, several species of soil dwelling microorganisms have shown As volatizing potential (Frankenberger and Arshad, 2002). This includes species of Penicillium and Aspergillus, which can volatilize both organic and inorganic As compounds, and Pseudomonas, which are capable of volatilizing inorganic As.

Visootviseth and Panviroj (2001) reported that the fungal species Penicillium sp. were capable of volatilizing 25.8–43.9 mg of As during a 5 day cultivation period. Edvantoro et al. (2004) showed that augmenting contaminated soils (1390 mg As kg⁻¹ soil) with methylating fungi (Penicillium sp. and Ulpcladium sp.) significantly increased the As volatilization rates (up to eight-fold increase). During As volatilization, some species of fungi and bacteria methylate inorganic As species to relatively less toxic volatile methylarsenicals (Rodriguez et al., 2003).
Fig 2.4: Arsenic volatilization pathway (Frankenberger and Arshad, 2002)

Cernansky et al. (2009) studied the production of volatile derivatives of As using pure cultures of different fungal strains under laboratory conditions. The average amount of volatilized As for all fungal strains ranged from 0.026 to 0.257 mg of AsIII and 0.024 to 0.191 mg of AsV, respectively. Approximately 23% of As was volatilized from all culture media originally enriched with approximately 4 and 17 mg L⁻¹ of AsIII. The average amount of biovolatilized As from culture media originally enriched with 4 and 17 mg L⁻¹ of AsV was 24% and 16%, respectively. The order of ability of As biovolatilization is *Neosartorya fischeri* > *Aspergillus clavatus* > *A. niger*. Genetically engineered *B. idriensis* strains could remove 2.2%–4.5% of As from soil system by biovolatilization in 30 days (Liu et al., 2011). The rate of biovolatilization of AsV was observed to be 23% while for AsIII it was 26% by *Staphylococcus* sp. in 3 days from culture media (Srivastava et al., 2012).
Most of the previous reports related to As volatilization involved either aerobic (Pseudomonas, Bacillus, and Alcaligenes) or anaerobic (Clostridium, Desulfovibrio, and Methanobacterium) bacteria (Bentley and Chasteen, 2002). Thus, the natural presence of soil dwelling As volatizing organisms offers the possibility to stimulate bioremediation of As contaminated soils.

2.5 Genes and enzymes involved in arsenic transformation

Arsenic resistance mechanisms have been reviewed extensively (Silver and Phung, 1996; Mukhopadhyay et al., 2002). Typically, the minimal set of genes that confers resistance to both AsIII and AsV are known as the arsR, arsB and arsC genes.

According to Mateos et al. (2006) the most common microbial As defense mechanism is based on the presence of the As resistance operon (ars), which codes for: (i) a As responsive regulatory protein, ArsR; (ii) AsIII permease, a membrane-located AsIII efflux pump, ArsB; and (iii) an enzyme involved in AsV reduction, arsenate reductase, ArsC. Recently two additional genes arsA and arsD, are found in ars operons of Gram-negative bacteria and the gene order becomes arsRDABC. ArsA is an intracellular ATPase protein that binds to the membrane ArsB (Tisa and Rosen, 1990). ArsD has been found to act as an AsIII binding protein or metallo-chaperone (Lin et al., 2007) that sequesters intracellular AsIII and delivers it to ArsA before being pumped out of the cell through the action of the ArsAB membrane efflux pump.

Two more totally different enzyme systems involved in As transformation have been identified and genetically characterized. These are the periplasmic respiratory arsenite oxidase (Anderson et al., 1992; Anderson et al., 2001) and the respiratory arsenate reductase (Alkorta et al., 2004). The arsenite oxidase system converts highly toxic AsIII to relatively less toxic AsV (Muller et al., 2003; Silver and Phung, 2005), while respiratory arsenate reductase functions as a terminal electron acceptor, allowing heterotrophic anaerobic growth in the absence of oxygen. Both the oxidase and reductase contain homologous molybdoprotein center and Fe-S cage and both are coupled to inner membrane respiratory chains, where the oxidase acts as an initial electron donor while the reductase is the terminal electron accepter in an anaerobic respiratory process (Anderson et al., 2001). Arsenite serves as electron donor in the energy metabolism of several chemolithotrophic AsIII oxidising bacteria; therefore, genes encoding the respective proteins are usually designated aro (Santini and Vanden-Hoven, 2004). In case of many heterotrophic AsIII oxidising bacteria, the
contributing genes may not have an effect on energy metabolism, but have function in AsIII detoxification. Therefore, the genes are usually designated *aox* (Muller et al., 2003) or *aso* (Kashyap et al., 2006).

