UNIVERSITY OF NAIROBI

Department of Civil and Construction Engineering

Anaerobic Co-Digestion of Water Hyacinth (*Eichhornia crassipes*) with Ruminal Slaughterhouse Waste under Mesophilic Conditions

By

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This research work is dedicated to my Daughter, Aurel and my Son Jovany, as a motivation to them in pursuing education even to a higher level than this.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

AD	Anaerobic Digestion
AOAC	Association of Official Analytical Chemists
BMP	Bio Methane Potential
BOD	Biochemical Oxygen Demand
C/N	Carbon/Nitrogen
C/P	Carbon/Phosphorus
CH_4	Methane
COD	Chemical Oxygen Demand
CWs	Cellulose Waste
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetra-acetic Acid
EPA	Environmental Protection Agency
F/M	Food to Microbe
FISH	Fluorescence *In Situ* Hybridization
FLP	Fragment Length Polymorphism
FS	Fixed Solids
GC	Gas Chromatography
GCFID	Gas Chromatogram with Flame Indication Detector
HCL	Hydrochloric Acid
K	Potassium
LCFA	Long Chain Fatty Acids
MGRT	Minimum Guaranteed Retention Time
MSW	Municipal Solid waste

N	Nitrogen
ND	No Detection
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
PCR	Polymerase Chain Reaction
pH	Potential Hydrogen
RNA	Riboneucleic Acid
RSW	Ruminal Slaughterhouse Waste
RT	Retention Time
SCOD	Soluble Chemical Oxygen Demand
SDS	Sodium Dodecyl Sulphate
SSCP	Single-stranded Conformation Polymorphism
T	Temperature
TCOD	Total Chemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TMA	Trimethylamine
TOC	Total Organic Carbon
TP	Total Phosphorus
TS	Total Solids
TSS	Total Suspended Solids
UV	Ultraviolet
V	Volume
VFAs	Volatile Fatty Acids
VOC	Volatile Organic Compounds
VS	Volatile Solids

VSS	Volatile Suspended Solids
WH	Water Hyacinth

ABSTRACT

Water hyacinth (*Eichhornia crassipes*), an invasive aquatic weed with large biomass poses serious socio-economic and environmental challenges in fresh water bodies such as Lake Victoria in East Africa. Efforts towards its control and removal can be complemented by biogas production for use as energy source. However, knowledge of chemical and nutritional composition of its largely complex lignocellulosic biomass is important in determining its conversion into biogas. The complex structure of lignocellulosic biomass can affect its biodegradability and limit biogas production. On the other hand, co-digestion with complimentary substrates can potentially make it more amenable to biodegradation and improve biogas production. Biomethanation involves a combination of physicochemical conditions in anaerobic reactors and the action of microbial community. Understanding the dynamics of the microbial community can provide insights on how co-digestion influences biogas generation.

This study evaluated synergy in co-digestion of WH from Lake Victoria with ruminal slaughterhouse waste (RSW) from Dagoretti slaughterhouses in Nairobi. The study characterized WH and RSW as co-substrates in biogas production by conducting proximate, crude fiber, elemental and biochemical analysis. It evaluated influence of processes parameters that included pH, temperatures, reaction times and substrate mix proportions on biogas production for WH and RSW substrates digested separately and in co-digestion. The study also investigated the dynamics of microbial communities in digestion of single substrates and in co-digestion at critical stages of biogas production by isolation, identification, DNA extraction, 16S rRNA gene amplification and sequencing using the Basic Local Alignment Search Tool (BLAST) technique.

Water Hyacinth had significant concentrations of cellulose, hemicellulose and carbohydrates of 331,200, 231,800 and 447,800 mg/L, respectively and lesser concentration of lignin of 99,400 g/L, which is desirable in biomass for biogas production. The concentration for C, N, P and K in WH were 15480, 1654, 51 and 137 mg/L, respectively compared to 26,220, 1,390, 34 and 7,475 mg/L for RSW. The concentration of potassium for WH was below the optimum range for biogas production of 200 - 400 mg/l while that for RSW was in the inhibitory range. Both biomass exhibited phosphorous deficiency at C/P of 310 and 656 for WH and RSW, respectively against optimum ratio of 100 and 150 for hydrolysis and acidogenesis stages, and methanogenesis stage, respectively. The C/N ratio for WH was 9.4 that tended towards ammonia toxicity at the lower limit of 8 to 20 C/N ratio for optimal biogas production while that for RSW was 18.8, which bordered on nitrogen deficiency. The results indicated potential for complementing of nutrients in co-digestion of WH with RSW substrates, for enhanced biogas production.

Profiles of biogas production exhibited distinct acclimatization, lag and active biomethanation phases. Water hyacinth and RSW digested separately experienced lag phase of 7 and 20 days, respectively in which pH dropped from 7.5 to 7.0 for WH and to acidic pH of 6.0 for RSW. The pH drop was attributed to rapid generation of volatile fatty acids by hydrolysis and acidogenesis process without corresponding consumption by acetogenesis and methanogenesis processes. Depressed pH inhibited methanogenic organisms resulting in lag in biogas yield. However, co-digestion of WH with RSW led to a consistent and improved biogas production that was attributed to collation of processes parameters restraining of pH drop alkaline to levels that were conducive for biomethanation. A WH: RSW co-digestion ratio of 70:30 exhibited the most consistent and largest biogas yield over a residence time of 60 days. Co-digestion of WH with

30% RSW proportion at 24°C improved WH biogas yield by 75% from 8.05 to 14.1 L/Kg biomass and the proportion of methane component by 9% from 59 to 68% suggesting synergisms in the co-digestion.

