CHAPTER II
LITERATURE REVIEW

2.1 Biogas

Biogas is the name of the mixture of carbon dioxide (CO_2) and inflammable gas methane (CH_4) which is produced by bacterial conversion of organic matter under anaerobic (oxygen free) conditions. It originates from methanogens (methane producing bacteria) in the process of bio-degradation of organic material under anaerobic conditions. Biogas is fully capable of replacing other rural energy sources like wood, hard coal, kerosene, plant residues, and propane (Olugasa *et al.*, 2014).

2.1.1 Advantages of Biogas

2.1.1.1 Biogas is Renewable Source Energy

Biogas is considered to be a renewable source of energy. Since it often produced from materials that form sewage and waste products, the only time it will be depleted is when we stop producing any waste.

2.1.1.2 Biogas is Non-polluting

It is also considered to be non-polluting in nature. The production of biogas does not require oxygen, which means that resources are conserved by not using any further fuel.

2.1.1.3 Biogas is Reduction of Landfills

It also uses up waste material found in landfills, dump sites and even farms across the country, allowing for decreased soil and water pollution.

2.1.1.4 Biogas is Cheaper Technology

Applications for biogas are increasing as the technology to utilize it gets better. It can be used to produce electricity and for the purpose of heating as well. Compressed Natural Gas (CNG) is biogas that has been compressed and can be used as a fuel for vehicles. Production can be carried out through many small plants or one large plant.

2.1.1.5 Biogas is Large Number of Jobs

Either way, work opportunities are created for thousands of people in these plants. These jobs are a blessing in rural areas, which are the targeted grounds for the use of biogas. In fact, biogas can easily be decentralized, making it easier to access by those living in remote areas or facing frequent power outages.

2.1.1.6 Biogas is Little Capital Investment

Biogas is easy to set up and require little capital investment on a small scale basis. In fact, many farms can become self-sufficient by utilizing biogas plants and the waste material produced by their livestock each day. A single cow can provide enough waste material within a day to power a light bulb the entire day.

2.1.1.7 Biogas is Reduces Greenhouse Effect

It also reduces the greenhouse effect by utilizing the gases being produced in landfills as forms of energy. This is a major reason why the use of biogas has started catching on. It recycles most forms of biodegradable waste and works on simple forms of technology (www.biogas-renewable-energy.com).

2.2 Cassava Wastewater

Cassava, also called yuca or manioc, is a perennial woody shrub of Euphorbiaceae (Spurge family), which is a native of South America and grows in tropical and subtropical areas. As the third largest source of carbohydrates for human food in the world, the cassava has become a good starchy material in food and fermentation industry (Fubao *et al.*, 2010).

Cassava residues, a waste byproduct with a large amount of lignocellulosic components are generated during the cassava-based bioethanol production. Direct discharge of these residues to the environment will cause serious environmental pollution problem and huge waste of the lignocellulosic resources. Anaerobic digestion of cassava residues is another choice. However, due to their complex lignocellulosic structure, direct digestion of these residues will result in low hydrolysis efficiency and consequent reduction of the methane yield. Lignocellulosic material consists of mainly three different types of polymers.

2.2.1 Type of Lignocellulosic

2.2.1.1 Cellulose

Cellulose exists of D-glucose subunits, linked by b-1,4 glycosidic bonds (Fengel and Wegener, 1984). The cellulose in a plant consists of parts with a crystalline (organized) structure, and parts with a, not well-organized, amorphous structure. The cellulose strains are 'bundled' together and form so called cellulose fibrils or cellulose bundles. These cellulose fibrils are mostly independent and weakly bound through hydrogen bonding (Perez *et al.*, 2005).