![Arsenic resistance operon system](image)

**Fig 2.5: Arsenic resistance operon system**

Arsenite oxidising bacteria are phylogenetically diverse (Battaglia-Brunet et al., 2006) but all perform AsIII oxidation by arsenite oxidase, an enzyme that belongs to the dimethylsulfoxide (DMSO) reductase family of the molybdopterin-containing proteins (AroA/AsoA/AoxB), and a Fe–S Rieske protein (Ellis et al., 2001; Santini and Vanden-Hoven, 2004; Kashyap et al., 2006).

Anderson et al., 1992 reported that the enzyme is located on the outer surface of the inner membrane and the arsenite oxidase transfers electrons to the periplasmic electron carriers amicyanin or cytochrome c. Genes encoding these protein subunits have successfully been characterized in some AsIII oxidising bacteria such as *Herminiimonas arsenicoxydans* (Muller et al., 2003), *Rhizobium* sp. NT-26 (Santini and Vanden-Hoven, 2004) and *Agrobacterium tumefaciens* 5A (Kashyap et al., 2006). Recently, AsIII oxidase gene *arsA* was identified in *Alkalilimnicola ehrlichii* MLHE1 (Zargar et al., 2010), *aoxB* from β-proteobacteria ULPAs1 (Muller et al., 2003) and *Agrobacterium tumefaciens* (Kashyap et al., 2006). The similarity of the arsenite oxidase enzymes in phylogenetically and physiologically diverse AsIII oxidising microorganisms has lead the investigators to focus on the *aoxB* gene as a potential molecular marker to understand the ecology of AsIII oxidising microorganisms (Hamamura et al., 2009).

Bacterial methylation of inorganic As has been studied extensively in methanogenic bacteria. The pathway proceeds by reduction of AsV to AsIII followed by methylation in the
presence of coenzyme M (CoM), a low molecular weight cofactor found in all methanogenic bacteria. Anaerobic biomethylation of As by bacteria proceeds only to dimethylarsine, which is stable in the absence of oxygen. In anaerobic environments, dimethylarsine can react with disulfide bonds on particulates in soil thus reducing the concentration of soluble As (Bentley and Chasteen, 2002).

There is an additional aspect of microbial As biochemistry concerning As methylation. Qin et al. (2006) reported a mechanism of AsIII resistance through methylation and subsequent volatization. Heterologous expression of \textit{arsM} from \textit{Rhodopseudomonas palustris} was shown to confer AsIII resistance to an As sensitive strain of \textit{Escherichia coli}. ArsM catalyzes the formation of a number of methylated intermediates from AsIII, with trimethylarsine as the end product. The net result is loss of As, from both the medium and the cells. Because ArsM homologues are widespread in nature, this microbial-mediated transformation is proposed to have an important impact on the global As cycle.

2.6. Arsenic mitigation approaches in soil – biota ecosystem

2.6.1 Soil organics and arsenic transformation

Arsenic in soil was observed to have a significant relationship with soil organic fraction either native or added. Application of organic manure proved to be beneficial in reducing the As uptake and its accumulation in the plant bodies (ICAR, 2005).

Das et al. (2005) reported that the extractable As content in the soil significantly decreased with the application of varying levels of organic matter and, however, such depressive effect was found more pronounced with well-decomposed farm yard manure than that of vermicompost. Such reduction in extractable As content might be associated with formation of insoluble arseno-organic complexes and their adsorption on to organic colloids (Das et al., 2008). In a pot culture experiment with rice, application of FYM helps to reduce the As accumulation in both soil and plant (Mukhopadhyay and Sanyal, 2002).

Mukhopadhyay and Sanyal (2004) reported that there was an ability of the native soil organic fraction to adsorb As, thereby moderating its toxicity in the soil/crop system and its entry in the food chain. In a pot-culture study, Ghosh et al. (2004) found that application of FYM helped to moderate the As accumulation in both soil and plant due to the formation of organo-As complexes with humic/fluvic colloids of native soil and of incorporated organic manures.
Singh (2007) observed that the addition of organic amendments and microbial inoculants favoured improvement in microbial population which helped in transformations of inorganic As to less toxic gaseous organic form and reduced As toxicity.

Biswas et al. (2011) in an incubation experiment studied the effect of native arsenic transforming bacteria on total and available soil arsenic in presence and absence of FYM. A significant reduction in total soil arsenic was observed in all the inoculated treatments. *Pseudomonas putida* adopt better in arsenic polluted soil and reduce available arsenic in soil. FYM @ 10 t ha\(^{-1}\) reduced available soil arsenic but had no significant contribution in reduction of total soil arsenic. Sinha et al. (2011) reported that the organic manures added as soil amendment significantly reduced the accumulation of As in sesame seed to a maximum extent of 65.5% (vermicompost), 50% (Phosphocompost), 42% (mustard cake) and 40% (FYM) compared with the control counterpart.

