CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Background

2.1.1 Anaerobic Wastewater Treatment and Process

Anaerobic wastewater treatment is the biological treatment of wastewater without the use of air or elemental oxygen. Many applications are directed towards the removal of organic pollution in wastewater, slurries and sludges. The organic pollutants are converted by anaerobic microorganisms to a gas containing methane (CH_4) and carbon dioxide (CO_2), known as "biogas" (Equation 2.1)

$$\text{Organic pollutants} \xrightarrow[\text{- methane producing bacteria (MPB)}]{\text{- acidogenic bacteria}} CH_4 + CO_2 \qquad (2.1)$$

Equation 2.1 expresses the conversion of organic pollutants to biogas by anaerobic microorganisms.

The anaerobic process consists of three step. Insoluble organic materials are first hydrolyzed to soluble organics by extracellular enzymes of microbial origin. In the second step, the short chain fragments are converted to simple organic compounds, predominantly organic acids, by a group of facultative bacteria. The third step consists of the conversion of the volatile acids to CH_4, CO_2 and other trace gases.

When anaerobic reactor is working properly, the three steps are in dynamic equilibrium; that is, the volatile organic acids are converted to CH_4 at the same rate that they are formed. The hydrolytic extracellular enzymes are abundant in microbial systems. The acid-producing bacteria can function over a wide range of environmental conditions, whereas the methane producing bacteria (MPB) are quite sensitive to such conditions. As a result when anaerobic reactor is stressed by shock loads, temperature fluctuations, or an inhibitory material, methane bacteria begin to lag and organic acids cannot be converted to CH_4 as rapidly as they form and the pH drops. As a result the methane producing bacteria (MPB) are further inhibited. The changes in gas production and volatile acid concentration occur.

2.1.1.1 Factors Controlling Anaerobic Process

Anaerobic process is affected by temperature, alkalinity and pH, competition of methane producing bacteria (MPB) with sulfate-reducing bacteria (SRB), and the presence of toxicants.

2.1.1.1.1 Temperature

The bacteria can be very sensitive to changes in their environment. Temperature is a prime example. CH_4 production has been documented under a wide range of temperatures. Anaerobic process is carried out in the mesophilic range at temperatures from 25°C to up to 40°C with the optimum at approximately 35°C. Thermophilic digestion operates at temperature ranges of 50°C - 65°C. One drawback is its higher sensitivity to toxicants. Because of their slower growth as compared with acidogenic bacteria, methanogenic bacteria (or methane producing bacteria) are very sensitive to small changes in temperature, which leads to a decrease of the maximum specific growth rate while the half-saturation constant increases. Thus, a mesophilic digester must be designed to operate at temperatures between 30°C and 35°C.

2.1.1.1.2 Alkalinity and pH

Alkalinity is a measure of the amount of carbonate in a solution. Acidity or basicity of a solution is indicated by pH. An acidic solution has more hydrogen or hydronium ions than hydroxide ions. A basic solution has more hydroxide than hydronium ions. At a pH of 7 there are equal amounts of hydroxide and hydronium ions. A pH greater than 7 indicates a basic solution and a pH less than 7 indicates an acidic solution. Alkalinity is important because as acid is added to solution, carbonates will contribute hydroxide ions which tend to neutralize the acid. This is known as the buffering effect of alkalinity. Just as the bacterial population responsible for CH_4 production flourishes in the absence of oxygen and over a relatively narrow temperature range, it also flourishes over the narrow pH range of 6.5 to 8.0. As the acid-forming bacteria produce acid, the methane-forming bacteria utilize the acid and maintain a neutral pH. Since the reaction rate involving the acid-forming bacteria proceeds much faster than the reaction involving methane-forming bacteria, a larger population of methane-forming bacteria must be nurtured and maintained. Digester start-up is an especially critical step. When the digester

is initially fed with substrate, acid-forming bacteria quickly produce acid. The methane-forming bacteria population may not be sufficient to consume the acid produced and maintain a neutral pH. If the pH drops below 6.5, the methane-forming bacterial population begins to die and the bacterial population becomes further unbalanced. The digester acidifies and produces no biogas. In order to allow the methane-forming bacterial population to grow, digesters are initially fed with very small amounts of substrate and are often buffered by raising the alkalinity.

2.1.1.1.3 Toxicants

A wide range of toxicants is responsible for the occasional failure of anaerobic digesters. Inhibition of methanogenesis is generally indicated by reduced in CH_4 production and increased in concentration of volatile acids.

2.1.1.2 Process Microbiology

Consortia of microorganisms, mostly bacteria, are involved in the transformation of complex high molecular weight organic compounds to CH_4. Furthermore, there are synergistic interactions between the various groups of bacteria implicated in anaerobic digestion of wastes. Although some fungi and protozoa can be found in anaerobic digesters, bacteria are undoubtedly the dominant microorganisms. Large numbers of strict and facultative anaerobic bacteria are involved in the hydrolysis and fermentation of organic compounds. There are four categories of bacteria that are involved in the transformation of complex materials into simple molecules such as CH_4 and CO_2. These bacterial groups operate in a synergistic relationship in as much as group1 has to perform its metabolic action before group 2 can take over, etc.

2.1.1.2.1 Group 1: Hydrolytic Bacteria

Consortia of anaerobic bacteria break down complex organic molecules (proteins, cellulose, lignin, and lipids) into soluble monomer molecules such as amino acids, glucose, fatty acids, and glycerol. The monomers are directly available to the next group of bacteria. Hydrolysis of the complex molecules is catalyzed by extracellular enzymes such as cellulases, proteases, and lipases. However, the hydrolytic phase is relatively slow and can be limiting in anaerobic digestion of waste.

2.1.1.2.2 Group 2: Fermentative Acidogenic Bacteria

Acidogenic (i.e., acid-forming) bacteria convert sugars, amino acids, and fatty acids to organic acids (e.g., acetic, propionic, formic, lactic, butyric, or succinic acids), alcohols and ketones (e.g., ethanol, methanol, glycerol, acetone), acetate, CO_2, and H_2. Acetate is the main product of carbohydrate fermentation. The products formed vary with the type of bacteria as well as with culture conditions (temperature, pH and redox potential).