The morphologies of microbial colonies isolated from reactor sludge were dominated by short and long rods bacilli, but also cocci, and streptococcus mainly in WH samples. About 77% of the isolates were Gram positive that indicated dominance of the Firmicutes phyla that includes the *bacillus* genus, while 23% were Gram negative. Molecular analysis identified a shift in microbial community during the acidic lag phase from *Bacillus* genus to *Lysinibacillus* and *Solibacillus* genera. Recovery of alkaline conditions resulted in re-emergence of diverse species of *Bacillus* genus including *Bacillus aerophilus, Bacillus pumilus, Bacillus glycinifermentans, Bacillus cereus, Bacillus thuringiensis, Bacillus licheniformis* and *Bacillus aquimaris,* which were associated with active biomethanation. Consequently, consistent and improved biogas production in co-digestion of WH with RSW was attributed to collation of processes parameters that restrained pH to alkaline levels allowing growth of *Bacillus sp.* associated with active biomethanation.

CHAPTER 1: INTRODUCTION

1.1 Background of Study

Water hyacinth, an aggressive aquatic plant, has the potential for doubling its population every 5 to 15 days producing up to 140 million daughter plants annually (World Bank, 1996; Tao et al., 2016). The weed has invaded and proliferated in fresh water bodies such as Lake Victoria in East Africa, causing major socio-economic and environmental disruptions including blockage of fishing grounds, transport routes, and water intakes, and increase of waterborne diseases (Mailu, 2001; Gichuki et al., 2012). Management and removal of the weed has been expensive and only partially successful (Guerena et al., 2015). However, efforts for its control and removal can be complemented by its adoption as biomass in biogas production. Generally, biomass is potentially available for exploitation and use in energy production with less capital-intensive technologies than those for fossil fuels (Anjanabha and Pawan, 2010). Consequently, exploitation of water hyacinth (WH) has the potential to mitigate over reliance on fossil fuel for energy, and its associated environmental degradation including climate change (Budiyano et al., 2010b).

Knowledge of the characteristics of biomass is important in its conversion into fuels and other valuable chemicals, and would be useful in process commercialization. Lignocellulose biomass from plants is grossly composed of holocellulose and lignin bound together by ionic bonds strongly attracted into microfibrils (Luciana & Orlando, 2009). The complex structure of the lignocellulose limits the degradation process which demands large reactor volumes for long hydraulic retention times (Adney et al., 1991).

Energy crops are largely lignocellulosic in nature and are often rich in carbon, while poor in nutrients like phosphorus, nitrogen and trace elements (Saini et al., 2015). A carefully

chosen co-substrate such as slaughterhouse waste has the potential for compensating limitations of lignocellulosic biomass facilitating stable and efficient anaerobic digestion process that enhances biomethanation (Horváth et al., 2016).

Simultaneous processing of substrates through co-digestion may result in greater biogas production than with individual feedstock (e.g. Rao and Baral 2011; Dias et al., 2014: Li et al., 2011). Co-digestion holds numerous advantages for microbial digestion that include reduced concentration of toxic compounds, increased nutrients concentration, improved substrate loading, supply of buffer capacity and hygienic stabilization of enzymes (Tufaner and Avsar, 2016). The benefits are essential in ensuring consistency in the digestion process in addition to enhancing biogas yield (Morales-Polos et al., 2018).

Slaughterhouse waste has significant concentration of nutrients that can complement the digestion of other substrates such as water hyacinth (Wei wu, 2010). However, most of the slaughterhouse waste components with the exception of ruminal waste also have large concentration of proteins, which make them susceptible to ammonia toxicity (Callaghan et al., 2002; Edstrom et al., 2003; Cuetos et al., 2010; Chen et al., 2008). Similarly, volatile fatty acids (VFAs) have a tendency of accumulating in the reactors resulting in continual pH drop that suppresses the vibrancy of methanogenic community (Amani et al., 2011). Rumen contents have limited protein concentration and occur in the largest proportion in the waste; therefore, would be the desirable component for co-digestion with water hyacinth. Furthermore, rumen slaughterhouse waste (RSW) contains cellulolytic anaerobic bacteria that are suitable as inoculum for degradation of cellulose (Aurora, 1983; Castillo, 1995).

The anaerobic digestion system entails numerous biochemical steps that include hydrolysis, fermentation, acetogenesis and methanogenesis where groups of bacteria and archaea transform organic substrate into biogas (Meyer and Edwards, 2014). Improvements of bio digestion for increased and stable biogas production requires a deeper understanding of reactor operating parameters and the dynamics of microbial organisms involved (Walter et al., 2012). This study evaluated synergy in co-digestion of WH with RSW through characterization of substrate, evaluation of biogas production and establishment of dynamics of microbial communities.

1.2 Statement of Problem

Proliferation of water hyacinth in fresh water bodies causes major socio-economic and environmental disruptions including blockage of fishing grounds, transport routes, and water intakes and increase of waterborne diseases among other challenges (Mailu 2001; Gichuki et al. 2012). Management and removal of the weed from has been expensive and only partially successful (Guerena et al. 2015); for example, use of mechanical methods requires large energy inputs and lacks economic motivation (Petrell and Bagnall, 1991). Conversely, the use of the biomass in biogas generation can be a viable approach to control and management of the weed. However, such use demands process optimization. Therefore, there is need to characterize the substrates and determine the impact of operating parameters including temperature, pH and retention time on biogas yield. Additionally, use of methods such as co-digestion with other substrates to improve digestion of lignocellulosic biomass requires evaluation of mix proportion of co-substrates. Because microbial communities are key players in anaerobic digestions, there is also need to understand their dynamics in the co-digestion.

1.3 Objectives

The general objective of this study was to evaluate synergy in co-digestion of WH with RSW as feedstock in biogas generation. The specific objectives were to:

1. Characterize WH from Lake Victoria and ruminal slaughterhouse waste (RSW) from Dagoretti slaughter houses as substrates for biogas production in co-digestion.
2. Evaluate the impact of temperature, retention time, pH and mix proportions of substrates in co-digestion of WH with RSW on biogas production.
3. Establish the dynamics of microbial communities in co-digestion of WH with RSW.

