CHAPTER 2

THEORIES

2.1 Anaerobic Digestion

The transformation of complex macromolecules, for example, protein, carbohydrate (polysaccharides), and lipids present in wastewater or solids into end products, such as methane and carbon dioxide, is accomplished through a number of metabolic stages mediated by several groups of microorganisms. In case of a completely anaerobic breakdown, the end-products will be methane, carbon dioxide, water, and new bacterial cells; however, incompletely breakdown may occur under inappropriate environmental conditions or the presence of inhibitors, leading to the formation of intermediate products forming, especially VFA, alcohols, and ammonia. Generally, the conversion of anaerobic digestion process can be represented by the following equation (Tchobanoglous *et al.*, 1993):

$$\text{Organic matter} + H_2O + \text{nutrients} \rightarrow \text{new cell} + \text{resistant organic matter} + CO_2 + H_2O + NH_3 + SO_4^{2-} + \text{heat} \qquad [2.1]$$

2.1.1 Process of Anaerobic Digestion

There are mainly three stages in the process of anaerobic digestion. The first stage of the process involves the hydrolysis of solids such as cellulose and protein. This process results in the production of simple or soluble organic compounds (volatile substances and alcohols) that can be absorbed by bacteria. The second stage of the process is acidogenesis, as it involves the conversion of the volatile acids and alcohols to fatty acid substrates, such as acetic acid or acetate (CH_3COOH) and hydrogen gas, that can be utilized by methane forming bacteria. The third or final stage of the process is methanogenesis, which involves the production of methane and carbon dioxide. The last two steps are biological, whereas the hydrolysis step is enzymatic (Henze *et al.*, 2002).

2.1.1.1 Hydrolysis

Complex organic compounds such as proteins, carbohydrates, and lipids are transformed into simple soluble products such as amino acids, sugars, long chain fatty acids, and alcohol, by the action of extracellular enzymes excreted by the fermentative bacteria group. This step is commonly known as hydrolysis or liquefaction. The process will convert protein into amino acid, carbohydrates into simple sugars and fat into long chain fatty acid. Hydrolysis can be a rated-limiting step in the overall anaerobic digestion process, especially from waste containing lipids and/or a significant amount of particulate matter (e.g. sewage sludge, animal manure, and food waste). For such wastes, the rate of methane production in a digester is proportional to the net rate of particulate solubilization. The hydrolysis rate depends on substrate and bacteria concentration as well as environmental factors such as pH and temperature (Polprasert, 1996). The fraction of cellulose and other complex compound such as hemicellulose, starch, and pectin is very slow to be converted and can be a rate-limiting step in the anaerobic digestion process. Cellulose is generally resistant to hydrolysis due to its structure and the lignin barrier. Other factors such as accessible surface, pore size distribution, degree of polymerization and moisture content also affect the rate of cellulose digestion (Tsao, 1984). Since an approximate chemical formula for the mixture of organic waste is $C_6H_{10}O_4$ (Themelis and Verma, 2004); hydrolysis reaction of organic waste to a simple sugar can be represented by the following equation:

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2 \qquad [2.2]$$

The following describes the hydrolysis of the major polymers found in MSW

a. Lignocellulose: is the collective term for the three major components of plant tissue namely, cellulose, hemicelluloses, and lignin. In general, processed MSW contains 40-50% cellulose, 12% hemicellulose, 10-15% lignin by dry weight (Wang *et al.*, 1994). The sources of these components are paper, paper board, yard waste, and food waste. The cellulose and hemicellulose fractions are biodegradable and make up over 90% of the biochemical methane potential of MSW. Cellulose is contained in the plant cell wall and surrounding it is a heavily lignified material called the middle lamella. The middle lamella contains lignin and hemicellulose in the proportion of 70% to 30%. Hemicellulose is more readily degraded than cellulose by anaerobic digestion.

b. Starch: is readily biodegradable. Two types of molecules exist in nature, amylase, and amylopectin. An enzyme system for complete hydrolysis of starch is made up of a multiple number of enzymes that act synergistically.

c. Proteins: are hydrolyzed by proteolytic enzyme to peptides, amino acids, ammonia, and carbon dioxide. In anaerobic digesters, proteins serve as a source of carbon and energy for bacterial growth and the ammonia released during hydrolysis serves as the main source for anaerobic reactions.

d. Lipids: Fatty acids are the main constituents of the lipid fraction of waste. This group contains both highly hydrophobic and highly hydrophilic regions. Simple lipids (fats and oils) are esters of fatty acids with glycerol. The long chain fatty acids undergo an intracellular beta-oxidation mediated by a variety of enzymes resulting in the production of organic acids, such as acetic acid and propionic acid along with hydrogen.

2.1.1.2 Acidogenesis

The monomer products from hydrolysis will be changed to various intermediate products by acetogenic bacteria, mainly VFA, such as acetic, propionic and butyric acids, and also H_2 and CO_2. This process is carried out by bacteria that utilize CO_2 and organic acid as carbon source, ammonia as nitrogen source and sulfide as sulfur source. This group of bacteria has a rather slow growth rate with a minimum doubling time of 1.5 to 4 days (Verink *et al.*, 1993). The final product of this step depends on both the initial substrate and the environmental condition, for example, at high partial pressure of H_2, only propionate, lactate, and ethanol will be formed. The simple reactions in this stage are represented in the following equation and in Figure 2.1.

