CHAPTER II
LITERATURE REVIEW

2.1 Anaerobic Digestion

Anaerobic digestion involves bacterial fermentation of organic wastes in the absence of free oxygen. The fermentation leads to the breakdown of complex biodegradable organics in four stages (Rapport *et al.*, 2008) (Figure 2.1):

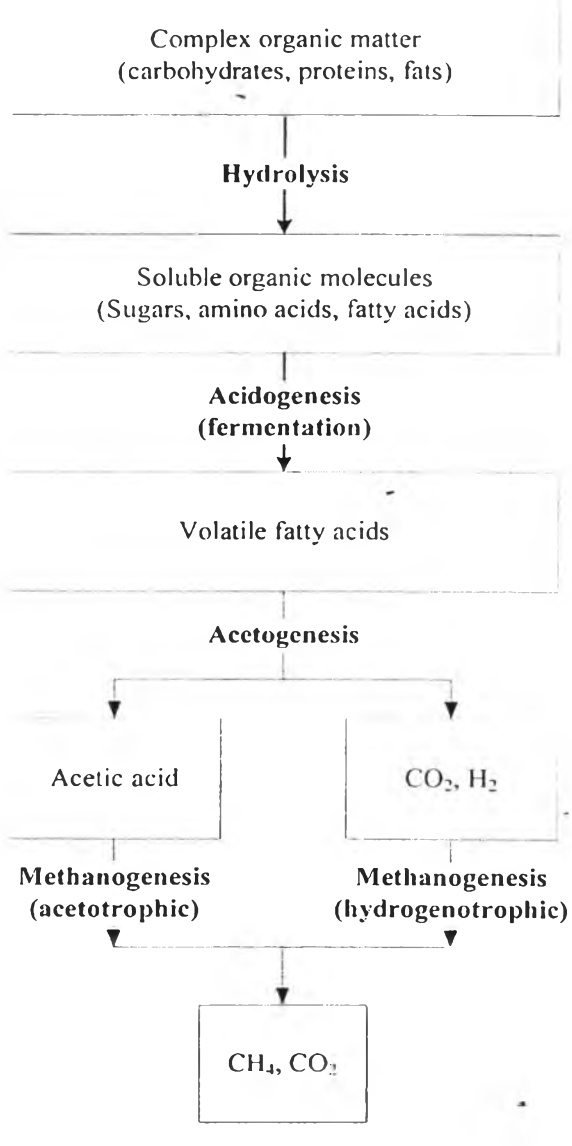

Figure 2.1 Steps involved in anaerobic digestion (Rapport *et al.*, 2008).

Step 1. Large protein macromolecules, fats, and carbohydrate polymers (such as cellulose and starch) are cracked into water soluble monomers (amino acids, long-chain fatty acids, and sugars). This is brought about by exoenzymes (hydrolase) present in facultative and obligatory anaerobic bacteria.

Step 2. These products are then fermented during acidogenesis to form short-chain (C_1–C_5) 'volatile fatty acids', principally lactic, propionic, butyric, and valeric acid.

Step 3. In acetogenesis, homoacetogenic microorganisms consume these fermentation products and generate acetic acid, carbondioxide, and hydrogen.

Step 4. Methanogenic organisms, which are strictly anaerobic, consume the acetate, hydrogen, and some of carbon dioxide to produce methane. Three biochemical pathways are used by methanogens to achieve this:

acetotrophic pathway;	$4CH_3COOH$	$\rightarrow$	$4CO_2 + 4CH_4$	(2.1)
hydrogenotrophic pathway;	$CO_2 + 4H_2$	$\rightarrow$	$CH_4 + 2H_2O$	(2.2)
methylotrophic pathway;	$4CH_3OH + 6H_2$	$\rightarrow$	$3CH_4 + 2H_2O$	(2.3)

Methylated substrates other than methanol can also be converted. Acetotrophic pathway is the primary one; hence, theoretical yield calculations are often made using this pathway (Rapport *et al.*, 2008). These four steps are principally involved. If the process is properly controlled in reactors so that it proceeds optimally as per these stages, the principal end product, the biogas, contains 40 - 70% (by volume) of methane gas but more often than not it is in the 55 - 65% range. Biogas compositions depend on wide variety of substrates that are used to generate biogas shown in Figure 2.2. Besides methane gas, the remaining of biogas compositions are carbon dioxide, traces of ammonia, hydrogen sulfide, and hydrogen gas (Abbasi *et al.*, 2012).

