CHAPTER 2
LITERATURE SURVEY

2.1 ARSENIC

2.1.1 SOURCES

2.1.1.1 Natural Sources

Arsenic is a ubiquitous element which is ranked twentieth in crustal abundance. The concentration of arsenic in natural reservoirs have been calculated (**Table 2-1**, page 38) and calculated arsenic rates of transfer or fluxes were shown in **Table 2-2**, page 39 (Bhumbla and Keefer, 1994 cited by Mackenzie et. al., 1979). Approximate ratios of those reservoirs showed that more than 99% of the total arsenic in the environment is present in rocks. The concentrations in the rocks depend on the rock type, and it was found that sedimentary rocks contain much higher concentrations of arsenic than igneous or metamorphic rocks. The amount of arsenic in oceans higher than in soil that are higher than in waters that are higher than in biota (plants, animals, man and microbes) which is higher than in the atmosphere. The average concentration of arsenic is soil was 7.2 part per million, ppm, (Bhumbla and Keefer, 1994 cited by anonymous, 1978). Because of the difference in abundance of arsenic in soils and rocks, the total amounts if arsenic in reserviors is much smaller in soil than in rocks. Arsenic was possibly brought to the surface of the earth during mining and smelting and increases the potential for soil contamination. The most common of arsenic minerals is arsenopyrite, (FeAsS), and also including orpiment, (As_2S_3), realgar, (AsS), and enargite, (Cu_3AsS_4); those have been mined and act as sources of arsenic. Nowsday, most arsenic is produced as a by-product of the extraction of copper (Cu), lead (Pb), silver (Ag) and gold (Au) from their ores.

2.1.1.2 Anthropogenic

Arsenic is released into the environment by certain activities of human being, those are :

i) Mining activities and smelters

Arsenic is a natural contamination in lead, zinc, gold and copper ores and able to be released during the smelting process. Especially, smelting of Cu is the largest single anthropogenic input, representing about 40% of the anthropogenic total, and the next most significant contamination is coal combustion at about 20% of the total (O'Neill, 1995). The stack dust and flue gases from smelters often contaminate soil with arsenic downwind from the operation. There are widely different quantities of anthropogenically atmospheric emission, depending upon the industrialization of a country and the degree of pollution control.

ii) Agricultural material

From the late 1800s to the mid 1900s, inorganic arsenicals, usually as lead, calcium (Ca), magnesium (Mg) and zinc (Zn) arsenate, zinc arsenite or paris green (acetoarsenite) were used extensively as pesticides in orchards. Sodium (Na) arsenite was used as a herbicide and non-selective soil sterilant, while arsenic acid was used as a cotton desiccant. Organic arsenicals were also used as silvicide, herbicides, and desiccants. (Bhumbla and Keefer, 1994). In addition, those agricultural uses contribute the arsenic-containing compounds discharging into the environment.

iii) **Sewage sludges**

Arsenic can be found in sewage sludges. The levels of As in the sewage sludges reflect the degree of industrialization of the area served by the sewage system. It is mainly derived from surface run-off bringing in atmospherically deposited As plus residues from pesticide usage. Phosphate detergents add small quantities, and industrial effluents, particularly from the metal processing industry, can add significant quantities.

2.1.2 PHISICAL AND CHEMICAL PROPERTIES

Being chemicallly nonmetal or metalloid, arsenic is the third member of nitrogen (N) group of elements (N, P=phosphate, As, Sb=antimony, Bi=bismuth) and possesses five electrons in the outer shell. Atomic number and the atomic weight of As is 33 and 74.2916, respectively. Its properties are similar to P and Sb, with oxidation state of +3, +5, 0 and -3 corresponding to the inorganic compounds, arsenate, arsenite, arsenic metal, and arsine gas. It exists in several allotropic forms. The most common is a gray, crystalline material with low heat and electrical conductivity. Condensation from vapor produces a black form that is converted to the gray form by heating to 360 °c (degree celsius). The melting point of As is at 613 °c (under normal pressure), but it will melt at 817 °c under 28 atm (atmosphere). Arsenic is soluble in oxidizing acids, i.e., nitric acid or hot sulfuric acid, and other mineral acids with the aid of oxidizing agents. The solution resulting from sulfuric acid dissolution will contain arsenous arsenic or As(III). Solution obtained from other acids in conjunction with an oxidizing agent will contain arsenic or As(V) with varying amounts of sample dissolution. Reaction of arsenic with sodium hydroxide will result in solutions of arsenous arsenic.

In natural waters, the valence and the species of arsenic are depend on oxidation-reduction conditions and the pH of the water. The soluble arsenite species, i.e., H_3AsO_3, $H_2AsO_3^-$, and $HAsO_3^{2-}$ are found in a system including oxygen and sulfur. The soluble arsenate species are H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, and AsO_4^{3-}. In the pH range 4-10, the predominant form of arsenite is H_3AsO_3 and of arsenate are $H_2AsO_4^-$ and $HAsO_4^{2-}$. At neutral pH value the rate of oxidation of As(III) to As(V) with oxygen was found to be very slow, but proceeded measurably in several days in strong alkaline or acid solutions (Ferguson and Gavis, 1972).

2.1.3 USES

Arsenic compounds have been used for many years in the technological age. It is increasingly used as a doping agent in solid-state devices such as transistors. Gallium arsenides is used as laser material to convert electricity directly into coherent light. Arsenic trioxide (As_2O_3) and arsenic trisulfide are also used in bronzing, pyrotechnics, for hardening and improving the spherically of gunshot. In pharmaceutical processes, arsenic have been exploited in the production of antimicrobial agent, such as the first specific antibiotic named Salvorsan 606 and the African sleeping sickness drug named Melarsen. In addition, chromate copper arsenate (CCA) and zinc arsenate are also used as wood preservatives. When these compounds are applied under pressure they react with the wood to create water insoluble compounds. The preserved timber is resistant to both fungal and insect attack (WHO, 1981).

For agricultural application, arsenic compounds have been widely used as pesticides for over a hundred years, but their uses was now declining, having probably halved in the decade from 1970 to 1980. The

phytotoxic effect of As compounds made them attractive as herbicides and as desiccants to allow cotton to be easily harvested after defoliation. However, there has been concern about the build-up of As residues in soils and lake sediments that have occurred after the use of large quantities of inorganic As compounds. Consequently, other pesticides have been replaced As compounds, such as, lead arsenate [$Pb_5(PbOH)(AsO_4)_4$] and calcium arsenate [$Ca_3(AsO_40_2$], which were commonly used in orchards to control insect pests, and sodium arsenite which was extensively employed as a herbicide to clear aquatic weeds and defoliate seed potatoes.

Worldwide usage has been estimated to be 8,000 tons of As per year as herbicide, 1,200 tons of As per year as cotton desiccant and 1,600 tons of As per year in wood preservatives (Chilvers and Peterson, 1987). Generally, the pesticides was applied in the range of 2-4 kilogram (kg) of As per ha (hectare), but larger quantities of dimethylarsinic acid may be used with application rates being up to three times greater (National Academy of Science, 1977). Besides, small quantities of organoarsenic compounds are used as animal feed additives, i.e., phenylarsenic compound (4-aminophenyl arsenic acid), arsenilic acid and carbarsone, at the rate of 10-50 mg/kg (milligram per kilogram) of feed, to promote growth in chickens, turkeys and pigs. Thereafter, the compounds are rapidly excreted, often with little chemical change having apparently taken placed.

2.1.4 TOXICITY OF ARSENIC

2.1.4.1 Toxic effects on man and animals

Arsenic is a highly toxic element and has threatened community health. Its metalloid properties and complex chemistry, arsenic is found in both organic and inorganic compounds. In general, inorganic

arsenic compounds exhibit higher toxicity than organic ones and As (III) compounds are up to 200 times more toxic than As (V) compounds (Williams and Silver, 1984). Owing to trivalent arsenical has a strongly preferential binding to sulhydryl groups (SH-group) of the enzymes, and this is thought to be a primary mode of toxicity, causing inactivation of many enzymes containing sulhydryl groups, i.e., lactate dehydrogenes, cytochrome oxidase and glucose oxidase. Compounds of pentavalent arsenic are less toxic because they do not react with sulhydryls groups but act as analogue of inorganic phosphate, i.e., in cellular membrane transport systems and enzymes system, such as, kinase. Spontaneously, Arsenylated sugars hydrolyzed, resulting in loss of free energy in glycolytic energy metabolism (Summers and Silver, 1978). Fortunately, elemental arsenic is found to be a relatively low level of toxicity but continuous exposure to elemental arsenic dust may caused health effect possibly (Casarett and Doull's, 1991).