2.1.1.2.3 Group 3: Acetogenic Bacteria

Acetogenic bacteria convert fatty acids (e.g., propionic acid, butyric acid) and alcohols into acetate, hydrogen, and carbon dioxide, which are used by the methane-forming bacteria. This group requires low hydrogen tensions for fatty acid conversion; and therefore a close monitoring of hydrogen concentrations is necessary. Under relatively high H_2 partial pressure, acetate formation is reduced and the substrate is converted to propionic acid, butyric acid and ethanol rather than methane.

2.1.1.2.4 Group 4: Methane Producing Bacteria (MPB)

This group is composed of both gram-positive and gram-negative bacteria with a wide variety of shapes. Methanogenic microorganisms grow slowly in wastewater and their generation times range from 2 days at 35°C to as high as 50 days at 10°C. About two thirds of CH_4 is derived from acetate conversion by methane producing bacteria (MPB). The other third is the result of carbon dioxide reduction by hydrogen.

2.1.1.3 Biochemical Kinetics

Two basic approaches are used to determine the rate of substrate removal that occurs in biological processes, and both approaches utilized relationships applied in the fermentation industries (Reynolds and Richards, 1996).

The first approaches uses a modification of chemical kinetics, and its equations can be derived from the Michaelis-Menten relationship for substrate utilization. The second approach uses the Monod relationship for microbial growth kinetics.

The first approach employs a formulation that has been developed for industrial fermentations. It has been found that most enzyme-catalyzed reactions

involving a single substrate are zero order with respect to the substrate at relatively high substrate concentrations and are first order with respect to the substrate at relatively low substrate concentrations. When this occurs in a stirred laboratory vessel having no inflow or outflow (that is, a batch reactor), the substrate utilization is zero order for a period after the inoculation and later becomes first order. An explanation of this occurrence is that at high substrate concentrations, the surface of enzymes is saturated with substrate concentrations; thus the reaction is independent of the substrate concentration. At relatively low substrate concentrations, the portion of the surface of the enzymes that is recovered with the substrate is proportional to the substrate concentration. This phenomenon is explained is explained by the Michaelis-Menten concept.

The Michaelis–Menten equation gives the relationship between the rate of product production, dp/dt, and substrate concentration, S. The rate of product production may be expressed as the specific rate of product production, (dp/dt)/X, where X is the cell mass concentration. Since the specific rate of substrate utilization, (dS/dt)/X, is proportional to the specific rate of product production, it may be incorporated into the Michaelis –Menten formulation as follows:

$$\frac{(dS/dt)}{X} = k_s \frac{(S)}{(Km + S)} \qquad (2.2)$$

Where

$\frac{(dS/dt)}{X}$ = specific rate of substrate utilization, mass/ (mass microbes)(time)

dS/dt = rate of substrate utilization, mass/(volume)(time)

k_s = maximum rate of substrate utilization, mass/ (mass microbes)(time)

Km = substrate concentration when the rate of utilization is half the maximum rate, mass/volume

S = substrate concentration, mass/volume

From Equation (2.2), there are two limiting and cases. If S is relatively large, Km may be neglected, and the reaction is zero order in substrate.

$$\frac{(dS/dt)}{X} = k_s = k, K \qquad (2.3)$$

Where k or K is the rate constant for a zero-order reaction. If, on the other and, S is relatively small , it may be neglected in the denominator, and the reaction is first order in substrate, or

$$\frac{(dS/dt)}{X} = \frac{k_s (S)}{Km} = kS = KS \qquad (2.4)$$

Where k, or K is the rate constant for a first–order reaction in substrate. Equations. (2.3) and (2.4) represent the specific rate of substrate utilization, (dS/dt)/X. The equations for the specific rate of substrate are modifications of zero-order and first-order chemical equations with a term added for the cell mass, X. These equations have been used for the design of various biological treatment processes. To summarize the approach using the Michaelis-Menten concept, high substrate concentrations yield zero-order in substrate utilization, and low substrate concentration yield first-order in substrate utilization.

The Monod equation is similar to the Michaelis-Menten equation except that it relates growth to substrate concentration and is

$$\mu = \frac{\mu_{max} S}{(Ks + S)} \qquad (2.5)$$

where

μ = growth rate constant

μ_{max} = maximum value of the growth rate constant, $time^{-1}$

S = substrate concentration in solution, mass/ volume

Ks = substrate concentration when the growth rate constant is half the maximum rate constant, mass/ volume

Monod observed that the microbial growth that occurs is represented by

$$dX/dt = \mu X$$

where

dX/dt = rate of cell production, number/time or mass/ time

X = number or mass of microbes present

μ = growth rate constant, $time^{-1}$

These kinetic expressions are used to describe the growth of microorganisms and the waste utilization of substrate. It is very important to express them in terms of the specific growth rate.

Monod found the relationhip between the growth rate constant, μ, and the substrate concentration, S. When the growth rate constant is $\mu_{max}/2$, the value of S is Ks. At high substrate concentrations, the growth rate constant is μ_{max}. At lower substrate concentrations, it is as shown in equation (2.5). The similarities between the Michaelis-Menten and the Monod relationships can be seen by comparing Equation (2.2) and (2.5). The Monod equation has also been used to derive design equations for biological treatment processes.

2.1.2 Sulfate Reduction and Metal Ion Precipitation

Sulfate-reducing bacteria are biotechnologically relevant to SO_4^{2-} removal or heavy metal precipitation in wastewater or waste and to the elimination of SO_2 during offgas purification. An overview of applications of sulfate-reducing microorganisms in environmental biotechnology is given by Lens et al. (2002). SO_4^{2-} is the terminal electron acceptor and is reduced to sulfide, with reducing equivalents derived from the degradation many other organic compounds (Widdel, 1988). Alternatively, some sulfate reducers can also use molecular hydrogen. Sulfate reducers gain energy in an anaerobic electron transport chain (Hansen, 1994), leading to sulfide, a weak dibasic acid, which dissociates according to Equation 2.6.

$$H_2S \overset{K_1}{\longleftrightarrow} H^+ + HS^- \overset{K_2}{\longleftrightarrow} 2H^+ + S^{2-}$$

$$K_1 = 1.02 \times 10^{-7},\ K_2 = 1.3 \times 10^{-13} \quad (25\ ^0C) \qquad (2.6)$$

The total dissociation is described by Equation 2.7:

$$K = \frac{[H^+][S^{2-}]}{[H_2S]} = K_1K_2 = 1.3 \times 10^{-20} \qquad (2.7)$$

For precipitation of heavy metal ions, sulfide ions are necessary (Equation 2.8):

$$M^{2+} + S^{2-} \longleftrightarrow MS \quad \text{or} \quad 2M^+ + S^{2-} \longleftrightarrow M_2S \qquad (2.8)$$

The concentration of sulfide is pH-dependent. At acid pH only those metal sulfides

of very low solubility can be precipitated and removed by sedimentation. Thus, at acid pH, CdS, CuS, and PbS form precipitates, whereas at a more alkaline pH, ZnS, FeS, NiS, and MnS form precipitates.