1.4 Hypothesis

1. Water hyacinth and ruminal slaughterhouse waste do not have complementary properties for enhancement of biogas production.
2. Co-digestion for WH and RSW cannot alter substrates digestibility, stabilize pH, enhance biogas yield and improve biogas properties.
3. Anaerobic co-digestion of WH with RSW does not modify microbial community for enhanced biomethanation.

1.5 Scope of study

This study evaluated co-digestion of WH with RSW under varied conditions. The study characterized water hyacinth and ruminal slaughterhouse waste to determine proximate, fiber, elemental and biochemical characteristics of the two substrates. Water hyacinth and ruminal slaughterhouse waste were obtained from Lake Victoria and Nairobi's Dagoretti slaughterhouses, respectively. The study conducted anaerobic digestion of WH with RSW as separate substrates and in co-digestion at various mix

proportions in mesophilic temperatures range of 20-40ºC and obtained pH variation and biogas production profiles with reaction time.

The study applied DNA extraction and 16S rRNA gene amplification to identify dominant microorganisms at various stages of biogas production for the WH and RHW substrates separately and in co-digestion.

1.6 Definition of Terms

Acidogenesis: This refers to the second step of anaerobic digestion where large organic molecules gets converted to volatile fatty acids.

Anaerobic Digestion (AD): This is a combination of processes in which anaerobic microbes degrade organic material in oxygen free environment to generate biogas. The term also refers to the entire anaerobic treatment process.

Batch-feed: A process of feeding the reactor with biomass once, then processing and eventually emptying in one instance. It is the exact opposite of continuous feeding.

Biodegradable: Refers to substance that is easily broken down into primary molecules by the action bacteria, fungi, and other microorganisms.

Biogas: This is a gaseous substance produced by anaerobic digestion or fermentation of organic material. The gas is generally methane and carbon dioxide with some trace gases.

Degradation: Is the progressive decomposition of organic matter that occurs through a defined process with the end products having fewer carbon atoms than the originally fed feedstock. Such decomposition is carried out by microorganisms.

Digestate: A material either solid or liquid in nature that remains after a completed anaerobic digestion. The material is often still rich in nutrient concentration.

Digester: A closed chamber where anaerobic digestion of organic substance occur. The chamber may be a tank, cylinder or silo and the term is synonymous with reactor.

Feedstock: Refers to organic input material suitable for either aerobic or anaerobic processing. Such material may be of varied physical and chemical properties but which is easily degradable.

Hydraulic Retention Time (HRT): Refers to the duration taken by substrate, either liquid or solid, stays in a reactor. It can be arrived at by dividing the volume of the reactor by the flow.

Mesophilic: Temperature range between 20– 45°C in which microbial processes occur. It is considered the moderate temperature range of anaerobic digestion.

Methane: This is a gaseous component of biogas with the chemical formula CH_4. It is a group-14 hydride and the simplest alkane, and is the major component of natural gas.

Methanogenesis: This is the last step in anaerobic digestion where acetic acid and hydrogen are finally converted into biogas.

Molecular techniques: Methods involving manipulation of DNA, RNA, protein, and lipids

Morphological techniques: Analysis of the organisms with respect to the form, structure, and their specific features.

Organic Loading Rate (OLR): Is the volume of feedstock injected into the digester capacity at a given time. The unit is kg substrate (VS)/ m^3 reactor and day.

Phylogenetic analysis: Is a system of carrying out an estimation of evolutionary correlation. In molecular phylogenetic analysis, the sequence of a common gene or protein is applicable in determination of evolutionary relationship for various species.

Polymerase Chain Reaction (PCR): A molecular biology technique widely used to rapidly make millions to billions of copies of a specific DNA sample. This ultimately allows taking of a very small sample of DNA which can be amplified for detailed analysis.

Primers: Is a strand of DNA bases that facilitates replication of DNA. Primers are generally used in Polymerase Chain Reaction and each primer is single stranded DNA relating to a specific piece of template DNA.

Ruminal slaughterhouse waste: This is the proportion of slaughterhouse waste that originates from the first compartment of a ruminant animal. It is also the chamber where regurgitation of food for rumination takes place and it is also where cellulose is digested by symbiotic microorganisms. The material is also called paunch.

Scrubber solution: Is a diverse group of chemical solutions that can be used to remove some particulates and/or gases from industrial exhaust streams.

Solids Retention Time (SRT): Is the average duration for which solid material remains in a reactor.

Thermophilic: Temperature range between 50– 65°C in which microbial activity occurs. It is considered the upper temperature range for anaerobic digestion.

Total Solids (TS): This is the residue that remains when water or sludge sample is filtered and dried at 105 °C. It is measured in mg /L (mass per volume) or as a percentage of wet weight.

Volatile Solids (VS): Are substances that can easily convert from solid phase to vapor phase without going through a liquid phase.

Water hyacinth: Is a free-floating aquatic plant (or hydrophyte) originally from Amazon basin and is often highly invasive.

CHAPTER 2: LITERATURE REVIEW

2.1 Biogas as an Alternative Source of Energy

Energy demand and consumption is growing worldwide, while reliance on fossil fuels such as coal, oil and natural gas is becoming unsustainable. Exploitation of alternative energies such as biofuels, has the potential of minimizing the use of fossil fuels thereby averting environmental pollution and associated health risks (Kumar and Mohan, 2005). Furthermore, the rising prices for traditional fuels cause a major concern for most economies prompting the need to explore alternative energy sources including bioenergy (e.g. Berndes et al., 2003). Apart from the energy crisis, there is a growing concern about global climate change alongside related global warming emanating from such use of fossil fuels (Tu et al., 2011). Exploitation and application of bioenergy holds the potential of minimizing emissions of greenhouse gases (GHGs) and hence mitigating the associated global warming and climate change (Bilgen et al., 2008). The raw materials potentially available for biogas generation include but not limited to; municipal waste, green waste, agricultural waste, food waste, cattle manure and plant materials anaerobic digestion.

