CHAPTER II

LITERATURE REVIEWS

2.1 Background

2.1.1 Landfill status:

The main problem from landfill leachate migration is contamination of groundwater by heavy metals. Pollution Control Department (PCD), 1998 showed that groundwater has been significantly contaminated by heavy metals and other harmful substances in the areas around numerous waste sites in Northern and Central Thailand. This problem may be caused by inappropriate management of landfill system with respect to both design and operation, as well as no proper separation of the wastes. The contamination of household hazardous waste in municipal waste is one cause to this problem. According to a survey conducted by Public Health Research Institute of Thailand (Public Cleaning Bangkok Metropolitan Admin (BMA), 1997), the contamination of household hazardous waste in municipal solid waste in Bangkok in 1993 was 22 tons per day and the trend would increase. The household hazardous wastes are oil, tires, used oil, automobile battery, household battery, mobile battery, etc.

The major sources of heavy metals in landfills are the co-disposed industrial wastes, incinerator ashes, mine wastes and household hazardous substances such as batteries, paints, dyes, inks, etc. (Forstner, 1991). Common heavy metals in landfills are iron, cadmium, copper, zinc and nickel (Flyhammer, 1998). These heavy metals are toxic and thus liable to produce serious problems on health of population and on maintenance of the biosphere. Consumer batteries contribute significantly to this problem. Heavy metal concentrations in leachate do not appear to follow degradation patterns of organic indicators such as COD, BOD, nutrients, or major ions. Heavy metal release is a function of characteristics of leachate such as pH, ORP, and concentration of complexing agents (Forstner, 1991).

2.1.2 Acid Mine Drainage status:

One of the most important problems affecting mining companies around the world is the treatment of acid mine drainage (AMD) also known as acid rock drainage (ARD). AMD is characterized by its high acidity, high concentration of metals (for instance, Cu, Fe, Zn, A1, Pb, As, Cd, etc.) and high concentration of dissolved sulfates. Typically, the pH is lower than 3 and the sulfate concentration is higher than 3,000 part per millions (ppm) (Lyew et al., 1994). Therefore, the treatment of mining effluents is aimed at water neutralization and removal of the dissolved metals and sulfates.

The techniques traditionally used for the treatment of residual acid mine waters have been based on chemical methods of neutralization and precipitation. These techniques, even though quick and effective, present several disadvantages, such as the need for building additional treatment plants, the high cost of the chemical reagents used and the generation of an important volume of sludges which need to be relocated. Result in high operational and maintenance costs, which have to be store in controlled of hazardous waste disposal sites for sludge.

A possible alternative to the chemical treatment of these effluents is bioremediation using anaerobic sulfate-reducing bacteria (SRB), taking advantage of the fact that these microorganisms grow in mining environments. In fact, several SRB have been isolated from the bottom of mining tailing. Likewise it has been proven that aquatic ecosystems contaminated by acidic waters have been capable of naturally recovering the initial situation thanks to the presence of species with the capacity to buffer the medium. The anaerobic bacterial processes, as in denitrification and biological reduction, are implied in these phenomena (Gyure et al., 1990). SRB are useful to abate AMD due to two fundamental reasons. Firstly because of their capacity to reduce sulfate to sulfide. These sulfides react additionally with certain metals dissolved in the contaminated waters, such as copper, iron and zinc, forming insoluble precipitates. On the other hand, the system acidity is reduced by their own action of sulfate reduction and by the carbon metabolism of the bacteria (Garcia et al., 2001).

The use of sulfate-reducing bacteria (SRB) to treat AMD has been investigated in laboratory and field studies. The treatment of AMD by SRB is based on the ability of SRB to reduce sulfate to sulfide, which binds readily with most metals to form an insoluble precipitate. The metals are, therefore, removed from solution in a stable form. The metabolism of the SRB also generates alkalinity, which contributes towards neutralizing the acidity of the AMD. The survival of the SRB in the presence of AMD is partly due to the fact that they inhabit the sediment, and not the water column. This is a consequence of the anaerobic nature of these bacteria and their requirement for an anoxic environment.

One problem facing the development of such a treatment process is the ability to monitor SRB activity directly in the sediment without disturbing their habitat to take samples, as this may affect the experiment and the continuing survival of the bacteria. Furthermore, enumeration of the SRB is problematic since this requires anaerobic techniques and is complicated by the tendency of these bacteria to aggregate on the surface of the particles in the sediment. A possible solution is the measurement of either impedance or conductivity which is nondestructive methods that, once installed, will not disturb the system (Lyew., 2001)

2.1.3 Problem statement

The existing chemical treatment technologies are stationary, which required space and time to construct with high operating and maintenance cost. Portable or mobile treatment equipments will be the solution, as it will provide the advantages of mobility, cost effectiveness and ease for operation.

Alternative treatment technique was bioremediation as it had been taken into consideration for the treatment of heavy metal in contaminated soil and wastewater effluent. For example, Sulfidogenic bacterial activity had described as a potential bioremediation tool for the removal of heavy metals during downstream processing from a range of industrial plants. Therefore, the further study on the utilization of heavy metal by bacteria or microorganism to treat the discharge water and soil should be conducted.