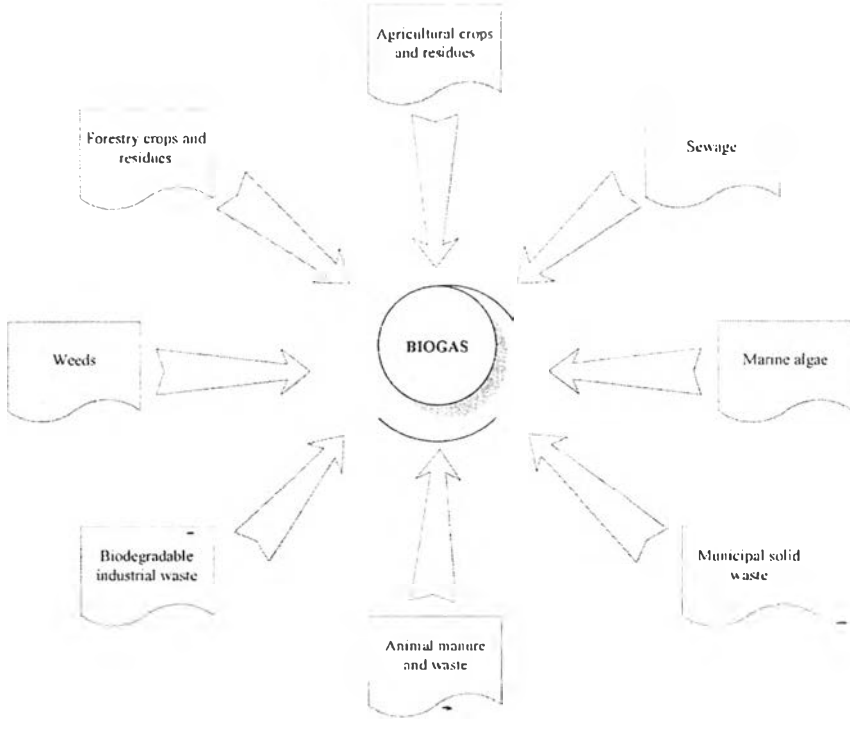

Figure 2.2 Examples of substrates which can be anaerobically digested to generate biogas (Abbasi *et al.*, 2012).

Biogas, in theory, should contain equal volumes (50 - 50) of methane and carbon dioxide gas. However, acetogenesis typically produces some hydrogen, and for every four moles of hydrogen consumed by hydrogenotrophic methanogens, a mole of carbon dioxide is converted to methane. Fats and proteins can yield larger amounts of hydrogen leading to higher typical methane content for these substrates. In certain conditions, these molecules can also get converted to products other than methane. Therefore, the overall biogas yield and methane content vary for different substrates, biological consortia, and digester conditions (Rapport *et al.*, 2008).

If ignited, biogas burns cleanly (i.e. gives off no soot or foul smell) similar to LPG (liquefied petroleum gas) or CNG (compressed natural gas). Biogas has a good calorific value, though lesser than LPG and CNG (Hill *et al.*, 2011) (Table 2.1).

Table 2.1 Comparison of the calorific values of various fuels (Hill *et al.*, 2011)

Fuel	**Calorific value (CV) (approximate)**	**Indirect emission factor ($kgCO_2e/GJ$, net CV basis)**
Petrol	10,800 kcal/kg	12.51
Natural gas	8,600 $kcal/m^3$	5.55[a]
Liquefied natural gas	13,140 kcal/kg	20.00
Liquefied petroleum gas	10,800 kcal/kg	8.00
Kerosene	10,300 kcal/kg	13.34
Diesel	10,700 kcal/kg	14.13
Compressed natural gas	8,600 $kcal/m^3$	8.36
Biogas	5,000 $kcal/m^3$	0.246[b]

[a] Natural gas European Union mix

[b] Direct CO_2 emissions (emission factor, gCO_2e/kWh)

Factors, which influence anaerobic digestion of an organic substrate, are as follows:

- Specific Surface of the Substrate

The greater the specific surface of the substrate, the more efficiently the microorganism–substrate contact; consequently, the faster the digestion. If the substrate is in the form of large pieces of solids, it should be comminuted (Abbasi *et al.*, 2012).

- C/N Ratio

The relative proportions of carbon and nitrogen present in an organic material is expressed in terms of the carbon/nitrogen (C/N) ratio. The C/N ratio in the range of 16:1–25:1 is considered to be optimum for anaerobic digestion.

If the C/N ratio is too high, nitrogen is consumed rapidly by the methanogens to meet their protein requirement and is no longer available to react on the left-over carbon content in the material. As a result, the biogas production gets depressed.

If the C/N ratio is too low, nitrogen is liberated and accumulates in the form of ammonia. This increases the pH of the material. When the pH value rises higher than 8.5, it begins to exert a toxic effect on the methanogenic bacteria.

To maintain the C/N level of the digester material at optimum levels, materials of high C/N ratio can be mixed with materials of low C/N ratio (Siddiqui *et al.*, 2011).

- Dilution

Water should be added, if necessary, to the raw material to generate slurry, which is neither too thick nor too thin. If a material is diluted too much, the solid particles may settle down in the digester and may not get degraded properly. If the slurry is too thick, it may be difficult to stir and may impede the flow of gas to the upper part of the digester. Different systems can handle different levels of slurry density, generally in the range of 10–25% of solids (Abbasi *et al.*, 1992).