The effects of arsenical compounds on human and other higher mammals are both acute and chronic or long-term. Occasionally, the most of arsenic has caused chronic poisoning rather than acute one. Arsenic has been specified as carcinogen for skin and lung cancer by International Agency for Research on Cancer (IARC) and as one of priority pollutants and hazardous waste constituents by United State of Environmental Protections Agency (USEPA).

In Japan, there were two major evidences of As poisoning in human, i.e., powdered milk for infants and soysauce were contaminated. In the former instance, more than 12,000 cases of infant poisoning were recorded, with 130 deaths. The survivors were threatened by severe damages, including retarded growth and brain dysfunction.

In Taiwan, artersian well water was contaminated with quite high concentration of arsenic for more than several decades, high prevalence of chronic arsenism, for example, tissue wastage or Blackfoot disease caused by peripheral circulate disorders, hyperpigmentation, skin carcinomas, and including gastrointestinal cancers, has been observed. Maximal permissible level off As in public drinking water supplies is not more than 0.05 mg/l (Milligram per Liter). Ocurring of Blackfoot disease would be found in the patient who has drunk drinking water containing arsenic concentration above 0.35 mg/l (Hsia and Lo,1990).

In West Bengal, India, the investigate showed that high levels of arsenic in water caused by leaching of arsenic from underground sources into thousands of village wells. Possibly, more than 1 million Indians are drinking arsenic-containing water, and tens of millions more could be at risk in other areas that have been not yet tested. Anyway, estimated 200,000 Indians have already had arsenic-induced skin lesions, and many of them may also have hyperkeratoses or hardend patch of skins that might develop further to cancers (Bagla and Kaiser,1996).

As mention earlier, there was arsenic contamination at Amphor Ron Phibun, Changwat Nakhon Si Thammarat (in the southern part of Thailand) especially, the area of tin (Sn) deposits which also contain both of As and wolfram (W) minerals. In this case, mining has been point as a principal cause of surface-and groundwater contamination. A variety of mine-waste products, including arsenic-floting residues, waste-rock piles, and dredged alluvium provide potential sources of arsenic contamination. Public health problems from As-containing water supplies in the area were first highlighted in 1987. One year later, research on the extent, distribution

and epidemiology of arsenism were initiated by the Ministry of Public Health, and a preliminary survey confirmed approximately 1,000 cases of As- induced skin disorders, including 20 cases of arsenical melanomas. In the school-age population, As concentrations in hair and finger nails were found to be alleviated (up to 3.1 mg/kg and 56 mg/kg, respectively) in 80% of pupils, and also strong spatial correlation in drinking water (principally derived from shallow wells) was confirmed. In 1992, a follow up study of 2,400 school pupils showed that 89% of the pupils had As-containing blood in excess, with 22% of them had incidences of arsenical skin manifestations (Williams et al.,1996). Additionally, incidences of chronic arsenism which were related to mining have been shown before, for example, mining in many gold and base-metal-producing countries including Chile, Argentina and Mexico. (Williams et al.,1996 cited by Borgano and others, 1980, Astolfi and other, 1981, and Cerbrian and other, 1983, respectively).

2.1.4.2 Toxic Effects on Plants

Arsenic is chemically similar to phosphorus, an essential plant nutrient, it behaves very much like phosphate in the plant-soil system, but it is phytotoxic. In place of phosphate or phosphorus, arsenate becomes a toxicant in plant. Normally, arsenate is absorbed in a manner similar to the phosphate uptake mechanism.

Arsenic is accumulated in plant grown on soil contaminated by arsenical pesticides and areas around mining and smelter. Most of arsenic is accumulated to root because it is not readily translocated to shoot. Some higher plants can tolerate arsenic in high concentrations , i.e., ***Cynodon dactylon*** , ***Agrostis tenuis*** and ***A. stolonifera***. Arsenic is extremely toxic in sensitive or nontolerant plants, but adding of high concentration of

phosphate can alleviate the toxicity. In case of the concentrations of phosphate and arsenate were almost equal or arsenate was more concentrated, there was no significant alleviation of toxicity at low level of phosphate. Fortunately, when the phosphate concentration is increased, phosphate is able to compete with arsenate for the absorption site and then inhibite arsenate absorption (Blumbla and Keefer, 1994).

2.2 PHYSICO-CHEMICAL METHODS OF ARSENIC REMOVAL

Some conventional methods have been used for arsenic removal from water and wastewater, i.e., conventional coagulation, lime softening, sulfide precipitation, adsorption by alumina and activated carbon, ion exchange and reverse osmosis. Summary of treatment methods and removal achieved as follow:

2.2.1 CONVENTIONAL COAGULATION

Additions of various water-soluble chemicals that promote coagulation are found to greatly enhance precipitation process of heavy metals. Coagulation is the addition and rapid mixing of the certain coagulant (s) to neutralize charges and collapse colloidal particles and thereafter agglumeration and settlement of the heavy metals will occur.

Coagulation treatment of arsenic removal process is more widely studied. It is believe that the process also is the function of the oxidation state, pH of the water, initial arsenic concentration as well as type and dose of the coagulant. Some extensively-used coagulants are ferric sulfate, $Fe_2(SO4)_3$, ferric hydroxide, $Fe(OH)_3$, ferrous sulfate, $FeSO_4$, ferric chloride, $FeCl_3$, calcium hydroxide, $Ca(OH)_2$, aluminium hydroxide, $Al(OH)_3$,

aluminium sulfate, $Al_2(SO_4)_3$, cupric sulfate, $CuSO_4$ and calcium carbonate, $CaCO_3$. Ferric sulfate or alum used in the system effectively removed 90% or more As(V) but poorly remove arsenic (III). Prior to coagulation, oxidation of As(III) by chlorine produced removals similar to arsenic (V), this evidence was found by many groups of investigations (Logsdon, Sorg, and Symons, 1974 cited by Guha and Chaudhuri, 1990; Merill et al.,1986).

Three coagulants were tested for arsenic removal, i.e., ferrous sulfate, ferric chloride, and alum. The highest removals (82%) was achieved by ferric chloride. The percentages of arsenic removal by alum and ferrous sulfate were only 32 and 24, respectively. Oxidation of arsenic by chlorine or potassium permanganate firstly, and then followed by coagulation with ferric chloride, the removal of arsenic in the system is increased from 90% to 98.7% . In contrast, oxidation of arsenic was done in the same manner prior to coagulation with copper sulfate, alum or ferric sulfate, the removal of arsenic by those agents was found to be less than 38% (Shen, 1973).

2.2.2 LIME SOFTENING

Lime softening is also effective in arsenic removal. The degree of removal is dependent on the pH value and valence of the arsenic contaminant. The evidence showed that As (V) was more readily removed from hard water than As (III) and removals of both forms increased possibly resulting from increasing pH of the system. For example, 95% As (V) removal was found at pH 10.6 - 11.4 after adding of excess lime softening (Logsdon and Symons, 1973 cited by Hsia and Lo, 1990).