$$C_6H_{12}O_6 + 2H_2O \rightarrow \underset{\text{(acetic acid)}}{2CH_3COOH} + 2CO_2 + 4H_2 \qquad [2.3]$$

$$C_6H_{12}O_6 \rightarrow \underset{\text{(butyric acid)}}{2CH_3CH_2CH_2COOH} + 2CO_2 + 2H_2 \qquad [2.4]$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow \underset{\text{(propionic acid)}}{2CH_3CH_2COOH} + 2H_2O \qquad [2.5]$$

Afterwards, another group of bacteria catabolizes propionic and other organic acids molecules into acetic acid according to the following equations:

$$2CH_3CH_2CH_2COOH + 2CO_2 + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \qquad [2.6]$$

$$2CH_3CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \qquad [2.7]$$

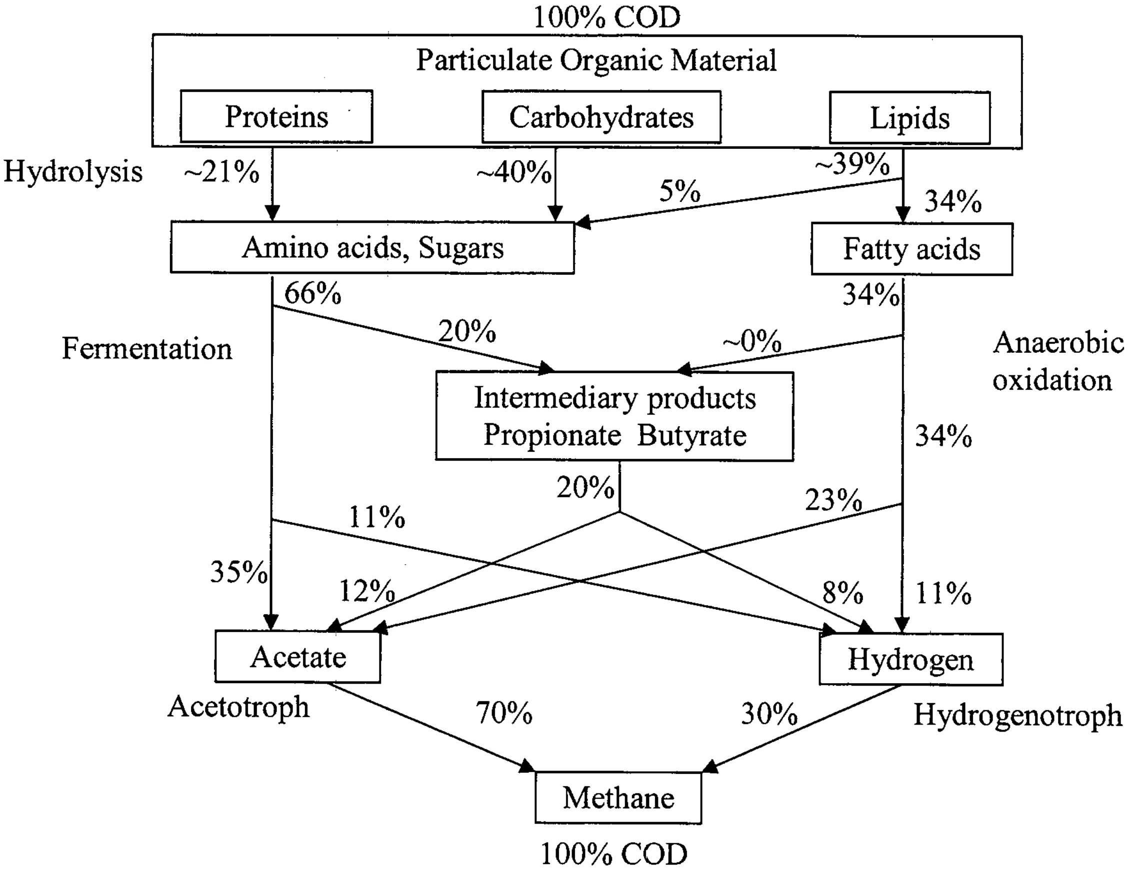

Figure 2.1 Scheme of the biodegradation steps of complex matter (Source: Gujer and Zehnder, 1983)

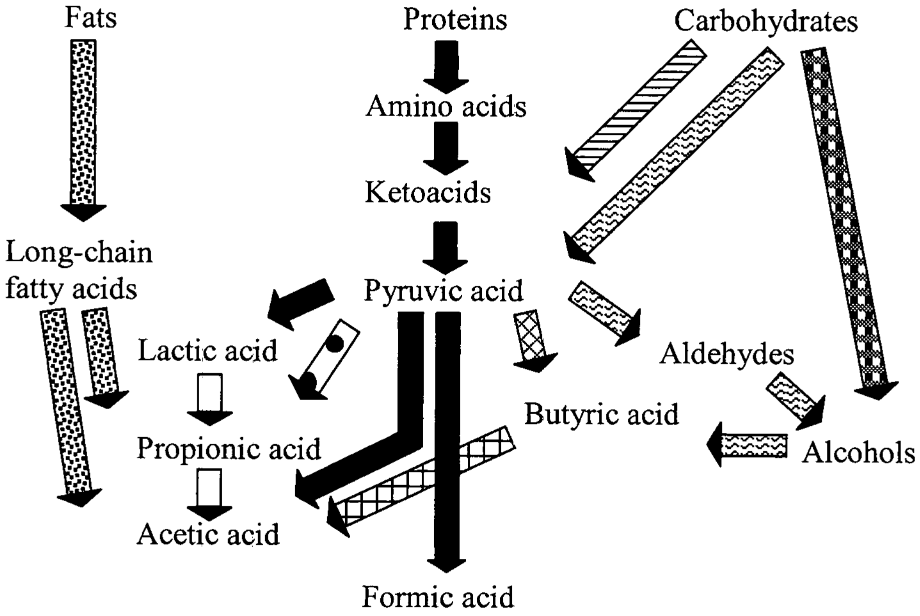

Figure 2.2 Acid step in anaerobic process (Source: Henze *et al.*, 2002)