- pH

Optimum biogas production is achieved when the pH value of the input mixture is between 6.7 and 7.5 (Daisy and Kamaraj, 2011). During the initial period of digestion, large amounts of organic acids are produced and the pH of the mixture decreases. As digestion continues and the concentration of ammonia increases, due to the digestion of nitrogen, the pH value increases. When the methane gas production stabilizes, the pH remains between 7.2 and 8.2. When plant material is fermented in a batch system, the acetogenesis/fermentation stage is rapid, producing organic acids, which reduce the pH and inhibit further digestion (Abbasi *et al.*, 1991). In general, a drop in the pH and a rise in the proportion of carbon dioxide in the biogas are indicators of a disturbance in the digestion process (Abbasi *et al.*, 2012). The ammonification counteracts the reduction of the pH resulting from the acidification step of anaerobic digestion. In most cases, the pH is increased by the addition of lime and sodium hydroxide. The amount of chemicals required to increase the pH is strongly influenced by the composition and the buffer capacity of the wastewater to be treated (Fricke *et al.*, 2007).

- Temperature

Different species of methanogenic bacteria function optimally in three different temperature ranges: 50–65 °C, 20–40 °C, and <1.2 °C. The concerned bacteria are called thermophilic, mesophilic, and psychrophilic, respectively. Large-scale anaerobic digestion is generally carried out in the mesophilic mode with lesser number of digesters operating in thermophilic mode and much lesser in the psychrophilic mode. The mesophilic temperature considered to be most suitable for anaerobic digestion is 35 °C. In thermophilic digestion, 55 °C is considered to be ideal. Although thermophilic anaerobic digestion process is generally more efficient than the mesophilic process, it is more difficult to control and also needs extra energy inputs, leading to a less favorable energy balance than mesophilic anaerobic digestion (Abbasi *et al.*, 2012).

- Loading Rate

This is an important process control parameter especially when the digestion is carried out in continuous mode. Overloading can easily lead to system failure. This can happen if there is inadequate mixing of the waste with slurry. It may cause a significant rise in volatile fatty acid concentration, leading to sharp drop in pH. When this happens, feed rate to the system has to be reduced for a while till the process re-stabilizes (Abbasi *et al.*, 2012).

- Retention Time

Retention time is the duration, for which organic material (substrate) and microorganisms ('solids') must remain together in a digester to achieve the desired extent of degradation. Shorter the substrate retention time (HRT) required to achieve this objective in an anaerobic reactor, more efficient the reactor. But to achieve low substrate retention time, it is necessary to simultaneously achieve high microorganism; solids retention time (SRT).

The ratio of the quantity of substrate and to the quantity of bacteria available to consume that substrate is called the 'food-to-microorganism ratio' (F/M). This ratio is the controlling factor in all biological treatment processes. A lower than adequate F/M ratio will result in a greater percentage of the substrate being converted to biogas. The only way, in which F/M ratio can be kept adequately low even as we

aim to reduce HRT (to enhance digester efficiency), is to find away, by which SRT is kept high. In other words, to find ways by which the substrate passes through the digester quickly but microorganisms pass through much more slowly. This situation can ensure that, at any given time, more quantities of microorganisms are present in a digester than substrate (hence low F/M ratio).

In conventional low-rate digesters and in the continuously stirred tank reactors (CSTRs), there is no provision to retain 'solids' (microorganisms). Hence, the solids pass out of the digesters at the same rate as the substrate-to-be-degraded does. In other words, in these systems, HRT = SRT. On the other hand, in high-rate digesters, retention of microorganisms by way of attached growth or suspended growth systems enables SRT >>HRT. In a typical high rate anaerobic digester, SRT is about three times higher than the HRT (Abbasi *et al.*, 2012).

- Toxicity

Mineral ions, especially of heavy metals, and detergents are among the materials that inhibit the normal growth of bacteria in a digester. Small quantities of minerals (sodium, potassium, calcium, magnesium, ammonium, and sulfur) stimulate the bacterial growth, but higher concentrations may be inhibitory. Heavy metals such as copper, nickel, chromium, zinc, and lead are essential for bacterial growth in very small quantities, but higher quantities have a toxic effect. Detergents such as soap, antibiotics, organic solvents also inhibit the bacteria. Recovery of digesters following inhibition by toxic substances can only be achieved by cessation of feeding and flushing the contents or diluting the contents to push the concentration of inhibitory substances to below the toxic level (Chen *et al.*, 2008).

- Mixing/Agitation

Mixing is required to maintain fluid homogeneity, hence process stability, within a digester. The objectives of mixing are to combine the incoming material with the bacteria, to stop the formation of scum, and to avoid pronounced temperature gradients within the digester. Very rapid mixing can disrupt the bacterial community, while too slow a stirring can cause inadequate mixing and short circuiting. The extent of mixing required is also dependent on the content of the digestion mixture (Abbasi *et al.*, 2012).