2.2.3 SULFIDE PRECIPITATION

Effectively, this method achieves a high degree of separation of various heavy metals from industrial wastewater. The high reactivity of sulfides (S^{2-} and HS^-) with heavy metal ions and the very low solubility of heavy metal sulfides over broad pH range are more attractive, comparing to the coagulation method. This process is advantageous in terms of metals removal efficiency, solid and liquid separation, sludge-thickening and sludge -dewatering capabilities and sludge stability for disposal by landfill. Also, the valence state of the metal is important in the precipitation process. When the neutralized wastewater is then treated with sodium bisulfide to form metal-sulfide precipitation, the reactions of the 2-valent metal and 3-valent metal are shown respectively, (Whang et. al., 1982).

$$M^{+2} + HS^- + OH^- \text{ ------ } MS + H_2O$$

$$2M^{+3} + 3HS^- + 3OH^- \text{ ------ } M_2S_3 + 3H_2O$$

For example, the sulfide of 2-valent metals are zinc sulfide, ZnS, cadmium sulfide, CdS, nickel sulfide, NiS, cupric sulfide, CuS, etc., and the sulfide of 3-valent metal is arsenic trisulfide. In the actual waste experiment showed that optimal sulfide dosage and pH range (slightly alkaline) were performed, the removals of Cd, Zn and Cu were found to be greater than 99% and the percentages of removals arsenic and selenium(Se) were 98 and more than 92, respectively (Bhattachryya, Jumawan and Grieves, 1979).

2.2.4 ADSORPTION

Adsorption process is the the adhesion of molecules or particles (organic and inorganic compounds) to the surface of a solid adsorbent

without any chemical reaction. Activated carbon and activated alumina were found to be suitable for arsenic removing (Gupta and Chen, 1978). Activated alumina and bone char could remove arsenic from water, but an irreversible change in the chemical structure of the char was investigated. This evidence cause problem of regeneration resuling in limitation of bone char use. Activated alumina can be regenerated by sodium hydroxide followed by sulfuric acid and may be economic for arsenic treatment (Bellack, 1971). Adsorption of As(V) by activated alumina is high than As (III) for ten times (Viraraghavan, Jin and Tonita, 1992). The advantages of activated alumina over anion exchange resin include it low cost and its preference for arsenic in the presence of sulfate and chloride anions.

Activated carbon is widely used to adsorb undesirable organic and inorganic substance in either granular or powdered form, depending upon the application and the process economics. It was determined that lignite-based activated carbon has a better capacity for removal of arsenic than bituminous-based activated carbon and the adsorption capacity of lignite-based powdered activated carbon is superior to lignite-based granular activated carbon (Viraraghavan, Jin and Tonita, 1992).

Recently, arsenic (V) removal from aqueous solution by lanthanum compounds has been found, i.e., lanthanum hydroxide (LH), lanthanum carbonate (LC) and basic lanthanum carbonate (BLC). Two mechanisms of As removal by those compounds are proposed, i.e.,
(i) adsorption by exchange of CO_3 and/or OH group with As ions in the neutral to alkaline pH range where Lanthanum does not dissolve and
(ii) precipitation of insoluble lanthanum arsenate, $LaAsO_4$, in the acid pH range (Tokunaga, Wasay and Park, 1997; Misra et. al., 1998).

2.2.5 ION EXCHANGE

This process is a reversible exchange of ions between liquid and solid phase. Ions held by electrostatic forces to charged functional groups on the surface of an insoluble solid are replaced by ions of similar charge in a solution. Ion exchange is stoichiometric, reversible and selective in removal of dissolved ionic species. Materials used for ion exchange should have ion-active sites throughout their entire structure, high capacity, selectivity for ionic species, capability of regeneration, chemical and physical stability, and low solubility.

Ion exchange is widely used in treatment of hazardous wastewater. Some common applications are desalting, ammonium removal, and treatment of heavy metal in wastewater (Wentz, 1989). Studies on arsenic removal from water by ion exchange are rare. However, the limited data showed that arsenic can be removed by anion exchange resins but not by cation resins. Because arsenic as an anion and cannot be removed with cation resins, neither the common household water softener nor the conventional zeolite (inorganic sodium aluminosilicates) softening system can remove arsenic from water. Anion exchangers exhibit arsenic removal of 55-58 % by equilibrium test (Calmon, 1973, cited by Hsia and Lo, 1990).

2.2.6 REVERSE OSMOSIS

By the process of osmosis, solvent flows through a semi-permeable membrane from a dilute to more concentrated solution. Normally, the solvent flows in the direction that will reduce the concentration of the higher concentrated solution. The osmotic pressure of the solution is the pressure which when applied to the solution, will just prevent the passage of the

solvent through the semi-permeable membrane. In reverse osmosis, a differential pressure that exceeds the osmotic pressure is applied to the membrane, causing the solvent to flow from the stronger (higher concentrated) solution to the weaker (low concentrated) one. Reverse osmosis is very effective in the removal of most of dissolved solids, and also, this treatment should be effective in arsenic removal (Sorg and Logsdon, 1978). However, the important disadvantage of this method: is membrane does not resist to oxidizing agents (Dainichi, 1998).

It is concluded that those methods of arsenic removal are considerably more expensive; using a large-net volume of substances and generally narrower in application. Besides, some of those conventional methods are not easy for operation and maintenance and very often, it is difficult to remove trace arsenic.

2.3 ARSENIC RESISTANCE IN MICROORGANISMS

Increasing in heavy metal contamination in the environment causes increasing interest in understanding the mechanisms of resistance to heavy metals in microorganisms.The use of microbial cells and/or products in bioremediation procedures has been widely considered as one of the appropriate alternatives for environmental clean-up. The possibility of recovering valuable metals from low-grade ores or mines by using microorganisms has also resulted from an enhanced interest in analyzing the interactions between microbes and heavy metals.

2.3.1 ARSENIC RESISTANCE IN ALGAE

Some of both freshwater and marine algae have the highest resistance and/or accumulation capabilities of arsenic. Efficiently, those

organisms take up inorganic arsenic compounds in the aqueous phase, and convert them into nonvolatile and nontoxic organoarsenic compounds, for example, methanearsonic acid or dimethylarsinic acid . It may be a probable mechanism for detoxification and transformation of inorganic arsenic into lipid-soluble compounds which could be explained as an adaptive mechanism to compensate for limitation of nitrate availability (Tamaki and Frankenberger, 1992). It is know that arsenic can replace nitrogen in choline molecule, and arsonium phosphatides are formed. The newly formed products functions efficiently as structural lipid (Wrench and Addison, 1981,cited by Tamaki and Frankenberger, 1992). Certain strains of marine algae are resistance to high level of arsenic, e.g., ***Tetraselmis chuii***, a green flagellate unicellular algae, can survive in an arsenic concentration as high as 1 g/l (gram per liter). The proposed mechanism of tolerance or resistance is the biosynthesis of arsenolipids with an arsenocholine moiety (Bottino et. al., 1978, cited by Cervantes et. al., 1994).

In Japan, algae strains, isolated from samples collected from metal-polluted sites, were tested. In most of the sites, high concentration of As, Cu, Sn or Au contaminated from old mines and a geothermal electric power plant were determined. The algae strains were screened for arsenic tolerance. ***Chlorella vulgaris*** was isolated and found to survive in the culture medium containing 10,000 μg As(v)/g (microgram per gram) dry weight. Growth of ***C. vulgaris*** increased with increasing in arsenate concentration up to 2,000 μg As(v)/g, and accumulation of arsenic also increased with increasing in arsenic concentration in the medium, e.g., maximum accumulation of arsenic in logalithmic growth phase of the organism in the medium containing 10,000 μg As(v)/g was found to be about 50,000 μg/g dry cell weight. It was proposed that the organisms might remove inorganic-arsenic compounds

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from freshwater by the aid of bioaccumulation (Maeda et. al., 1988 and Maeda et. al., 1993). The analysis of this algae found that arsenic was combined with a protein (molecular weight of around 3000) in the arsenic-accumulated living cells. Analysis of the amino acid of the arsenic-bound protein showed that no metallothionein-like protein was inductively biosynthesized in ***C. vulgaris*** after exposure of arsenic. In contrast, when the same alga was exposed to zinc and cadmium, metallohionein-like proteins were inductively synthesized. It means that the algae strains may have another detoxifying process for arsenic tolerance, e.g., methylation. (Maeda et. al., 1992, cited by Maeda, 1994). Additionally, other freshwater alga are found to be highly resistant to arsenic and are capable of arsenic metabolizing into various methylated forms, i.e., ***Chlamydomonas reinhardtii, Anabaena variabilis, Cryptomonas erosa, Scenedesmas sp., Ankistrodesmus sp.***(Tamaki and Frankenberger, 1992).

