CHAPTER II

LITERATURE REVIEWS

2.1 Backgrounds

2.1.1 Anaerobic wastewater treatment process

Anaerobic wastewater treatment process is happening by co-operation of many kinds of bacteria to digesting organic compound in anaerobic condition. The process can be divided into 3 steps as follows (Saipanich, 1987):

1. Hydrolysis
2. Acidogenesis (Non-Methanogenic)
3. Methane Formation

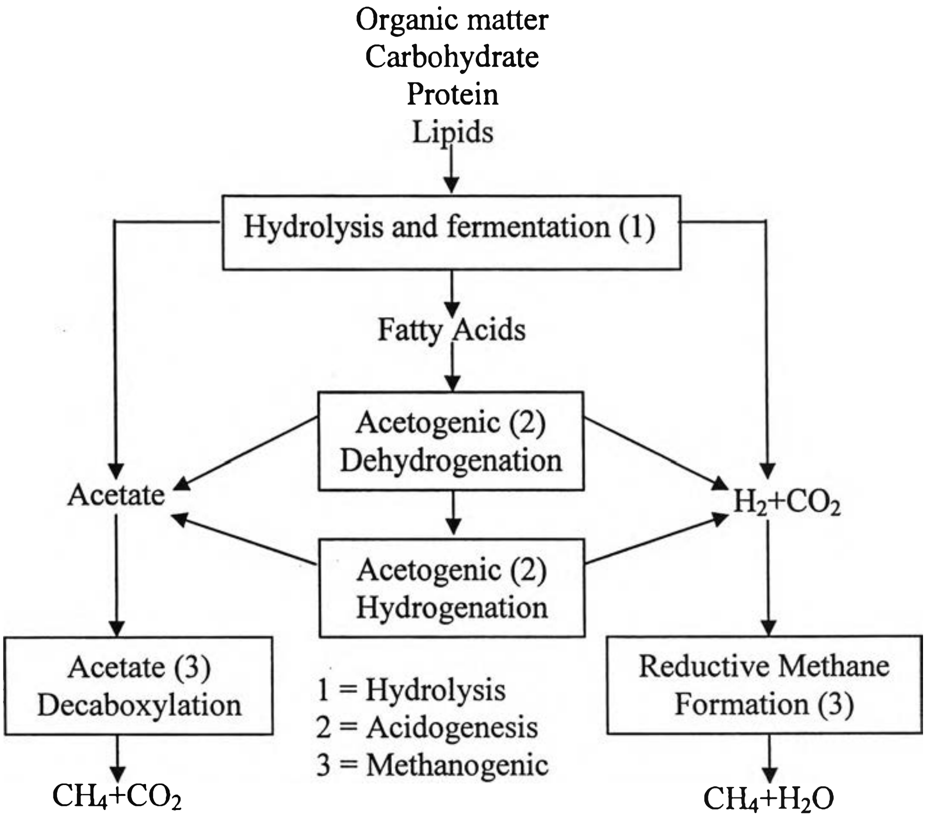

Figure 2.1 Process of anaerobic digestion (McInerney et al., 1979)

2.1.1.1 Hydrolysis

The complex organic compound that has large molecule for example carbohydrates, proteins and lipids will be digested by hydrolysis reaction to small molecule and soluble in water such as glucose, fatty acids, glycerol and amino acids as shown in figure 2.2. The microorganism releases enzyme to catalyst hydrolysis reaction and COD does not decrease in this step.

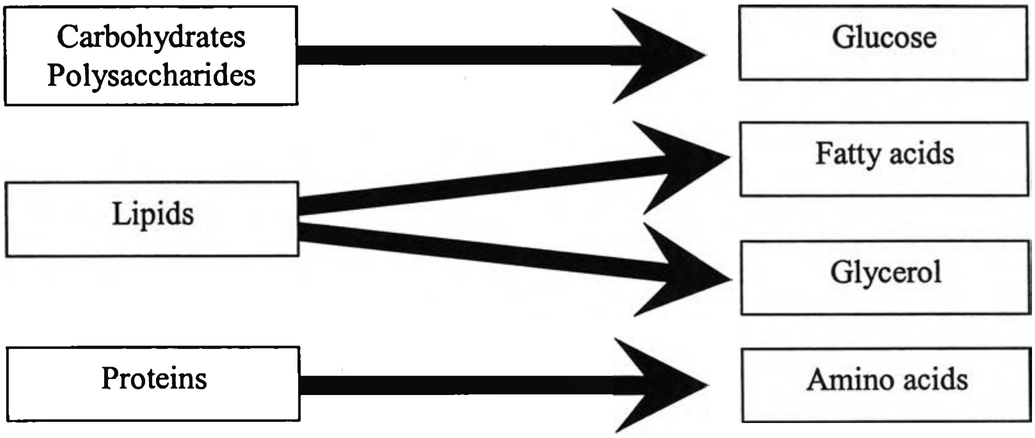

Figure 2.2 The digestion organic compounds by hydrolysis process (McInerney et al., 1979)

2.1.1.2 Acidogenesis

The simple organic digested from hydrolysis will be absorbed into acid production bacteria cell to build new cell and use as energy. In this step, organic compound will be changed to volatile fatty acids. The organic acid has low molecular weigh (Carbon atom less than 5 such as alcohol carbon dioxide) will be formed into internal cell digestion process.

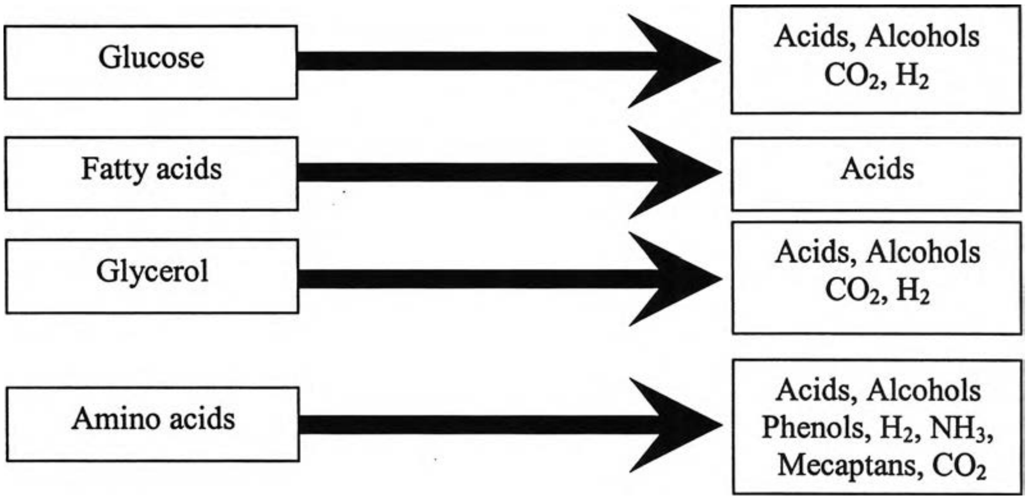

Figure 2.3 The digestion of organic compound by acidogenesis process (McInerney et al., 1979)