2.1.1.3 Methanogenesis

In the methanogenesis stage, methane is formed mostly from acetate, and carbon dioxide and hydrogen gas by three main groups of methane-forming bacteria. About two thirds of methane is derived from acetate conversion or the fermentation of an alcohol, such as methyl alcohol, and one third is the result of carbon dioxide reduction by hydrogen.

$$CH_3CH_2OH + CO_2 \rightarrow CH_3COOH + 2H_2 \qquad [2.8]$$

$$2CH_3COOH \rightarrow 2CH_4 + 2CO_2 \qquad [2.9]$$

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O \qquad [2.10]$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad [2.11]$$

Methanogens are very sensitive to changes and prefer a neutral to slightly alkaline environment. If the pH is allowed to fall below 6, methanogenic bacteria cannot survive. Methanogenesis is the rate-controlling portion of the process because methanogens have a much slower growth rate than acidogens.

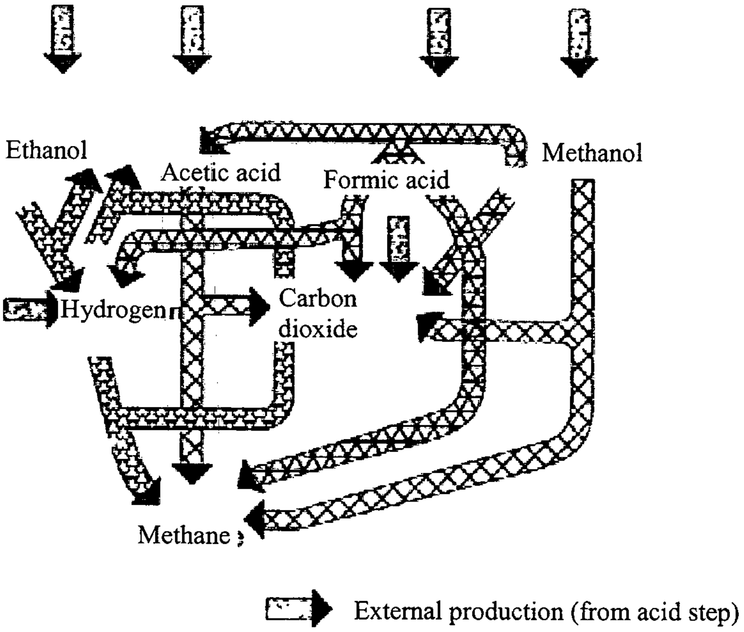

Figure 2.3 Methane step in anaerobic process

(Source: Henze *et al.*, 2002)

Table 2.1 The three stages of anaerobic digestion of solids

Stage	Activity	Enzymes Used
First	Hydrolysis: Solubilization of particulate and colloidal wastes	Exoenzymes
Second	Acid forming: Conversion of soluble organic acids and alcohols to acetate, carbon dioxide, and hydrogen	Endoenzymes
Third	Methanogenesis: Production of methane and carbon dioxide	Endoenzymes

(Source: Gerardi, 2003)

2.1.2 Microorganisms in Anaerobic Digestion

Anaerobes are inactive in the presence of free molecular oxygen conditions. They can be divided into two subgroups: oxygen-tolerant species and oxygen-intolerant species or strict anaerobes such as methane-forming bacteria. There are three important bacteria groups in anaerobic digestion. These groups include acetate-forming (acetogenic) bacteria, the sulfate-reducing bacteria, and the methane-forming bacteria.

2.1.2.1 Acetate-Forming Bacteria

Acetate-forming (acetogenic) bacteria, also called acidogen or acid former, grow in a symbiotic relationship with methane-forming bacteria. The acetate produced is a substrate for the methane-forming bacteria. For example, when ethanol is converted to acetate, carbon dioxide is used, and then acetate and hydrogen are produced (Equation 2.8). When acetate-forming bacteria produce acetate, hydrogen is also produced. If the hydrogen accumulates and significant hydrogen pressure occurs, the pressure results in the termination of activity of acetate-forming bacteria and loss of acetate production because acetate-forming bacteria can survive only at very low concentrations of hydrogen in the environment. However, methane-forming bacteria also utilize hydrogen in the production of methane (Equation 2.11) and significant hydrogen pressure may not occur.

2.1.2.2 Sulfate-Reducing Bacteria

Sulfate-reducing bacteria are also found in anaerobic digestion along with acetate-forming bacteria and methane-forming bacteria. If sulfates are present, sulfate-reducing bacteria multiply. Their reproduction often requires the use of hydrogen and acetate, which are also substrate for the methane-forming bacteria; consequently, a competition between both bacterial groups occurs in the system. However, in the case of a substrate-to-sulfate ratio above 3, methane-forming bacteria are favored.