- Pathogens

Pathogenic bacteria (e.g., *Salmonella*, *Escherichia coli*, *Listeria*) and viruses present in municipal solid waste can pose risk of infection to the workers handling the waste. Such pathogens are sensitive to temperature, hence most effective pathogen control occurs when anaerobic digestion is performed at thermophilic temperatures and at long retention times. For certain types of wastes, a separate pasteurization step before or after anaerobic digestion at 70 °C for 60 min has been stipulated by the European Union Animal Byproducts Regulation (Abbasi *et al.*, 2012). Pasteurization (70 °C) is an effective alternative to sterilization (130 °C); however, bacterial spores are not reduced in the former. Moreover, pasteurized digestate is prone to recontamination (Weiland, 2010).

- Light

Light does not kill methanogens but strongly inhibits methanation. Hence, light should be blocked from entering the anaerobic digestion chamber (Abbasi *et al.*, 2012).

- Solid Residue/Slurry

After the anaerobic degradation is nearly complete, the solid residue or digestate is removed and is normally cured aerobically and screened for items such as glass shards, and plastic pieces before being disposed on land. The purity of the material fed into the system dictates the quality of the slurry that is produced (Abbasi *et al.*, 2012).

2.2 Cassava Wastewater

Cassava wastewater is acidic with a high organic matter content (soluble carbohydrates and proteins) and suspended solids (lipids and non-soluble carbohydrates-starch or cellulose fibers). Besides, it also has very high COD and BOD (www.fao.org).

Cassava residue is lignocellulose that the main component of lignocellulose is cellulose, a beta (1–4)-linked chain of glucose molecules. Hydrogen bonds between different layers of the polysaccharides contribute to the resistance of crystalline

cellulose to degradation. Hemicellulose, the second most abundant component of lignocellulose, is composed of various 5- and 6-carbon sugars such as arabinose, galactose, glucose, mannose, and xylose. Lignin is composed of three major phenolic components, namely p-coumaryl alcohol (H), coniferyl alcohol (G) and sinapyl alcohol (S). Lignin is synthesized by polymerization of these components and their ratio within the polymer varies between different plants, wood tissues, and cell wall layers. Cellulose, hemicellulose, and lignin form structures called microfibrils, which are organized into macrofibrils (Figure 2.3), that cause high structural stability in the plant cell wall (Robin, 2008). As a result, it is difficult for anaerobic microorganisms to digest cassava residue by hydrolysis because the microorganisms cannot digest lignin.

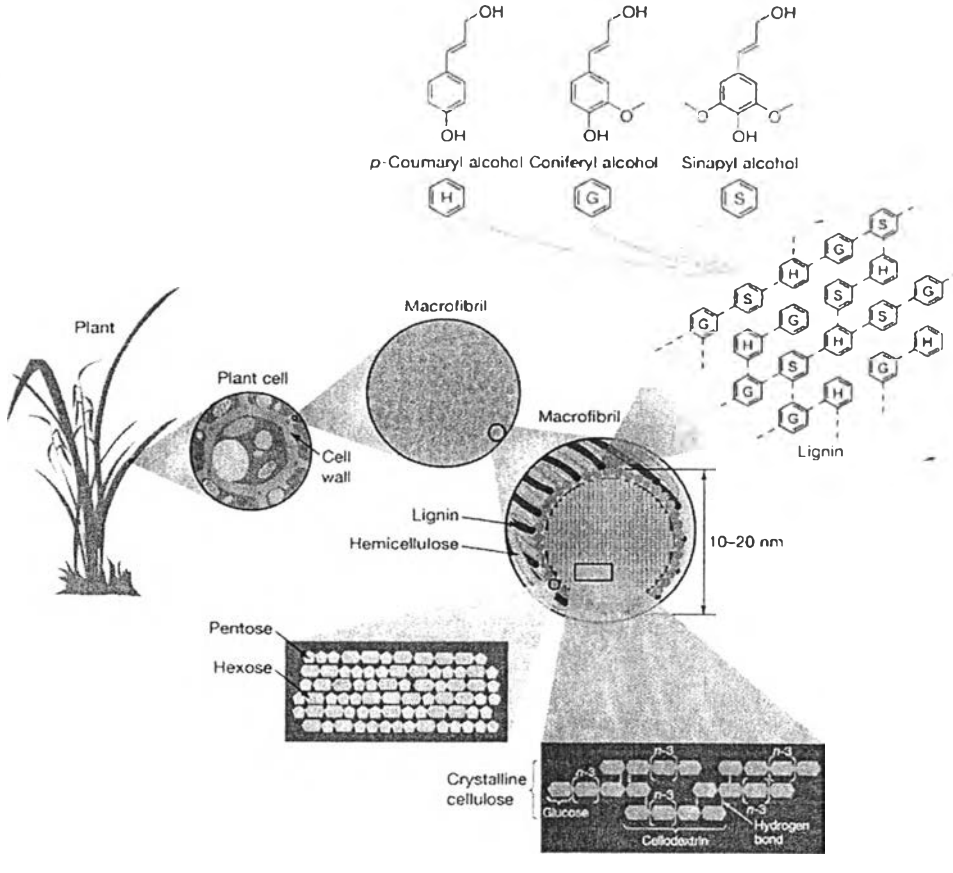

Figure 2.3 Structure of lignocelluloses (Robin, 2008).