2.2.1.2 Hemicellulose

Hemicellulose is a complex carbohydrate structure that consists of different polymers like pentoses (like xylose and arabinose), hexoses (like mannose, glucose and galactose), and sugar acids. The dominant component of hemicellulose from hardwood and agricultural plants, like grasses and straw, is xylan, while this is glucomannan for softwood (Fengel and Wegener, 1984; Saha *et al.*, 2003).Hemicellulose has a lower molecular weight than cellulose, and branches with short lateral chains that consist of different sugars, which are easy hydrolysable polymers (Fengel and Wegener, 1984). Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives the whole cellulose, hemicellulose and lignin network more rigidity (Laureano-Perez *et al.*, 2005). The solubility of the different hemicellulose compounds is in descending order: mannose, xylose, glucose, arabinose, and galactose. The solubility is increase with increasing temperature. The solubility's of higher molecular polymers could not be predicted, because of unknown melting points (Gray *et al.*, 2003). The solubilization of hemicellulose compounds into the water starts around 180 °C under neutral conditions (Bobleter *et al.*, 1994; Garrot *et al.*, 1999) however mention that already from 150 °C parts of the hemicellulose solubilize. The solubilization of lignocellulose components not only depends on temperature, but also on other aspects like moisture content and pH (Fengel and Wegener, 1984). The xylan of hemicellulose can be extracted quite well in an acid or alkaline environment, while glucomannan can hardly be extracted in an acid environment and needs a stronger alkaline environment than xylan to be extracted (Balaban and Ucar, 1999; Fengel and Wegener, 1984; Lawther *et al.*, 1996). Xylan appears to be the part that can be extracted the most easily. Of cellulose, hemicellulose and lignin the hemicelluloses are themost thermal - chemically sensitive (Levan *et al.*, 1990; Winandy *et al*, 1995). During thermal–chemical pretreatment firstly the side groups of hemicellulose react, followed by the hemicellulose backbone (Sweet and Winandy, 1999).

2.2.1.3 Lignin

Lignin is, after cellulose and hemicellulose, one of the most abundant polymers in nature and is present in the cellular wall. It is an amorphous heteropolymer consisting of three different phenyl propane units (p-coumaryl, coniferyl and sinapyl alcohol) that are held together by different kind of linkages. The main purpose of lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress. The amorphous heteropolymer is also non-water soluble and optically inactive; all this makes the degradation of lignin very tough (Fengel and Wegener, 1984). Lignin, like hemicellulose, normally starts to dissolve into water around 180 °C under neutral conditions (Bobleter, 1994). The solubility of the lignin in acid, neutral or alkaline environments depends however on the precursor (p-coumaryl, coniferyl, sinapyl alcohol or combinations of them) of the lignin (Grabber *et al*, 2005). Therefore, in order to effectively utilize these residues and obtain maximum methane yield, a promising pretreatment method must be applied to treat these materials prior to anaerobic digestion (Zhang *et al.,* 2011).

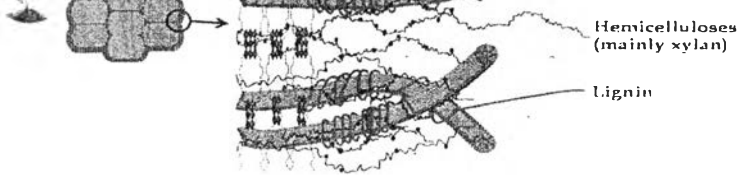

Figure 2.1 Structure of lignocellulosic plant biomass (Tomme *et al.*, 1995).

2.2.2 Pretreatment Lignocellulosic

Pretreatment methods are either physical or chemical. Some methods incorporate both effects (Mcmillan 1994; Hsu, 1996). For the purposes of classification, steam and water are excluded from being considered chemical agents for pretreatment since extraneous chemicals are not added to the biomass. Physical pretreatment methods include commination (mechanical reduction in biomass particulate size), steam explosion, and hydrothermolysis. Commination, including dry, wet, and vibratory ball milling (Millett *et al.*, 1979; Rivers and Emert, 1987; Sidiras and Koukios, 1989), and compression milling (Tassinari *et al.*, 1980, 1982) is sometimes needed to make material handling easier through subsequent processing steps. Acids or bases that promote hydrolysis and improve the yield of glucose recovery from cellulose by removing hemicellulose or lignin during pretreatment. The most commonly used acid and base are H_2SO_4 and NaOH, respectively.