2.2 Waste Composition in Thailand

These solid waste sources are industrial, agricultural, mining and animal wastes as shown in Table 2.1. The industrial wastes are generally particular to the individual industry. They may include scrap metals, lumber, plastics, bales of waste paper, boards and rags, used-up drums and containers, slags, sludges, etc. The agricultural wastes consist of residues from most crop harvestings, horticulture wastes and orchard prunings. The animal wastes are generated from feedlots, dairies, chicken ranches, pig factories and other confined animal raising operations. The waste is generally manure, bedding material, punch manure from slaughterhouses, and carcasses. The major components of mining wastes are the byproducts of mining operations and processing of minerals and fossil fuels. Hazardous wastes include explosive, flammable, volatile, radioactive, toxic, and pathological wastes. These wastes require special care in storage, collection, transportation, treatment, and disposal to prevent harm to human health, animals, and property. These wastes can increase serious irreversible or incapacitating illness, or pose potential hazards to human health or environment when improperly managed.

Today, solid waste is accepted as a major problem of our society. Many waste disposal sites in Thailand are sources of environment pollution because waste is still largely disposed of without effective safety and environmental control measures. This situation poses a serious risk to the environment and to human health, especially via the path of groundwater contamination.

Landfills are the most widely used method of solid waste disposal in Thailand and the world today. This is primarily due to their ability to be designed, installed and operated at lower costs than other solid waste management alternatives. However, landfills have often been designed and operated without an appropriate understanding of their consequences within a particular environmental setting. Indeed, few other waste management systems afford so little opportunity for operational control as the indigenous processes of waste stabilization proceed.

Table 2.1 Waste Composition in Thailand (PCD, 1998)

Types of Waste	**Major Sources**	**Major Constituents**
Municipal Solid Waste	Residential	Kitchen Waste (51%)
	Commercial/Tourism	Plastic and Foam (22%)
	Agriculture	Paper (13%)
		Glass (3%)
Infectious Waste	Hospitals (93%)	Tissue samples
	Educational and	Blood and other liquids
	Labs (7%)	Surgical wastes and syringes
Industrial Hazardous Waste	Metals industries (33%)	Filter materials, waste
	Electronic industries (28%)	Sludge (35%)
	Plastic industries (8%)	Fuel, oil and grease (28%)
	Chemicals and Petroleum	Liquid organic compounds (8%)
	Industries (7%)	
Industrial Non-Hazardous Waste	Metals industries (36%)	Metals and metal alloys (30%)
	Food industries (13%)	Parts of wood (16%)
	Furniture (7%)	Animal parts (13%)
Community Hazardous Waste	Automotive stations (54%)	Recyclable waste oils (27%)
	Residential (19%)	Lead acid batteries (21%)
	Agriculture (10%)	Other toxic chemical (8%)
	Gas station (10%)	Other waste oils (6%)

2.3 Advantages and Disadvantages of anaerobic process of biological pre-treatment of wastewater.

Anaerobic biological treatment processes have been used in the treatment of sewage sludge for more than a century. The advantages of using anaerobic processes include: (i) the excessive biomass production is only about 1/3–1/5 of the typical aerobic process allowing the subsequent sludge treatment cost to reduced; (ii) with a low assimilated biomass, the amount of nutrients required for biomass synthesis is low; (iii) no aeration is required, thus power consumption can be reduced; (iv) under standard temperature and pressure, removal of 1 kg COD can produce $0.35m^3$ methane; (v) even with no feeding for a period of time the system can remain dormant. When feeding is restored, the system can return to normal operation within a short period of time; (vi) unlike aerobic systems, anaerobic systems are not restricted to electron acceptor limitations (i.e., molecular oxygen), thus the system can be loaded under a much higher concentration than aerobic systems; (vii) under anaerobic conditions the formation of metal sulfides were effectively eliminated the majority of heavy metals.

The anaerobic process has some disadvantages: (i) the reaction rate is only 1/4–1/10 of a typical aerobic process; (ii) the low growth rate from the low reaction rate requires a longer start-up time and takes a longer time to recover from fluctuations such as pH, temperature, and organic loads; (iii) with a low growth yield, a high biomass concentration is not easy to maintain when the influent concentration is low; (iv) anaerobic treatment is operated under reduced conditions and produces volatile acids, mercaptans, and hydrogen sulfide causing odor problems; (v) due to the low growth rate, anaerobic microorganisms are more sensitive to inhibitory or toxic compounds.

2.4 Characteristics of Leachate

The characterization of leachate provides important information necessary for the control of landfill functions and for the design and operation of leachate treatment facilities, facilitates risk analysis of leachate impact on the environmental should liners leak, permits comparison of the impact of alternative landfill design or operating protocol on the environment, and discloses the interaction of leachate parameters.

Material is removed from the waste mass via mechanisms that include leaching of inherently soluble material, leaching of soluble products of biological and chemical transformation, and washout of fines and colloids. The characteristics of the leachate are highly variable depending on the composition of the waste, rate of water infiltration, refuse moisture content, and landfill design, operation, and age.

Organic containments of leachate are primarily soluble refuse components or decomposition products of biodegradable fractions of waste. Organic compounds detected in nineteen municipal solid waste (MSW) landfill leachates or contaminated groundwater plumes emanating from landfills included organic acids, ketones, aromatic compounds, chlorinated aromatic compounds, ethers, phthalates, halogenated aliphatic compounds, nitro-aromatic compounds, phenols, heterocyclic compounds, pesticides, sulfur substituted aromatic compounds, polyaromatic hydrocarbons, polychlorinated biphenyls, and organophosphates (Brown and Donnelly, 1988).

2.5 Indicator Parameters that describe the quality of Landfill Leachate and Wastewater

There are certain traditional indicator parameters that can be used to indicate and to describe the presence, intensity, and properties of landfill and wastewater. Both gas and leachate parameters are monitored and analyzed for this purpose.