Presently, various pretreatments of lignocellulose before hydrolysis in anaerobic digestion have been used to remove the barriers and make cellulose more accessible to hydrolysis (Figure 2.4). But the pretreatments have been viewed as one of the most expensive processing steps (Balat, 2011). There are various pretreatment processes for lignocellulosic materials shown in Table 2.2.

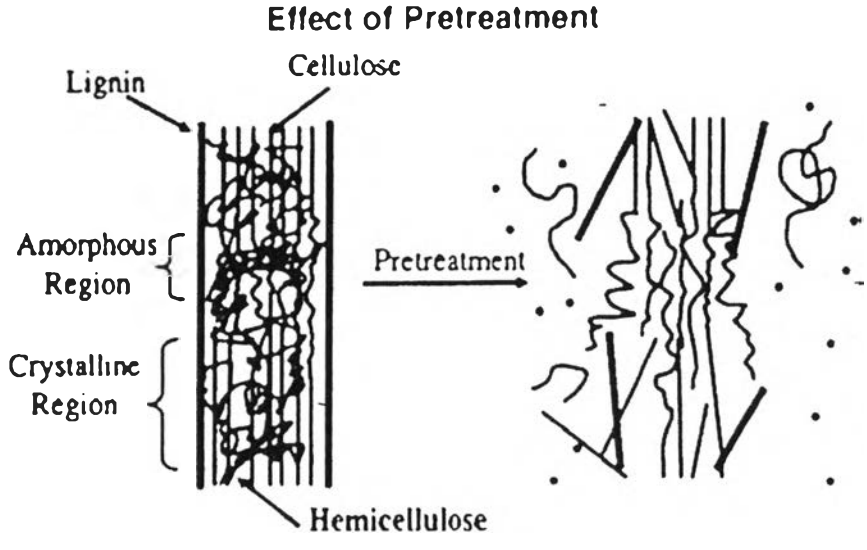

Figure 2.4 Pretreatment of lignocellulosic material (Balat, 2011).

Table 2.2 indicates that the biological pretreatment uses simple equipment and low energy for operation of process. Thus, this method is cheaper than the others but the rate of hydrolysis is very low.

2.3 Anaerobic Hydrolysis and Microaeration

Complex waste (water) has been proven to be degradable under anaerobic conditions. During the digestion process in the conversion of the complex organic molecules into mono- and dimer components, also called the hydrolysis, is often the rate-limiting step. Design and optimization of the anaerobic conversion of complex waste (water) is essential (Sanders, 2001).

Table 2.2 Advantages and disadvantages of various pretreatment processes for lignocellulosic materials (Balat, 2011)

Pretreatment process	Advantages	Limitations and disadvantages
Mechanical comminution	Reduces cellulose crystallinity	Power consumption usually higher than inherent biomass energy
Steam explosion	Causes hemicellulose degradation and lignin transformation; cost-effective	Incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms
CO_2 explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass
Pyrolysis	Produces gas and liquid products	High temperature; ash production
Biological	Simple equipment degrades lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low

Aerobic and anaerobic (including anoxic) digestion are the two major biological treatment methods for waste (water). Under aerobic conditions, organic components are oxidized to carbon dioxide and under anaerobic to carbon dioxide and methane. When comparing the two treatment methods with respect to sustainability, anaerobic digestion by far is the favorite because hardly if any energy input is needed, the methane produced can be used as a substitute for fossil fuels, and the production of excess sludge is much lower. Moreover, the technology of anaerobic treatment is much less complex, and anaerobic systems are applicable at any site and any size (Sanders, 2001).

Most of the substrate in complex waste (water) is present as particulate matter. Although less common, some of the complex waste (water) contains a significant amount of dissolved substrate requiring hydrolysis (Levine *et al.*, 1991).