Pyruvic acid usually is formed by the digestion of acid production bacteria for the first time. After that pyruvic will be digested and changed to organic acid molecule which is larger than acetic acid for example propionic acid, butyric acid etc. These acids will be digested by hydrogen production acetogenic bacteria and changed to hydrogen acid as shown in figure 2.3 (Jeris et al., 1962).

$$CH_2CHOHCOO^{-}+2H_2O \text{ ---------}\rightarrow CH_3COO^{-}+HCO_3+H^{+}+2H_2$$

The digestion may be processed without pyruvic acid for example Acetic acid is formed by the combination of carbon dioxide and hydrogen with *Clostridum Aceticum* (Thimann, 1963).

$$2CO_2 + 4H_2 \text{ ---------------}\rightarrow CH_3COOH + 2H_2O$$

The process of digestion which produces the acid will affect the decrease of the value of COD, or there will be no decrease if the hydrogen is not produced. The decrease of COD is due to the loss of efficiency of bacteria during the change of organic form. When there is a production of hydrogen, the electron sent with the hydrogen ion which produces gas will decrease the electron of organic form which causes the decrease of oxidation condition as shown in figure 2.4.

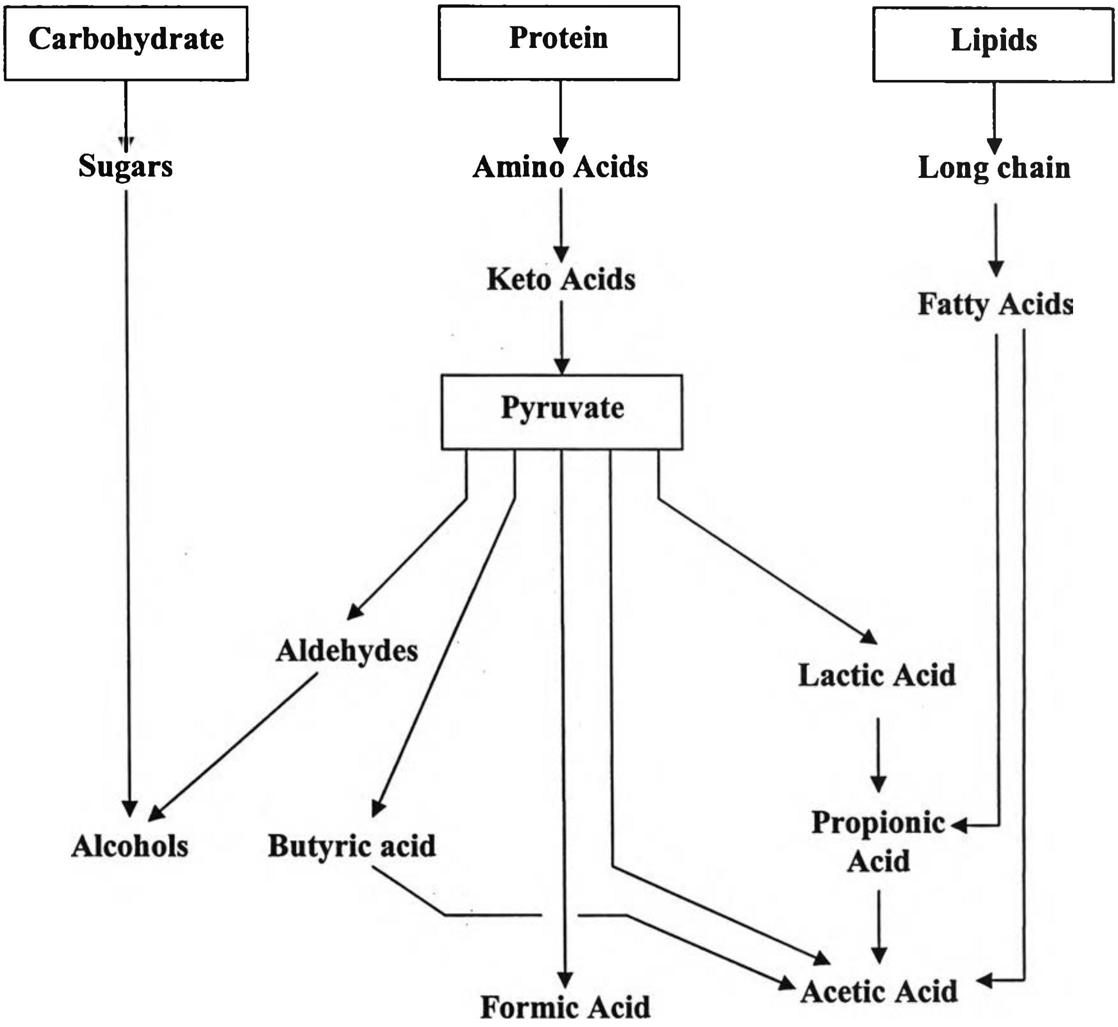

Figure 2.4 The organic digestion by acid production bacteria (Jeris et al., 1962)

The bacteria at this step will mostly live in the conditions both with and without oxygen (Facultative anaerobic bacteria)(Kroeker et al., 1979). However, the bacteria which live in the condition without oxygen (Obigate anaerobic bacteria) are very few.

2.1.1.3 Methanogenesis

The bacteria which produce methane will digest the productions and also digest the organic. The digestion of organic acid will reduce the COD value in the wastewater and form the methane. More than 90% of the chemical power in the COD forms will be changed to methane.

Most methane will drive from the biochemical reaction of the digestion of acetic acid. (McCarty, 1982)

ACETATE DECARBOXYLATION

$CH_3COOH \text{--------}\rightarrow CH_4 + CO_2$

Methane also derives from the biochemical between hydrogen and carbon dioxide.