Biological oxygen demand (BOD) indicates the concentration of compounds in the groundwater that can be readily biodegradable. Chemical oxygen demand

(COD) is a chemical parameter indicative of the organic strength of leachate in terms of the amounts of oxygen needed to obtain oxidation of the chemically oxidizable fractions contained within the waste. The concentration of volatile organic acids (VOA) is closely related to the biodegradability portion of the leachate constituents, since during the Acid Forming phase, the majority of the COD is composed of VOA. Alkalinity indicates the natural buffering capacity of the water due to dissolved carbonates. pH and oxidation-reduction potential (ORP) are physical-chemical parameters and indicative of the acid-base and oxidation-reduction condition, respectively. Availability of essential nutrients, nitrogen and phosphorus, are assessed through the analyses of leachate ammonia nitrogen and orthophosphate, which are the readily available forms of both elements (Chian and DeWalle, 1976).

2.6 Characteristics of Zinc

Zinc chemistry

Pure zinc is a crystalline metal, insoluble in hot and cold water and soluble in alcohol, acids, and alkalies. It is extremely brittle at ordinary temperatures, but becomes malleable between 120° and 150° C (248° and 302° F) and may be rolled into sheets between heated rollers. Zinc is unaffected by dry air; in moist air it is oxidized and becomes coated with a carbonate film that protects it from further corrosion. Zinc melts at about 420° C (about 788° F), boils at about 907° C (about 1665° F), and has a specific gravity of 7.14. Zinc (Zn) is the first element in Group IIB in the periodic table; it has an atomic number of 30, an atomic weight of 65.38, and a valence of 2. The average abundance of Zn in the earth's crust is 76 ppm; in soils, 25 to 68 ppm; in streams, 20 μg/L, and groundwater, < 0.1 mg/L. The solubility of zinc is controlled in natural waters by adsorption on mineral surfaces, carbonate equilibrium, and organic complexes (Standard Methods for the Examination of Water and Wastewater, 1980). Zinc pictured as shown in Figure 2.1, comes in form as a bar and as a powder, is a metallic element.

Zinc is one of the most common elements in the earth's crust. It is present in nearly all foods. Also found naturally in air, soil, and water, it is commercially used to produce such products as dry cell batteries, brass, bronze, fungicides, pigments and coating to protect other metals from corrosion. Zinc combines with elements such as chlorine, oxygen, and sulfur to form compounds. Some of the most familiar compounds are zinc chloride, zinc oxide, zinc hydroxide, zinc sulfate, and zinc sulfide. These substances are used by the drug industry as ingredients in common products like sun blocks, diaper rash ointments, and deodorants. Some zinc compounds also compounds also end up in residual waste and require management at hazardous waste treatment and disposal facilities. Zinc is an essential growth element for plants and animals but at elevated levels it is toxic to some species of aquatic life. The United Nations Food and Agriculture Organization recommended level of zinc in irrigation waters is 2 mg/L. The U.S. EPA secondary drinking water standard of maximum concentration limit (MCL) is 5 mg/L. Concentrations above 5 mg/L can cause a bitter astringent taste and opalescence in alkaline waters (Standard Methods for the Examination of Water and Wastewater, 1980).

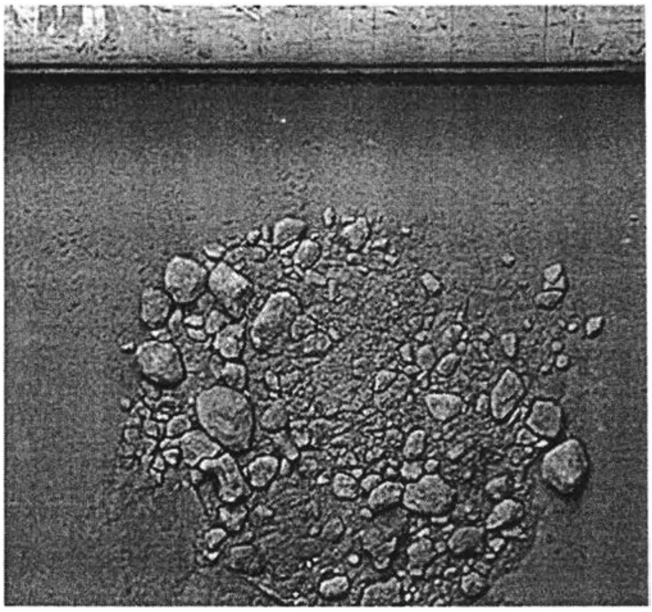

Figure 2.1: Zinc powder

Zinc can negatively affect health in large doses. It results in such physical symptoms as stomach cramps and nausea. Ingesting high levels of zinc for several months may lead to anemia, damage to the pancreas, and decreased levels of cholesterol. It is not known if high levels of zinc affect the ability of woman to reproduce or cause birth defects in humans. Inhaling large amounts of zinc may cause a specific short-term disease called metal fume fever. Inhalation can occur from exposure to zinc dust, or smelting or welding fumes. However, very little is known about the long-term effects of breathing zinc dust or fumes. Consuming too little zinc is also a concern. Without enough zinc in the diet, people may experience loss of appetite, decreased sense of taste and smell, impaired immune function, slow wound healing, or skin sores.

2.7 Heavy Metal Removal technologies

2.7.1 Immobilization technologies

Metals dissolved in groundwater and wastewater can be removed by adding chemicals that cause the metals to form insoluble compounds. These solid precipitates can be removed by flocculation, sedimentation, and/or filtration. Contaminants may also be removed by coprecipitation. Coprecipitation is an adsorption phenomenon wherein a trace constituent is adsorbed onto a bulk-precipitated solid, or coagulated and enmeshed in the bulk precipitate. Coprecipitation can remove contaminants to levels below the solubility level. Hydroxide and sulfide precipitation are commonly used to remove metals from wastewater.