2.1.3 Source of Heavy Metals

The presence of significant quantities of heavy metals in sewage is due to the industrial, commercial and domestic activities. In a more detailed way it is possible to separate the heavy metal sources in two categories

1) Specific sources which are the industries with high concentrations of heavy metals in their effluents.
2) Non specific sources which are domestic sewage, septage (if it is discharge into the network), groundwater and surface water (runoff, street refuse etc.).

In addition to industrial point sources, heavy metals are also contributed from urban storm water runoff, water distribution system corrosion (Klein et al., 1974) and domestic sewage (Atkins and Hawley, 1978). Cd, Cr, Cu, Pb, Ni, Zn are the six most commonly found heavy metals (Table 2.1) in U.S. municipal wastewaters (USEPA, 1980). Except for nickel and lead, the majority of heavy metals in a municipal wastewater influent are predominately in the particulate form and can be readily precipitated in a wastewater treatment plant. The amount, or percentage, of a particular heavy metal precipitated depends on several factors; pH, concentration of organic material, and the presence of other metals and or pollutants (Nelson et al., 1981). In general, sludge will concentrate the metal by a factor of 2,000 to 10,000 times over that of the surrounding liquid heavy metal concentration (Kalinski, 1981).

2.1.4 Basic Fundametals in Biological and Toxicity Control

Understanding how to prevent biological inhibition or toxicity in an anaerobic reactor is a complex phenomenon that is slowly being understood. Much of the work prior to the 1960's is considered erroneous and misleading because of inadequate experimental techniques and general lack of understanding (Kugelman, I.J. and Chin, K.K., 1971). A major aspect of biological inhibition and toxicity control is in understanding the basic fundamentals of the subject.

First, for any material to be biologically inhibitory or toxic, it must be in solution (soluble form as opposed to non-soluble (particulate) form). If the substance

is not in solution, it is not possible for the material to pass through the cell wall and therefore cannot affect the organism.

Second, toxicity is a relative term. There are many soluble organic and inorganic materials which can be either stimulatory or inhibitory or toxic. A good example of this is the effect of heavy metals on the anaerobic process.

Third is acclimation. When potential inhibitory or toxic materials are slowly increased within the environment, many biological organisms can rearrange their metabolic resources, thus overcoming the metabolic block produced by the normally inhibitory or toxic material. Under shock load conditions sufficient time is not available for this rearrangement to take place.

Finally, there is the possibility of antagonism and synergism.

- Antagonism is defined as a reduction of the toxic effect of one substance by the presence of another.
- Synergism is defined as an increase in the toxic effect of one substance by the presence of another.

These are an important consideration when designing for potential metal toxicity.

2.1.5 Inhibitory of Heavy Metals and Sulfide

Heavy metals, at some concentration levels, can be inhibitory or toxic to the anaerobic digestion process. Following is a discussion of the impact of metals and sulfide, the product of SO_4^{2-} reduction.

2.1.5.1 Heavy Metals

Table 2.2 summarizes those substances which are typically considered when discussing heavy metal toxicity in municipal wastewater systems (USEPA, 1981). Even though trace amounts of many of the elements listed are necessary for maximum biological development (Wood, D.K. and Tchobanoglous, G., 1975), the concentrations which can develop in the wastewater sludge pumped to an anaerobic digester can cause problems. Heavy metal toxicity has frequently been cited as the cause of many anaerobic process failures.

Table 2.1 Source of six common heavy metals found in influents of municipal wastewater treatments (USEPA, 1980).

Element	Average Percent Contributed,	Average Percent Contributed, Domestic	Average Concentration of Metal in Wastewater Non Domestic Treatment Plant Influent, (mg/l)
Cadmium	15	85	0.0055
Chromium	17	83	0.128
Copper	81	19	0.141
Lead	52	48	0.040
Nickel	23	77	0.106
Zinc	55	45	0.430

Table 2.2 Elements normally thought of when discussing heavy metal toxicity in anaerobic reactor (USEPA, 1981).

Consistently Found	Frequently Found	Occasionally Found
Cadmium	Arsenic	Aluminum
Chromium	Iron	Cobalt
Copper	Manganese	Molybdenum
Lead	Mercury	Selenium
Nickel	Silver	Tin
Zinc		

Table 2.3 Soluble heavy metal concentrations inhibitory to the anaerobic process.

Heavy Metal	Soluble Concentration, (mg/l)
Arsenic	0.5 to 1.0
Cadmium	0.01 to 0.02
Chromium (hexavalent)	1.0 to 1.5
Copper	0.5 to 1.0
Nickel	1.0 to 2.0
Zinc	0.5 to 1.0

Due to the ease in which heavy metals take part in complex-type reactions with ammonia, carbonates and sulfides, it has been difficult to define a particular total heavy metal concentration which is inhibitory or toxic Mosey, 1976). Table 2.3 summarizes some information found in the literature. Except for chromium, heavy metal toxicity in anaerobic digesters can be controlled by precipitation with sulfide (Lawrence and McCarty 1965, Masselli et al., 1967, and Regan, T.M. and Peters,

M.M., 1970). The reason for using sulfide precipitation is the insolubility of heavy metal sulfides (Langes, 1969). Approximately 0.5 milligram of sulfide is required to precipitate 1.0 milligram of heavy metal. If sufficient sulfide is not available from natural sources, then it must be added in the form of SO_4^{2-} which is reduced to sulfide under anaerobic conditions. A potential drawback of using sulfide saturation is sulfide toxicity, production of hydrogen sulfide gas (H_2S) or the generation of weak sulfuric acid which will cause corrosion problems.