2.2 Biogas Production by Anaerobic Digestion

Anaerobic digestion (AD) entails a number of controlled biological processes for degradation of organic materials in an environment free of oxygen. The process involves the action of diverse bacteria that catalyze series of composite microbial activities (McInerney et al., 1980). The application of AD in processing industrial and domestic organic wastes and biogas generation is economically and environmentally attractive considering reduction of greenhouse gas emissions (Berndes et al., 2003; Barton et al., 2008). Apart from energy generation, AD process also lowers the quantity of disposable material thereby minimizing possible land and groundwater contamination. Accordingly, the technology offers solution

to numerous ecological and agrochemical challenges (Budiyano et al., 2010). The AD in turn presents a very lucrative platform to utilize certain quality of biomass in energy generation and meeting part of energy demands. The process has attracted an increasing interest despite its inefficiencies resulting from inadequate knowledge linking the process parameter and microbial community dynamics that is essential in enhancing reactor performance and biomethanation (Lee et al., 2009; Pycke et al., 2011).

As a biological process, AD is greatly influenced by operating parameters including; temperature, pH, nutrients concentration and balance such as carbon/phosphorus (C/P) and carbon/nitrogen (C/N) ratios (Demirel and Scherer, 2008; Akuzawa et al., 2011; Esposito et al., 2012; Kim et al., 2013; Agbo et al., 2013). Organic material can be digested to methane through anaerobic decomposition, a process that is highly dependent on reciprocated syntrophic reactions of specific microorganisms (Akuzawa et al., 2011). The generation and multiplication of the microorganisms is significant in determining the rate of degradation (Kangle et al., 2012). Other factors which influence AD biological process include presence of inhibitors, substrate type and its characteristics, substrate particles size, and availability of micro elements (Esposito et al., 2012). An appropriate operating environment and their continual monitoring during the process is important in optimizing anaerobic activities while co-digestion of several solid or liquid organic wastes can greatly augment essential nutrient balance and enhance biogas production (Tufaner and Avsar, 2016; Kiros et al., 2017).

2.3 Overview of Anaerobic Digestion

Anaerobic digestion is a combination of microbial activities where organic matters is degraded in oxygen-free environment, with biogas as a product. Such processes occur naturally e.g in soils, lakes, swamps and ocean beds. The process also occur in sanitary landfills marshy areas and digestive system of ruminants and termites, forming biogas.

Anaerobic digestion can also occur in waste treatment lagoons with recovery of biogas (Asikong et al., 2013). In energy production, anaerobic digestion is exploited in biodigesters to produce biogas. Four different biochemical steps have been identified in biogas generation; namely, hydrolysis, acidogenesis, acetogenesis and methanogenesis (Asikong et al., 2013).

2.4 Substrates for biogas production

Diverse organic matter including cattle manure, food wastes, wastewater form treatment plants and sewage sludge are suitable as substrates in a biogas process. For example, palm oil mill effluent (POME), a byproduct of palm oil mills has COD of 16,000 to 110,000 mg/L with a potential to generate up to 20 m^3 of biogas per ton fresh fruit bunches (Azmi et al., 2014).

Disposal of slaughterhouse waste generates serious environmental concerns everywhere around the world (Bandaw and Herago, 2017). Anaerobic digestion potentially an economic method for slaughterhouse waste disposal given the environmental concerns and added advantage of biogas production. The technology assist reduce greenhouse gas release from use of fossil fuels and it is useful in pollution suppression in abattoirs (Medina-Herrera et al., 2014). Slaughterhouse waste has high COD and is highly moist, characteristics which are desirable for anaerobic digestion feedstock. The biogas potential for slaughterhouse waste ranges from 120 to 160 m^3 biogas per ton of slaughterhouse biomass (Cu et al., 2015). However, relatively low C:N of slaughterhouse waste of 4:1 can lead to ammonia toxicity; such constraint can be solved through co-digestion with feedstock of high C:N like poultry waste, animal manure, domestic waste, and agricultural wastes (Afazelia et al., 2014). Amongst anaerobic treatment methods, up-flow anaerobic sludge blanket (UASB) is popularly

applied in biogas generation for abattoir wastes. This is particularly common in developing countries (Medina-Herrera et al., 2014).

Animal manure contains valuable nutrients that can be resourceful in renewable energy generation (Leenstra et al., 2014). In an attempt to protect the environment and utilize farm wastes, anaerobic digestion of animal manure has recently become attractive as a value added approach through biogas generation (Bandaw and Herago, 2017). The process is however impacted by the diversity of parameters like temperature and pH of the feedstock. Optimal performance of most biogas plants is reportedly at neutral pH and temperature of around 35ºC (Jayaraj et al., 2014). The C/N ratio of the feedstock offers optimal results where it is maintained between a minimum, 20:1 and maximum of 30:1 (FAO, 2004). Animal manure has a C/N of 25:1 making it a potential feedstock for anaerobic digestion and biomethanation.

Food residuals forms another untapped renewable energy feedstock that often find its way into landfills where it rots in uncontrolled environment without releasing its trapped energy advantage. The resultant effect translates to transmission of greenhouse gases into the atmosphere (FAO, 2004). Anaerobic digestion presents a suitable method of disposal of organic wastes, a method with techno-economic advantage which supports its viability and environmental sustainability (Alzate et al., 2019). Application of this technology can save landfill space and mitigate possible climate change. Food waste can economically be exploited for its considerable proportion of carbon that can efficiently translate to green energy and organic fertilizer form its residue. In biogas generation, such garbage qualifies as a feedstock for biogas generation and can either be used alone or co-digested with agricultural crop residues,

cattle manure, slaughterhouse wastes, poultry litter or sewage sludge (Medina-Herrera et al., 2014).