Hydroxide precipitation, lime (CaO), caustic (NaOH), or, less commonly, magnesium hydroxide ($Mg(OH)_2$) are added to the wastewater to adjust the pH and precipitate the metal as the hydroxide.

The solubility of a metal in groundwater depends on the concentrations of organic material and other ions in the water; high concentrations of organics or of anions such as Cl^- or SO_4^{2-} will complex with the metal can cause it to be more soluble than it would be in pure water at the same pH. As a result, the solubilities shown in Figure 2.3 represent the minimum solubilities observed. Treatment systems relying on precipitation can rarely reach these low levels.

Either lime or caustic soda maybe used for hydroxide precipitation. Lime is less expensive than caustic soda by 40% to 60%. However, more sludge is typically formed from treatment by lime than by caustic soda, so sludge disposal costs are higher.

Sulfide precipitation can be achieved by two processes: in the insoluble sulfide process and the soluble sulfide process.

The insoluble sulfide precipitation process was prepared with slurry of ferrous sulfide (FeS) and added to wastewater or groundwater to precipitate heavy metals as the sulfide salts. Ferrous ions dissociated from FeS will precipitate as ferrous hydroxide at the operating pH of 8 to 9.

$$FeS \rightarrow Fe^{2+} + S^{2-} \quad \text{..........(2.1)}$$

$$M^{2+} + S^{2-} \rightarrow MS \quad \text{..........(2.2)}$$

$$Fe^{2+} + 2OH- \rightarrow Fe(OH)_2 \quad \text{..........(2.3)}$$

Only those metals whose sulfides are less soluble than ferrous sulfide will precipitate; most priority pollutant metals will precipitate.

The soluble sulfide precipitation process is similar, except that sodium bisulfide is used rather than ferrous sulfide.

$$M^{2+} + HS^{-} + OH^{-} \rightarrow MS + H_2O \quad \text{..........(2.4)}$$

Metal sulfides are less soluble than hydroxides, as shown in Figure 2.2. As a result, sulfide precipitation may be preferred form of treatment when discharge limits are very low or there are concerns over the classification of the waste sludge. Sulfide sludge can be more difficult to flocculate than hydroxide sludge; however, the sludge can generally be thickened more easily. A final operational concern is the level of soluble sulfide remaining in the effluent.

2.7.2 Solubility of metals

Solubility of metals in leachate depends on pH, redox-potential, and solubility of the deposited metal species, concentration of complexing agents (NH_3/ NH_4^+, organic acids) and ionic strength (Forstner, 1991). Metal solubilities in the leachate increase as pH decreases. The highest metal concentrations are observed during the acid formation phase of waste stabilization when pH values are low. Therefore, methanogenic conditions and neutral pH must be established within landfill site so as to form insoluble metals in the reducing atmosphere before the co-disposal commences (Greedy, 1993, Campbell, 1994). Under methanogenic conditions, soluble metals precipitate as insoluble sulfides, carbonates, hydroxides and possibly phosphates in landfills (Pohland, 1991). However, in the presence of sulfides, most of heavy metals except chromium form extremely insoluble sulfide salt (Pohland, 2000).