2.1.5.2 Sulfides

Sulfides within the anaerobic digester result from three sources: 1) they are present in the influent wastewater stream and enter with the raw sludge; 2) they are biologically produced within the digester by the reduction of SO_4^{2-} and other sulfur containing inorganic compounds; and/or 3) they are degradation products of sulfur containing organic materials such as proteins.

Soluble sulfide concentrations above 200 milligrams per liter have been shown to be toxic to the anaerobic digestion process (Lawrence, A.W. and McCarty, P.L., 1965). The soluble sulfide concentration within an anaerobic digester is a function of the incoming source of sulfur, the pH, the rate of gas production and the amount of heavy metals to act as complexing agents.

2.1.6 Several Processes to Safeguard in Anaerobic Reactor

There are several processes which help to safeguard the anaerobic system.

1) Precipitation of the heavy metals as insoluble sulfides.

The arrangement in an increasing order precipitation is Cu>Pb>Cd>Zn>Ni. The sulfide necessary to effect the formation of the metallic sulfide precipitate in the system can originate from several sources (Section 2.1.5).

2) Precipitation of the metals as sparingly soluble carbonate salts.

This gives protection only against some of the heavy metals and only if the pH value of the system is high enough (pH $\geq$ 7.2 for Cd and $\geq$ 7.7 for Zn)

3) Reduction of the metals to a lower valency state.

This increases the equivalent weight of the metal, thus reducing the weight of sulfide necessary for the neutralization.

The remaining available concentration of heavy metals in the system is the reason for the toxicity in the process.

There is no universal definition of the toxicity of heavy metals and therefore it is not possible to determine toxic concentrations of heavy metals in reactor. It should be emphasized that for the design of an anaerobic system or for the operation of an anaerobic reactor, it is more useful to determine heavy metal levels (concentrations and forms) which cause inhibition in the influents.

2.2 Literature Reviews

The previous work was divided into 3 main categories.

2.2.1 Studies on Various Anaerobic Bacteria and Heavy Metals

Wong and Cheung (1995) found that sewage sludge collected from 2 different plants gave different amount in gas yield. Sludge from the plant which contained higher content of heavy metals, including Cr, Cu, Ni and Zn had a lower gas yield than the other. In their experiment, they dosed different concentrations of the four metals to the sewage sludge of less amount of metals and found that gas yields were severely inhibited at the highest concentration of each metal tested. The degree of toxicity of the four metals tested was in the order of Cr>Ni>Cu>Zn. In addition, the addition of pig manure to both sludges raised their biogas yields.

Invanitsa and Bukhtiyarov (2000) studied the resistance of a range of marine heterotrophic bacteria, including Pseudomonas and Cytophaga strains, isolated from coastal and open regions of the Black Sea to heavy metals. The accumulation of heavy metals by some of the strains was also studied. The accumulation of metals varied with metal and with strain. The order of metal accumulation for all isolates was Zn>Ni>Fe>Co>Cd. The pattern of accumulation by individual strains varied between unamended seawater with added metal ions. The resistance of the bacteria to heavy metals also varied considerably with metal and microbial type. There were marked differences in resistance of the marine isolates with the year of isolation. They also found that *Pseudomonas sp.10* efficiently reduced SO_4^{2-} through a dissimilative process in anaerobic respiration. The extent of SO_4^{2-} reduction varied with carbon source.

Mori et al. (2000) isolated the pure culture of *methanobacterium thermoautotrophicum* KHT-2 and *desulfotomaculum sp.* RHT- 3 from leachate at a sea-based landfill site in Japan. The sensitivity of both methane producing bacteria and sulfate-reducing bacterium to Cd and Cu was studied. Strains KHT-2 could not grow at 0.5 mM Cd or 1.0 mM Cu. However, strains KHT-2 and RHT-3 were cultured together in the presence of the heavy metals, strain KHT-2 could grow at high heavy metal concentrations after insolubilization of the metals by strain RHT-3.

2.2.2 Studies on Sulfate Reduction

The high SO_4^{2-} content causes major trouble during the anaerobic digestion. First, sulfate stimulates the growth of sulfate reducing bacteria (SRB) which out-compete methane producing bacteria (MPB) for substrates (H_2 and acetate) (Kristjason et al., 1982). Moreover, SRB consume hydrogen below a minimum threshold for hydrogen metabolism by methanogenic bacteria. Besides, they release sulfide, a very toxic compound. Its free soluble form, H_2S, readily permeates the cell membrane and denatures native proteins inside the cytoplasm producing sulfide and disulfide cross-links between polypeptide chain. Concentrations at which methanogenesis is inhibited by 50% vary between 50 and 250 mg H_2S/l. Sulfides are also involved in the precipitation of non-alkalinity metal in digesters, thus reducing their availability for MPB. In addition to these unfavorable conditions, the overall process failure of high sulfate-content anaerobic digestion seems to be driven by the COD:S ratio (Isa et al., 1986 a, b; Mizuno et al., 1994; Parkin et al., 1990; Valviline et al., 1994).

Visser et al. (1993) assessed the short-term temperature increases on the performance of 30°C mesophillic UASB reactor treating SO_4^{2-} containing wastewater, with respect to process stability, process recovery and particularly the competition between SRB and MPB and acidogenic bacteria. Their experiment was performed in methanogenic stage using acetate, propionate, butyrate and sulfate as a synthetic medium. Three temperature shocks in the range from 45 °C to 65°C during 8-9 hour period were applied. The temperature shocks of 45°C did not show any detrimental effect. However, temperature shocks of 55 °C and 65°C gave a serious drop in the treatment efficiency. Recovery after temperature shock was quite fast for propionic and butyric acid oxidation as well as SO_4^{2-} reduction, but slow for acetic

acid oxidation and methanogenesis. The competition between MPB and SRB was influenced by temperature shock. Exposure to high temperatures favoured SRB over MPB. In additional batch experiment conducted in serum bottles at 55 °C. A decay rate was about 1-2 h^{-1} for acid oxidizing bacteria, MPB and SRB. At 65 °C the decay rate exceeded 10 h^{-1} for all bacteria.