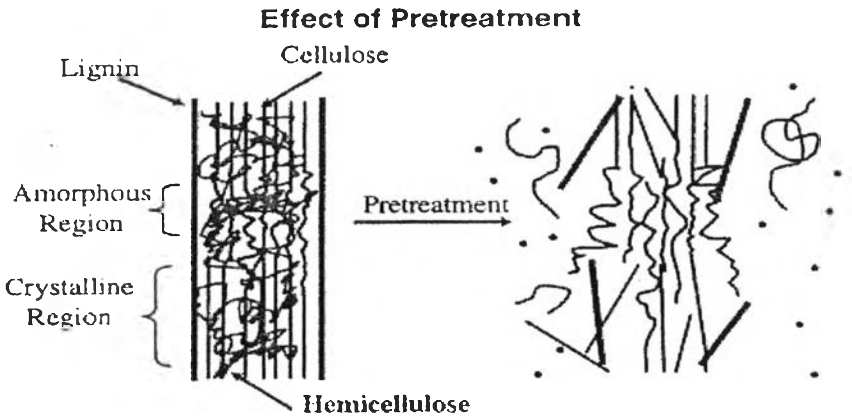

Figure 2.2 Schematic of pretreatment on lignocellulosic material (Hsu *et al.*, 1980).

Table 2.1 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

Table 2.1 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 Digestion

Anaerobic digestion (AD) is a biological process in which organic matter is decomposed by an assortment of microbes under oxygen free conditions and produces biogas. That technology for methane production is a more efficient method for energy generation from biomass compared to other biological and thermo-chemical conversion processes. Another benefit of anaerobic digestion is the reduction of natural methane emissions from the self-decomposition of biomass in landfills or other open environments. Because the global warming potential of methane is estimated to be 20 times higher than carbon dioxide, while the AD system capture and utilizes the methane for energy production.The AD process can be divided into four steps (Zheng *et al.*, 2014)

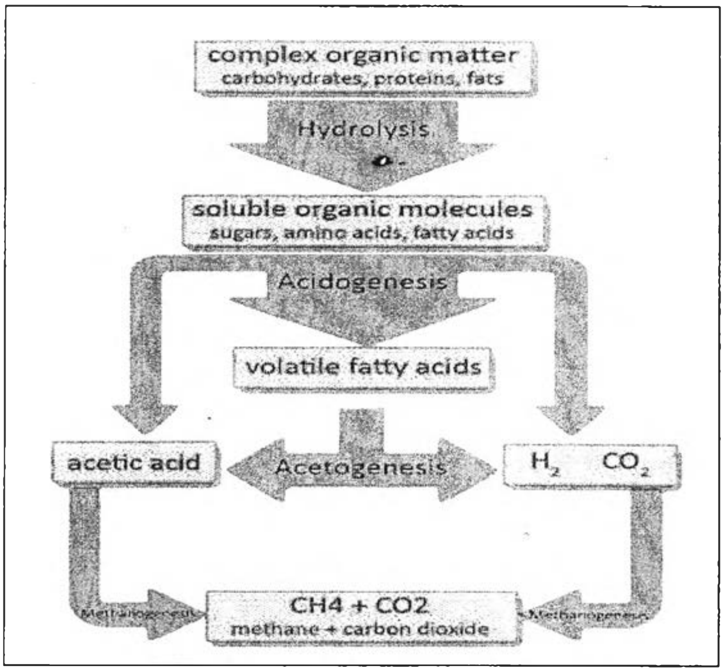

Figure 2.3 Steps involved in anaerobic digestion (Zheng *et al.*, 2014).

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.

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 acids 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).

2.4 Micronutrients

Micronutrients are essential elements needed by life in small quantities. They include micro minerals and Vitamins. Micro minerals or trace elements include at least iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc, and molybdenum. They are dietary minerals needed by microorganism in very small quantities as opposed to macro minerals which are required in larger quantities. (www.ncbi.nlm.nih.gov.com).

2.5 Chelants

Chelation is the formation or presence of two or more separate coordinate bonds between a polydentate (multiple bonded) ligand and a single central atom. Usually these ligands are organic compounds and are called chelants, chelators, chelating agents, or sequestering agents, the resulting complexes called chelate compounds. Those are divided biodegradable and non-biodegradable chelate.