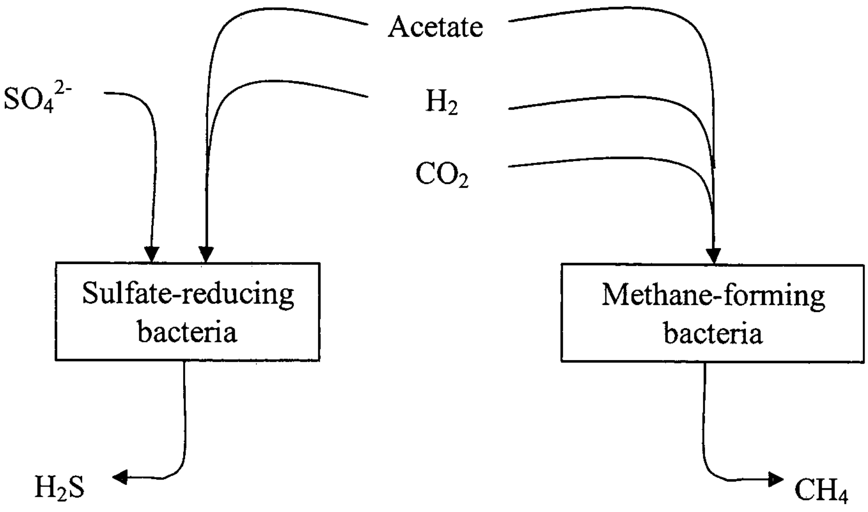

Figure 2.4 Using of acetate and hydrogen by sulfate-reducing bacteria and methane-forming bacteria (Source: Gerardi, 2003)

2.1.2.3 Methane-Forming Bacteria

There are three principal groups of methane-forming bacteria.

1) Hydrogenotrophic Methanogens

The hydrogenotrophic methanogens use hydrogen to convert carbon dioxide to methane (Equation 2.11). These organisms help to maintain the low partial hydrogen pressure in an anaerobic digester that is required for acetogenic bacteria.

2) Acetotrophic Methanogens

The acetotrophic methanogens use acetate to produce methane and carbon dioxide (Equation 2.12). The carbon dioxide produced from acetate may be converted by hydrogenotrophic methanogens to methane as shown in Equation 2.11. However, some hydrogenotrophic methanogens can use carbon monoxide to produce methane (Equation 2.13).

$$4CH_3COOH \rightarrow 2H_2 + 4CO_2 \quad [2.12]$$

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2 \quad [2.13]$$

The acetotrophic methanogens reproduce more slowly than the hydrogenotrophic methanogens and are adversely affected by the accumulation of hydrogen. Therefore, the maintenance of a low hydrogen pressure in an anaerobic digester is favorable for the activity of both acetate-forming bacteria and acetotrophic methanogens.

3) Methylothrophic Methanogens

The methylothrophic methanogens grow on the substrates that contain the methyl group (-CH_3) such as methanol (CH_3OH) and methylamines [$(CH_3)_3$-N] as shown in Equations 2.14 and 2.15, respectively. Group 1 and 2 methanogens produce methane from CO_2 and H_2 while group 3 methanogens produce methane directly from the methyl group and not from CO_2.

$$3CH_3OH + 6H \rightarrow 3CH_4 + 3H_2O \qquad [2.14]$$

$$4(CH_3)_3\text{-}N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3 \qquad [2.15]$$

Under optimal conditions, the reproduction times of methane-forming bacteria may be from a few days to several weeks. Therefore, if the solid retention time (SRT) is short, the population size of methane-forming bacteria is greatly reduced, resulting in poor digester performance or failure of the digester. Generally, methane-forming bacteria are very sensitive to pH. They will only survive between pH 6.5 and pH 8.5. Overloading of solid waste feed to the system results in the rapid growth of acid former and produces more acids than the methane forming bacteria can digest. Consequently, the digestion system may fail. To help ensure that the pH will remain in the optimal range, alkalinity is also frequently monitored. Generally, a range of between 2,000 to 3,500 mg/l $CaCO_3$ is acceptable (Girovich, 1996).

Methane-forming bacteria are strict anaerobes and are extremely sensitive to changes in alkalinity, pH, and temperature. Therefore, operational conditions in the digester must be periodically monitored and maintained within optimum ranges. In addition to alkalinity, pH, and temperature, several other operational conditions should be monitored and maintained within optimum ranges for acceptable activity of methane-forming bacteria. These conditions are gas composition, HRT, oxidation-reduction potential, and volatile acid concentration (Table 2.2).

Table 2.2 Operational conditions for acceptable activity of methane-forming bacteria and methane production

Condition	Optimum	Marginal
Alkalinity, mg/l as $CaCO_3$	1500-3000	1000-1500 3000-5000
Gas composition		
Methane, % volume	65-70	60-65 & 70-75
Carbon dioxide, % volume	30-35	25-30 & 35-40
HRT, days	10-15	7-10 & 15-30
pH	6.8-7.2	6.6-6.8 & 7.2-7.6
Temperature, mesophilic	30-35°C	20-30°C & 35-40°C
Temperature, thermophilic	50-56°C	45-50°C & 57-60°C
Volatile acids, mg/l as acetic acid	50-500	500-2000

(Source: Gerardi, 2003)

2.2 Reactor Design

The basic requirements of an anaerobic digester design are: to allow for a continuously high and sustainable organic load rate, a short HRT (to minimize reactor volume) and to produce the maximum volume of methane. There are three main groups of reactors in use today. Batch reactors are the most simple. These are simply filled with the feedstock and left for a period that can be considered to be the HRT, after which they are emptied. The second type are one-stage continuously fed systems, where all the biochemical reactions take place in one reactor, and, finally, the third type are the two-stage (or even multi-stage) continuously fed systems where the hydrolysis/acidification and acetogenesis/methanogenesis processes are separated.