CARBONDIOXIDE REDUCTION

$CO_2 + 4H_2 \text{-------}\rightarrow CH_4 + 2H_2O$

Anaerobic digestion of organic equations (Balch, 1979)(Caulter, 1957)(McCarty, 1982).

$CnHaOb + (n\text{-}a/4\text{-}b/2)\ H_2O \text{------}\rightarrow (n/2\text{-}a/8+b/4)$

$CO_2 + (n/2+a/8\text{-}b/4)\ CH_4$

The relationship between CH_4 and decreased COD is 1 pound of digested COD or BOD can produce 5.62 ft^3 CH_4 or 1 g of digested COD or BOD can produce 0.351 l. CH_4 (STP). Of the 70% methane produced is by acetic acid digestion as shown in figure 2.5.

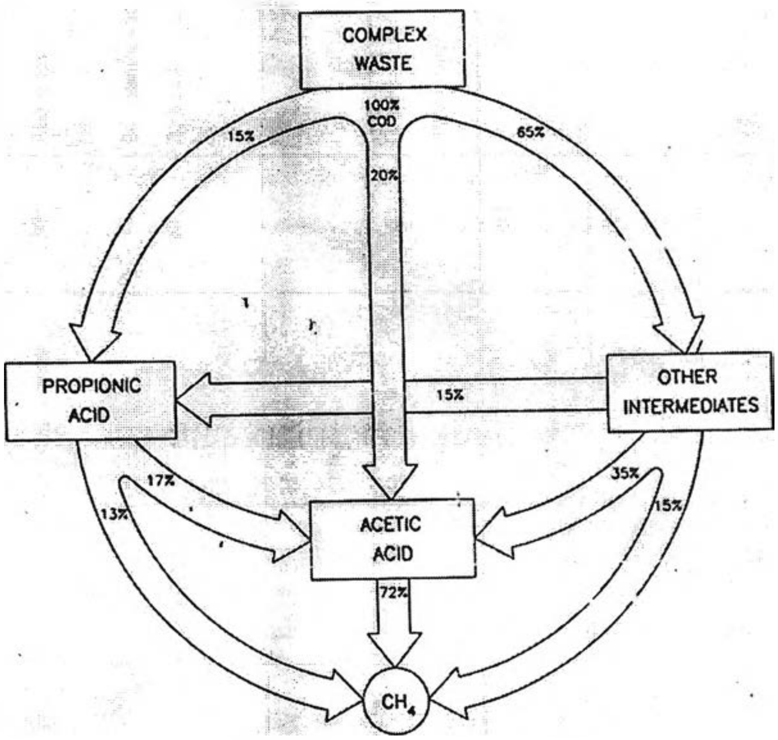

Figure 2.5 The change of organic by methane production bacteria (Jeris et al., 1962)

At present, it is accepted that the methanogenic bacteria can grow only in the narrow pH period, and the factor from the environment will directly effect the growth. All kinds of methanogenic bacteria are obligate anaerobic bacteria and it could be summarized that methane is produced from the process of acetate decarboxylation and carbon dioxide reduction, as 70% of the methane will be produced from the process of acetate decarboxylation and the methanogenic bacteria.

2.1.2 Factor affecting the anaerobic wastewater treatment process

Factors such as temperature, pH, volatile fatty acids, alkaline, necessary nutrient and toxic affect the growth and the efficiency of the organic digestion of bacteria. In order to control the process at highest efficiency, it is necessary to control the factors and condition appropriately. (Saipanich, 1987)

2.1.2.1 Temperature

The temperature has the most influence on the organic digestion of bacteria. As the bacteria has water as its main factor, so most bacteria can live at the temperature of lower than 99 degree Celsius. The result of the temperature which has effect on the growth rate can be divided into 3 ranges as follows (Saipanich, 1987):

1. Psychophysics range in the temperature range of 5-15°C
2. Mesospheric range in the temperature range of 35-37 °C
3. Thermophilic range in the temperature range of 50-55°C

In each temperature range, there will be different bacteria. It will be noted that the measophilic range is the range in which the bacteria has an organic digestion rate much higher than the range of the psychrophilic range, but a bit less than the range of thermophilic range. Some power is still required to control the lower range of psychrophilic range and they will be the flexibility of the change of the temperature as shown in figure 2.6