Harada et al.(1994) studied the interaction between SRB and MPB in UASB reactors fed with low strength starch or glucose synthetic waste of 500 mg COD/l, but different SO_4^{2-} levels of 30-600 mg/l. The digested sewage sludge from MSW was used as seed. The mass balances of COD and sulfur over the experimental period of 180 days operation indicated that the higher the level of SO_4^{2-}, the less CH_4 production caused since a greater electron flow was distributed to SRB. They found that although the COD removal efficiencies of all reactors were similar, the COD reduction by SRB increased in the order of increasing SO_4^{2-} level. In the case of glucose as the test substrated, the specific methanogenic activities (SMA) increased with an increase in SO_4^{2-} level, while it was not affected by SO_4^{2-} when acetate was used.

Genschow et al. (1996) studied a long term biological SO_4^{2-} reduction in anaerobic two-stage pilot plants treating tannery wastewater with the objective to reduce most of the SO_4^{2-} in the first stage. They found that the removal of SO_4^{2-} in the first stage was approximately 30%. They also studied the effect of pH on SO_4^{2-} reduction and found that a pH of 7 was the most effective value for all process-acidification, desulfurization and CH_4 production. Compare to pH 5, 6 and 7 in the influent, no significant influence on COD removal and volume of gas were observed.

Percheron et al. (1997) studied on the start-up of anaerobic digestion of sulfate wastewater using cultures with two different kinds of sludges, a sludge acclimated to molasses wastewater and sludge originated from a digester treating wine distillery wastewater. The sludges did not show the same behavior in reducing TOC and acetate as well as in producing CH_4. They concluded that using an appropriate sludge, the effluent was biodegradable.

Singh and Viraraghavan, (1998) reported that SO_4^{2-}concentration in the municipal wastewater varied from 50-100 mg/l (as S). With the UASB reactor at 48 hour HRT and 20^0C, they found that about 70 to 80 % of SO_4^{2-} in the influent was

reduced, and 30 to 40% of the total COD was consumed in SO_4^{2-}reduction resulting in a low recovery of CH_4.

Mizuno et al. (1998) investigated the behavior of SRB in acidogenic phase of anaerobic digestion. A 10,000 mg COD/l of sucrose was used as substrate. The SO_4^{2-} concentration was varied from 0 (control), 600, 1200, and 2400 mg/l. The SO_4^{2-} removal efficiencies were over 90% at SO_4^{2-} concentrations of 600 and 1200 mg/l at HRTs longer than 8 hours. The results showed that SRB could grow under acidogenic condition, and almost all of SO_4^{2-} could be removed in the acidogenic phase.

Moosa, et al. (2002) performed the experiment of SO_4^{2-} reduction in continuous anaerobic bioreactors and used the data to develop a kinetic model, incorporating terms for the effects of initial and residual concentrations of SO_4^{2-} and biomass. They found that the increase in initial concentrations of SO_4^{2-} in the range of 1000-10,000 mg/l enhanced the reduction rate from 7-170 mg/l-hour. The initial concentration of SO_4^{2-} did not have a significant effect on maximum specific growth rate (μ_m), decay coefficient (K_d) on bacterial yields (Y_x/sulfate and Y_x/acetate).

Knobel and Lewis (2002) developed a comprehensive mathematical model to describe the anaerobic treatment of high sulfate wastewaters. The model could be able to simulate SO_4^{2-} reduction and could be applicable for a number of carbon sources; account for pH, sulfite, hydrogen and fatty acid inhibition; and could be valid for a number of popular reactor types including a dynamic batch, steady state CSTR and dynamic CSTR. However, data for the model development and calibration were taken from literature. The inhibition of CH_4 from SO_4^{2-} reduction and that of simultaneously with metal toxicity were not considered.

A lot of research work has been done during the last years and a lot of promising results were presented. Unfortunately these result sometimes are contradicting or not comparable because of different process configuration. Too many factors affect the degradation and each research work is carried out under more or less different conditions. Even if the proposition of studies are qualitative the same, no quantitative results can be derived. Hence, quantitative comparison is not possible. The investigation of anaerobic degradation is connected with some problems. There are also still too many differences between laboratory scale and full scale applications.

2.2.3 Toxicity of Heavy Metals in Anaerobic Process

Chiu-Yue Lin (1992) studied the effect Cr, Cd, Pb, Cu, Zn and Ni on VFA degradation in anaerobic digestion using seed from sewage sludge digester. The experiments were carried out in serum vials by a batch test. The relative toxicity of heavy metals to degradation of acetic acid (HAc), propionic acid (HPr), and n-butyric(HBu) was Cd>Cu>Cr>Zn>Pb>Ni, Cd>Cu>=Zn=Cr>Pb>Ni, and Cd> Cu>Cr>Zn>Pb>Ni, respectively. Cd and Cu were the most and Pb and Ni were the least toxic heavy metals to VFA-degrading organisms. The sensitivity of the VFA degradation to the metallic inhibition was HPr > HAc = HBu for Cr, HAc > HPr = HBu for Cd and Pb, HPr > HAc = HBu for Zn, HAc = HPr = HBu for Cu and HAc > HPr > HBu for Ni.

Leighton and Forster (1997) studied a two-phase, thermophilic anaerobic reactor operated with a starch-based feed. The results were analyzed to determine the extent of metal binding in the acidogenic first-stage reactor and the degree of protection that this afforded to the traditionally more sensitive methanogenic phase. An examination of the gas production by the methanogenic stage, in relation to the amount of metal reaching this stage, suggested that the phase separation did not offer any real protection from the toxic effects of heavy metals.

Leighton and Forster (1998) studied the effect of Cu, Ni, Zn, Pb on a thermophilic methanogenic upflow sludge blanket reactor and found that all the metal caused a reduction in the COD removal which was reversed when the metal dosing ceased. Ni and Pb had the greatest impact. Pb also had the greatest effect on biogas production and, on the basis of the VFA production, appeared to act immediately on acidogenesis and slower on the methanogenic bacteria.