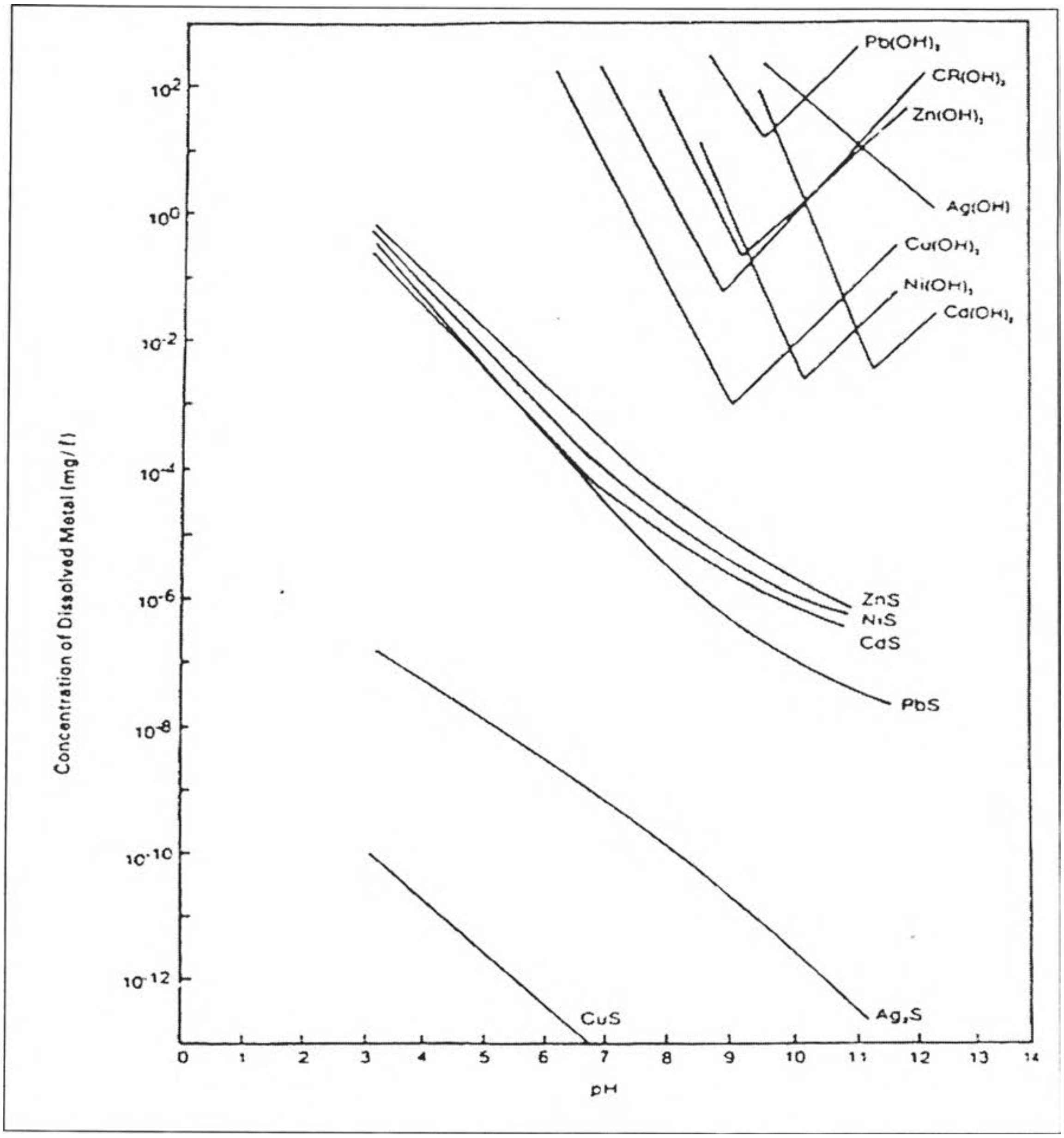

Figure 2.2: Solubilities of metal hydroxides and sulfides. (USEPA, 1995)

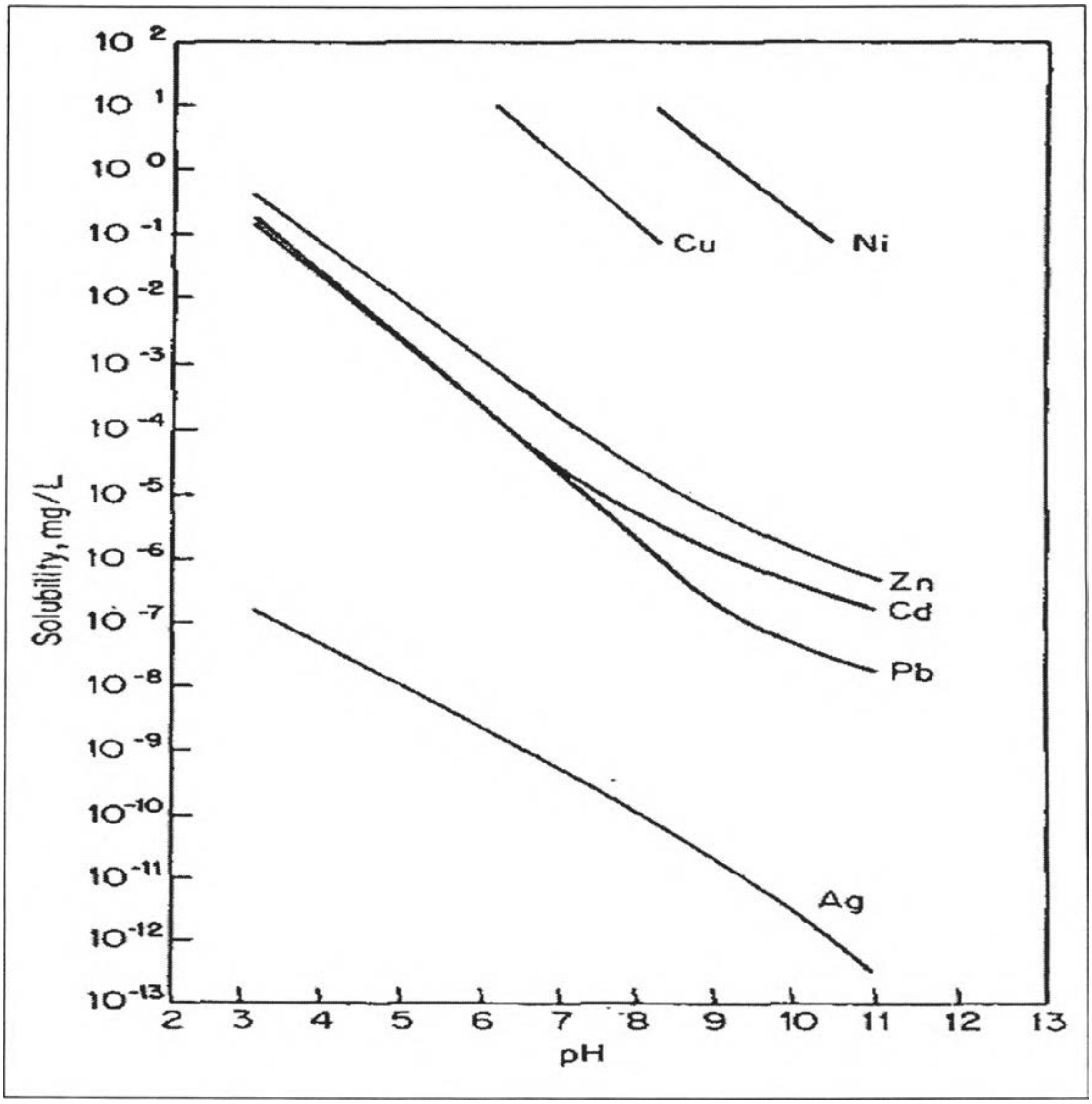

Figure.2.3 Solubility of metal ions as a function of pH (Freeman, (1989) and cited in Chewha, (2003)).