Solid waste digesters are also divided into ''wet'' or ''dry'' types. Wet reactors are those with a TS value of 16% or less, whilst dry reactors have between 22% and 40% TS, and those that fall between wet and dry are considered semi-dry (Mata-Alvarez, 2003). The dry reactor technology is mainly used with MSW or vegetable wastes rather than with manures. Their high water content also means a larger overall volume to treat a similar mass of feedstock when compared to ''dry'' or ''semi-dry'' technologies. If using a wet reactor for treatment of dry feedstocks, pre-treatments of pulping and slurrying are required before digestion. Using either fresh or recycled process water to obtain less than 15% TS has the advantage that any inhibitors of methanogenesis which may be present in the fresh material, such as excessive short chain fatty acids or ammonium, are diluted, but there is also the possibility that these inhibitors will spread quickly through the reactor if dilution is insufficient.

Table 2.3 Comparison of wet and dry fermentations

Process Mode	Dry	Wet
TS content	high 25-45%	Low 2-15%
Reactor volume	minimized	increased
Conveyance technique	expensive	simple
Agitation	difficult	easy
Scumming	little risk	high risk
Short circuit flow	little risk	high risk
Solid-liquid separation	simple	expensive
Variety of waste components	small	great

(Source: Jördening and Winter, 2005)

Multi-stage systems attempt to separate the hydrolysis/acidification processes from the acetogenesis/methanogenesis processes, as these do not share the same optimum environmental conditions (Liu *et al.*, 2006). Multistage reactors usually only have two stages. A multi-stage system can improve the stability of the process compared to one-stage systems, particularly when digesting easily hydrolysable feedstocks (Bouallagui *et al.*, 2005). Instability can be caused by fluctuations in OLR, heterogeneity of wastes or excessive inhibitors. Multi-stage systems provide some protection against a variable OLR as the more sensitive methanogens are buffered by the first stage. Thus, the material passing from the first stage to the second stage has become homogenized and therefore more stable (Mata-Alvarez, 2003). However, although multi-stage digesters are generally found to have a higher performance than single-stage digesters, they are more expensive to build and maintain. For instance, in a comparison of one- and two-stage thermophilic reactors treating cattle manure, it was found that the two-stage digester had a 6–8% higher specific methane yield and a 9% more effective VS removal than the conventional single stage reactor (Nielsen *et al.*, 2004). Liu *et al.* (2006) found a 21% increase in methane yield in a two-stage reactor when compared to a single-stage reactor, both operating on MSW. Given the presence of a complete mix, one-stage reactors are more likely to suffer from short-circuiting than multi-stage systems. Short-circuiting is where the feedstock passes through at a shorter retention time than is required, reducing biogas yield and preventing complete sanitization of the material. The use of a pre-chamber in the reactor can reduce the effect of short-circuiting, which is effectively a similar approach to a multi-stage digester.

Anaerobic digesters are capable of treating insoluble wastes and soluble wastewaters. Insoluble wastes such as particulate and colloidal organics are considered to be high-strength wastes and require lengthy digestion periods for hydrolysis and solubilization. Digester retention times of at least 10-20 days are typical for high-strength wastes. Several anaerobic digester processes and configurations are available for the treatment of insoluble wastes and soluble wastewaters (Table 2.4). Each configuration has an impact on the SRT and HRT. Minimal HRT is desired to reduce digester volume and capital costs. Maximal SRT is desired to achieve process stability and minimal sludge production.

Table 2.4 Types of anaerobic digesters

Characteristic	Application
Bacterial growth system	Suspended
	Fixed film
Temperature	Psychrophilic
	Mesophilic
	Thermophilic
Configuration	Single-stage (phase)
	Two-stage (phase)

(Source: Gerardi, 2003)

2.2.1 Bacterial Growth-Suspended

In suspended growth systems, the bacteria are suspended in the digester through intermittent or continuous mixing action. The mixing action distributes the bacteria or biomass throughout the digester. Because completely mixed anaerobic digesters do not incorporate a means for retaining and concentrating the biomass, the SRT is the same as the HRT. Completely mixed anaerobic digesters are designed for relatively long HRTs. Feed sludge can be added to the digester on a continuous or intermittent basis. Advantages and disadvantages of completely mixed suspended growth digesters are listed in Table 2.5.

Table 2.5 Advantages and disadvantages of the suspended growth anaerobic digesters

Advantages	Suitable for the treatment of particulate, colloidal, and soluble wastes Toxic wastes may be diluted Uniform distribution of nutrients, pH, substrate, and temperature
Disadvantages	Large digester volume required to provide necessary SRT Treatment efficiency may be reduced due to loss of particulate and colloidal wastes and bacteria in digester effluent

(Source: Gerardi, 2003)

2.2.2 Bacterial Growth-Fixed film

Anaerobic fixed-film (sludge blankets) systems provide a quiescent environment for the growth of an agglutinated mass of bacteria. Because bacterial growth requires relatively long periods of time to develop, the media used in fixed-film systems hold the bacteria in the digester for relatively long periods and provide for long SRTs and short HRTs. The bacteria grow as fixed films of dendritic masses on the supportive media within the openings or voids of the supportive media. Fixed-film systems usually use gravel, plastic, and rock as the supportive media. The openings make up approximately 50% or more of the media.

Fixed-film systems operate as flow-through processes, that is, wastewater passes over and through a bed of fixed film of bacteria growth and through entrapped clumps of bacterial growth, providing good contact between the wastes and the bacteria. Soluble organic compounds in the wastewater are absorbed (diffuse into) by the bacteria, whereas insoluble organic compounds are adsorbed (attach) to the surface of the bacteria. The flow of wastewater through fixed-film systems may be from the bottom to the top (up flow) or from the top to the bottom (down flow). Because the bacteria (solids) in fixed-film systems remain in the digester for long SRTs, the systems allow methane-forming bacteria to acclimate to toxicants such as ammonia, sulfide, and formaldehyde. Therefore, anaerobic fixed-film systems with long SRTs and short HRTs may be used to treat industrial wastewater containing toxicants.