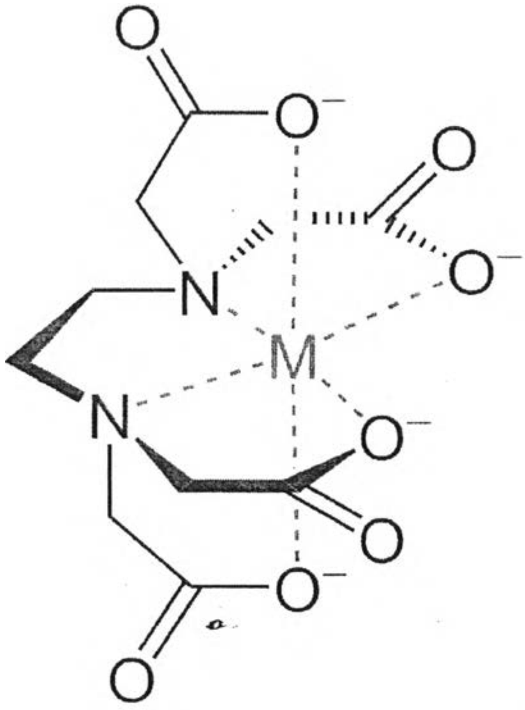

Figure 2.4 Chemical structures of EDTA chelate (www. illumin.usc.edu.com).

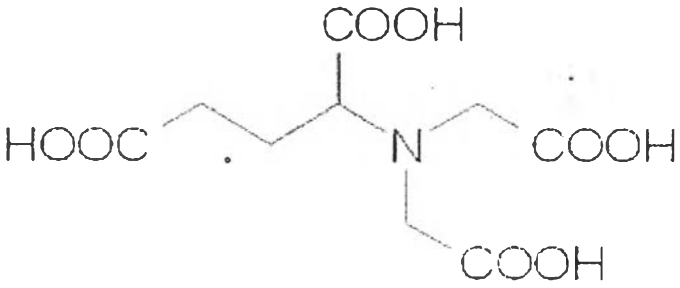

Figure 2.5 Chemical structures of GLDA chelate (www.akzonobel.com).

Chelation therapy is the use of chelating agents to detoxify poisonous metal agents, such as mercury, arsenic, and lead, by converting them to a chemically inert form that can be excreted without further interaction with the body. Although they can be beneficial in cases of heavy metal poisoning, chelating agents also can be dangerous (www.boundless.com). Chelants are used in domestic products such as laundry detergents, household cleaners, personal care products, and industrial applications such as pulp bleaching, photographic development, water treatment, metal cleaning and agriculture. EDTA are non-biodegradable chelants that are used extensively in domestic and industrial applications. There are environmental concerns that once in river systems chelants can extract heavy toxic metals from mud and sediment and cause remobilization of the metals in the environment that may have adverse long term effect. A major advantage of GLDA is excellent properties with regard to environmental acceptability. It is completely safe for the environmental. It is not expected to be harmful to aquatic organisms. Produced from naturally occurring raw materials, GLDA is widely regarded as a good basis for microorganisms to feed upon (www.akzonobel.com)

2.6 Literature Review on Improvement of Gas Production by Added Micronutrient

Moriizumi *et al.* (2012) studied five types of plants that produce ethanol from cassava in Thailand were examined to identify the best option with respect to greenhouse gas (GHG) emissions reduction. We specified the five plant types for evaluating the impacts of biogas utilization and the co-location of ethanol production at existing cassava starch factories and assessed the life-cycle GHG emissions of these plant types. The results showed that the amount of GHG emission reduction in cassava ethanol production depended on the plant type. Substantial GHG savings are achieved by ethanol plants that use biogas produced during wastewater treatment as fuel. Using biogas for steam generation is more effective in reducing GHG emissions than using it for electricity generation. Plants co-located with starch factories have advantages in relation to GHG emissions when compared with stand-alone plants. However, the impact of co-location is limited because cassava starch factories are not energetically self-sufficient. Improvement analysis suggested that boiler efficiency and the energy source for steam generation are key factors for reducing GHG emissions.

Zheng *et al.* (2014) studied Pretreatment of lignocellulosic biomass for enhanced biogas production. Lignocellulosic biomass is an abundant organic material that can be used for sustainable production of bioenergy and biofuels such as biogas (about 50-75 % CH_4 and 25-50 % CO_2). Out of all bioconversion technologies for biofuel and bioenergy production, anaerobic digestion (AD) is a most cost-effective bioconversion technology that has been implemented worldwide for commercial production of electricity, heat, and compressed natural gas (CNG) from organic materials. However, the utilization of lignocellulosic biomass for biogas production via anaerobic digestion has not been widely adopted because the complicated structure of the plant cell wall makes it resistant to microbial attack. Pretreatment of recalcitrant lignocellulosic biomass is essential to achieve high biogas yield in the AD process. A number of different pretreatment techniques involving physical, chemical, and biological approaches have been investigated over the past few decades, but there is no report that systematically compares the performance of these pretreatment methods for application on lignocellulosic biomass for biogas production. The result show anaerobic digestion is an effective biological process for treating a broad range of biomass feedstock for low cost production of biogas. However, AD efficiency is highly dependent on the type of feedstock. For example, digestion of livestock manure is more efficient than digestion of lignocellulosic biomass because of the complexity of lignocellulose. Consequently, pretreatment (including physical, chemical, or biological) is commonly employed to decrease