2.7.3 Zinc Removal

Zinc is present in wastewater streams from steelworks, rayon yarn and fiber manufacture, ground wood-pulp production, and recirculating cooling water systems employing cathodic treatment. Zinc is also present in wastewaters from the plating and metal-processing-industry. Zinc can be removed by precipitation as zinc hydroxide with either lime or caustic. The disadvantage of lime addition is the concurrent precipitation of calcium sulfate in the presence of high sulfate levels in the wastewater. An effluent soluble zinc of less than 0.1 mg/l has been achieved at pH 11.0.

The major attenuation mechanisms for zinc are adsorption, cation exchange, and precipitation. Zinc is a common cation in soil systems. As is true with other cations, the pH of the leachate-soil system is crucial factor in zinc removal, reflecting the influence of dominant hydrolysis species on both the affinity for soil colloids and the solubility of zinc.

The attenuation of zinc was found to increase rapidly for a pH change from 2 to 8 with a significant rise around 6 to 8. Precipitation of zinc with a variety of anions including sulfide, phosphate, carbonate, and silicate has also been found to be important in zinc immobilization. Experimental results suggest that the removal of zinc is also dependent on clay type and cation-exchange capacity. Organic matter improves zinc immobilization, and zinc chelates are most stable at pHs between about 5 and 7.5. Soil material favoring attenuation of zinc includes clays, organic material, hydrous metal oxides, and free lime. Zinc attenuation will be most favored by an alkaline condition. In general, mobility of zinc in a clayey environment is low.

2.8 Characteristics of sulfur bacteria

The bacteria that oxidize or reduce significant amounts of inorganic sulfur compounds exhibit a wide diversity of morphological and biochemical characteristics. One group, the sulfate reducing bacteria, consists mainly of single-celled forms that grow anaerobically and reduce sulfate, SO_4^{2-}, to hydrogen sulfide H_2S. One member of this group, *Desulfonema*, is multicellular and exhibits gliding motility. A second group, the photosynthetic green and purple sulfur bacteria, grows anaerobically in the light and uses H_2S as a hydrogen donor for photosynthesis. Members of the third colorless filamentous group are myxotrophic and utilize organic sources of carbon but may get their energy from the oxidation of reduced sulfur compounds. The sulfide is oxidized to sulfur or sulfate. A forth group, the aerobic sulfur-oxidizer, oxidizes reduced sulfur compounds aerobically to obtain energy for chemoautotrophic growth.

The sulfur bacteria of most importance in the water and wastewater field are the sulfate reducing bacteria, which include *Desulfovibrio*, and the single-celled aerobic sulfur-oxidizers, of the genus *Thiobacillus*. The sulfate reducing bacteria contribute and to taste and odor problems in water. *Thiobacillus*, by its production of sulfuric acid, has contributed to the destruction of concrete sewers and the acid corrosion of metals.

2.9 Heavy metal precipitation in soil by the use of sulfate reducing bacteria (SRB)

Singhal et al. (2004), studied in a laboratory using continuous flow was conducted to investigate the potential for creating columns with low permeability waste containment barriers by stimulating bacterial growth and assessing the relative significance of bioaccumulation, metal precipitation and gas formation in reducing the hydraulic conductivity of soils at the base of landfills. Several physical, geochemical, and biological interactions were observed during leachate transport in soils that resulted in a reduction of its permeability. Experimental observations suggest simultaneous reduction of manganese and iron accompanied by sulfate degradation and methane production, and that methanogenic processes were primarily responsible for acetic acid degradation. An increase in the substrate concentration resulted in rapidly increasing the pH, inorganic carbon (total dissolved carbonate), and attached biomass at the column inlet, leading to enhanced precipitation of Fe^{2+}, Mn^{2+}, and Ca^{2+} at the column inlet.

Foucher et al. (2001), used a process of sulfate-reducing bacteria to treat acid-mine drainage has been tested at the laboratory pilot scale. A fixed-bed bioreactor, fed with an H_2/CO_2 mixture, was used in conjunction with a gas stripping column. This process includes an H_2S biological production step using hydrogen followed by a chemical sulfide precipitation step. Two main parameters appear to influence the rate of biological H_2S production; the availability of hydrogen and the physiological state of the bacteria. The biological activity was particularly enhanced when the micro-organisms were fed with real effluent that had been pretreated through the precipitation of metal sulfides. This real effluent contains traces of metals that may enhance the bacterial affinity for sulfate.

Komnitsas et al. (2004), Limestone and red mud permeable reactive barriers are efficient in decontaminating acidic leachates generated at mining sites and loaded with quite high concentrations of hazardous heavy metal ions. Experimental column studies confirm that most of the contaminants, at the concentrations and the experimental conditions tested, can be effectively removed over a long period and provide useful information for the design of permeable reactive barriers (PRBs) installed in the field for the treatment of acidic leachates and the subsequent prevention of groundwater contamination.

Limestone, when attacked by acidic solutions, releases Ca^{2-} ions, adds alkalinity in the system and initiates clean up of leachates mainly due to precipitation and co-precipitation of heavy metal ions and also act as buffer for the system. Clean up efficiency will be much better regarding both reactive media is anticipated if solutions with lower acidity and heavy metal ion concentration are treated.

2.10 Heavy metal precipitation in wastewater by the use of sulfate reducing bacteria (SRB)

Kaksonena et al. (2003), studied on the treatment of metal containing wastewaters with SRB has promise as an alternative over chemical methods. From this study, they found out that; 1. Lactate supplementation of acidic metal containing wastewater stoichiometrically to sulfate concentration of the water selectively enriches SRB in suspension and biofilm reactors. 2. High recycle rate of fluidize bed reactor (FBR) allows treatment of wastewater with high concentrations of zinc (230 mg/l) and with pH of 2.5 without inhibitory effects because of its dilution effect. 3. Low fluidization rate (10%) in the FBRs enables faster biofilm formation during culture enrichment phase, whereas higher fluidization (20–30%), i.e. recycling rate is advantageous at increased loading rates. 4. Metals precipitate in the reactors as sulfides.