Codina et al. (1998) studied on the research methodology of microbial toxicity assays. The inhibition of methanogenic activity by heavy metals using anaerobic domestic sludges and sewage was studied in serum bottles. The concentrations exhibiting at 50% reduction in the analyzed bacterial activity (EC_{50}) were used as the indicators of the relative toxicity. A comparison of the values of EC_{50} indicated that the relative toxicities were Zn>Cr>Cu>Cd>Ni>Pb. The CH_4 proportion in the atmosphere of vials was monitored over time. The most adequate time for the best

CH_4 production and the additional time of sample as well as that of metals to be tested to the sludge were also evaluated. Statistical analysis revealed no significant differences (>95%) in relation to the time of CH_4 analysis. The determination made after 48 hours of incubation was the selected time for the toxicity test in this study. The addition of the sample to be tested to the sludge at the beginning of the assay was the most efficient procedure for the methanogenic activity inhibition assay to detect heavy metal toxicity. When metals were added at the beginning of the incubation period, the bacteria were in active growth and they were more sensitivity to the toxic stress.

Chirwa and Wang (2000) investigated the simultaneous removal of Cr (VI) and phenol in an anaerobic consortium of bacteria supplemented with a Cr(VI)– reducing organism, *E.Coli ATCC 33456*. Optimum Cr(VI) reduction was observed at a phenol concentration of 200 mg/l and at an initial Cr(VI) concentration of 2.0 mg/l. Higher phenol-degrader/E-coli ratios resulted in higher rate of Cr(VI) reduction. The results indicated that E-coli utilized metabolites formed from phenol degradation as electron donors for Cr (VI) reduction.

Drzyzga et al. (2002) demonstrated the bioremediation of chloroethane and nickel-contaminated sediment in a single anaerobic step under sulfate-reducing conditions. SO_4^{2-} was used as supplementary electron acceptor, and lactate (with and without methanol) was used as electron donor. Dehalogination of tetra- and trichloroethanes to ethene and ethane was achieved. A few weeks after SO_4^{2-} addition, production of sulfide increased, the Ni concentration in the effluent of one nickel-spiked column was greatly reduced more than 94%.

Jong and Parry (2003) removed SO_4^{2-} and heavy metal from mildly acidic mine water by a mixed population of SRB in the upflow anaerobic packed bed reactor containing silica sand. Effluent pH above 7.2 and greater than 80% SO_4^{2-} removal efficiencies were attained due to the activity of SRB. Metal removal efficiencies of more than 97.5% for Cu, Zn and Ni were achieved.

Although many studies have appeared that discuss the effect of heavy metal(s) and SO_4^{2-} on anaerobic wastewater treatment processes, little attention has been paid to the prevention of the system operating problems that may result from $SO_4^{2-}$and heavy metal(s). In this study, the toxicity to anaerobic treatment systems of SO_4^{2-} and heavy

metal(s) were studied. Results were complied and evaluated. Optimum ratios of SO_4^{2-}, COD, and heavy metals have been determined that facilitate, in anaerobic bioreactors, simultaneous lowering of COD and SO_4^{2-} with precipitation of metal sulfides, along with CH_4 production. A predictive model to prevent system impairment from those toxicants was proposed.

2.2.4 Sources of Mixed Microbial Assemblages

As seen at its outset, the resistance of bacteria to heavy metals varied considerably with metal and microbial types. Bacterial assemblages (or communities) from different locations, through natural selection, were different potentials under anaerobic conditions to interact with SO_4^{2-} and heavy metal combinations in water. Here are some sources which were recommended for bacterial-mixed culture having high ability in tolerating to SO_4^{2-} reduction and heavy metals.

2.2.4.1 Mixed-Liquor in Septic Tanks

The septic tank is the essential first part of an onsite sewage treatment system. Raw sewage flows into the tank from the house or the building sewer. The liquid flowing out is called septic tank effluent. The mixed liquor in septic tank is the liquor mixed with anaerobic biomass and stay in the tank. There are three distinct zones or layers in a septic tank. At the top is the floating scum layer which collects wastes such as soap or detergent scum, cooking fats, and any other material that floats. Most of the material in the scum layer does not decompose under the bacterial action in a septic tank. The center zone is called the clear zone which is liquid that contains suspended solids and bacteria. It is important that the tank have a deep clear zone. In this study, the bacteria were collected from this zone. At the bottom of the tank is the sludge layer which consists of decomposing and partially decomposed solids which sink to the bottom of the tank. The decomposition process continually goes on in the sludge layer.

Studies of metal removal by anaerobically digested sewage sludge were well documented. In Thailand it is hard to find the anaerobically digested sewage sludge, the mixed liquor in septic tank was used to represent anaerobically digested sewage sludge in this study.

2.2.4.2 Coastal Area

The coastal and marine sediments consist of the vast populations of the globally significant bacterial species. Aquatic environments tend to be oligotrophic in nature, with the indigenous population adapted to low nutrient requirements. The microorganism in coastal ecosystems contribute to the biogeochemical transformations of metals and organic matter. It is possible that anaerobic processes can contribute to considerable flux of the elements in the environments. Most often bacteria transform the toxic metal to an innocuous state. Heavy metal such as Hg and Pb have been found to be widely distributed in the marine and estuarine environments.

In coastal freshwater and marine sediments the terminal stages of anaerobic breakdown of organic matter are thought to be mediated by sulfate reducing bacteria (SRB) and methane producing bacteria (MPB) acting in competition for the common resources, such as acetate and hydrogen. The vast majority of microbial diversity remains to be discovered. Black sea and an island in the Antarctic are the locations of examples. With the advent of the molecular detection techniques, examination of the structure and dynamics of complex microbial communities has become possible. General observations about MPB and SRB community structure were widely studied.

In this study, sediment from the eastern seaboard of Thailand, City of Pattaya, was used as the representative of sediment from coastal area.

2.2.4.3 Sludge from Alcohol Production Plant

A liquid waste from alcohol production by fermentation has been reported to have a low pH and contain a high amount of organic matter, metals and SO_4^{2-} which is the main inhibitory inorganic constituent. During anaerobic digestion, SO_4^{2-} is reduced to sulfide, which is the major source of sulfur to methanogenic bacteria. It has been found that optimization of condition for different groups of bacteria in the reactor is complicated. These bacteria are acclimated with the heavily polluted food industry waste containing SO_4^{2-} and some extent of metals. Thus, the bacterial assemblage from alcoholic production waste which has a precise processes in bacterial characterization for a small group of potentially mixed microbial degraders was selected for this study.