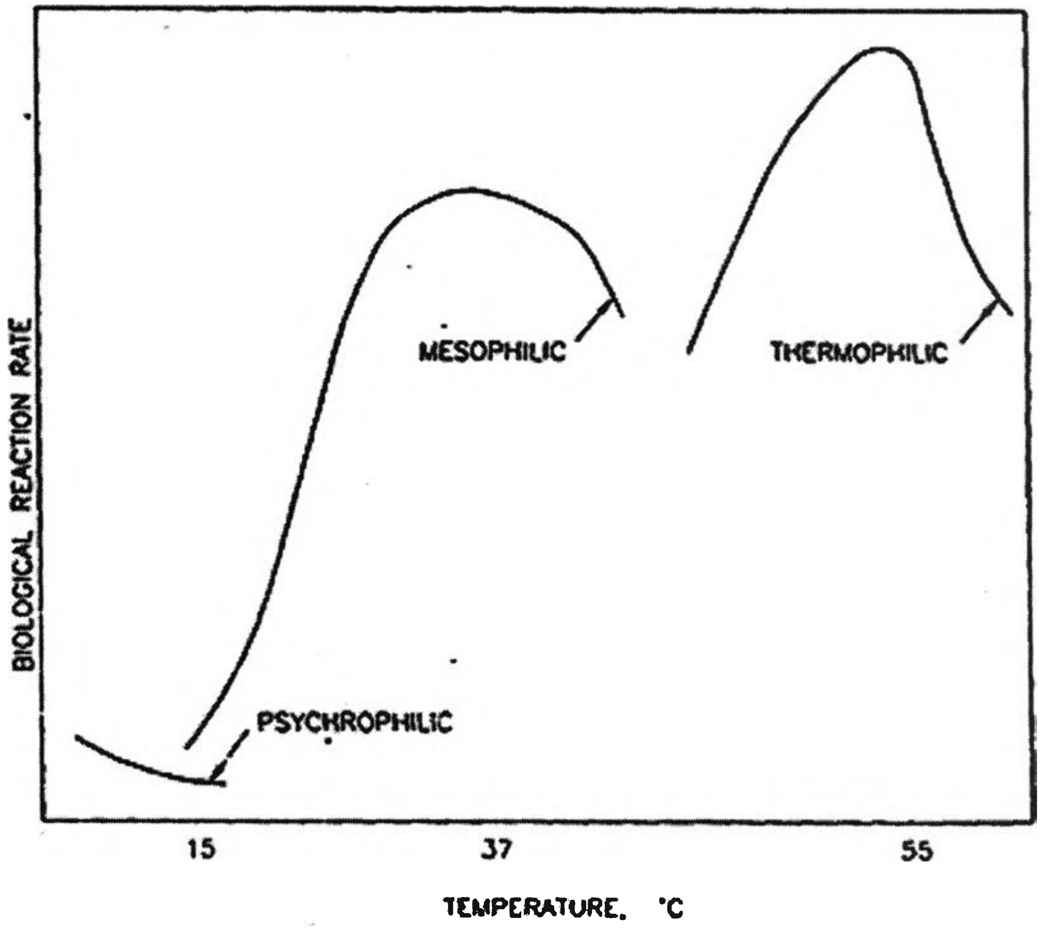

Figure 2.6 The influence of the temperature against the rate of organic digestion of the bacteria in the anaerobic condition.

In Thailand, most of the temperature of the wastewater will be in the temperature range of 28-30°C. Moreover, in the reactor, the temperature will increase about 3-5°C which happens from the reaction of the bacteria. Therefore, ours will be arranged in the range of mesophilic range without providing heat and temperature controller. The temperature between 30-38°C can treat the wastewater quite well and quite economical (McCarty, 1982). However, the temperature of higher than 60°C, the efficiency of the system will decrease (Pfeffer, 1967). However, at the digestion rate will increase twice at the temperature of 30°C when comparing to that of the temperature of 20 °C (Saipanich, 1987).

2.1.2.2 pH

The pH has an effect on the bacteria in respect of growth. The different pH will have the different quantity of hydrogen ion which will make the difference of electro chemical gradient of the delivery of nutrient and the eradication of the waste from the changing cell. As the low pH will have much hydrogen ion, it is difficult to percolate in and out through the cell. This will be the cause of inhibiting the growth and the death of the bacteria (Saipanich, 1987).

In the process of anaerobic digestion, if pH is lower than 6.5 the efficient work of the bacteria producing methane will decrease a lot, and if lower than 5.0, it will inhibit the growth and the death. For the bacteria producing acid, it can resist the acid condition of lower than 4.5 without any danger. Because of the nature of this kind of bacteria, the organic acid will be produced when the organic is digested. Moreover the pH is higher than 8.0, the production of methane will decrease as well (Saipanich, 1987). The suitable range of pH for anaerobic digestion process should be 6.6-7.4 (McCarty, 1964).

2.1.2.3 Volatile fatty acids and total alkalinity

Volatile fatty acids are produced from the anaerobic digestion process i.e. acetic acid, butyric acid, propionic acid etc. But, most will be acetic acid in the condition of pH as neutral. These volatile fatty acids will be in the form of acetate, butyrate and propionate. In the system of balance condition, the rate of producing acid and the rate of using acid for producing methane of bacteria will be equal. However, if it is in the input stage, the rate of producing acid higher, it will result the accumulation of volatile fatty acids which will decrease the pH and will cause failure for the system in the long run. The volatile fatty acids has a direct toxic in the bacteria with the concentration over 2000mg./l. But, it may be tested for the concentration up to 5000 mg./l. (Saipanich, 1987).

Therefore, the control of pH is the important means in controlling the system, which the change of pH will depend on the buffer capacity of the system. Of the system has sufficient buffer capacity, the immediate change of pH will be difficult, which will cause the system work efficiently (Saipanich, 1987).

In 1969 water pollution control federation (WPCF) has accepted the use of VFA as the control of the action of anaerobic wastewater treatment system, by the following summary (Mitchell,1974);

VFA/Alk<0.3-0.4 well action system
VFA/Alk >0.8 failure system

2.1.2.4 Nutrients

Nutrients which are necessary for bacteria in the anaerobic wastewater treatment process is carbon, nitrogen, phosphorus with appropriate ratio: COD:N:P equal to 100:1.1:0.2 (Saipanich, 1987).

2.1.2.5 Toxic substances

Any chemical substances which enter into the system when the concentration value increase up to one level will effecting or stability of the decreased system as shown in figure 2.7. The substances will be considered as toxic. The toxicity to the system is available from inhibiting the growth of bacteria to the destruction of bacteria. Therefore, it's necessary to control the concentration of all substances, and to prevent the limit which will affect the system (McCarty, 1964).

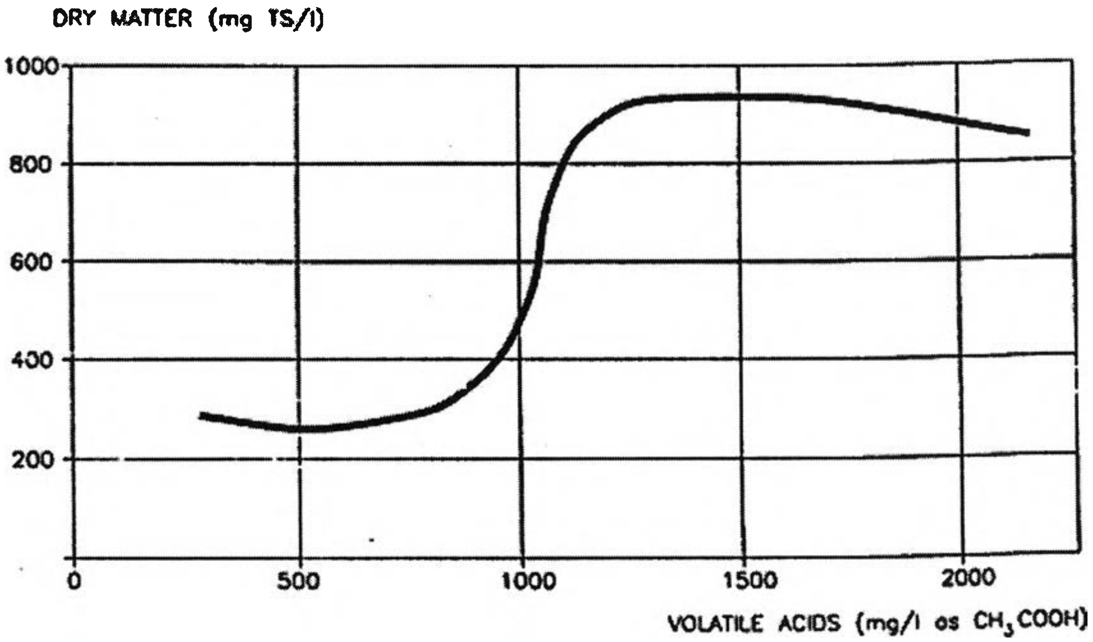

Figure 2.7 Result of organic acid against the capacity of the precipitation of the organic precipitation in the process of anaerobic digestion (Kotze et al., 1969)