Several plant materials have been tested as biomass for biogas generation (Zonta et al., 2013). Such plant materials include sugarcane, cassava, corn wastes, agricultural wastes like rice stalk, maize cobs, wood and wastes such as saw dust, pulp wastes, and paper mill waste. Other plant materials are largely manufacturing and industrial residues such as palm oil extraction residues, sugar mill refinery like bagasse and molasses, municipal solid wastes and rice mill waste in form of rice husk (Alzate et al., 2019). However, the plant materials derived from crops are reportedly less digestible compared with animal wastes, which is attributed to their slow acclimatization during the hydrolysis of cellulosic and lignocellulosic components. Water hyacinth is a highly reproductive plant with massive biomass that can be exploited for biogas production.

Plant biomass is characterized by lignocellulosic matter that consists of rigid matrix of cellulosic microfibrils lodged in the soft matrix of hemicellulose and lignin (Alemdar and Sin, 2008). The internal cell content mainly comprise soluble carbohydrates, organic acids, fats, polypeptides, and ashes. The external part of the cell is largely comprised of a rigid layer outside the cell membrane that largely provide structural support and protection to the cell. It is generally comprised of celluloses, hemicelluloses, cutin, lignin, and silica. In most plants, cell wall comprises between 65-85% of the plants dry mass and is largely composed of polysaccharides, proteins, and lignin (Ali et al., 2019). The most commonly available plant biomass, in considerable quantities are agricultural crop residues in form of sugarcane bagasse, maize straw, grass straw and rice straw, which are available in millions of tons (Sarkar

et al., 2012). Water hyacinth does not compete with agricultural plants for the arable farmland. It is also highly prolific and grows fast in aquatic environment without requiring attention, which makes it a suitable biomass for biogas production. To optimize biogas from plant materials, pre-treatment techniques like size reduction, microwave irradiation, heat treatment, and biological pretreatment are recommended. Similarly, to balance the C/N ratio, co-digestion of plant biomass with readily degradable substrates like poultry litter, sewage sludge or cattle manure is encouraged.

2.5 Water Hyacinth

2.5.1 General information

Water hyacinth is a perpetual vegetative fresh water free-floating hydrophyte plant from the *Pontederiaceae* family. Depending on the age and habitat, the plant's physical characteristics can vary considerably based on their leaves, flowers and color. The leaves are generally deep green, glossy and ovate while the flowers are purple bluish. The roots are hairy, long and featherlike, which in deep clear water may grow up to one metre long. In shallow marshy water, the roots can grow and hold in the mud or sediment making the plants static (Sharda and Lakshmi, 2014).

The seeds of the plant are approximately 0.9 to 1.6 mm long contained in a capsule set up that is nearly oval, with rough ridges from one end to the other. The seeds are versatile and may remain dormant in mud and hash environment for up to 20 years. The dispersal method for the seeds is mainly by birds, wind and water transport. However, the plants can also reproduce and propagate vegetatively, through stolons. The reproduction can be very rapid, where a single plant under ideal conditions can produce up to 3,000 others plants in a period of 50 days. Thus, in approximately one year the multiplication can cover an area of up to 600 m^2. In turbulent waters, such as floods and storms, the plants large mats can break up and relocate to new locations

(Sylvina et al., 2014). The flowers are bluish-purple or dark blue in color and are supported by a stalk approximately 150mm above the foliage with a yellow center having petals that are darker purple on the upper part. Flowers, commonly eight on each stalk but can range from 3 to 35 on upright stems, can self-fertilize and aggressively reproduce seeds with minimal external pollination agents (Sharda and Lakshmi, 2014). The characteristic and nature of the plants can vary depending their growth conditions (Julien et al., 1999). The plant can tolerate annual temperatures between 21 to 27°C with a pH of 5.0 to 7.5.

Water hyacinth is among the leading populous aquatic plant with a major hindrance to nation's development activities in and around water bodies (Kriticos and Brunel, 2006; Ndimele et al., 2011). The plant is categorized as a significant risk to biodiversity and human activities. Some of the environmental impacts associated with the plant include massive water loss due to evapotranspiration, deteriorated physical and chemical water properties which in turn adversely affect the flora and fauna community, curtailing of oxygen and light diffusion and hampering water flow and aeration (Gopal, 1987). However, its fast growth provides great potential for its adoption as a raw material for biogas generation guaranteeing raw material supply. Moreover, the plant is rich in nitrogen and other desirable nutrients with high fermentable matter content (Jagadish et al., 2012).

2.5.2 Suitability of Water Hyacinth Plant for Biogas Production

An ideal biofuel crop should have certain properties which are essential in biogas production (Anjanaba and Pawan, 2010). Water hyacinth possesses these attributes as follows.

1) Grows naturally, vegetative and preferably perennial: Water hyacinth naturally grows on fresh water bodies and requires no care (Njoka 2004).
2) High holocellulose and low lignin per unit volume: Water hyacinth has 11-43% holocellulose and 8-14% lignin, which suggests its viability as a biomass crop for biogas production (Lara-Serrano et al. 2016).
3) Easily degradable: Water hyacinth is rich in carbohydrates at approximately 40%. Plants rich in carbohydrates have high biodegradability potential (Ferrer et al., 2010)
4) No competition with crops for arable land, space, light and nutrients: Water hyacinth grows in water therefore it does not pose any challenge to crops for arable land.
5) Hardy and resistant to pests, insects and disease: Attempts for biological eradication of water hyacinth through the use of pests has not been fruitful due to its versatility and resistance to pests, insects and diseases (Njoka 2004).
6) Not susceptible to genetic manipulation through cross pollination with arable food crops: Water hyacinth is an aquatic plant.

Water hyacinth biomass contains cellulose, 20%, and hemicellulose, 33% (Anjanaba and Puwan, 2010). The water hyacinth biomass has relatively high carbon to nitrogen ratio, a characteristic desired in substrates for biogas production (Subhabrata et al., 2013).