the recalcitrance of lignocellulosic feedstock to anaerobic digestion for increased biogas yield. Pretreatment can decrease crystallinity of cellulose, increase accessible surface area, and reduce lignin content, depending on the functioning mode of the pretreatment methods.

Zhang *et al.* (2011) studied; the pretreatment of cassava residues by thermal-dilute sulfuric acid (TDSA) hydrolysis was investigated by means of a statistically designed set of experiments. A three factor central composite design (CCD) was employed to identify the optimum pretreatment condition of cassava residues for methane production. The individual and interactive effects of temperature, H_2SO_4 concentration and reaction time on increase of methane yield (IMY) were evaluated by applying response surface methodology (RSM). After optimization, the resulting optimum pretreatment condition was 157.84 °C, utilizing 2.99 % (w/w TS) H_2SO_4 for 20.15 min, where the maximum methane yield (248 mL/g VS) was 56.96 % higher than the control (158 mL/g VS), which was very close to the predict value 56.53 %. These results indicate the model obtained through RSM analysis is suit to predict the optimum pretreatment condition and there is great potential of using TDSA pretreatment of cassava residues to enhance methane yield.

Zhang *et al.* (2011) studied Enhancement of methane production from cassava residues by biological pretreatment using a constructed microbial consortium. A stable thermophilic microbial consortium with high cellulose-degradation ability was successfully constructed. That several species of microbes coexisted in this consortium was proved by DGGE (denaturing gradient gel electrophoresis) and sequence analysis. The cooperation and symbiosis of these microbes in this consortium enhanced their cellulose-degradation ability. The pretreatment of cassava residues mixing with distillery wastewater prior to anaerobic digestion was investigated by using this microbial consortium as inoculums in batch bioreactors at 55 °C. The experimental results showed that the maximum methane yield (259.46 mL/g-VS) of cassava residues was obtained through 12 h of pretreatment by this microbial consortium, which was 96.63 % higher than the control (131.95 mL/g-VS). In addition, it was also found that the maximum methane yield is obtained when the highest filter paper cellulase (FPase), carboxymethyl cellulase (CMCase) and xylanase activity and soluble COD are produced.

Lijo *et al.* (2014) studied this study aims to analysis the potential environmental benefits and impacts associated to the anaerobic mono-digestion of two different substrates (pig slurry and maize silage). The Life Cycle Assessment methodology was applied in two full-scale Italian biogas plants (Plant A is pig slurry and Plant B is maize silage) in order to calculate the environmental profile of both systems with the aim of identifying the most suitable alternative from an environmental point of view. The study also includes credits due to avoided processes such as electricity production from the grid and mineral fertilization as well as the conventional management of pig slurry regarding Plant A. The main outcomes show the importance of the feedstock composition on the environmental performance of these systems. While the assessment of Plant A ended up in environmental benefits in all impact categories as a consequence of credits related to replaced processes, its capacity for bioenergy production was limited. On the contrary, the use of maize silage as substrate provided a larger production capacity but it was also associated to negative environmental impacts. In this system, the cultivation of maize showed up as the largest responsible of the environmental impacts, specifically due to diesel fuel consumption in agricultural activities as well as on-site emissions linked to the application of fertilizers. A sensitivity analysis proved that the environmental profile of these bioenergy systems could be improved through surplus heat use as well as technological improvements such as the replacement of the traditional dehumidification unit by a chiller.