Most natural carbohydrates are macromolecules like polysaccharides. These polysaccharides are predominantly simple and derived sugars linked together by glycosidic bonds. Most polysaccharides are insoluble in water and they can form colloidal suspensions. Polysaccharides found in complex organic waste (water) are cellulose, hemi-cellulose, pectin, and starch. Cellulose is the most abundant polysaccharide in complex organic waste. Cellulose is a linear polymer that consists of D-glucose units linked together through β-1,4 bonds. A considerable fraction of the cellulose in organic household waste is incorporated in a lignocellulosic complex with lignin. Starch consists of two types of polysaccharides, viz. the linear amylose (~20%) and the branched amylopectin (~80%). In amylose, the glucose units are linked together through α-1,4 bonds. In amylopectin, the glucose units are linked together through α-1,4 bonds, but also through α-1,6 bonds. Amylose is soluble in water, where as amylopectin is not. In some research, soluble starch is used, in which case the substrate only consists of amylose. Proteins can be divided into two general groups i.e. globular and fibroid proteins. Fibroid proteins have a fibrous structure and are the most important building material for animal tissue. Collagen and elastin (in connective tissue, ligamentsand tendons), keratin (in skin, hair, feathers, horns and hoofs), and myosin (inmuscles) are fibroid proteins. Due to their structure and biological function, fibroid proteins are water-insoluble and rather stable at changing pH and temperature. Globular proteins are water-soluble or form colloidal

suspensions. These proteins have a more regulatory function (enzymes, hormones, antibody's) and are rather sensitive to changes in pH and temperature. The bulk of the fats in complex waste (water) are triglyceride esters also called triacylglycerols or neutral lipid. About 90% of these triglycerides are composed of glycerol and myristic (C14: 0), palmitic (C16: 0), stearic (C18: 0), oleic (C18: 1), and linoleic (C18: 2) acids. Lipids are water insoluble and, due to their hydrophobic nature they will easily attach to particles in the waste (water) (Sanders, 2001).

The main intermediates and end-products of the anaerobic digestion process are volatile fatty acids, hydrogen, and biogas, respectively. Because the methanogenic bacteria are very sensitive to a drop in pH that could be caused by accumulation of volatile fatty acids, the digestion of complex waste obviously is a delicate balance between the rate of hydrolysis, acidogenesis, and methanogenesis (Sanders, 2001).

Veeken *et al.* (2000) studied the anaerobic hydrolysis rate of organic solid waste at fixed volatile fatty acid (VFA) concentrations ranging from 3 to 30 g COD/L and fixed pH values between 5 and 7. For separate control of both VFA and pH, a special completely mixed reactor was designed. Results showed that hydrolysis of the organic solid waste followed first-order kinetics. Using a statistical analysis found that the hydrolysis rate constant was pH dependent but was not related to the total VFA and undissociated VFA concentrations.

After that, Denise *et al.* (2012) studied the effect of pH control and hydraulic flush on hydrolysis and VFA production and profile in anaerobic leach bed reactors digesting a high solids content substrate. The results showed that buffering at pH~6.5 improved hydrolysis (volatile solid (VS) degradation) and VFA production by ~50%. Butyric and acetic acid were dominant, when reactors were buffered, while only butyric acid was produced at low pH. Hydraulic flush enhanced VS degradation and VFA production by ~15% and ~32%, respectively. Most Probable Number (MPN) of cellulolytic microorganisms indicated a wash out when hydraulic flush was applied, but pH control helped to counteract this. The highest VS degradation (~89%), VFA yield (0.84 kg COD/kg VS_{added}) and theoretical methane potential (0.37 m^3 CH_4/kg VS_{added}) were obtained when pH control and hydraulic flush were applied.

Jenicek (2011) described microaerobic condition that it was condition between aerobic and anaerobic systems such as aerobic system with low oxygen concentration and anaerobic system with limited O_2 supply. Besides, he indicated the potential benefits and drawbacks of microaerobic conditions were as follows:

Potential benefits of microaerobic conditions;

- Augmentation of microbial species diversity
- Improvement of biogas composition (hydrogen sulfide removal)
- Detoxification of the digester (sulphide removal)
- Improvement of organic compounds biodegradability

Potential drawbacks of microaerobic conditions;

- Dilution of biogas by nitrogen if air is used
- Lower methane production (not in all cases)
- Lack of full scale experience
- Oxygenophobia of digester operators

Oxygen is considered as a potential toxic compound during anaerobic digestion, especially, for the end-of-food-chain microorgamisms, the acetogens and principally the methanogens, which are usually regarded as strict anaerobes. However, some previous studies showed that microaeration can be used in anaerobic digestion.

Jenicek *et al.* (2008) studied effects of microaerobic conditions for anaerobic digestion of solid wastes containing slowly biodegradable compounds or high level of sulphur compounds. Results showed that in the operation of microaerobic desulphurization in the anaerobic mesophilic digester, the efficiency of hydrogen sulfide removal from biogas was very good and stable – average of 99.0% is a realistic value at high initial concentration (4,000-8,000 mg/m^3). The presence of the limited amount of oxygen in the digester does not destroy the digestion process even in the systems where the oxygen is not consumed by prompt sulphide oxidation. The VSS/TSS ratio of the digested sludge decreased due to the better efficiency in VSS degradation, including the decrease of the soluble COD concentration, ammonia, nitrogen, and phosphate concentration in the sludge liquor. Besides, the decrease of the relative methane content in biogas was caused by the presence of

nitrogen remaining in the biogas from the dosed air. Therefore, microaerobic conditions (that means controlled dosing of small amount of air or oxygen into digester) was an efficient tool to increase the biodegradability of treated material and/or to increase the activity of methanogenic bacteria. After that, Jenicek *et al.* (2010) studied results of microaerobic experiments for anaerobic digestion at both high and low sulphide concentrations and showed that anaerobic bacteria including methanogens can be active also in this system. In a mixed culture, even strict anaerobes can survive without inhibition, if the facultative microorganisms are able to consume the present oxygen quickly and fully. Besides, the microaerobic conditions were predominantly used for hydrogen sulphide removal from biogas.