2.2.3 Single and Two Stage Configuration

A typical single-stage digester consists of only one tank or reactor. Digester operations consist of sludge addition and withdraw, mixing, heating, gas collecting, and supernating. Single-stage digesters are more easily upset than two-stage digesters. This is because of the presence of the simultaneous activities of two groups of bacteria, the acid-forming bacteria and the methane-forming bacteria. Because acid-forming bacteria grow more quickly than methane-forming bacteria and are more tolerant of fluctuations in operational conditions, an imbalance between acid production rate and methane production rate often occurs. This imbalance may cause a decrease in alkalinity and pH that result in digester failure.

Two-stage digester systems consist of at least two separate tanks or reactors. A two-stage system yields improved efficiency and stability over a single-stage system. A two-stage system is capable of obtaining methane production and solids reduction similar to those of a single-stage system at a smaller HRT. In addition, toxicants are removed in the first stage. In some two-stage systems acid production occurs in the first stage and methane production occurs in the second stage (Figure 2.5).

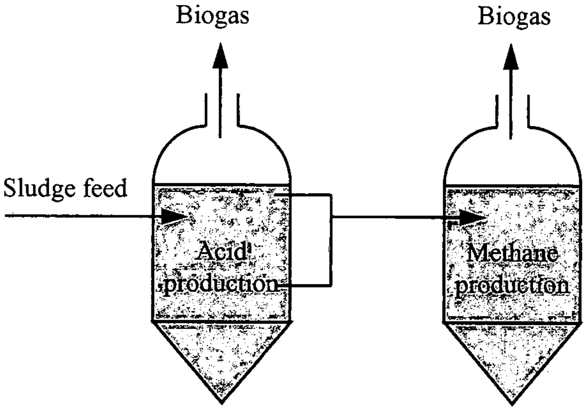

Figure 2.5 Two-stage digester system

(Source: Meta-Alvarez, 2003)

Table 2.6 Advantages and disadvantages of the two-stage system

Criteria	Advantages	Disadvantages
- Technical	- Design flexibility	- Complex
- Biological	- More reliable for cellulose-poor kitchen waste - Only reliable design (with biomass retention) for C/N<20	- Smaller biogas yield (when solids not methanogenized)
- Economical & Environmental	- Less heavy metal in compost (when solids not methanogenized)	- Larger investment

(Source: Meta-Alvarez, 2003)

2.3 Volatile Acids Inhibition

There are several indicators of unstable anaerobic digesters (Table 2.7). These indicators are either increases or decreases in specific operational values. The indicators include decreases in biogas production and methane production, decreases in alkalinity and pH, a decrease in VS destruction, and increases in volatile acid concentration and percent carbon dioxide in the biogas.

Table 2.7 Indicators of unstable anaerobic digesters

Indicator	Decrease	Increase
Biogas production	x	
Methane production	x	
Alkalinity	x	
pH	x	
Volatile solids destruction	x	
Volatile acid concentration		x
Percent CO_2 in biogas		x

(Source: Gerardi, 2003)

Some organic acids are known also as volatile acids or volatile fatty acids. These acids occur as substrates and products in the anaerobic digester. Many serve as substrate for methane-forming bacteria, and they are the products of the fermentative activities of facultative anaerobes and anaerobes. Volatile acid production in an anaerobic digester results in the production of methane. Although volatile acids vary in length, most volatile acids produced in an anaerobic digester are low-molecular-weight, short-chain acids, for example, formate (1 carbon unit), acetate (2 carbon units), propionate (3 carbon units), and butyrate (4 carbon units). These short-chain acids are known as volatile acids because they can vaporize or evaporate at atmospheric pressure. Of these acids, acetate is the predominant acid produced in an anaerobic digester. Approximately 85% of the volatile acid content of an anaerobic digester is acetate. As wastes are degraded, new bacterial cells or sludge are produced. The cellular growth or amount of sludge produced is expressed as net biomass yield (as percentage of chemical oxygen demand (COD) removed).

Table 2.8 Main cause of elevated VFA in anaerobic process

- trace metal limitation-caused restriction of metabolism
- toxicity impairment of metabolism
- kinetic overload
- single stage CSTR configuration
- mass transfer limitation
- hydraulic short circuiting
- nitrogen or phosphorus limitation
- thermodynamic impairment of propionate conversion due to elevated H_2 concentrations

(Source: Speece, 1995)

The higher the VS feed to the digester, the larger the amount of volatile acids formed in the digester. The larger the amount of volatile acids in the digester, the greater the impact of volatile acids on digester alkalinity and pH. Therefore, feedstock that has a high volatile content should be transferred slowly to an anaerobic digester. The presence of a relatively high concentration of short-chain volatile acids such as acetate causes a decrease in the concentration of alkalinity and a drop in pH. Propionate is perhaps the most toxic of the volatile acids and may exert toxicity at concentrations <5 mg/l. Because the chemical composition and structure of several long-chain fatty acids are similar to those of the lipid components in the cell wall of acetoclastic bacteria and methane-forming bacteria, the fatty acids dissolve in the cell wall. Once dissolved in the cell wall, the acids inhibit the activity of the bacteria, even at very low concentrations.