Yu *et al.* (2014) studied Biogas production and methanogenic archaeal community in mesophilic and thermophilic anaerobic co-digestion processes. Over 258 Mt of solid waste are generated annually in Europe, a large fraction of which is bio waste. Sewage sludge is another major waste fraction. In this study, bio waste and sewage sludge were co digested in an anaerobic digestion reactor (30 % and 70 % of total wet weight, respectively). The purpose was to investigate the biogas production and methanogenic archaeal community composition in the anaerobic digestion reactor under mesophilic (35-37 °C) and thermophilic (55-57 °C) processes and an increasing organic loading rate (OLR, 1-10 kg VS $m^{-3}d^{-1}$), and also to find a feasible compromise between waste treatment capacity and biogas production without causing process instability. In summary, more biogas was produced with all OLRs by the thermophilic process. Both processes showed a limited diversity of the methanogenic archaeal community which was dominated by Methanobacteriales and Methanosarcinales (e.g. Methanosarcina) in both mesophilic and thermophilic processes. Methanothermobacter was detected as an additional dominant genus in the thermophilic process. In addition to operating temperatures, the OLRs, the acetate concentration, and the presence of key substrates like propionate also affected the methanogenic archaeal community composition. A bacterial cell count 6.25 times higher than archaeal cell count was observed throughout the thermophilic process, while the cell count ratio varied between 0.2 and 8.5 in the mesophilic process. This suggests that the thermophilic process is more stable, but also that the relative abundance between bacteria and archaea can vary without seriously affecting biogas production.

Chen *et al.* (2008) studied Inhibition of anaerobic digestion process. That is an attractive waste treatment practice in which both pollution control and energy recovery can be achieved. Many agricultural and industrial wastes are ideal candidates for anaerobic digestion because they contain high levels of easily biodegradable materials. Problems such as low methane yield and process instability are often encountered in anaerobic digestion, preventing this technique from being widely applied. A wide variety of inhibitory substances are the primary cause of anaerobic digester upset or failure since they are present in substantial concentrations in wastes. Considerable research efforts have been made to identify the mechanism and the controlling factors of inhibition. This review provides a detailed summary of the research conducted on the inhibition of anaerobic processes. The inhibitors commonly present in anaerobic digesters include ammonia, sulfide. light metal ions, heavy metals, and organics. Due to the difference in anaerobic inocula, waste composition, and experimental methods and conditions, literature results on inhibition caused by specific toxicants vary widely. Co-digestion with other waste, adaptation of microorganisms to inhibitory substances, and incorporation of methods to remove or counteract toxicants before anaerobic digestion can significantly improve the waste treatment efficiency.

Wang *et al.* (2011) studied Bio hydrogen and methane production by co-digestion of cassava stillage and excess sludge under thermophilic condition. The hydrogen and methane production by co-digestion of cassava stillage (CS) and excess sludge (ES) was investigated in this study. The improved hydrogen and subsequent methane production were observed by co-digestion of CS with certain amount of ES in batch experiments. Compared with one phase anaerobic digestion, two phases anaerobic digestion offered an attractive alternative with more abundant biogas production and energy yield, e.g., the total energy yield in two phase obtained at VS_{CS}/VS_{ES} of 3:1 was 25 % higher than the value of one phase. Results from continuous experiments further demonstrated that VS_{CS}/VS_{ES} of 3:1 was optimal for hydrogen production with the highest hydrogen yield of 74 mL/g total VS added, the balanced nutrient condition with C/N ratio of 1.5 g carbohydrate COD/g protein–COD or 11.9 g C/g N might be the main reason for such enhancement. VS_{CS}/VS_{ES} of 3:1 was also optimal for continuous methane production considering the higher methane yield of 350 mL/g total VS added and the lower propionate concentration in the effluent.

Achu and Bjornsson (2012) studied High methane yields and stable operation during anaerobic digestion of nutrient-supplemented energy crop mixtures. The feasibility of digesting energy crops supplemented with macro and micronutrients instead of manure, without the commonly applied long hydraulic retention time (HRT), was investigated in long-term, single-stage continuous stirred tank processes. The crops used were mixtures of sugar beets, maize and whole crop triticale. The organic loading rate (OLR) measured as a total solid (TS) was 1.5-5.5 kg m^{-3} d^{-1} and the HRT from 30 to 40 days. The results showed high methane yields, comparable to those in batch digestion, and high stability. The digestion of beets only was most stable, and showed the highest average TS based methane yield

(383 ±26 m^3 kg^{-1}) at an OLR of 4.5 kgm_3 d_1 and a HRT of 40 days. No significant difference in methane yield was found for all the crop mixtures during stable operation. Nutrient addition therefore showed the same stimulatory and stabilising effects as manure with high methane yields achieved at relatively short HRTs.