Diels et al. (2003), studied on the moving bed sand filter technology that allows a homogeneous treatment of wastewater containing heavy metals. Due to the continuous washing system, the filter bed resistance and flow rate are always stable and self-regulating, providing a stable output and steady-state operation.

The use of active bacteria in a biofilm allows the system to remove the heavy metals not only by biosorption but also by bioprecipitation processes. The biopreciptation is induced by the presence of functional groups and crystallization foci together with the physico-chemical microenvironment created by the biofilm. The biofilm generates steep pH gradients at the cell surface that allow the crystallization to occur.

Jong et al. (2003), This investigation demonstrated microbial sulfate reduction and subsequent precipitation of Cu, Zn, Ni, Fe and As by a mixed population of SRB in an upflow anaerobic packed bed reactor (UAPB reactor) containing silica sand. After an initial lag phase or adaptation period, sulfate reduction began and pH increased, redox potentials dropped and dissolved concentrations of Cu, Zn, As, Ni and Fe were significantly reduced in SRB inoculated systems supplied with lactate. In continuous-flow column experiments containing SRB, effluent pH above pH 7.2 and greater than 80% sulfate removal efficiencies were attained due to the activity of SRB. Conversely, pH levels remained low pH~4.5 with no sulfate reduction detected in systems containing no SRB. Metal removal efficiencies of more than 97.5% for Cu, Zn and Ni, and >82% for Fe were achieved in the column experiments.

The treatment process also removed >77.5% of the initial concentration of As, but was ineffective in removing Mg and Al. The results presented here have relevance to SRB found in natural systems and also to efforts to use similar systems to remediate water quality in mildly acidic metal and sulfate contaminated water.

Jong et al. (2004), batch experiments showed that metal sulfide solid phases produced by sulfate reducing bacteria can remove Pb(II), Cu(II), Cd(II), Zn(II), Ni(II), Fe(II), and As(V) from aqueous solution to low residual levels. The adsorption process was found to be dependent on initial metal concentrations, pH, and adsorbent dosage, with pH having the greatest effect. The percentage of adsorption increased with increasing pH and adsorbent dose, but decreased with increasing initial dissolved metal concentration. The characteristics of adsorption of these metals by bacterially produced metal sulfide (BPMS) were similar to those previously observed for (hydr)oxides.

The adsorption process was rapid with maximum adsorption achieved within a 24-h period. Desorption experiments indicated that there was a delay in desorption and the reversibility of adsorption was low, consistent with a high-affinity type of adsorption governed by chemisorption mechanisms. The pH of the solution was the most important parameter controlling adsorption of Cd(II), Cu(II), Fe(II), Ni(II), Pb(II), Zn(II), and As(V) by BPMS.

Lyew et al. (2001), the establishment of the SRB in the columns was indicated by the presence of a black deposit in the gravel bed and on the walls of the reactor, a decrease in sulfate concentrations, a strong sulfurous odor in water samples taken from the sediment, an increase in the pH to above 6, and a decrease in the ORP to below -300 mV. Once established, the SRB were able to treat varying loads of fresh AMD as shown by decreases in sulfate and metal concentrations and by increases in pH to acceptable levels. These phenomena were not observed in the control column, which did not contain SRB.

Furthermore, the only carbon source added to the AMD was lactate which favors the growth of the SRB and the harsh nature of the AMD does not encourage the growth of other microorganisms. The changes occurring in the inoculated column were, therefore, assumed to be due to SRB activity. By extension, the changes in the conductivity observed in the SRB column were also assumed to be due to SRB activity, and this is further supported by the fact that little or no change in conductivity was observed in the control column.

Garcia et al. (2001), the microbiological diversity associated with mining environments is a very well proven fact. One of the communities appearing in these environments is that formed by anaerobic sulfate-reducing bacteria (SRB) which can be used for the decontamination of acid mine drainage waters. Results from experiment showed that this option is effective for the precipitation of the dissolved metals (copper and iron), for the reduction and removal of sulfates and even for the alkalizing of the waters. In addition, the possible application of a mixed culture of SRB, isolated from the system studied; to treat a real AMD was considered. The different variables were analyzed: pH range in which the bacteria remain active, growth potential in the simultaneous presence of up to 200 ppm of copper and 30 ppm of iron, and efficiency of reducing up to 9,000 ppm of sulfate from AMD.

2.11 Limitations on the lifetime and treatment efficiency of reactor

The lifetime of SRB reactors is limited by the amount of reducing equivalents readily available for sulfate-reducing bacteria. Once the source of reducing equivalents is exhausted from the matrix, sulfate-reducing bacteria will no longer efficiently reduce sulfate to sulfide and treatment efficiency initially decreases. Physical supports that have good hydraulic conductivity and the ability to flush out the precipitates are required for extended (years to decades) use of bioreactors.