2.3.2 ARSENIC RESISTANCE IN FUNGI

Some strains of fungi are resistance to high concentrations of heavy metals by binding of the metal ions by proteins or chelation by phytochelatin peptides. On the other hand, yeast posses multidrug resistance (mdr) transporters to cope with toxic inorganic ions. Neither of these mechanisms seem to be related to arsenic resistance in yeast and fungi.

Biological methylation of arsenic was first recognized in the early nineteenth century when several people died in their sleeping from arsenic poisoning. Gosio, an Italian scientist, found that a fungi living on the arsenical pigments of wallpaper (Scheele's green and Schweinfurter green) emitted a volatile arsenical with a charaeteristic garlic-like odor. In 1935, Challenger demonstrated ***Scopuloriopsis brevicaulis***, the bread mold, was

capable of synthesizing trimethylarsine from inorganic arsenic salts, and a metabolic pathway was proposed for the production of trimethylarsine from arsenite. It was demonstrated that methyl transfer to arsenic must occur by nucleophillic attach by some reduced arsenic species, in particular, on the carbon-sulfur bond of S-adenosylmethionine (SAM) as **Fig. 2-1**, page 43 (Challenger,1945).

Three different fungal species, i.e., ***Candida humicola***, ***Gliocladium roseum*** and ***Penicillium sp.***, were capable of converting methanearsonic and dimethylarsinic acids to trimethylarsine (Cox and Alexander, 1973b, cited by Cullen and Reimer, 1989; Iverson and Brinkanan, 1978).. In addition, ***C. humicola*** used arsenate and arsenite as substrates to produce trimethylarsine. Cell-free homogenates of ***C. humicola*** transformed arsenate into arsenite, methanearsonic and dimethylarsinic acid (Cullen, McBride and Pickett, 1979). Although trimethylarsine formation from inorganic arsenic and methanearsonic acid is inhibited by the presence of phosphate, its synthesis from dimethylarsinic acid is increased in the presence of phosphate (Cox and Alexander, 1973 a). The yeast, ***Rhodotorula rubra*** , did not accumulate arsenite, but transported some of it into the culture medium and methylated the remainder first to methylarsonic acid and then to dimethylarsinic acid. Finally, the latter compound was methylated further and volatile alkylarsines were formed (Vidal and Vidal, 1980). In 1992, Huysmans and Frankenberger isolated ***Penicillium sp*** from agricultural evaporation pond water capable of trimethylarsine production from methanearsonic acid and dimethylarsinic acid. The occurring of arsenic methylation found to via transfer of the cabonium ion from S-adenosylmethionine (SAM) to arsenic. Inhibition of

arsine production by methionine as a methyl donor. (Cullen et. al., 1977, cited by, Cullen, McBride and Reimer, 1979).

Soil fungal species may play a major role in the transformation and movement of arsenic chemicals used in agriculture, the methylation of arylarsonic acid is also important because of their wide use as food supplements for swine, turkeys and poultry. ***C. humicola*** is capable of methylating benzene arsonic acid to produce volatile dimethylphenylarsine. (Cullen et. al., 1983, cited by, Tamaki and Frankenberger, 1992). Also, methylphenylarsinic acid and dimethylphenylarsine oxide are reduced by ***C. humicola*** to dimethylphenylarsine. Arsanilic acid, which contains an amino group at the para position of phenylarsonic acid, was not converted to a volatile arsine but it has been reported that soil treated with arsanilic acid can be lose their arsenic component. The evidence of depletion of arsenic from diluted solution of the highly effective wood preserving fungicide, chromate-copper-arsenate, by volatilization was shown in adaptive strain of ***C. humicola*** in arsenic methylation (Cullen et. al., 1984). It has also been demonstrated that a variety of soils have potentially produce alkylarsines (Woolson, 1977). Soil amended with inorganic and methylated arsenic herbicides produce dimethylarsine and trimethylarsine (Woolson and Kearney, 1973; Woolson, 1977; Baker et. al., 1983; Hassler, Klein and Meglen, 1984;).

2..3.3 ARSENIC RESISTANCE IN BACTERIA

Numerous species of bacteria are able to transform arsenic compounds. The processes of transformation are methylation ,oxidation and reduction. The transformation reactions may be a part of specific detoxification processes (Silver and Keach, 1982).

2.3.3.1 Methylation and Demethylation

Methylation and demethylation are important mechanisms in transporting and cycling of arsenic in the environment. Although the exact nature of the process is still further investigated, the environmental conditions, which promote microbiological activities, would likely enhance the methylation and volatilization processes. Also, those metabolic processes affect the toxicity of individual arsenic species (Hamasaki et. al., 1995).

In 1935, Challenger demonstrated that the bread mold, ***S. brevicaulis,*** was capable of synthesizing of trimethylarsine from inorganic arsenic. Thereafter, it has been demonstrated that ***Methanobacterium*** **strain M.O.H.** reduced and methylated arsenate under anaerobic conditions to dimethylarsine. In 1971, McBride and Wolfe found that methylcobalamin (methyl B_{12} vitamin) acts as the methyl donor in the biosynthesis of dimethylarsine from arsenate or arsenite in cell extract of the bacterium. Adenosinetriphosphate (ATP) and hydrogen are essential for the formation of dimethylarsine. The pathway of anaerobic biomethylation of arsenic by ***Methanobacterium sp.*** was shown **Fig. 2-2** on page 44 (McBride and Wolfe, 1971).

Seven years later, two pure bacterial cultures, ***Aeromonas*** and ***Flavobacterium*** **sp.**, isolated from lake water and another bacterium ***Escherichia coli***, commonly found in the intestine of an organism and in polluted water, had the capability of arsenic methylation when grown in a medium of 0.5% nutrient broth, 0.1% glucose and 10 mg/L arsenic

compound (as As) at 20 °C under aerobic condition (MaBride et. al., 1978). After that, five bacterial species, i.e., ***Proteus* sp.**, ***E. coli*, *Flavobacterium* sp.**, ***Corynebacterium* sp. and *Pseudomanas* sp.** were capable of arsenate methylation in buffered-salt medium. Results showed that five bacterial species were able to reduce sodium arsenate to arsenite and methylate to ultimately yield dimethylarsine. ***Proteus sp.*, *E. coli*** and ***Pseudomonas sp.*** also formed monomethylarsine as an end product, and ***Corynebacterium*** and ***Pseudomonas sp.*** were able to produce trimethylarsine. However, only ***Pseudomonas sp.*** was able to produce all four metabolites (Shariatpanahi et. al, 1981).

New arsenic methylating bacterium belonging to the Flavobacterium-Cytoghaga group was isolated from soil containing 1.5 ppm arsenic. A volatile product of the methylation of both arsenate and arsenite, trimethylarsine (TMA) was formed exclusively. The highest product (approximate 5 ppm) or TMA had been formed in a medium containing 50 ppm As as arsenite (Honschopp et. al., 1996). Interestingly, another study indicated that arsenic methylation is pH-dependent with the highest rate occurring at pH 3.5 . It is suggested that arsenic mobilization from sediments to the overlying water phase was enhanced by acidification (Baker et. al., 1983).

Based on the microbial population and arsenic volatilization relationship in shale, it would appear that fungal growth is predominantly responsible for arsenic volatilization in this material at higher nutrient levels, in contrast, bacteria would play a more important role under conditions of lower nutrient availability. (Hassler, Kline and Meglen, 1984; Sanford and Kline, 1998) Woolson (1977) demonstrated dimethylarsine and

trimethylarsine was produced from arsenate-treated soil under both aerobic and anaerobic conditions. The summary of microbial production of alkylated arsines was shown **Table 2.3** on page 40-41.

Demethylating activity has also been reported in two isolates of ***Actinomycetes*** (Von et. al., 1968). A few studies have been conducted to examine organoarsenical demethylation. Woolson and Kearney (1977) showed that dimethylarsinic acid was degraded to arsenate in soil under aerobic condition but not anaerobic one. ^{14}C – dimethylarsenic acid was degraded in a ecosystem model to arsenate, arsenite, and $^{14}CO_2$. Arsenate was the predominant from after 59 days incubation. Certain amount of arsenic was lost from the systems, probably as alkylarsine. Andrea (1979) suggested that biological demethylation may be the dominant process responsible for the regeneration of inorganic arsenic from methylated arsenicals and the evident was confirmed (Lemmo et. al., 1983).