### 2.6.2 Physical and chemical factors on volatilization

Volatilization rates can be affected by a variety of chemical and physical factors. Soil pH, moisture, and temperature, as well as the addition of organic matter, heavy metals, and phosphorus to soils, all have effects on the rate of volatilization. Some of these conditions affect volatilization rates by simply promoting or inhibiting microbial growth. Temperature has a large effect on most biological processes, including volatilization. Frankenberger and Arshad (2002) found that the optimal temperature for volatilization in *Penicillium* was 20°C. Soil moisture level has a distinct effect on volatilization rates as well (Gao and Burau, 1997). Increases in soil moisture level above this value led to a decrease in volatilization rates. Environmental pH is also an important factor for volatilization rates. Most of the studies shown the process to occur most efficiently in acidic conditions, around pH of 5.0 (Baker, 1983). Arsenic species are generally more mobile under acidic conditions, making them more readily available to volatilizing organisms (Cox, 1975). In order to develop As bioremediation technologies based on natural attenuation, it is critical that the environmental conditions that control microbial As volatilization be understood (Frankenberger and Arshad, 2002).

### 2.6.3 Effects of organic amendments on volatilization

Organic amendments have been shown to affect the rates of volatilization in soils, presumably by stimulating microorganisms. The use of organic material to promote volatilization is desirable because it could recycle organic wastes, improves soil quality, and
is compatible with a society that is demanding natural bioremediation methods. Studies have shown that soils with ~11% organic matter have the highest rates of As volatilization (Akins and Lewis, 1976). Additions of glucose directly to soil showed increased As volatilization under aerobic but not anaerobic conditions (Frankenberger and Arshad, 2002). Additions of fresh manure to soil may also increase the bioavailability of As species that appear to be linked to higher rates of volatilization (Walker et al., 2006).

### 2.6.4 Bioremediation approach by using arsenic volatilizing microbes

Remediation of As contaminated soils and ground water is necessary for providing safe environment to the mankind. Researcher’s worldover were directed to generate new technologies for removal of As from water and soil by means of various physical and chemical remediation process. A number of treatment method such as coagulation, membrane separation, and adsorption/precipitation, anion exchange to remove As from waters, and stabilization/solidification technologies to retain As in contaminated soils, have been developed. However the application of these methods have been limited because of high material costs, generation of sludge, high energy requirements, and problems related to disposal (Wang and Zhao, 2009). These technologies are expensive and have limited use as poverty and contamination coexist in most As contaminated areas in the world (Zhu and Rosen, 2009).

However mitigation of As toxicity in soil – biota ecosystem may be achieved with the following approaches.
Bioremediation of As from the contaminated environments shows a great potential for future developments due to its environmental compatibility and possible cost effectiveness. It relies on microbial activity to resist, accumulate and transform As species into its less toxic form via oxidation, reduction, mobilization, biomethylation or volatilization. Considering the limitations of conventional mitigation processes, relatively low cost, eco-friendly bioremediation techniques using microorganisms can be a promising alternative (Singh et al., 2008).

Bioremediation, which involves the use of microbes to detoxify and degrade environmental contaminants, has received increasing attention in recent times to clean up a polluted environment (Gadd, 2000; Farhadian et al., 2008). Bioremediation, being in situ treatment, provides a safe and economic alternative to commonly used physicochemical strategies (Bai et al., 2008). However, it seems not feasible at present. On one hand, it is difficult to obtain such valuable microbes from numerous kinds of microorganisms for heavy metal bioremediation. As well as, the adaptation abilities and the remediation efficiencies of reported microorganisms are not enough for practical application (Guo et al., 2010). Little literature is available on removal of As by application of As volatilizing soil bacteria accelerated by easily available organic manure.

Attempts made to assess the capabilities of microorganism in decontaminating soil matrices have often dealt with genetically engineered organisms earlier (Liu et al., 2011) or in simulated in situ situations of anthropogenic contamination.

Biswas et al. (2011) reported that the native As transforming isolates *Pseudomonas putida* adopt better in As polluted soil and reduced extractable soil As. Considering the transformation of As in soil, accumulation of As in different plant parts *P.putida*, *P.mendocina*, *P.syringae* and *C.koseri* were found to be more promising bio-inoculants, those can be used for bio-remediation of soil As in future. But the strains were not characterized based on their As transformation pathway.

So the most pressing challenge is how to translate the fundamental knowledge to combat the noxious effects of As on human and other ecosystem. Exploration of these bioagents with advance and applicable form are still in a small scale and new conceptual investigations will surely help to solve this problem.