Table 2.1 Concentration of ion and heavy metals affecting the anaerobic treatment. (Saipanich, 1987)

Toxic substance	Maximum conc. that without effect to bacteria (mg/l, ppm)
Zn	5.0
S	100.0
SO_4^{2-}	500.0
Cu	1.0
Cr^{6+}	5.0
Cr^{3+}	2,000
Total Chromium	5.0
Ni	2.0
Cd	0.02
Ammonia	1,500
Na	3,500
K	2,500
Ca^{2+}	2,500
Mg^{2+}	1,000
Aerylonitrite	5.0
Benzene	50
CCl_4	10
Chloroform	0.1
Pentachlorophenol	0.4
Cyanide	1.0

2.1.3 Sulfate reducing bacteria (SRB)

2.1.3.1 SRB information

Sulfate-reducing bacteria (SRB) are the main group of taxonomically diverse micro-organisms which are classified as strictly anaerobic and which are distributed within two domains: *Archaea* and *Bacteria*. SRB perform dissimilatory reduction of sulfur compounds such as sulfate, sulfite, thiosulfate and sulfur itself to sulfide. Some species from the *Desulfovibrio* genus can grow with nitrate or fumarate as alternative electron acceptors. Compounds frequently used as a carbon source and electron donors and oxidized to acetate and CO_2 are lactate, pyruvate, malate, high molecular weight fatty acids or simple aromatic compounds, such as benzene or phenol. SRB can also degrade saturated hydrocarbons (Iwona, 2003).

SRB are usually lumped into two nutrient categories. Those that can use lactate and those that cannot. The latter generally use acetate and are difficult to grow in the laboratory on any medium. Lactate, acetate, and other short chain fatty acids usable by SRB do not occur naturally in the environment. Therefore, these organisms depend on other organisms to produce such compounds.

SRB reduce sulfate to sulfide, which usually shows up as hydrogen sulfide or, if iron is available, as black ferrous sulfide. In the absence of sulfate, some strains can function as fermenters and use organic compounds such as pyruvate to produce acetate, hydrogen, and carbon dioxide. Many SRB strains also contain hydrogenase enzymes, which allow them to consume hydrogen. Most common strains of SRB grow best at temperatures from 25° to 35°C. A few thermophilic strains capable of functioning efficiently at more than 60°C have been reported (Roberge, www.corrosion-doctors.org).

2.1.3.2Competition between MPB and SRB

Sulfides can be formed during the anaerobic decomposition of solid waste either from sulfur-containing amino acids or by reduction of inorganic sulfur compounds. Dissimilar microbial sulfate reduction is a process in which certain bacteria use sulfate as the electron acceptor in the oxidation of organic matter. It is known that sulfate reduction and methane production can occur in the same environment (Oleszkiewicz,1997).

Sulfate reducing bacteria (SRB) have a thermodynamic advantage over the methane producing consortia. Therefore, SRB out compete the methane-producing consortia for available substrates and sulfide toxicity will be more severe for methane producers. On the other hand, they play an important role in the removal of heavy metals in anaerobic systems (Oleszkiewicz,1997).

The reactions for SRB are as follows:

$8H^+ + 8e^- + SO_4^{2-} \rightarrow S^{2-} + 4H_2O$

Oxidation of 64 g. COD is required for the reduction of 96 $SO_4^{2-}$to 32 g.S^{2-}.

The reactions for SRB are as follows:

$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$ $\Delta G^0 = -153$ kJ/mole

$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$ $\Delta G^0 = -72$ kJ/mole

The reactions for MPB are as follows:

$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ $\Delta G^0 = -136$ kJ/mole

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ $\Delta G^0 = -31$ kJ/mole

Competition between methane production bacteria (MPB) and sulfate reducing bacteria (SRB) for predominance in an anaerobic consortium is based on subtle interrelationships still not clarified. This anomalous competition is not controlled by one or two characteristic, for rarely if ever does one organism manifest superior characteristics in every facet of metabolism. Even in microbial kinetics, seldom does one organism have an exceptionally high k_{max} along with a strong affinity for the substrate, which would be revealed in correspondingly low values for K_s (Oleszkiewicz,1997).

The microbes with high specific utilization rates (k_{max}) not always predominate. Obviously additional factors such as K_s, trace metal requirements, and toxicity exert great influence. Even in less than ideal environmental conditions within an anaerobic reactor, SRB may still function sufficiently well enough to cause difficulties an indication of the tenacity of these organisms for survival.

This study focused on sulfate reducing process. It was necessary that the condition of SRB (sulfate reducing bacteria) was more advantageous than MPB (methane production bacteria) in the reactor, in order to control the most reaction in the reactor to become sulfate reducing process.

Table 2.2 The factor which control MPB/SRB competition (Oleszkiewicz,1997)

Factors influencing MPB/SRB competition
- Substrate concentration in feed
- Sulfate concentration in feed
- Maximum specific utilization rate- (k_{max})
- Half velocity Constant – (K_s)
- Thermodynamics/free energy of the reaction
- Nutrient availability
- Adhesion properties
- Proximity of cells (Biofilms vs Dispersed cells)
- Temperature
- Substrate type
- Long term shifts

2.1.4 Sulfur

2.1.4.1 Mass balance of sulfur

It is rather difficult to close a material balance on sulfur in an anaerobic treatment process. Sulfur sinks, or problem of sulfide disappearance, can be caused by the conditions enumerated in table 2.2

Table 2.3 The possible sulfur sinks (Oleszkiewicz,1997)

Possible sulfur sinks
- SO_4^{2-} in influent and effluent
- H_2S in off-gas
- total sulfide(TS) in effluent
- Microbial synthesis of sulfur
- Internal precipitation of sulfide production of other sulfur compounds

From an operational viewpoint, H_2S in the off-gas becomes the factor of overriding concern because it determines the H_2S and the Dissolved sulfide (DS) concentration in the aqueous phase for a given temperature and pH.