2.3.3.2 OXIDATION

Oxidation of As(III) represents the potential process of detoxification which allows microorganisms to tolerate high levels of arsenite. Several examples of bacterial oxidation of arsenite to arsenate were being reported as early as 1918 by Green bacterium, isolated from arsenical cattle dips in South Africa. This organism is grew in 1% arsenic trioxide medium and oxidized the arsenite to arsenate. Now, it was lost, and described as ***Bacillus arsenoxydan***. In 1940s, Turner and Legge investigated that the spontaneous oxidation of arsenite in cattle dips in Australia by different bacterial isolates. Most of those fell into the classes of ***Pseudomonaceae*** or ***Achromobacter***. They also discovered that a soluble enzyme, arsenite dehydrogenase(named by the investigations) affected the

oxidation of arsenite. The activity was inducible and appeared to be coupled to electron transport via cytochrome to oxygen (Summers and Silver, 1978). Isolated ***Alcaligenes*** strains was able to oxidize arsenite and oxygen acted as a final electron acceptor (Osborne and Ehrich, 1976; Phillips and Taylor, 1976). In 1992, Anderson et. al. purified and characterized arsenite oxidase from a selected strain of ***Alcaligenes faecalis*** as shown in **Fig. 2-3**, page 45. The enzyme was located on outer surface of the inner membrane and exhibit arsenite oxidation activity in the presence of azurin and cytochrome C as electron acceptor. Molecular mass of the purified protein was 85,000 and occurred as a monomer containing several metal centers including probably both (4Fe-4S) HiPIP (high potential iron protein) and Rieske type (2Fe-2s) centers (Anderson, Williams and Hille, 1992 cited by Cervantes et. al., 1994). In extreme environments, e.g., acid mine water, arsenic concentrations was found to be as high as 2.0 to 13 mg/L and the major inorganic species is arsenite. It was demonstrated that oxidation of arsenite by heterotrophic bacteria play an important role in detoxifying the environment catalyzing as much as 78 to 96% of the arsenite to arsenate (Wakao et. al., 1988).

2.3.3.3 Reduction

Occurring of microbial reduction of arsenate has been found in a variety of bacterial species. For example, cell extracts of ***Micrococcus lactilyticus*** and whole cells of ***M. aerogenes*** were known to reduce arsenate to arsenite with H_2 as a reductant (Woolfolts and Whitely, 1962), and various strains of ***Pseudomonas sp.*** and ***Alcaligenes sp.*** reduce arsenate and arsenite to arsine (Cheng and Focht, 1979). The reduction reaction may be one of mechanisms of arsenic resistance. Arsenate is transported through cell membranes in competition with the transport of

phosphate ions, but arsenite is transported through a phosphate-independent mechanism (Wood and Wang, 1983; Belliveau et. al., 1987). Any-way some bacteria posses two distinct phosphate transport pathways (Willsky and Malamy, 1980), i.e., one system take up both phosphate and arsenate at equal rates (named the Pit, for Pi transport, whereas the other one the Pst, for phosphate-specific transport, system) is highly specific for phosphate and transports arsenate poorly (Cervantes et. al., 1994; Tamaki and Frankenbergar, 1992). If biologically available phosphate is scarce, arsenate is able to interfere with microbial phosphate uptake and metabolism. Bacteria can alleviate the competition in two ways: by inactivating nonspecific phosphate-uptake system (the Pit system) and inducing more- specific ones (the Pst system); or by inducing a system of arsenate detoxification, e.g., resistance mechanism (Beveridge et. al., 1997). The mechanism of arsenic resistance by the production of specific protein encoded by similar ***ars*** operons. Those operons are located on the chromosome and/or on plasmids. For the latter genetic material, arsenic resistance determinants ***(ars)***, isolated from both gram- negative and gram-positive bacterial species, have been found to be very homologous and generally consist of either three or five genes that has been organized into a single transcription unit (Silver and Walderhaug, 1992). In gram negative bacteria, the well-studied ***ars***-containing plasmid, R773, isolated from ***E. coli*** (Chen et. al., 1986; Silver et. al, 1981); other plasmids, the operon consists of five genes named ***ars*** **RDABC** and IncN plasmid R46, found originally in ***Salmonella typhimurium*** (Bruhn et. al., 1996).

The first two, ***ars*** R and ***ars*** D, encode regulatory proteins. The ***ars*** A and ***ars*** B genes encode the actual resistance, Ars A protein is an arsenite-stimulated ATPase which is part of a complex with the

membrane-bound Ars B protein. The Ars B protein forms the transmembrane pathway by which arsenicals are extruded from the cell by an ATP-dependent process (Silver et. al., 1993). The pump which extrudes salt of arsenite exhibits no evolutionarily relationship to other families of transport ATPase. The last gene of the operon, ***ars*** C, encodes a reductase that catalyzes the conversion of As (V) to As (III). The arsenate reductase of these arsenic- resistance systems does not appear to be involved in energy conservation when the reduction of arsenate to arsenite is catalyzed (Ji and Silvar, 1995).

Contrarary, in gram positive bacteria, the well-studied ***ars***-containing plasmids isolated from ***Staphylococcus*** species, i.e., plasmid pI258 and pSX267), the ***ars*** RBC is conserved, where the ***ars*** D and ***ars*** A genes are absent (Broer et. al., 1993 and Rosenstein et. al., 1992). In this case, the Ars B protein is believed to drive the efflux of intracellular arsenite ions through cellular membrane (Ji and Silver, 1992). Operons in the chromosome found in ***E. coli*** contain only three genes; ***ars*** RBC, which similar to ***ars*** operons of two ***Staphylococcol*** plasmids (Diorio et. al., 1995; Silver, 1996; Xu et. al., 1998). Models of arsenic resistance mechanisms and energy coupling in ***E. coli*** and ***S. aureus*** was shown in **Fig. 2-4,** page 46 (Cervantes et. al., 1994).

2.4 NOVEL MECHANISM OF ARSENIC

2.4.1 ARSENIC AS THE ELECTRON ACCEPTOR IN BACTERIA

Some microorganisms can utilize certain metals and metalloids as electron acceptors for respiration, i.e., iron or Fe(III), manganese or Mn(IV), uranium or U(VI) and Se(VI) (Lovely and Phillips, 1988; Nealson and

Saffarini, 1994; Oremland et. al., 1994). Certain groups of bacteria are able to grow anaerobically by reducing arsenate to arsenite and arsenate reduction does appear to support growth. There were numerous researches done to support the above evident. For example: ***Sulfurospirillum arsenophilus*** strain MIT-13, a newly bacterial isolate, was studied in the medium containing arsenate. In the absence of oxygen, they grew normally and was found to use arsenate as a terminal electron acceptor and lactate as the electron donor and carbon source. The lactate was presumably oxidized to acetate, but not completely to CO_2 (Ahmann et. al., 1994). ***S. barnesii***, strain SES-3, which was isolated first as a sulfate reducing bacterium, was similarly found to reduce arsenate to arsenite and also reduce Fe (III) and thiosulfate under an anoxic condition or absence of oxygen. In the medium, lactate which was oxidized to acetate acted as the electron donor and carbon source (Laverman et. al., 1995). ***Chrysiogenes arsenatis*** strain BAL-1^T, a newly isolated-strictly-anaerobic organism, was able to grow on acetate which act as the electron donor only it arsenate, nitrate, or nitrite was present in the medium, and all of then as the terminal electron acceptor (Macy et. al., 1996). ***Desulfutomaculum auripigmentum*** strain OREX-4, a newly arsenate reducing bacterium, was able to grow on lactate, with either arsenate or sulfate serving as the electron acceptor. Preferrably, the bacterium was capable to precipitating As_2S_3 when growth on arsenate and sulfate (Newman et. al., 1997a; Newman et. al., 1997b). ***Wollinella succinogenes***, a dose relative of strains MIT-13 and SES-3, is also known to use arsenate as a terminal electron acceptor (Newman, Ahman and Morel, 1998). Two alkalophilic-moderate halophiles, ***Bacillus selenitireducens*** strain MLS-10 and ***B. arsenicoselenatis*** strain E1-H, have been found to grow by dissimilatory reduction of arsenate to arsenite with the concomitant oxidation lactate to acetate and CO_2. Both strains were alkalophiles and had

optimal specific growth rates in the pH range of 8.5 – 10. Strain E1-H had a salinity optimum at 60 g/l NaCl, while strain MLS-10 had optimum growth at lower salinity range, 24-60 g/l NaCl (Blum et. al., 1998). The demonstration was shown that arsenate reduction in pure culture stoichiometrically follows the oxidation of lactate (Laverman et. al., 1995; Newman et. al., 1997), and the oxidation of acetate (Macy et. al., 1996). The reactions were shown as follow:

$$CH_3\text{-}CHOH\text{-}COO^- + 2\,HAsO_4^{2-} + 4H^+ \longrightarrow CH_3\text{-}COO^- + 2HAsO_2 + CO_{2(g)} + 3\,H_2O$$

$$2\,HAsO_4^{2-} + 2H_2AsO_4^- + 5\,H+ + CH_3COO^- \longrightarrow 4H_3AsO_3 + 2HCO_3^-$$