Kato *et al.* (1993) studied the effect of oxygen exposure on the methanogenic activity of anaerobic granular sludges and showed that the amount of oxygen that caused 50% inhibition of the methanogenic activity after 3 days of exposure ranged from 7% to 41% oxygen. These results indicated that methanogens located in granular sludge had a high tolerance for oxygen. The most important factor contributing to the tolerance was the oxygen consumption by facultative bacteria metabolizing biodegradable substrates. Uptake of oxygen by these bacteria creates anaerobic microenvironments, where the methanogenic bacteria are protected.

Zitomer and Shrout (1998) studied methanogenesis under oxygen-limited conditions and showed that the methanogenic activity can sometimes be even higher under microaerobic conditions in comparison with a purely anaerobic system.

Diaz *et al.* (2010) studied the removal performance of hydrogen sulphide in severely polluted biogas produced during the anaerobic digestion of sludge by employing pure oxygen and showed that the O_2 supply (0.25 Nm^3/m^3feed) to the bioreactor successfully reduced the hydrogen sulphide content from 15,811 mg/Nm^3 to less than 400 mg/Nm^3. Diaz *et al.* (2011) studied effect of the limited O_2 supply on the degradation kinetics of cellulose in batch-tests. Results showed that the performance of the digestion under anaerobic conditions showed a final methane production after 19 d of 316±11 mL CH_4/g VS_{fed}, while for the microaerobic conditions showed 327±6 mL CH_4/g VS_{fed}. Therefore, microaerobic conditions had shorter lag-phase time than the anaerobic conditions resulting in faster production of methane during the first steps of the degradation; specifically, the maximum methane

production found in the anaerobic test in 19 d was found in the microaerobic test before the day 15. Then, the impact of microaerobic conditions on the anaerobic degradation can be expected on the first steps of solubilisation of complex organic matter. Besides, oxygen did not inhibit methanogenesis, or compete for the consumption of volatile fatty acids, as the methane yield was not reduced in the microaerobic assays.

Lim and Wang (2013) studied effect of microaeration pretreatment on the anaerobic co-digestion of brown water and food waste in batch-tests. After 4-day pretreatment with 37.5 mL O_2/L_R-d added. The added oxygen was consumed fully by facultative microorganisms. Higher COD solubilization, microaeration pretreatment led to greater VFA accumulation and the conversion of other short chain fatty acids to acetate. This could be due to enhanced activities of hydrolytic and acidogenic bacteria and the degradation of slowly biodegradable compounds under microaerobic conditions. This study also found that the nature of inoculum influenced the effects of microaeration as a 21% and 10% increase in methane yield was observed when pretreatment was applied to inoculated substrates, and substrates without inoculum, respectively.

Lots of industrial wastewater have both high organic pollution and sulfate (SO_4^{-2}) concentrations. Although biological conversion of organics to methane may be an economical chemical oxygen demand (COD) removal option, significant inhibition of methane production results from reduction of sulfate to hydrogen sulfide (H_2S), which is inhibitory to methanogenic microorganisms. Therefore, sulfate-containing wastewater is often not amenable to conventional anaerobic treatment. Recently, limited aeration of recycle flow to hybrid and baffled reactors has been used to treat this wastewater and has been shown to reduce aqueous hydrogen sulfide concentrations by causing production of uninhibitory sulfur (S°) and thiosulfate ($S_2O_3^{-2}$) as well as gas stripping volatile hydrogen sulfide (Parkin *et al.*, 1991).

Therefore, Zitomer and Shrout (1999) studied effects of high COD and high-sulfate wastewater treatment by using aerated methanogenic fluidized bed reactors (FBRs) compared to strictly anaerobic FBRs. O_2 supply up to 28% of the COD load resulted in maximum specific oxygen utilization rates of 0.20 mg O_2/g VSmin, with

significant methane production. Results showed that under typically inhibitory sulfate loading, higher aeration caused increased effluent sulfate, increased hydrogen sulfide mass in the offgas, and lowered reactor hydrogen sulfide concentration including COD removal increased from 25% for a strictly anaerobic FBR to 87% for aerated FBR. In addition, aerated systems required significantly less alkalinity supplementation to maintain a pH value of 7, ostensibly because of stripping of acidic carbon dioxide. The potential pH increase associated with aeration also shifted sulfide speciation to less toxic bisulfide. The authors described the limited aeration of methanogenic FBRs as a method for increased COD removal when treating high COD and high-sulfate wastewater.