Figure 2.8 Show the mass balance for sulfide assuming no precipitation within the system. The H_2S composition in the gas phase as a function of feed COD, SO_4 precursors, and reactor pH in a completely-mixed reactor is also pictured. In this graph the intersection of the concentration of COD in the feed converted to gas (Methane and CO_2) with the concentration of sulfate converted to H_2S indicates the resulting fraction of H_2S in the gas (Oleszkiewicz,1997).

Reactor pH affects the sulfide ionization and thus strongly affects partitioning according to Henry's constant because the pH has to be incorporated; H_2S curves for both pH 7.0 and 7.4 are shown for examples The total gas production must be considered since H_2S partitions into the gas (according to its Henry's constant), requiring the fraction of CO_2 produced by the substrate to be included. This is the reason why two x-axis (abscissa) scales are shown, one for 25% CO_2 and the other for 50% CO_2 in the gas (Oleszkiewicz,1997).

At 35 °C, the stoichiometry for COD conversion to CH_4 is 0.395 L CH_4/g COD (6.3ft^3 CH_4/lb COD) converted to methane so that the CO_2 does not affect the stoichiometry. Likewise, 0.395 L H_2S/g COD (6.3 ft^3 H_2S/lb COD) also holds for H_2S at 35 °C. For a given concentration of sulfide precursors, H_2S composition in the gas decreases significantly with increasing feed COD concentration.

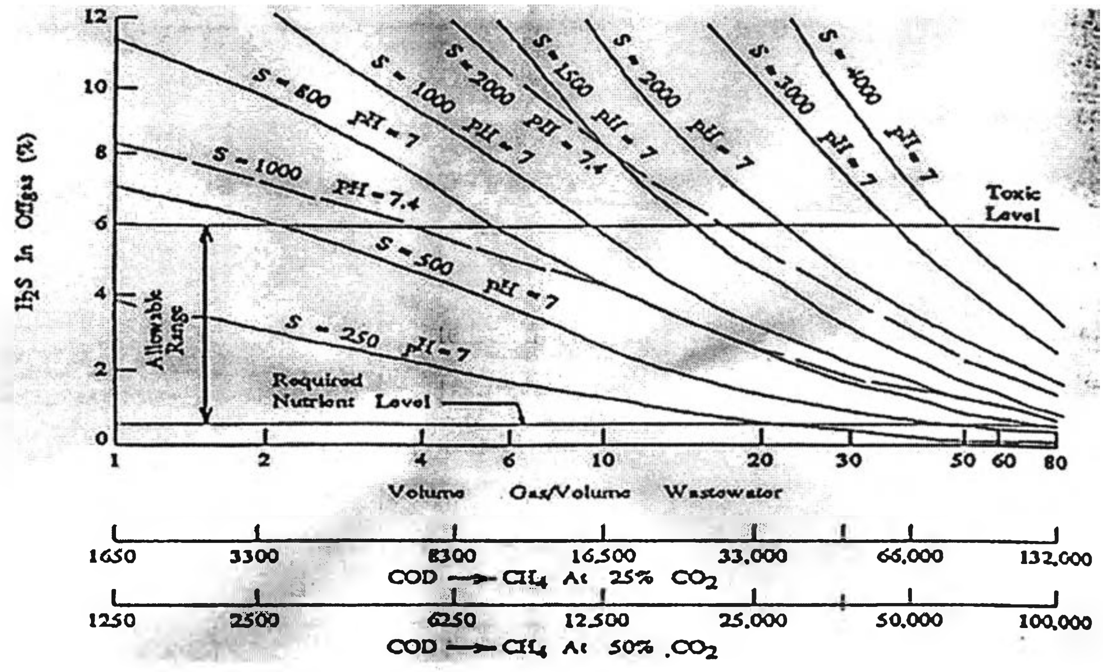

Figure 2.8 H_2S in reactor off-gas as a function of feed COD, CO_2 Production, sulfide precursors, and pH (Oleszkiewicz,1997).

Table 2.4 The ionization of H_2S for range of temperature (Oleszkiewicz,1997)

Temp °C	Ionization Constant, Kt
18	9.1×10^{-8}
25	11.2×10^{-8}
35	14.9×10^{-8}
45	19.4×10^{-8}
55	24.7×10^{-8}

Table 2.5 The solubility of H_2S vs temperature (Oleszkiewicz,1997)

Temp °C	H_2S (mg/l per atm of H_2S)
15	4410
20	3850
25	3380
30	2980
35	2650
40	2360
45	1200
50	1880
60	1480

2.1.4.2 Sulfide partition and metal precipitation

When organic sulfur compounds are decomposed by bacteria, the initial sulfur product is generally H_2S. Although a fraction of sulfide escapes in anaerobic systems in the biogas, the majority of sulfide remains dissolved in solution as either $H_2S(aq)$ or HS^-. $H_2S(aq)$ is in equilibrium with $H_2S(g)$ and when pH increases, $H_2S(aq)$ is converted to HS^-.

The dissolution of H_2S in water forms the following equilibrium (Veeken, 2003).

$$H_2S \leftrightarrow H^+ + HS^- \leftrightarrow 2H^+ + S^{2-}$$

$$H_2S \leftrightarrow H^+ + HS^- \qquad pK= -7.0 \qquad (1)$$

$$HS^- \leftrightarrow H^+ + S^{2-} \qquad pK= -13.9 \qquad (2)$$

Depending on the pH, the percentage of unionized H_2S drops from 90% at pH 6.0 to 50% at pH 7.0 and to 10% at pH 8.0.