2.5.3 Biochemical Composition of Water Hyacinth.

The mineral contents of the plant ranges from 12.4 to 26.8% dry weight (Promdee et al., 2012). Lignin, which is composed of phenyl propanoid groups ranges from 5.9 to 14.3% (Kumar et al., 2009). In plants, lignin, as a complex organic polymer offers the cell walls the structural support system by acting as a binder for hemicellulose the

cellulose components shielding it from chemical degradation (Anjanabha and Puwan, 2010). Lignin is resistant and not easily degradable into sugars. Degradation of lignin is a high-energy process; therefore, it is not suitable in biofuel production. Water hyacinth has relatively low lignin, which allows quick access to cellulose and hemicellulose for its conversion to fermentable sugar making it an attractive substrate applicable in producing ethanol and biogas (Ganguly et al. 2012). Hemicellulose content in water hyacinth ranges from 3 to 27.5%. However, Kumar et al. (2009) and Fileto-Pérez et al. (2013) observed up to 48.7 and 49.3% hemicellulose contents, respectively. Alkali solubility for water hyacinth ranges from 48.9 to 55.9%, whereas that for numerous wood species is lower than 45% (Bernabé-Santiago et al. 2013) suggesting potential for degradation. Biochemical characteristics of plants can be influenced by the habitat and the environmental conditions under which they grow, such as the ambient water quality (Sotolu, 2012). It is therefore important to establish site specific bio-chemical characteristics when working with water hyacinth from a different location.

Table 2-1 presents chemical properties of water hyacinth for water hyacinth collected from (TR) and Yuriria Lake (YL) in Mexico (Lara-Serrano et al., 2016).

Table 2-1. Characteristics of water hyacinth from El Tunal River (TR) and Yuriria Lake (YL) Sites (Lara-Serrano et al. 2016)

Property	**Sample Source**	**Plant Part**		
		Roots	Stem	Leaves
pH	TR	4.6±0.01	4.7±0.04	4.7±0.41
	YL	4.6±0.02	4.7±0.06	4.7±0.01
Ash (wt %)	TR	26.0±0.22	26.8±0.39	19.9±0.25
	YL	14.6±0.09	14.4±0.03	12.4±0.04
Alkali solubility (wt %)	TR	54.4±0.21	52.4±0.34	51.8±0.27
	YL	55.9±0.23	48.9±0.19	51.2±0.23
Total extractives (wt %)	TR	35.9±0.82	58.0±0.78	47.5±0.12
	YL	30.4±0.42	31.9±0.31	29.5±0.02
Lignin (wt %)	TR	13.4±0.53	4.5±0.08	11.5±0.91
	YL	8.8±0.17	5.9±0.18	14.3±0.27
Holocellulose (wt %)	TR	23.7±0.45	11.4±0.50	17.1±0.06
	YL	43.4±0.04	40.0±0.34	36.8±0.35
Cellulose (wt %)	TR	16.0±0.77	8.4±0.21	8.7±0.76
	YL	15.9±0.45	14.4±0.57	12.8±0.30
Hemicelluloses (wt %)	TR	7.7	3.0	8.4
	YL	27.5	25.8	24.0

2.5.4 Limitations of Water Hyacinth in Biogas Production

Although water hyacinth is low in lignin compared with many other plants, its lignocellulose nature may slow down hydrolysis process and conversion to biogas (Yadviva et al., 2004). The intricate structure of lignocellulose (Bajpai, 2017) can limit microbial degradation and result in slow digestion and reduced biogas yield (Li, 2011). Techniques available for improvement of bio-digestion include pre-treatment techniques categorized as physical, thermal, biological or chemical (Carrere et al., 2010) and dilution (Patil et al., 2011) and inoculums. A simple and inexpensive technology for enhancing microbial degradation of the biomass is correlating process

parameters by co-digestion with suitable selected substrates (Callaghan et al., 1999; Kumar and Sharma, 2017). One such substrate is slaughterhouse waste, which has significant concentration of nutrients that can complement those of water hyacinth (Wei wu, 2010). However, most of the slaughterhouse waste components with the exception of ruminal waste, have large concentration of proteins, which make them susceptible to ammonia toxicity (Callaghan et al., 2002; Edstrom et al., 2003; Cuetos et al., 2010; Chen et al., 2008). Ruminal slaughterhouse waste which has less proteins of concentration may be more suited for co-digestion with water hyacinth.

2.6 Slaughterhouse Waste

2.6.1. Challenges in Management of Slaughterhouse Waste

Slaughterhouse waste generally mean all waste produced from abattoirs from animals slaughtering process and other waste emanating from slaughterhouse operations (Weirs and Fischer 1978). Solid wastes in slaughterhouse include rumen contents, blood, bones, hides, skins, foetes, gall bladder, horns, urinary bladder, hoofs, meat trimmings, uterus, ear, rectum, snout, udder, hide and skin trimmings, condemned carcass, condemned meat, hair, oesophagus and poultry offals (FAO, 2004). World over, the challenge of treating and disposing slaughterhouse solid and liquid waste remains a persistent challenge that requires lasting solution. Solid waste from slaughterhouses can amount to as much as 45 to 50% of the live weights of animals (Munack, 2002). The solid waste component comprises rumen content, 56%; 18%; womb, inedible fats, 18%; large intestine, 15% and udder, 6% (Golbaz et al., 2017).

Disposal of slaughterhouse waste poses serious environmental challenges globally (Michael et al., 1988). Environmental Protection Agency (EPA) classifies wastewaters from slaughterhouses fall in the category of harmful discharges to the environment

(Walter et al., 1974). Most developing countries lack organized techniques for disposal of both solid waste and liquid effluent produced from abattoirs. In normal practice, the solid component is dumped to decompose in landfills or in exposed spaces whereas liquid component is illegally discharged into municipal sewer lines, open storm water drains and nearby water bodies. This action ends up endangering public health and adversely affecting terrestrial and aquatic life (Salminen and Rintala, 2002). Wastewater from slaughterhouses are characterized by high BOD in the range of 1500-2300 mg/L, high COD ranging from 4700-8000 mg/L, high total solids averaging 4000 mg/L, average pH of 6.7 and temperature between 20-25ºC (Mittal, 2004; Bazrafshan et al., 2012). Such properties may result in oxygen deficiency in water bodies hence destroying aquatic habitat. Despite the challenges in management of slaughterhouse waste, there are a number of techniques for beneficial use including conversion to biogas, production of fertilizer and processing as animal feed (Michael et al., 1988).