In sediment, the dissimulatory arsenate reduction was found to be an important process for transforming of arsenic. Reduction occurring in those slurries has been demonstrated linking to cellular energy generation. The respiratory inhibitors, i.e., uncouples dinitrophenol, rotenone and 2-heptyl-4-hydroxyquinoline N-oxide, individually, blocks arsenate reduction in bacteria. In addition, acetate was oxidized to CO_2 with the concomitant reduction of arsenate to arsenite. The reduction might be used as a technique for promoting the leaching of arsenic from arsenic-contaminated soil because of high solubility of arsenite than arsenate (Dowdle et. al., 1996).

Naturally, the strain MIT-13 is a strong arsenic-transforming organism found in the sediment and it is possibly suggested that dissimulatory arsenic reduction may contribute to arsenic flux from anoxic sediments in the most arsenic contaminated region of the Aberjona Watershed (Ahman et. al., 1997).

2.4.2 BIOCHEMICAL MODEL FOR ARSENATE RESPIRATION

Arsenic resistance appears to be widespread among bacteria. However, mechanism of arsenate and arsenite resistance has been investigated in depth only in organisms where resistance is conferred by protein encoded by ***ars*** aperons. Those operons might be located on chromosome or plasmid. The reduction of arsenate to arsenite controlled by arsenate reductase (know as the Ars C enzymes) which were not found to be involved in energy conservation. Recently, characterization of a respiratory arsenate reductase (Arr.) which is different from non-respiratory arsenate reductase was studied:

Purification and characterization of Arr. of ***C. arsenatis*** indicated that Arr. Enzyme consists of two subunits, ArrA and ArrB, with molecular masses of 87 and 29 kDa, respectively and heterodimer $\alpha_1\beta_1$ with a molecular mass of 123 kDa. The molecule contains molybdenum (Mo), iron (Fe), acid-labile sulfur (S) and zinc as cofactor constituents. The actual mechanism by which arsenate reduction is coupled to the cytoplasmic membrane is yet to be determined. **Table 2-4,** page 42 showed the comparison of different arsenate reductase between respiratory arsenate reductase of ***C. arsenatis*** and non-respiratory arsenate reductase of ***E.coli*** and ***S.aureus*** (Krafft and Macy, 1998).

Another study showed that the respiratory arsenate reductase of strain SES-3 is a multimeric integral membrane protein (molecular mass of the complex exceeding 100 kDa). The gene encoding the arsenate reductase has a presumptive chromosomal locus. It means that the ability of the

organism to respire arsenate is highly stable from one generation to the next, regardless of whether arsenate is present in the medium or not, and it is constitutive in nitrate-or selenate-grown cells. The composition of the enzyme may be Fe: S clusters and *b*-type cytochrome. **Fig. 2-5**, page 47 showed the model for growth on lactate and arsenate for strain SES-3. The reductase spans the cytoplasmic membrane, with the active site for the reduction of arsenate to arsenite facing the cytoplasm. An orientation would allow a proton motive force to be generated from the flow of electrons from a cytoplasmically oriented lactate dehydrogenase to the arsenate reductase, either through diffusion of H_2 (formed by as cytoplasmic hydrogenase) through the membrane to the outside. In the periplasm, the H_2 would be oxidized by a hydrogenase, allowing electrons to flow back to the arsenate reductase through membrane-bound electron carriers (Newman, Ahman, and Morel, 1998). There are other models would be verified by further studies.

2.5 ALTERNATIVE METHODS OF REMOVAL ARSENIC

Chemical and physico-chemical methods are available for extensive purification of the water, but they are still expensive for application on an industrial scale. Bioremediation may be more economical and usually significantly more favorable than other techniques. *The primary reason* is : there is no need for expensive costs of disposal of contaminated soil and high water-content wastes after using capable microorganisms isolated from natural environment. Those selected microorganisms are able to remove toxic metals and metalloids from contaminated waters and waste streams by converting them to form the products by precipitation or volatilization.

2.5.1 VOLATILIZATION

Volatilization of heavy metals occurs when living microorganisms methylate metals. Organometals and organometalloid could be found by the actions of certain groups of microorganisms. It was proposed that methylation process would be the mechanism of metal or metalloid detoxification. The most well-know example of volatilization is the methylation of mercury, whereby the mercury ion Hg(II) is converted to methylmercurial compound or dimethylmercury. Frequently, some metal, i.e., selenium, tellulium, arsenic and tin, are subject to be volatilized by bacteria and fungi. Arsenic methylation to methylarsenical products was shown **Table 2-3,** page 40-41. Although volatilization is important in metal transformation in the environment, particularly in soils and sediments, but the toxicity of some methylated metals and the difficulties in capturing volatilized metal have created further problems. Todate, little research and development effort on commercial scale employing the microbial mechanism of metal methylation has been devoted (Brierley, Brierley, and Davidson, 1989).

Due to the reaction of sulfides with reduced arsenic to form arsenic sulfides, which are apparently incapable of being biomethylated (Meada, 1994). However, microbial transformation may be useful in the implementing a bioremediation technique to remove arsenic from water or soil. Alkylarsine gas generated can be captured and concentrated on an effective trap, e.g., activated carbon. More effeciently, preferable techniques should be further studied (Huysmans and Frankenberger, 1992).

2.5.2 PRECIPITATION

2.5.2.1 Dissimilatory Sulfate Reduction

Precipitation of metal occurs when microorganisms produce metabolic products that are further excreted and resulted in the immobilization of metals. One of the best examples of metal precipitation is the production of sulfide by sulfate-reducing bacteria. The organisms are obligate anaerobic, using sulfate as terminal electron acceptor, as shown below.

$$SO_4^{2-} + 8\,H^+ + 8e^- \longrightarrow HS^- + 3H_2O + OH^-$$

The reducing power of sulfate reduction derives from the catabolism of organic substrates. An overall equation for the reduction of dissimilatory sulfate by lactate is:

$$2CH_3CHOHCOO^- + SO_4^{2-} + 8e^- \longrightarrow 2CH_3COO^- + S^{2-} + 2CO_2 + 2H_2O$$

The sulfide readily reacts with soluble heavy metal to form in soluble metal sulfide minerals. Solubility of most toxic metal sulfides are very low. It is possibly indicated that formation of sulfide by the SRB is responsible for removing soluble metals from metal-polluted waters (Brierley, Brierley and Davidson, 1989; White, Sayer and Gadd, 1997). The advantage of using bacterially generated sulfides rather than chemical sulfides obtained form commercial vendor, is its safety. Bacteria, which provide a low pressure and continuous sulfide source can be easily controlled for continuous processing of arsenic contaminated solutions and at the same time this technique can minimize the inherent risk of bulk chemical sulfide usage and storage (Belin, Dinsdale and Altringer,1993).