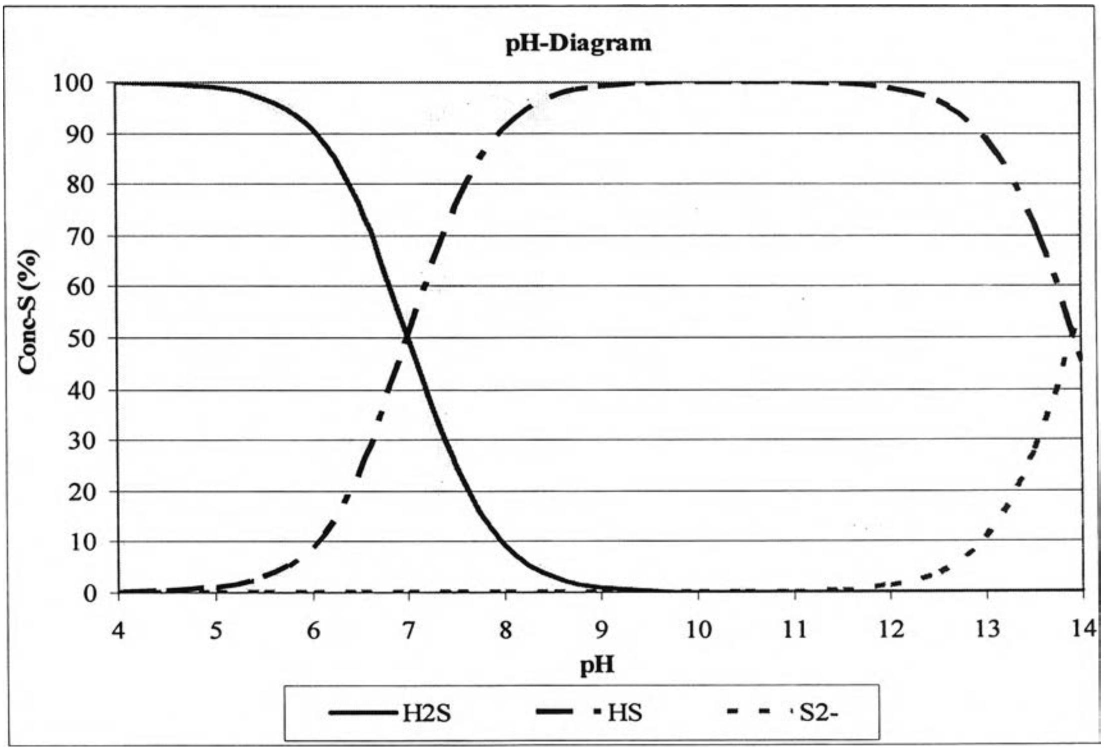

Figure 2.9 The relationship between pH and phase of sulfide

When sulfate transformed to sulfide, the heavy metal (Zn)can form with sulfide immediately like (1),(2)and(3) equations (Veeken, 2003).

$$Zn^{2+} + HS^- \leftrightarrow Zn(HS)^+ \qquad pK= -6.5 \qquad (1)$$

$$Zn^{2+} + 2HS^- \leftrightarrow Zn(HS)_2 \qquad pK= 14.0 \qquad (2)$$

$$ZnS(s) \leftrightarrow Zn^{2+} + S^{2-} \qquad pK= -24.7 \qquad (3)$$

2.1.5 Zinc

2.1.5.1 Zinc chemistry

Zinc is naturally occurring element found in the earth's surface rocks. Because of its reactivity, zinc metal is not found as the free element in nature. Zinc (Zn) appears in 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 two oxidation states, Zn(0) and Zn(+2) (Toxicological profile for zinc,2003). The average abundance of Zn in the earth's crust is 76 ppm; in soils, 25 to 68 ppm; in streams, 20µg/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 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, and pigments. 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 oxide, 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 MCL is 5 mg/L. Concentrations above 5 mg/L can cause a bitter astringent taste and opalescence in alkaline waters(Toxicological profile for Zinc, 2003).

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 (Toxicological profile for Zinc, 2003).

2.1.5.2 Heavy metals Removal

There are a number of technologies available for the removal of heavy metals from a wastewater. Chemical precipitation is most commonly employed for most of the metals. Common precipitants include OH^-, $CO_3^{2-}$and S^{2-}. Metals are precipitated as the hydroxide through the addition of lime or caustic to a pH of minimum solubility. Metals can also be precipitated as the sulfide or in some cases as the carbonate. Sulfide precipitation has several potential advantages as an alternative to hydroxide precipitation. The solubility of metal sulfides, shown in figure 2.10 is lower than those of corresponding metal hydroxides (Chewha, 2003).