2.4 Microbial Inhibition by Undissociated and Dissociated Organic Acids

In a microbial reaction, inhibition can be caused by substances either entering with the influent substrate or being produced by the anaerobic process itself. Regarding the latter, a simple classification could be:

- Substrate inhibition, where the substrate provokes enzymatic inhibition
- Production inhibition, which is caused by products that are final or intermediate in the chain of simultaneous biochemical reactions, such as H^+ (pH), H_2, NH_3, H_2S and volatile or long chain fatty acids (Meta-Alvarez, 2003).

An organic acid is a carbon-containing compound that donates protons, and it can be classified into specific sub-groups according to its chemical bonding or functional groups. Fatty acids are organic acids made of carbon, oxygen, and hydrogen, which are characterized by the presence of a carboxyl group, -COOH. Fatty acids are also known as volatile fatty, weak, or carboxylic acids. Since organic acids are weak acids, they do not readily donate protons $[H^+]$ in aqueous solutions. A weak acid partially dissociates in water, and has a reverse equilibrium reaction of hydrogen proton $[H^+]$ and acid anions $[A^-]$:

$$[HA] + [H_2O] \leftrightarrow [H^+] + [A^-] \qquad [2.16]$$

where [HA] is the molecular form of an acid. The dissociation equilibrium is defined as a measure of dissociation strength related to the equilibrium concentrations of $[H^+]$, $[A^-]$, and [HA] as follows:

$$pKa = -\log Ka \qquad [2.17]$$

where both pKa and Ka are the dissociation constants. Sometimes, dissociation constants are referred to as acidity constants, in which Ka is negatively and logarithmically related to pKa.

In general, the smaller the value of pKa (or the greater the value of Ka), the more the formation of $[H^+]$ is favored and the lower the pH. When the pH tends towards neutral (pH 7.0), acids generally have very small proportions of undissociated acid molecules [HA]. It is known that the microbial cell membrane is permeable to the undissociated acid molecules, which lower the intracellular pH when $[H^+]$ is dissociated inside the cell. Generally, considering two organic acids, the one with undissociated molecules at a given pH is more inhibitory to microorganisms (Sundberg and Jönsson 2005). However, strong acids, such as hydrochloric acid (HCl), completely dissociate into ion forms in aqueous solution. Strong acids lower pH values and exert the inhibitory effect on the exterior of the cell, but do not affect the intracellular pH to the same extent as weak acids (Presser *et al.*, 1997). Since the presence of organic acids and low pH values has inhibitory effects on microorganisms, it is assumed that the microbial inhibition is likely to be related to the concentration of undissociated acid molecules to which the membrane is permeable (Russell and Diez-Gonzalez, 1998). The lowering of intracellular pH resulting in the acidification of the cytoplasm of the cell is effective to limit microbial growth and activity. Therefore, the relationship between dissociated protons and anions and undissociated molecules at particular pH values is critical in the inhibition mechanism induced by the organic acid.

The [HA] can enter bacterial cells and release their dissociated protons $[H^+]$ inside. As these excess protons $[H^+]$ accumulate, the intracellular pH (the pH of the bacterial cell) decreases, and thereby becomes inhibitory or even lethal to the bacteria (Cherrington *et al.*, 1991). Organic acids can be bacteriostatic agents, which hamper the growth rate of bacteria, or bactericidal agents, leading to the loss of viability and eventually killing the bacteria. Figure 2.6 shows the transmembrane flux of undissociated acid molecules and the release of protons in the external and intracellular environment at acidic and neutral pH levels. When the extracelluar pH is about 4.50 or less, or the extracellular pH is lower than the pKa of the organic acid, the number of [HA] is relatively high in the external environment. [HA] molecules are then diffused into the intracellular environment of the bacterial cell from the external environment. The transported [HA] dissociate inside the cell and release protons $[H^+]$ and anions $[A^-]$. The dissociation inside the cell is promoted because at this time the intracellular pH is greater than the pKa of the acid. To maintain the pH gradient between the extracellular and intracellular environments, $[A^-]$ inside the cell have to regulate the pH gradient, and thus cross the cell membrane. Large amounts of protons still accumulate inside the cell, leading to a decrease in the intracellular pH, resulting in acidification of the cytoplasm of the microbial cell. Because $[A^-]$ has to be actively pumped, the energy of the cell is almost exhausted for further activity. Consequently, bacterial growth is inhibited or hampered. Only simply surviving or even dying bacteria are found under these conditions.