2.2.4.4 Acidic Sulfate Soil

Soil with enough sulfide (FeS_2, and others) to become strongly acidic when drained and aerated enough for cultivation are termed acidic sulfate soil. Before drainage, such soils may have normal soil pHs and are only potential acidic sulfate soils. They occur in many regions of the world, mostly along coastal areas where the land is inundated by saltwater (Bloomfield and Coulter, 1973, Jinpei et al., 1986). Baldi (2001) isolated *K. Oxytoca* strain BAS-10 from sediment under an iron mat formed in a stream receiving leached waters from pyrite (FeS_2) mine tailings and found that BAS-10's growth was not inhibited by 1 mm Pb-, Cd- or Zn acetate under anaerobic condition. This suggested a possible application of this strain for abatement of toxic metals. Thailand having extensive areas of acid sulfate soils covers 810,000 hectares. One of the most well known sources is that of Ongkarak district, Nakornayok province. The soil samples which contain a number of autotrophic microbes from this site were picked up to study.

2.2.4.5 Leachate

Leachate is the liquid that has seeped through solid waste in a landfill and has extracted soluble dissolved or suspended materials in the process. The composition of the leachate produced at a municipal solid waste (MSW) landfill is controlled by the composition of the solid waste stream. Despite waste stream restrictions, MSW landfill leachate still has a high probability of containing potentially significant concentrations of hazardous chemicals arising from household and commercial use of these chemicals and through illegal dumping. Municipal solid waste landfill leachate contains a wide variety of hazardous chemicals, conventional contaminants, and non-conventional contaminants. Conventional pollutants derived from a wide variety of MSW stream components are present in high concentrations in MSW landfill leachate. Their presence in a groundwater at elevated concentrations down groundwater gradient of an MSW landfill is an indication that the landfill leachate is contaminating the groundwaters, and can themselves cause considerable water use impairment.

Metals are infrequently found at high concentrations in leachate. A variety of heavy metals are frequently found in landfill leachates including Zn, Cu, Cd, Pb, Ni,

Cr, and Hg (Lee and Jones, 1991). These metals are either soluble components of the refuse or are products of physical processes such as corrosion and complexation. In several instances, heavy metal concentrations in leachate exceed US Toxicity Characteristic Leaching Procedure standards.

Nongkham is the landfill site located in Bangkok. The sediment in leachate storage pool at the Nongkham site was contaminated by Hg, Cd and Mn appoximatedly equivalent to 0.663, ND and 845.8 μg/g, respectively (Changpiyarat, 1992).

In the previous studies, most of the data reported concerning toxicity to the anaerobic processes involved either a single metal toxicity when using anaerobically digested sewage sludge, removal studies of heavy metals, or the toxicity from the reduction of SO_4^{2-}. There has been little study on the synergistic toxicity of combined metals in the digestion of biological waste and no systematic testing of synergism among metals has been conducted. Also, there is no consistent explanation for the effect of metal interaction. Moreover, the toxicity of heavy metals and that of sulfide occurring under sulfate reducing conditions may lead to intriguing positive results and may be of practical use in the field of hazardous waste management.

Little has been reported on the use of mixed microbial cultures from various sources as seed sludge, but there are some studies on anaerobically digested sewage sludge. The results obtained from this study provide new information on general criteria for the choice of microbial associations. Sources of seed that generate strains with high efficiency for heavy metal detoxification are reported. The results of this research can serve as an experimental basis for the development of new microbiological processes for decontamination of industrial effluents. This can contribute to environmental protection from heavy metals, SO_4^{2-} and sulfide.

To achieve explicit results from the experiments, the number of factors that could influence the results was reduced as part of the experimental design. In this study, the laboratory-scale single phase reactors are fed with readily biodegradable glucose synthetic waste. A non-ambiguous configuration is used. This can lead to a better understanding of the biochemical pathways and may deliver comparable results for limiting values in other less-controllable situations.

2.3 References

Atkins, E.D., and Hawley, J.R. 1978. Sources of metals and metal levels in municipal wastewaters, research report 80: M4V-1P5. Ontario Ministry of Environment, Pollution Control Branch Toronto Ontario.

Baldi, F. 2001. Gel sequestration of heavy metals by *Klebsiella Oxytoca* isolated from iron mat. FEMS Microbiolov Ecology 36:169-174.

Bloomfield, C., and Coulter, J.K. 1973. Genesis and management of acid sulfate soils. Advances in Agromy, 25:265-326.

Changpiyarat, W. 1992. The contamination of mercury, cadmium, manganese in sediment near solid waste disposal sites of Bangkok metropolitan administration. Master'sThesis. Major Field of Inter-Department Environmental Science, Faculty of Interdisciplinary, Gruadute Program, Chulalongkorn University.

Chirwa, E.N., and Wang, Y.T. 2000. Simultaneous chromium(VI) reduction and phenol degradation in anaerobic consortium of bacteria. Water Research 34: 2376-2384.

Chiu, Y.L. 1992. Effect of heavy metals on volatile fatty acid degradation in anaerobic digestion. Water Research 26:177-183.

Codina, J.C., Munoz, M.A., Cazorla, F.M., Garcia, A.P., Morinigo, M.A., and Vicenti, A.D. 1998. The inhibition of methanogenic activity from anaerobic domestic sludges as a simple toxicity bioassay. Water Research 32:1338-1342.

Drzyzga, O., Mamouni, R.E., Agathos, S.N., and Gottschal J.C. 2002. Dehalogenation of chlorinated ethenes and immobilization of nickel in anaerobic sediment columns under sulfidogenic conditions. Environmental Science & Technology 36:2630-2635.

Genschow, E., Hegemann W., and Maschke C. 1996. Biological sulfate removal from tannery wastewater in a two-stage anaerobic treatment. Water Research 30:2072-2078.

Hansen, T. A. 1994. Metabolism of sulfate-reducing prokaryotes, Antonie van Leeuwenhoek 66, 165–185.

Harada, H., Uemura, S., and Momonoi, S. 1994. Interaction between sulfate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low strength wastes containing different levels of sulfate. Water Research 28:355-367.

Isa, Z., Grusenmeyer, S., and Verstrate, W. 1986a. Sulfate reduction relative to methane production in high-rate anaerobic digestion: Microbiological aspects. Applied and Environmental Microbiology 51: 580-587.

Isa, Z., Grusenmeyer, S., and Verstrate, W. 1986b. Sulfate reduction relative to methane production in high-rate anaerobic digestion: Technicals l aspects. Applied and Environmental Microbiology 51:572-579.