2.6.5 Biochemical Composition for slaughterhouse waste

The composition of slaughterhouse may vary with factors such as the animal family, animal age, prevailing weather conditions, and the amount and properties of the feedstock (Pages-Diaz, 2015). Effluent emanating from slaughterhouse can be characterized as moderately strong and intricate wastewater comprised of 45% soluble and 55% coarse suspended organics. Generally, its composition is influenced by the amounts of blood, which contributes up to 3,500 mg/L nitrogen as ammonia (EPA, 2002). Blood, characterized with high COD averaging 375,000 mg/L is a crucial source of dissolved organic pollutants from abattoir wastewater (Tritt and Schchardt, 1992). Table 2-2 summarizes the properties of slaughterhouse waste water.

Table 2-2. Typical properties of abattoir wastewater (Tritt and Schchardt, 1992)

Property	Range	Average
BOD (mg/L)	150–8,500	3,000
COD (mg/L)	500–16,000	5,000
TOC (mg/L)	50–1,750	850
TN (mg/L)	50–850	450
TP (mg/L)	25–200	50
TSS (mg/L)	0.1–10,000	3,000
K (mg/L)	0.01–100	50
Color (mg/L Pt scale)	175–400	300
Turbidity	200–300	275
pH	4.9–8.1	6.5

2.6.2 Potency of slaughterhouse for biogas production

Organic matters contained in slaughterhouse waste are highly biodegradable either in aerobic or anaerobic condition. The potential methane yield is a factor of concentrations of carbohydrates, proteins, and fats. Because of large lipid and protein content, slaughter house waste components hold a large theoretical methane capacity averaging 980 $mLCH_4$ /gVS. However, despite the potential, the AD treatment suffers from slow biodegradation of lipids (Jeyaseelan and Matsuo, 1995). Excessive concentrations of proteins and lipid can destabilize the action and operation of responsible microbes. During the hydrolysis of lipids and glycerol, long chain fatty acids (LCFA) containing long carbon chains are produced by the action of extracellular lipases. High lipid content wastes are characterized by low alkalinity which makes their anaerobic digestion difficult (Rodríguez-Abalde et al., 2011). LCFAs can

potentially inhibit methanogenic activity and derail anaerobic digestion. Such hindrance can be associated with cell destruction and essential nutrients limitation (Jeyaseelan and Matsuo, 1995). However, after the microbes adapt to such environment, the system is able to recover from stagnation and efficiently start to degrade the LCFAs (Von Sachs et al., 2003).

Proteins are large biomolecules or macromolecules made up of long chains of amino acids bonded by peptide bonds (Rodríguez-Abalde et al., 2011). The hydrolysis of protein-rich feedstock in an anaerobic surrounding is dominated by proteolytic bacteria from the genus Clostridia which is also significantly relevant in subsequent degradation of amino acids (Strong and Gapes 2012).

Overall, total nitrogen concentrations in slaughterhouse wastewaters range from 59 to 330 mg/L whereas typical domestic wastewaters have a total nitrogen concentration ranging 25 to 70 mg/L with ammonia ranging between 4 to 13 mg/L (Bauer et al., 1979; Tchonaoglous and Burton, 1991; Sedlak, 1991; EPA, 2002). Because ammonia accumulation is a major limiting factor in anaerobic digestion, this characteristic is a concern in digestion of slaughterhouse waste. However, the rumen contents component has much less concentration of proteins, which makes it more amenable to anaerobic digestion. Rumen contents form approximately 60% of the slaughterhouse solid wastes, and, therefore, are sufficiently abundant for use as a co-substrate for water hyacinth.

2.6.3 Ruminal Slaughterhouse Waste Potential for Biogas Production

Rumen is obtained from digestive system of ruminant animals where the microbial fermentation similar to that in biogas digester occurs (Haryati et al., 2006). Therefore, the microorganisms in rumen can play a significant role in biomethanation process by

speeding up digestion of organic matter through fermentation of substrates. The degradation of rumen can result in significant pH drop that may destroy most microorganisms in the digester (Manyi-Loh et al., 2013); therefore, the process requires acidophilic microorganisms such as *Saccharomyces cerevisiae* to stabilize the digestion. For example, yeast increases degradation of cellulose by stimulating the multiplication of cellulolitic bacteria and fungi (Williams et al., 1991).

2.7 Factors Influencing Anaerobic Digestion

2.7.1 Temperature

Temperature is considered a critical environmental condition influencing anaerobic digestion. The reactor temperature influences biological growth, survival and activity of different types of microorganism in the reactor. However, increasing biodigester temperature involves greater energy consumption. Therefore, the choice and control of temperature in anaerobic digestion need to be optimized (Madigan et al., 2003).

The range of temperature applicable in anaerobic digestion is usually broad and can fall between 3 and 70°C. The process temperatures commonly fall into three categories; namely, psychrophilic, below 20°C; mesophilic, between 20 and 40°C; and thermophilic, above 40°C (Kossmann et al., 2007).