Fubao *et al.* (2011) studied Water-recycled Cassava Bioethanol Production Integrated with Two-stage UASB Treatment. Considering limited success in target hitting discharge from alcohol industry, this research attention was directed toward a recycling use of distillery spent wash (DS) in cassava bioethanol production by using a two-stage up flow anaerobic sludge blanket bioremediation (TS-UASBB). With the TS-UASBB, SO_4^{2-}, COD, N and P in the effluent from the DS degraded significantly and their concentrations were kept at 0.2 $g{\cdot}L^{-1}$, 2.0 $g{\cdot}L^{-1}$, 1.0 $g{\cdot}L^{-1}$ and 15 $mg{\cdot}L^{-1}$, respectively, in 13 batch processes for water-recycled ethanol fermentation. With the effluent used directly as dilution water, no heat-resistant bacteria were found alive. The thirteen-batch ethanol production individually achieved 10 % after 48 h fermentation. The starch utilization ratio and total sugar consumption were 90 % and 99.5 %, respectively. The novel water-recycled bioethanol production process with ethanol fermentation and TS-UASBB has a considerable potential in other starchy and cellulosic ethanol production.

Christy *et al.* (2014) studied the details about decomposition, process of anaerobic decomposition, diverse constraints behind anaerobic technology and different aspects for enhancement of biogas production through enzymes and microorganisms. The result show aerobic decomposition is found to be faster but their economics is not comparable with anaerobic decomposition. Anaerobic process have many advantages over the aerobic process because, low consumption of energy, low sludge production, smaller space requirements, reduction in waste volume, production of biofertiliser and valuable soil conditioners. The European Parliament Committee on Industry, External Trade, Research and Energy calls on to increase efforts within the research on new technologies of biogas as a biofuel. The success of biogas production will come from the availability at low costs and the wide variety of usable forms of biogas for the production of heat, stream, and electricity and for the utilization as a vehicle fuel. The success of biogas production will come from the availability at low costs and the wide variety of usable forms of biogas for the production of heat, stream, and electricity and for the utilization as a vehicle fuel.

Dorota (2011) studied the use of the strongly basic polystyrene and polyacrylate anion exchangers to remove heavy metal ions Cu(II), Zn(II), Co(II) and Pb(II) in the presence of a new generation chelating agent from wastewaters was evaluated. The increase of the sorption capacity of heavy metal ions in the presence of N,N-bis (carboxymethyl) glutamic acid (GLDA) could be achieved after optimization of phase contact time (1–180 min), shaking speed (140–180 rpm), concentration (7.5×10−4–2.5×10−2 mol/L), resin dosage (0.1–0.7 g), pH (2–12), temperature (293–333 K) and the presence of interfering ions (Ca(II), Mg(II), Cl^-, SO_4^{2-}, and NO_3^-). The experimental data were analyzed using the sorption kinetic models. In The M(II)–GLDA=1:1 system, the heavy metal ion sorption reached up to 56.67 mg/g for Cu(II) and 38.39 mg/g for Zn(II) on Amber lite IRA 402 and up to 51.10 mg/g for Cu(II) and 43.09 mg/g for Zn(II) on Amber lite IRA 458.The equilibrium sorption studies of Cu(II) and Zn(II) complexes with GLDA have been described by the Langmuir, Freundlich, Temkin and Dubinin–Radushkevich isotherm models.

Itsadanont (2013) studied The equilibrium solubilities of two model soap scums (calcium stearate and magnesium stearate: $Ca(C_{18})_2$ and $Mg(C_{18})_2$) were measured in aqueous solutions containing three different types of surfactants: methyl ester sulfonate (MES) as an anionic; alcohol ethoxylate (EO9) as a nonionic; and dimethyldodecylamine oxide (DDAO) as an amphoteric with and without a chelating agent [disodium ethylenediaminetetraacetate (Na_2EDTA)]. The solubility of calcium soap scum was generally higher than that of magnesium soap scum, the exception being some DDAO systems. The use of the DDAO surfactant with the Na_2EDTA chelating agent at high pH gives the highest solubilities of both studied soap scums. The soap scum solubility is on the order of 2,000 times that in water at high pH. The DDAO is the most effective surfactant under all conditions. The MES is more effective than the EO9 at low pH with the opposite trend observed at high pH. The synergism from added chelant is generally greater at higher pH and is greatest for DDAO followed by EO9.