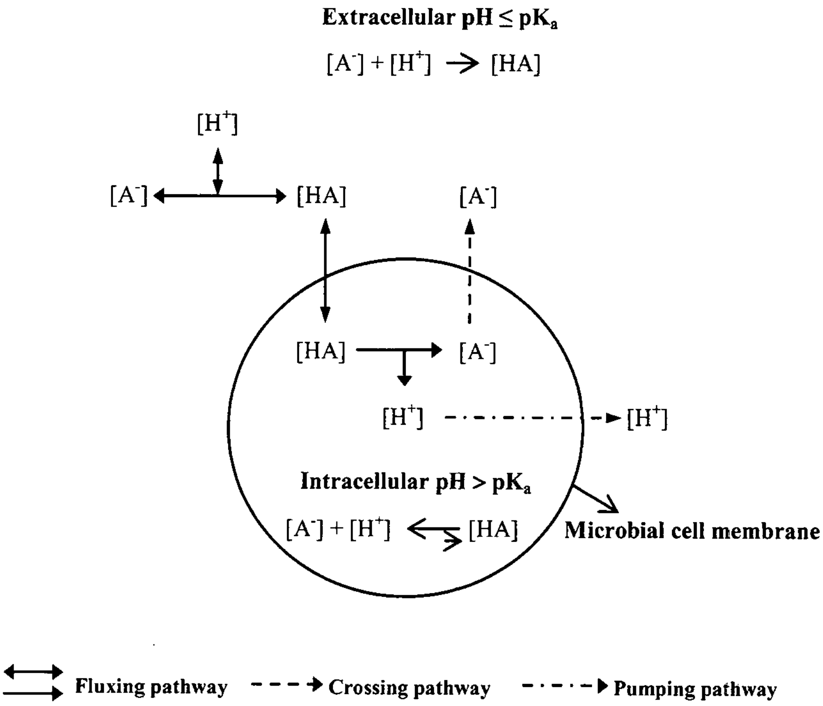

Figure 2.6 Transmembrane pathways of undissociated acid molecules and dissociated proton/anion in a bacterial cell at acidic and neutral pH

If the extracellular pH rises to about 5.00, or pH is approximately around the pKa of the organic acid, the proportion of undissociated acid molecules is less than at the acidic pH below 4.50. The [HA] molecules can still freely permeate the cell membrane and dissociate into [H^+] and [A^-] inside the cytoplasm. Because of the low concentrations of dissociated ions and smaller changes in the pH gradient between the extracellular and intracellular environments, the cell has sufficient energy and time to metabolize [A^-] as a carbon source, and to alkalinize the pH. Moreover, the cell has sufficient energy to pump out the unnecessary [H^+] to prevent intracellular acidity as well as the inhibition of growth or activity.

Thus, bacteria can better tolerate a slightly acidic pH. When the extracellular pH is above 5.00 and approaching neutral, or when the extracellular pH is greater than the pKa of the organic acid, the number of undissociated acid molecules is relatively small. The bacterial cell continuously pumps out unnecessary [H^+] and metabolizes [A^-] to regulate its intracellular pH, and to generate energy for optimal growth and activity. Hence, microbial growth is favored when the extracellular pH tends towards neutral, and results in high microbial activity. The maintenance of intracellular pH varies among bacteria. In some organisms, a 0.10 change of internal pH corresponds to a 1.00 change of external pH, whereas other organisms are much more sensitive to internal pH changes (Kabara and Eklund, 1991).

2.5 Biogas Production

The quantity and quality of gas produced during anaerobic digestion depends on the feed characteristics. Methane production can also be estimated from COD or ultimate biochemical oxygen demand (BOD_L) stabilization based on the fact that 1 kg COD destroyed produces 0.35 m^3 CH_4 (5.62 ft^3/lb COD destroyed) or 10-25 ft^3/lb volatile suspended solids (VSS) destroyed at standard temperature and pressure (STP). The typical gas production rates for different substrates are shown in Table 2.9.

Table 2.9 Typical digester gas production rates

Substrate	Specific gas production per unit mass destroyed	
	m^3/kg	ft^3/lb
Fats	1.2-1.6	19.2-25.6
Scum	0.9-1.0	14.4-16.0
Grease	1.1	17.6
Crude fibers	0.8	12.8
Protein	0.7	11.2
Carbohydrates	0.7	11.2

(Source: Khanal, 2008. adapted from WEF Manual of Practice 8, 1998)

Anaerobic digestion systems are rather complex processes that unfortunately often suffer from instability. Such instability is usually witnessed as a drop in the methane production rate, a drop in the pH and/or arise in the VFA concentration, causing digester failure. It can be caused by (a) feed overload, (b) feed underload, (c) entry of an inhibitor, or (d) inadequate temperature control (Lyberatos and Skiadas, 1999).

Biogas production is not as meaningful as methane production, because only methane production represents the final degradation of organic compounds. Although a decrease in methane production is associated with an unstable digester, a decrease in methane production also may be associated with a change (less substrate) in the composition of the feed sludge. Methane production and alkalinity may be correlated, and this correlation may be used as an indicator of an unstable digester. A decrease in methane production and a decrease in alkalinity indicate that toxicity is affecting methane-forming bacteria. A decrease in methane production and no significant change in alkalinity indicate that toxicity is affecting methane-forming bacteria and acid-forming bacteria. An increase in effluent volatile solids also will take place if toxicity to both groups of bacteria occurs.

The volume, rate, and composition of the biogas produced are indicative of digester performance. The amount of gas produced depends on several factors such as temperature, pH and alkalinity, hydraulic and organic loading rate, toxic compounds, substrate type, and TS/VS content. The rate and volume of methane produced during the anaerobic digestion of a waste can be used to determine its relative rate of conversion. Biogas is a mixture of various gases. Independent of the fermentation temperature, a biogas is produced which consists of 60%–70% methane and 30%–40% carbon dioxide. Trace components of ammonia and hydrogen sulfide can be detected. The caloric value of the biogas is about 5.5-6.0 kWh m^{-3}. This corresponds to about 0.5 L of diesel oil.

Table 2.10 Mean composition and specific yields of biogas in relation to the kind of substances degraded

Substance	Gas yield ($m^3 kg^{-1}$ TS)	CH_4 Content (vol. %)	CO_2 Content (vol. %)
Carbohydrates	0.79	50	50
Fats	1.27	68	32
Proteins	0.70	71	29
MSW	0.1-0.2	55-65	35-45
Biowaste	0.2-0.3	55-65	35-45
Sewage sludge	0.2-0.4	60-70	30-40
Manure	0.1-0.3	60-65	35-40

(Source: Rilling, 2005)