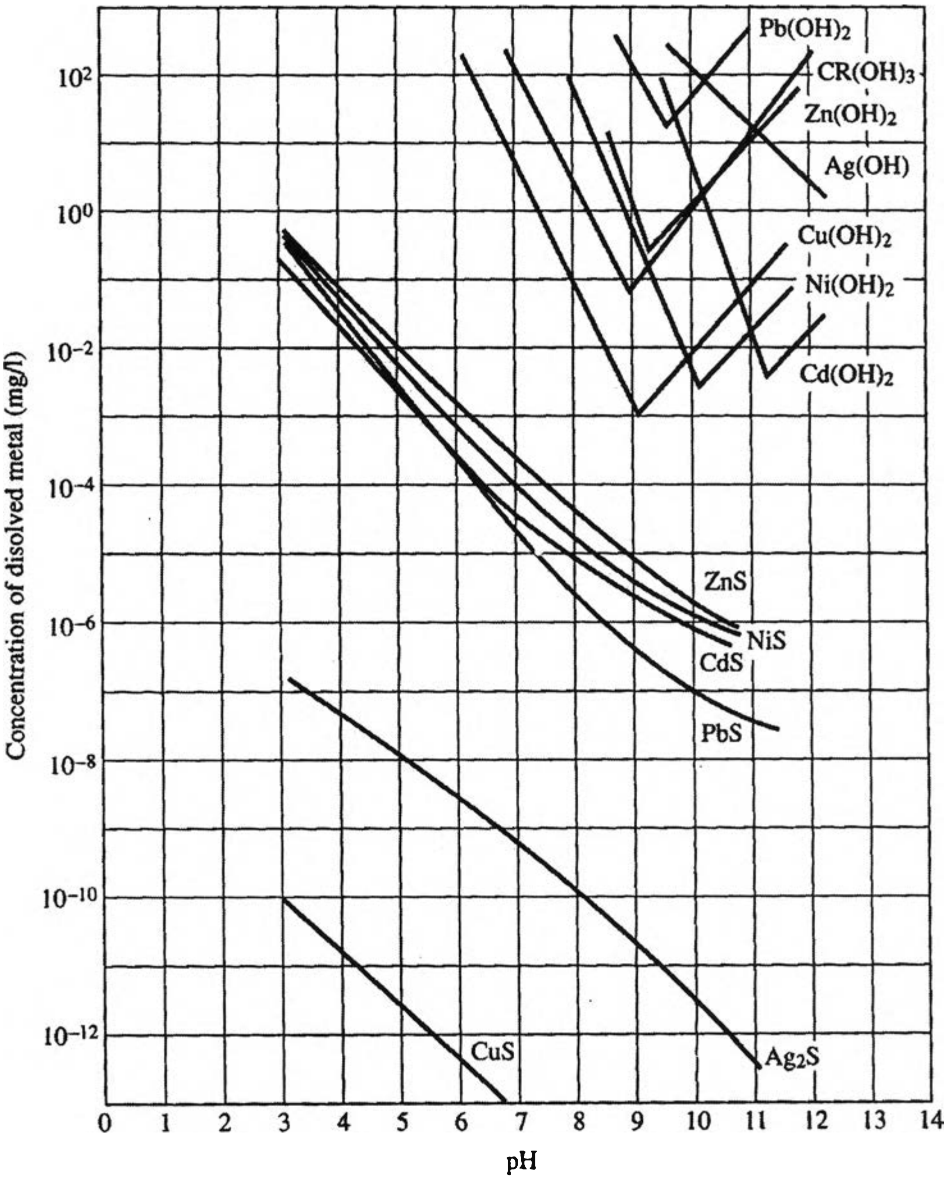

Figure.2.10 The solubility's of metal sulfides as a function of pH (Wesley, 2000)

2.1.5.3 Zinc Removal

Zinc is present in wastewater streams from steelworks, rayon yarn and fiber manufacture, ground wood-pulp production, and recirculation 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 (Toxicological profile for Zinc, 2003).

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 (Toxicological profile for Zinc, 2003).

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 (Chewha, 2003).

2.2 Related Studies

Veeken et al.(2003) studied precipitation of Zn^{2+} with S^{2-} at room temperature in a continuously stirred tank reactor of 0.5 l to which solutions of $ZnSO_4$ (800–5800 mg/l. Zn^{2+}), supply Na_2S, control pH at 6.5 and S^{2-} concentration in the reactor was controlled at set point values ranging from $3.2x10^{-19}$ to $3.2x10^{-4}$ mg/l.At $3.2x10^{-11}$ mgl/l of S^{2-}, the supplies of ZnSO4 and Na_2S solutions were stoichiometric for ZnS precipitation; removal of dissolved zinc was optimal with effluent zinc concentration <0.03mg/l. At S^{2-}levels higher than $3.2x10^{-11}$ mg/l the effluent zinc concentration increased due to the formation of soluble zinc sulfide complexes as confirmed by chemical equilibrium model calculations.

Foucher et al.(2001) treated Chessy acid-mine drainage by sulfate reducing bacteria. They study about sulfide precipitation and found copper and zinc could be selectively recovered while other metals, commonly present in acid-mine drainage, under different pH conditions. One improvement is the use of part of the bioreactor outlet stream to maintain pH at operating levels.

Garcia et al.(2001) used mixed population of SRB, isolated from the bottom of a pyrite tailing pond to decontamination of acid mine drainage waters by anaerobic pond. The results showed the potential of a mixed population of SRB, isolated from the bottom of a pyretic tailing pond is effective for the precipitation of the dissolved metals, for the reduction and removal of sulphates and even for the alkalising of the waters. SRB were able to remove up to 9,000 ppm of sulphate ion efficiently, to grow in the presence of up to 100 ppm of copper and 30 ppm of iron, and alkalise the medium, provided that this was not extremely acidic(pH>4).

Glombitza (2001) adapted methanol as a carbon source for mixed culture of sulfate reducing bacteria (SRB) in treatment of acid mine flooding water. The removal of heavy metals was close to 100%, the pH of the acid water increased from 3.0 to 6.9.

Jalali et al. (2000) used sulfate reducing bacteria for the removal of copper from copper sulfate solution. They found that copper can be removed effectively by SRB to less than 0.1 mg/l from sulfate solutions containing 150 mg/l or less initial copper concentration. Copper was seen to precipitate out more quickly in the presence of bacteria cells than without bacteria cells, sulfide not limiting. Thus, association of copper with the bacteria cells promotes precipitation kinetics.

Nagpal et al.(2000) studied the use of sulfate reducing bacteria to treat acidic wastewaters containing heavy metals. They use ethanol as electron donor for the sulfate reducing bacteria. They develop a process model that considers growth of SRB's on ethanol, and allows simulation of pH variations associated with SRB growth and product-formation kinetics. The result showed pH estimation is important as it strongly influences the rate of hydrogen sulfide removal from the liquid phase. An increase in pH of the media is expected from the stoichiometry of growth on ethanol accompanied by H_2S and CO_2 removal, and is dependent on the acetate and sulfide equilibrium.

Tuppurainen et al (2002) studied about zinc and sulfate removal from synthetic wastewater.Zinc and sulfate removal from synthetic wastewater was investigated by using four laboratory parallel up flow-mode reactors. All reactors were inoculated with anaerobically digested cow manure (referred as R1 to R4; R1 contained carriers to retain biomass, whereas R2–R4 were operated as suspended reactors). R1 and R2 were first fed with glucose and sulfate-containing feed for 48 days after which all four reactors were fed with wastewater containing 50 mg/1 of zinc in R1–R3 and 200 mg/1 in R4 and operated for 96 days. In all reactors, hydraulic retention time, organic loading rate, and sulfate load were 5–6 d, 0.2–0.4 kgCOD/m^{3}d and 3.3–3.8 g SO_4 /1d, respectively, whereas the zinc load in R1–R3 was 0.074–0.077 and in R4 0.282 g Zn /ld. During the runs, 30–40% of sulfate and over 98% of zinc was removed, and up to 150–200 mg H_2S was produced in all reactors. Effluent pH dropped in all reactors (feed pH 6.5) to 3–5 by the end of the experiment. No significant effects on zinc removal were observed, despite differences in operating conditions and feed.

It was only in the latter part of the runs (i.e. between experiment days 120–142) that zinc removal began to fluctuate, showing a negligible decrease in R3 and R4, whereas in R1 and R2zinc was removed below the limit of detection (<0.01 mg/l Zn). Qualitative X-ray diffraction analysis of the reactor sludge at the end of the runs indicated that the compounds precipitated were most probably ZnS, suggesting metal removal through sulfide precipitation; this was supported by the fact that sulfate was reduced and zinc removed simultaneously.