Ivanitsa, V., and Bukhtiyarov, A. 2000. Resistance level to heavy metals of microbial coenoses of the Black Sea shoresurface. Bulletin Odessa University, Series Biology, 5(N-1):61-66.

Jinpei, L., Danrui, C., and Kezheng, T. 1986. Tropical soil research laboratory, p.20. South China Agriculture University. International Rice Research Newsletter.

Jong, T., and Parry, D.L. 2003. Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. Water Research 37: 3379-3389.

Kalinski, A.A. 1981. Extracting heavy metals and toxic organics from sludge. Water Engineering and Management Reference Handbook 81: R148-151.

Klein, L.A., Lang, M., Nash, N., and Kirschner, S.L. 1974. Sources of metals in New York City wastewater. Journal Water Pollution Control Federation 46: 2653-2662.

Knobel, A.N., and Lewis, A.E. 2002. A mathematical model of a high sulphate wastewater anaerobic treatment system. Water Research 36: 257-265.

Kristjason, J. K., Schonheit, P., and Thauer, R. K. 1982. Different Ks values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: An explanation for the apparent inhibition of methanogenesis by sulfate. Archives of Microbiology 131: 278-282.

Kugelman, I.J., and Chin, K.K. 1971. Toxicity, synergism and antagonism in anaerobic waste treatment processes. In R.F. Gould (ed.), Anaerobic biological

treatment processes: Advances in Chemistry Series 105, American Chemical Society, Washington D.C.

Langes, 1969. Handbook of chemistry revised 10th edition (N.A. Lange, ed.) New York: McGrawHill Inc.

Lawrence, A.W., and McCarty, P.L. 1965. The role of sulfide in preventing heavy metal toxicity in anaerobic treatment. Journal Water Pollution Control Federation 37:392-406.

Lee, G. F., and Jones, R. A. 1991. Groundwater pollution by municipal landfills: Leachate composition, detection and water quality significance. Proc. National Water Well Associations's Fifth Outdoor Action Conference on Aquifer Restoration, Ground Water Monitoring, and Geophysical Methods 5: 257-271.

Leighton, I.R., and Forster, C.F. 1997. The adsorption of heavy metals in an acidogenic thermophillic anaerobic reactor. Water Research 31 :2969-2972.

Lens, P., Vallero, M., Esposito, G., and Zandvoort, M. 2002. Perspectives of sulfate reducing bioreactors in environmental biotechnology, Re/Views in Environmental Science & Technology 1 311–325.

Leighton, I.R., and Forster, C.F. 1998. The effect of heavy metals on the thermophilic methanogenic upflow sludge blanket reactor. Bioresource Technology 63:131-137.

Masselli, J.W., Masselli, N.W., and Burford, M.G. 1967. Sulfide saturation for better digester performance. Journal Water Pollution Control Federation 39:1369-1373.

Mizuno, O., Li, Y.Y., and Noike, T. 1994. Effects of sulfate concentration and sludge retention time on the interaction between methane production and sulfate reduction Water Science and Technology 30: 45-54.

Mizuno, O., Li, Y. Y., and Noike, T. 1998. The behavior of sulfate-reducing bacteria in acedogenic phase of anaerobic digestion. Water Research 32: 1626-1634.

Moosa, S., Nemati, M., and Harrison, S.T.L. 2002. A kinetic study on anaerobic reduction of sulphate, Part I: Effect of sulphate concentration. Chemical Engineering Science 57: 2773-2780.

Mori, K., Hatsu, M., Kimura, R., and Takamizawa, K. 2000. Effect of heavy metals on the growth of a methanogen in pure culture and coculture with sulfate-reducing bacterium. Journal of Bioscience and Bioengineering 90: 260-265.

Mosey, F.E. 1976. Assessment of the maximum concentration of heavy metals in crude sludge which will not inhibit the anaerobic digestion of sludge. Journal Institute of Water Pollution Control 75:10-15.

Nelson, P.O., Chung, A.K., and Hudson, M.C. 1981. Factors affecting the fate of heavy metals in the activated sludge process. Journal Water Pollution Control Federation 53: 1323-1333.

Parkin, G. F., Lynch, N.A., Kuo W.C., Van Keuren, E. L., and Bhattacharya, S.K. 1990. Interaction between sulfate reducers and methanogens fed acetate and propionate. Research Journal WPCF 62 : 780-788.

Percheron, G., Bernet, N., and Moletta, R. 1997. Start-up of anaerobic digestion of sulfate wastewater. Bioresource Technology 61:21-27.

Regan, T.M., and Peters, M.M. 1970. Heavy metals in digesters: Failure and Cure. Journal Water Pollution Control Federation 42:1832-1839.

Reynolds, T.D., and Richards, P.A. 1996. Biological concept. Unit operations and process in environmental engineering pp. 32-35. Boston: PWS Publishing Company.

Singh, K.S., and Viraraghavan, T. 1998. Start-up and operation of UASB reactors at 20^0C for municipal wastewater treatment. Journal of Fermentation and Bioengineering 85:609-614.

USEPA, 1980. Sources of toxic pollutants found in influents to sewage treatment plants 6: 75. Office of Water and Waste Management, Washington D.C., EPA Contract 68-01-3857.

USEPA, 1980. Fate of priority pollutants in publically owned treatment works: Interim report, Effluent Guidelines Division, Office of Water and Waste Management, Washington D.C. EPA 440/1-80-301.

USEPA, 1981. Data base for influent heavy metals in publicly owned treatment works, MERL, Cincinnati OH. EPA-600/S2-81-220.

Visser, A., Gao, Y., and Lettinga G. 1993. Effects of short-term temperature increases on the mesophilic anaerobic breakdown of sulfate containing synthetic wastewater. Water Research 27: 541-550.

Widdel, F. 1988. Microbiology and ecology of sulfate-and sulfur-reducing bacteria In A. J. B Zehnder (ed.), Biology of Anaerobic Microorganisms pp. 469–585. New York: Wiley.

Wong, M.H., and Cheung Y.H. 1995. Gas production and digestion efficiency of sewage sludge containing elevated toxic metals. Bioresource Technology 54: 261-268.

Wood, D.K., and Tchobanoglous, G. 1975. Trace elements in biological waste treatment. Journal Water Pollution Control Federation 47:1933-1945.