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 oxygen 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 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 % 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.

Fermoso *et al.* (2009) studied metal microbe interaction. Metals are important in all biochemical processes in any kind of living organism. Of the 95 naturally occurring elements of the periodic table, no less than 25 have an essential biologlcal function (Franzle and Markert, 2002). Some of these, e.g. zinc, nickel, cooper, selenium, cobalt, chromium, molybdenum, tungsten, manganese or iodine are required in small amounts and are termed essential trace elements. Most of them are part of active site of enzymes. Table 2.2 list the role of some essential trace elements in various enzymes catalyzing anaerobic reaction. The presence of high metal concentrations in bulk liquid does not mean that microorganisms take the metal up and incorporate it into the catalytic centers of their enzymes (Ermler, 2005). For instance, microorganisms take up metals also in the form of complexs, such as e.g. Vitamin B_{12} (Ferguson and Diesenhofer, 2004), Co-citratem (Krom, 2002) or bound to siderophores (Worms *et al.*, 2006). More in general, in between the presence of metals in the bulk liquid and the actual biological effects, complex process as biouptake and biosynthesis play an important role.

Table 2.2 Role of some essential trace elements in various enzymes involved in anaerobic reaction and transformation (Fermoso *et al.*, 2009)

Element	**Functions**
Cu	• Superoxide dismutase • Hydrogenase (Facultative anaerobes) • Nitrite reductase • Acetyl-CoA synthase
Co	• B_{12}-enzymes • CO-dehydrogenase • Methyltransferase
Fe	• Hydrogenase • CO-dehydrogenase • Methane monooxygenase • NO-reductase • Superoxid dismutase • Nitrite and Nitrate reductase • Nitrogenase
Mn	• Stabilize methyltransferase in methane producing bacteria
Mo	• Formate dehydrogenase • Nitrate reductase • Nitrogenase
Ni	• CO-dehydrogenase • Acetyl-CoA synthase • Methyl-CoM reductase (F_{430}) • Urease • Stabilize DNA, RNA • Hydrogenase

Se	• Hydrogenase • Formate dehydrogenase • Glycin reductase
W	• Formate dehydrogenase • Formylmethanofurandehydrogenase • Aldehydeoxydrogenase • Antagonist of Mo
Zn	• Hydrogenase • Formate dehydrogenase • Superoxide dismutase
V	• Nitrogenase • Chloroperoxydase • Bromineperoxydase

Schattauer *et al.* (2011) studied abundance of trace elements in demonstration biogas plants. The range of elements investigated and their measured concentrations are summarised in the Table 2.3.

Table 2.3 Recommended concentrations of trace elements in anaerobic digesters (Schattauer *et al., 2011*)

Element	Takashima and Speece (1990)	Sahm (1981)
B	-	0.001-11
Ca	>0.54-40	-
Co	>0.00059-0.12	0.06
Cr	-	0.005-52
Cu	-	0.06-64

Fe	>0.28-50.4	-
Mg	360-4800	-
Mn	-	0.005-55
Mo	>0.00096-0.048	0.05
Ni	0.0059-5	0.006
S	0.32-13	-
Se	0.079-0.79	0.008
W	0.018-18.3	-

Yu *et al.* (2015) studied effects of Dosing time of ferric chloride to disinhibit the excessive volatile fatty acids in sludge thermophilic anaerobic digestion system.

The result showed that the direct addition of $FeCl_3$ into the sludge thermophilic AD system was proposed to disinhibit the excessive VFAs. A dosing time gradient was adopted and 72nd hour dosing group was the best one for effective disinhibition of VFAs with the biogas production potential of 293.13 ± 11.38 mL/gVS. Acetic acid was the main inhibitor and $FeCl_3$ contributed to build a favorable substrate environment, except to remove excessive acetic acid. The advanced dosing was adverse to the methanogens growth, whereas the delayed dosing could not control the excessive VFAs; thus dosing at 72nd hour was suitable to the balance between hydrolysis–acidification and methanogenesis processes.