In the anaerobic treatment of wastewaters, sulfate ions could be reduced to sulfide in the reaction with hydrogen, acetates and propionates by SRB bacteria. On the other hand, MPBs produce carbon dioxide and methane gases also by utilizing hydrogen and acetate. Therefore, it is certain that SRBs and MPBs are in competition for utilization of hydrogen and acetates. Harada et al. (1994) notified that not only the free energies of SRB bacteria utilizing hydrogen and acetates are both greater than that of MPBs, but also the kinetic constants of SRBs show faster reaction rates as compared to MPB bacteria. The ratio of free energies of SRB to MPB in the reaction of hydrogen and acetates are 1.12 and 1.54, respectively. Also, the investigation of Monod constants ratios (µm/ks) for both hydrogen and acetates utilization reactions shows that these ratios are greater for SRBs than those of MPBs. The greater the ratio of µm/ks, the faster the reaction. Therefore, it can be concluded that the activities of SRBs are greater than MPBs and could out compete MPBs under standard conditions.

In addition to thermodynamic and kinetic factors, pH is another important factor in the activity of both groups of bacteria. It may affect the system by the presence of free H_2S in the system. In an aquatic environment, sulfide ions may exist in three forms: $S2^-$, HS^- and free H_2S. The equilibrium ionization constants for HS^- and H_2S are 1.3×10^{-13} and 10^{-7}, respectively. These show that the distribution of pH forms varies with different pH values so that the percentage of H_2S has highest change around neutral point (at pH 7). Free H_2S could cause severe toxicity for MPBs and prevent the growth of large stable granules in the system. The presence of dense and firm granules is necessary in the case of UASB reactors. Omil et al. (1996) showed that at low-pH values where the amount of free H_2S is high, MPBs are severely affected while SRBs are not. Although, at high-pH values the amount of free H_2S is low, but due to deviation from the optimum pH values for MPB bacteria, their activity also decreases. It has also been observed that at high-pH values, the total sulfide concentration is the factor affecting both MPB and SRB bacteria (Shayegan et al., 2004).

Nevertheless bacteria have certain specific environmental requirements that must be fulfilled: an anaerobic environment with a redox potential lower than - 100 mV and a pH of higher than 5.5 (Lyew et al., 1994, Garcia et al., 2001).

The column effectively treated the simulated acid mine drainage, found that severe plugging problems toward the end of the experiment. Treatment efficiency rapidly declined due to short circuiting and decreased residence time.

2.12 Related Studies

Chain and DeWalle (1976) reported that the formation of metal sulfides under anaerobic conditions effectively eliminated the majority of heavy metals in leachate. In addition, neutral or above neutral leachate conditions, promoted by leachate recirculation, enhance metal hydroxide precipitation.

Pohland (1986) investigated 2-year pilot-scale on the co-disposal of heavy metal sludge with municipal refuse, under the influence of leachate containment and recycles. The results are used to illustrate and describe the effects of various metal loadings on the normal progress of waste stabilization and to assess associated assimilative capacity. Evidence of metal precipitation as sulfides or hydroxides and subsequent removal by filtration and sorption in the refuse mass was demonstrated. Conversely, the mobilization of heavy of heavy metals was shown to be enhancing by complexation with humic-like substances.

Mott (1987) considered the precipitation in two landfill leachates. This investigation studied the partitioning of cadmium, copper, zinc, and nickel with iron and manganese solid phase formed from leachate constituents at pH 9. Two solid waste landfill leachates were studied in a preliminary study of in-situ precipitated hydrous ferric and manganese oxides in the removal of heavy metals from aqueous solutions by sorption-co-precipitation. For the conditions studied, the zinc removal rate was greater than 95%, cadmium was 89-90%, copper was 70-75%, and nickel was 24-36%.

Martenssen et al. (1999) investigated the effect of humic substances on the mobility of toxic metals in a mature landfill. Aeration increased the cation-exchange capacity, but decreased the buffering capacity and the metal-binding capacity. Leachates from the aerated landfill contained less than half the amount of aluminum, iron and molybdenum and more than double the amount of calcium, magnesium, sulfur and zinc of the leachates derived from an identical, but anaerobic, landfill. The leachate from the original landfill treated with leachate from the aerated landfill contained more than twice as much aluminium, boron, cadmium, chromium, copper, iron, lead, magnesium, nickel, phosphorus, potassium, sulfur and zinc than leachate from the identical anaerobic landfill. It was concluded that the mobility of deposited metals in landfills will change over time as the ageing of the landfills progresses and conditions become more oxidized.

Onay and Pohland (2001) found attenuation of nitrogen and sulfate in simulated landfill bioreactors. The characteristics of leachate from landfills vary according to site-specific conditions. Leachates from old landfill are often rich in ammonia nitrogen, posing potential adverse environmental impacts in the case of uncontrolled discharge. At landfills where leachate recirculation is practiced, leachate ammonia concentrations may accumulate to higher levels than during the single pass leaching. Using leachate recirculation with system modifications, separate aerobic and anoxic zones for nitrification and both autotrophic and heterotrophic denitrification can be provided. Results from simulated landfill bioreactors indicated that both nitrogenous and sulfur compounds can be attenuated through autotrophic denitrification, and leachate nitrate concentrations of 750 mg/L reduced to less than 1 mg/L by denitrification to nitrogen gas promoting this very common process in a landfill environment results in the reduction of both leachate ammonia and sulfate concentrations.

Erses and Onay (2003) studied the in-situ heavy metal attenuation in landfills under methanogenic conditions. Two landfill simulating reactors, one with leachate recirculation and the other without, were operated in a constant room temperature at 32 °C. These reactors were filled with shredded and compacted municipal solid waste having a typical solid waste composition of Istanbul region. After the onset of the methanogenic conditions, the selected heavy metals including iron, copper, nickel, cadmium and zinc were added according to amounts suggested for co-disposal. The results of the experiments indicated that about 90% of all heavy metals were precipitated from the reactors within the first 10 days due to the establishment of highly reducing environment and the formation of sulfide from sulfate reduction, which provided heavy metal precipitation.