Johansen and Bakke (2006) studied effects of microaeration on hydrolysis of primary sludge in 500 ml batch reactors operated at 37 °C with microaerobic inoculum and another with combination of microaerobic and anaerobic inoculum. Results showed that hydrolysis increased 50-60% during the 4-day experiment observed with a ratio of aerobic to anaerobic metabolism was 0.57. The ratio was higher than 0.5 indicated too high efficiency for methane production since a significant portion of the oxygen supplied was used for oxidation of methane potential. The ratio of extra hydrolysis to oxygen utilization was 0.388 g C/mg O_2 that the extra hydrolysed products were oxidized to carbon dioxide and incorporated into new biomass. The oxygen utilization to carbon dioxide production ratio was ~1:1 on a mol basis. The total hydrolysis increment happened by increased hydrolysis of carbohydrates and proteins. Lipids were only hydrolysed when anaerobic inoculum was added, but no effect of oxygen availability was detected. Hydrolysis was carried out by extracellular enzymes such as amylases, protease, α-glucosidase, and phosphatases. Increased hydrolysis under microaerobic conditions can be due to enhanced synthesis of these enzymes and/or due to synthesis or activation of a greater variety of extracellular hydrolytic enzymes. The authors suggested that supplying oxygen as a pretreatment to or directly into biogas process, reduced the methane potential as long as the sum of extra biomass accumulation and oxygen utilization was higher than the increased hydrolysis. Thus, we should optimize the O_2 supply in order to enhance the hydrolysis without oxidizing a large portion of the produced VFA.

Zhu *et al.* (2009) studied effects of microaeration and liquid recirculation on the hydrolysis of vegetable and flower wastes during two-phase solid–liquid anaerobic digestion. Five batches of waste treated under the following conditions: anaerobic, insufficient microaeration (aeration for 5 min every 24 hr), and sufficient microaeration (aeration for 5 min every 12, 4, and 1 hr). Results showed that hydrolysis was found to depend on the level of microaeration. Specifically, insufficient microaeration led to unstable and decreased performance. Conversely, sufficient microaeration promoted the hydrolysis of easily biodegradable carbohydrates and proteins. The hydrolysis efficiency under anaerobic conditions was comparable to the efficiency observed under sufficient microaeration, while the cumulative TOC (total organic carbon of hydrolytic effluent) of the anaerobic batch was 1.4–2.4 times higher than that of the micro-aerated batches. In addition, liquid recirculation did not have a negative effect on the development of microbial activity under anaerobic conditions, which resulted in the lignocelluloses having higher hydrolysis efficiency.

Botheju *et al.* (2009) studied effect of oxygen in anaerobic digestion by using model developed for the generally accepted anaerobic digestion; ADM 1 structure, which was developed by the Mathematical Modelling Task Group of the International Water Association (IWA). Results showed that under the oxygen load conditions of 22, 44, and 88 mg/L, the ADM1-Ox model simulations predicted the experimental methane potentials quite adequately. Both the experimental data and the simulations suggested a linear reduction of methane potential with respect to the increase in oxygen load within this range.

Botheju *et al.* (2010a) studied impacts of limited aeration in an anaerobic bio-gasification process by anaerobic bioreactors operated at 35 °C, both under semicontinuous and batch feed modes. Result showed that two series of batch experiments clearly indicated an increasing methane yield in the range of oxygenation loads of 0-16% (% O_2 of COD input). In the semicontinuous feed mode, four completely mixed bioreactors operated under oxygenation levels of 0, 1.3, 2.6, and 4% produced biogas at approximately equal level and constant rates. The methane generation rates at the low oxygenation levels of 1.3 and 2.6% were higher than the strict anaerobic condition, while the prolonged higher oxygenation level of

4% induced a slight negative impact on methane production at the later stage of the experiment. Accumulation of volatile fatty acids at the start up of the continuous feed reactors was lower for the aerated than the strict anaerobic reactor. The positive effect of oxygen on methane production had a much larger range in the batch feed mode compared to the semicontinuous feed mode. Besides, Botheju *et al.* (2010b) studied additionally in order to understand the impacts of partial aeration in anaerobic bio-gasification by laboratory scale bioreactor (5.5 L working volume) operated for more than 120 days duration at 35 °C under the organic loading rate of 0.33 kg COD/m^3 d, hydraulic retention time of 33 days with the oxygenation loads of 0, 2.5, 5.0, and 10.1% (% O_2 of COD input). Results showed that oxygenation under these operating conditions reduced the methane generation together with total biogas generation. A slight increase in carbon dioxide generation was noted. The accumulation of volatile fatty acids was extensively reduced by oxygen introduction. Therefore, the authors suggested that methane production can be optimized by some limited aeration in the first of two or more stages of anaerobic digestion. Partially aerated anaerobic digestion can be a useful and a stable process for enhanced waste treatment and resource recovery.