2.5.2.2 Arsenic Precipitation by Sulfate-Reducing Bacteria (SRB)

Sulfate reduction is an effective mechanism for precipitating a variety of metal contaminants as metal sulfides, e.g., precipitation of nickel by SRB (Hammack and Edemborn,1992), reduction of soluble U(VI) to insoluble U(IV) by ***Desulfovibrio desulfuricans*** (Lovely and Phillips, 1992), and precipitation of copper using ***Desulfuvibrio sp.*** (Panchanadikar and Kar, 1993) and etc.

Arsenic, one of metalloids, is able to precipitate by SRB. It was found that after 6 days of incubation, 96% of the initial 10 mg/l arsenic concentration was removed from solution in the bottles containing active mixed population of sulfate-reducing bacteria. The forming products were arsenous sulfide (AsS) or realgar and arsenic trisulfide (As_2S_3) or orpiment (Uhrie et. al., 1996). In microcosm experiments with arsenic contaminated sediments demonstrated that precipitation of dissolved As (III) occurred simultaneously with bacterially mediated sulfate reduction. Analysis of the precipitated samples from actively sulfidogenic, As (III)-removing microcosms suggested that certain amount of As(III) was precipitated as an arsenopyrite or FeAsS (Rittle, Drever and Colberg, 1995). The study in pack-bed bioreactor showed that sulfate-reducing bacteria was able to removed soluble arsenic from 0.6 mg/l to less than 0.1 mg/l (Somlev and Tishkov, 1994). In the 2-stage process, SRB generated sulfides from the stage-1, and followed by arsenic precipitation arsenic from wastewater in the stage-2 treatment reactor. The results indicated that arsenic concentration dropped from 70 ppm in the feed to 60 ppm after about 4 days of operation

and to less than 2 ppm after 19 days of operation (Belin, Dinsdale and Altringer, 1993). Latest, using SRB on an activated carbon support in an anaerobic up-flow column to treat arsenic contaminated petroleum wastewaters containing over 14 mg/l arsenic. Arsenic was removed to below 0.5 mg/l using retention times of 18 hours (Adam, Pickett and Nilsen, 1999). In addition, Lovely and Coates (1997) suggested that the precipitation of arsenic is preferable to solubilization, it might be possible to sequester arsenic in anaerobic sedimentary environments by enhancing sulfate reduction as well as As (V) reduction because sulfide and As (III) can combine as insoluble As_2S_3. The evident was found firstly in new bacterium, ***Desulfotomaculum auripigmentum,*** which was able to precipitate As(III) to form arsenic trisulfide. The precipitating product was resulted from bacteria reduction of As(V) to As(III) and S(VI) to S(-II). Electron microscopy of thin sections showed that the sulfide precipitate was forms both intra-and extracellularly (Newman, Beveridge and Morel, 1997; Newman et. al., 1997).

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Table 2-1 Calculated ratios of arsenic concentrations in natural reservoirs with respect to soils.

Reservoir	Approximate ratio with respect to soil
Rocks	25,000
Oceans	4
Soil	1
Biota (plant, man, microbes)	0.0005
Atmosphere	0.000001

Source : Bhumbla and Keefer, 1994

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Table 2-2 Calculated arsenic rates of transfer (Fluxes).

From:	To:	Approx. amount ($\times 10^8$ g/yr)
Land	Oceans	3,000
	Atmosphere	1,000
	Biota	300
Atmosphere	Oceans	2,000
	Land	1,000
Oceans	Sediments	2,500
	Biota	1,300
	Dissolved	1,000
Sediments	land	2,400
Mining, smelting		500
Terrestrial biota	Land	300
Volcanoes	Land	54
	Sediments	40
	Atmosphere	3

Source : Bhumbla and Keefer, 1994

Table 2-3 Microbial production of alkylated arsines.

Microorganisms	Product Formed	Conditions
Penicillium brevicaulis (*Scopulariopsis brevicaulis*)	TMA	Bread crumbs spiked with As_2O_3 or methyl-arsenate or cacodylate
P. notatum *P. chrysogenum* and *Aspergillus niger*	TMA	Sodium methylarsonate and sodium cacodylate
A. glaucus	TMA	Bread crumbs treated with arsenous and methylarsonic acids
Candida humicola	TMA	Arsenic sources, sodium arsenate, methanearsonic and dimethylarsinic acids
C. humicola	TMA	Substrate was chromate copper arsenate (wood preservation)
Gliocladium roseum *Pencillium* sp.	TMA	Methanearsonic and dimethylarsinic acids
Lenzites trabea	Volatile As derivative with garlic odor (probablyTMA)	Medium containing As trioxide
Methanobacterium strain M.O.H.	DMA	Cell-free extracts, anaerobic conditions in presence of CH_3-B_{12}, ATP, H_2

Table 2-3 Microbial production of alkylated arsines. (cont.)

Microorganisms	Product Formed	Conditions
Desulfovibrio vulgaris **strain 8303**	**Volatile As derivative with strong garlic odor indicative of as arsine**	**Cell-extracts stimulated by** CH_3-B_{12}
Aeromonas **sp.** ***Flavobacterium*** **sp. and** ***Escherichia coli***	**TMA**	**Nutrient broth, containing As (III) or methanearsonic acid or dimethylarsinic acid**
Flavobacterium **sp.** ***Proteus*** **sp. and** ***E. coli*** ***Pseudomonas*** **sp.** ***Corynebacterium*** **sp.**	**DMA** **DMA, MMA** **DMA, MMA** **DMA,MMA,TMA** **DMA, TMA**	**These five strains incubated in buffered salt medium and sodium arsenate**

Source : Tamaki and Frankenberger, 1992 and Shariatpanabi et. al., 1981

(TMA : Trimethylarsine ; DMA : Dimethylarsine; MA : Monomethylarsine)

Table 2-4 Comparison of different arsenate reductases.

	Arsenate reductase			
	Chrysiogenes arsenatis	***Sulfurospirillum barnesii***	***Escherichia coli***	***Staphylococcus aureus***
Function	terminal reductase of arsenate respiration		part of the arsenic resistance mechanism	
Localisation	periplasm	cytoplasmic membrane	cytoplasm	
Composition	two subunits (ArrA and ArrB)	multimeric integral membrane protein	one subunit (ArsC)	
Molecular mass	ArrA 87 kDa, ArrB 29 kDa	> 100 kDa	ArsC 16 kDa	ArsC 14.5 kDa
Structure	heterodimer $\alpha_1\beta_1$	-	monomer α_1	
Cofactors	MoCo, Fe-S,Zn	Fe-S,*b*-type cytochrome	none	
Km for arsenate	0.3 mM	0.2mM	8 mM	2 mM

Source : Newman, Ahmann, and Morel, 1998

Krafft and Macy, 1998

(Arr. = respiratory arsenate reductase, MoCo = molybdenum cofactor)

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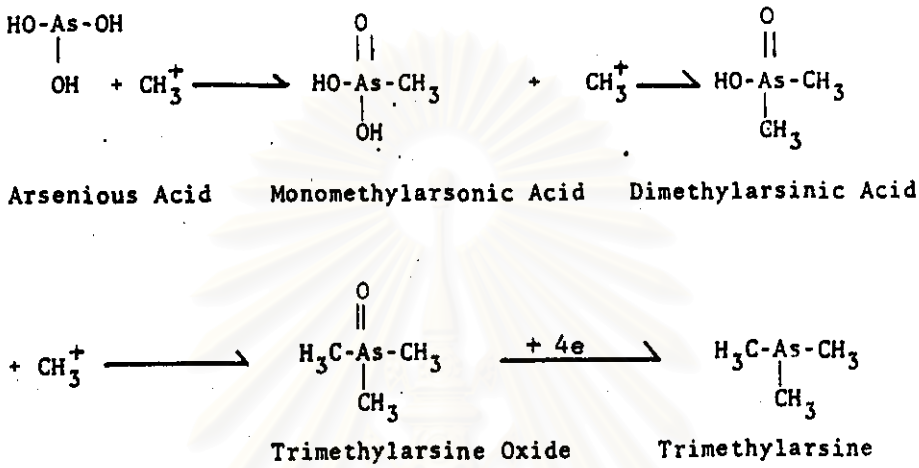

Figure 2-1 Challenger's proposed methylation pathway

Source : Tamaki and Frankenberger, 1992

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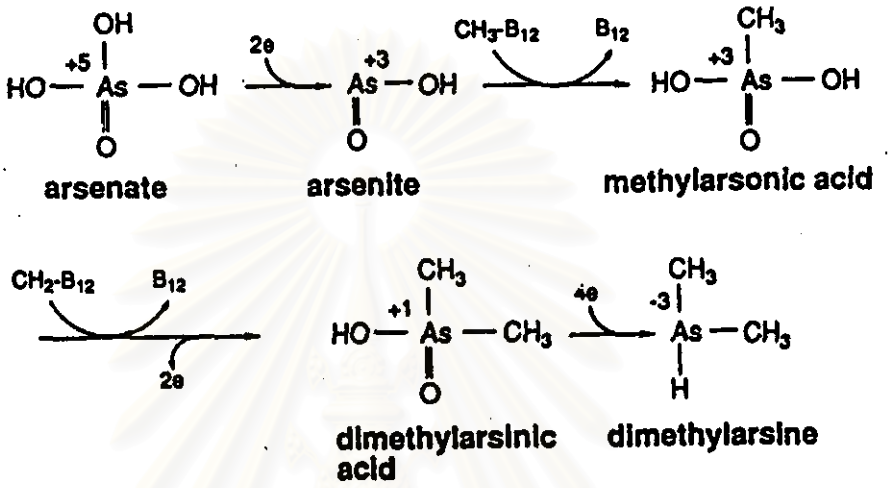

Figure 2-2 Anaerobic biomethylation pathway by *Methonobacterium sp.*

Source : McBride and Wolfe, 1971

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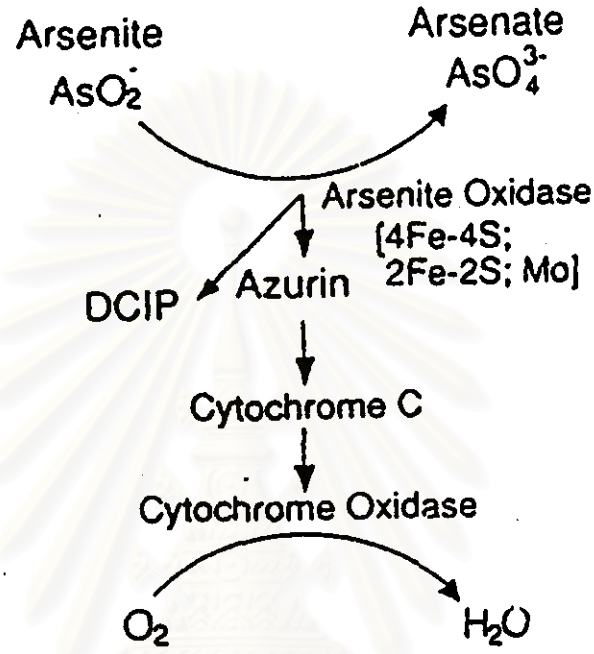

Figure 2-3 Proposed model for energy-coupling of arsenite oxidase to cytochrome oxidase.

Source : Cervantes et. al., 1994

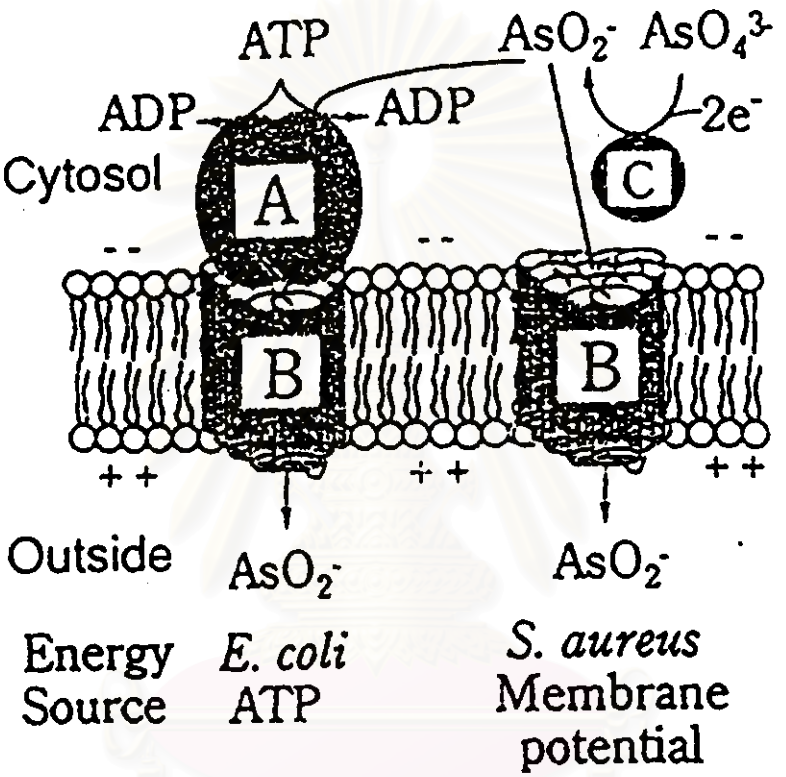

Figure 2-4 Model for arsenic resistance mechanisms and energy-coupling in ***Escherichia coli*** and ***Staphylococcus aureus***.

Source : Cervantes et. al., 1994

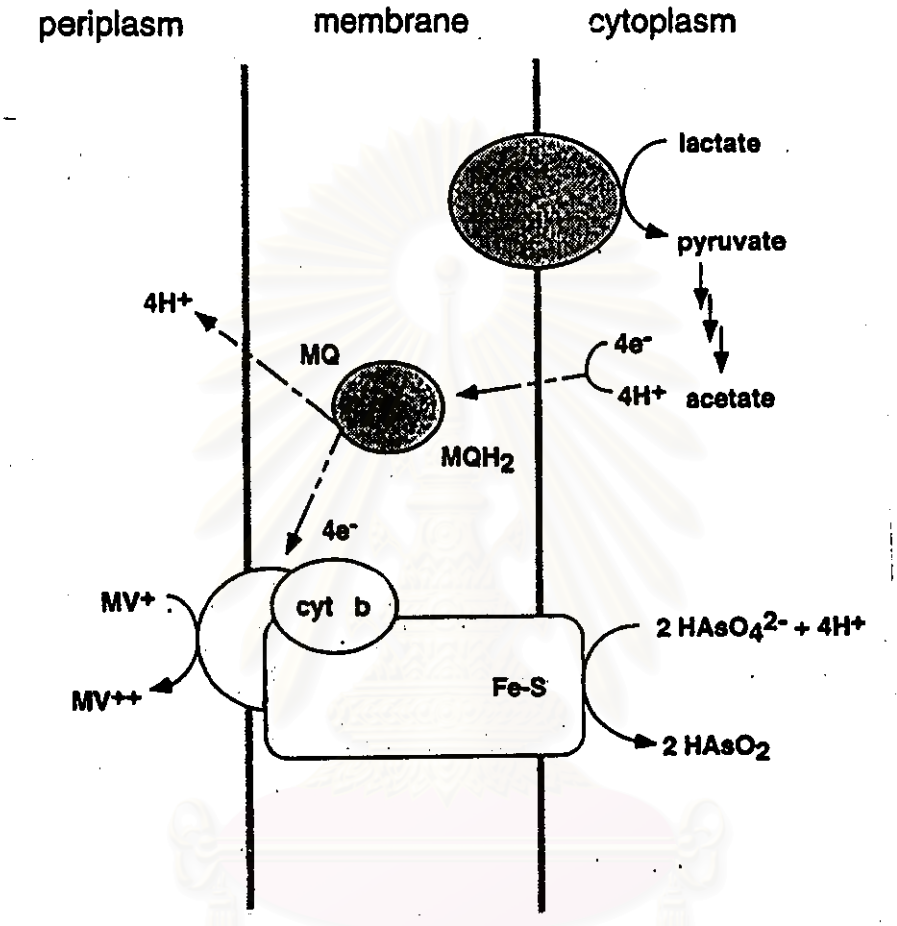

Figure 2-5 Biochemical model of arsenate respiration in ***Sufurospirillum barnesii*** (SES-3).

Source : Newman et. al., 1998