Conducive temperature for digestion of any feedstock is largely dependent on the feedstock properties and digester design; however, to achieve a stable and consistent biogas output, the temperature should always be kept constant throughout the retention period. Anaerobic digestion at thermophilic temperatures has many advantages including improved pathogen reduction, greater conversion rate, and shorter retention time. However, they require greater heat input. Furthermore, the digestion takes place at temperatures near the top limit for certain microbes associated with the process,

which may affect the survival of organisms particularly those sensitive to temperature variations (Ahring, et al., 2001). Therefore, thermophilic digestion is more susceptible to change in operating conditions and environmental fluctuations making it more challenging than mesophilic digestion (Shefali Verma, 2002). Additionally, raising temperature can raise concentration of free ammonia, which inhibits the process.

Methane generation process through anaerobic digestion is extremely sensitive to temperature alteration. Generally, fluctuations in temperature is limited to ± 2°C/h for psychrophiles, ± 1°C/h for mesophiles, and ± 0, 5°C/h for thermopiles (Kossmann et al., 2007).

2.7.2 pH value

The pH at any point of anaerobic digestion gives an indication of the state of the biodegradation process. Optimal anaerobic digestion occurs within a pH between of 6.5 to 7.5 (Lazor et al., 2010). Formation of volatile acids that occur at the onset of fermentation process, may cause the digester pH to decrease below 5. pH values below 6.5 are associated with toxicity to methanogenic bacteria with a potential for causing a lag in biogas generation (Kossmann et al., 2007).

Feedstock material that is nitrogenous in nature can liberate nitrogen as ammonium hydroxide during biomethanation process. This action can raise the pH of the substrate. When such situation occurs, injection of straw or other co-substrates would help ameliorate the pH (Karki, 2009). After fermentation activity stabilizes under anaerobic environment, the pH value is held in the narrow range of 7.2 - 8.2 by the buffer effect of increased ammonium concentration (Kossmann et al., 2007).

2.7.3 Retention time

The duration needed by enzymes in the reactor for biodegradation of substrates determines retention time. Longer retention times require bigger working volume and, therefore, larger investment and operation costs. Tremendous efforts have been put in the area of research to establish the possible effects of the holding duration on anaerobic digestion (Perot, et al., 1988; Elefsiniotis and Oldham, 1994; Zhang and Noike, 1994 and Bouzas, et al., 2002). Appropriate retention duration is proportional to the process temperature and substrate type. Retention time for substrates digested under mesophilic conditions range from 12 to 38 days whereas those operated under thermophilic conditions require shorter retention times approximated at 14 days (e.g. Shefali Verma., 2002). However, the advantage of the shorter retention time need to be weighed against greater heating costs.

2.7.4 Toxicity

A range of toxic substances can inhibit the multiplication and normal growth of microorganisms in anaerobic reactor. They include ammonia, nitrate/nitrite, heavy metals including Cu, Zn and Cd that are harmful to acidogenic microbes (Ahring and Westermann, 1983; Zinder and Koch. 1984). On the other hand, small proportions of mineral ions, such as S, Mg, Na, Ca, K and NH_3, are essential for stimulation of bacterial growth. Substances such as organic solvents, soap and antibiotics can limit the growth and propagation of methane producing bacteria thereby lowering biomethanation (Kossmann et al., 2007).

2.7.5 Feedstock Composition

Anaerobic microbes can digest various organic compounds broadly categorized as proteins, carbohydrates and lipids. The characteristic of feedstocks, especially the oxidative state of carbon determines the quality of biogas. The lower the carbon

content, the greater the methane content in biogas (Gujer and Zehnder, 1983). For an improved biomethanation, a well-balanced C/N ratio of any substrate is critical; for example, a C/N ratio of 10-20 is regarded as ideal for biomethanation under anaerobic digestion (Gomez et al., 2006). In scenarios where the ratio is not within this range, it could be adjusted by co-digestion with selected feedstock.

Large lipid concentration is potentially harmful during anaerobic digestion in a reactor. Although biomethane prospect for lipids of 1014 L/kg VS exceeds that of carbohydrates of 370 L/kg VS, their long-chain fatty acids (LCFA) are usually repressive because of absorption on the cell surface, which interferes with the cell transport mechanism. Additionally, the LCFA can be adsorbed to the biomass causing microbial flocculation (Zonta et al., 2013). Inhibition by LCFA can be avoided by reducing the concentration through dilution with an inoculum or by co-digestion with low lipid feedstock such as sludge, plant material and cattle manure (Palatsi et al., 2009).

Macronutrients such as sulfur, phosphorus, magnesium and potassium, can essentially be used to activate and develop anaerobic microorganisms needed in AD. Certain trace elements are equally vital in promoting enzymatic activities and cell reproduction. For example, iron and nickel are important for coenzyme F430 synthesis and electron carriage respectively. Other trace elements such as calcium can stabilize the cell wall and is useful in thermal stabilization of the endospores. Cobalt is a trace element rich in vitamin B_{12}, while zinc is also important in integration of numerous enzymes. Nevertheless, the elements are potentially harmful if present in large concentration and may disrupt AD process (Chen et al., 2009). To achieve best performance of the process, supplementing the deficient trace elements while diluting

excess ones by co-digestion is required (Choong et al., 2016 and Myszograj et al., 2018). Diversity in substrate characteristics makes it essential to assess the nutrient characteristics of feedstock in ensuring optimum biogas generation (Kossmann et al., 2007).

2.7.6 Carbon to Nitrogen (C/N) Ratio

Both nitrogen and carbon are significant in the generation and function of microbes. In anaerobic digestion, proportions of carbon in relation to nitrogen is presented by C/N ratio. The optimum C/N ratio for anaerobic digestion fall between 8–20 and is also dependent on the properties making up the substrate (Kossmann et al., 2007).

Very high C/N ratio above 20 is a recipe for aggressive depletion of nitrogen by methanogens resulting in digestion deficiency. Conversely, low C/N ratio less than 8 may cause ammonia accumulation in reactors resulting in pH values in excess of 8.5, which can be harmful to methanogenic bacteria. Therefore co-digestion of substrates containing high and low C/N ratios is a viable means of optimizing C/N ratios of reactor feedstock. An example, of such intervention is the mixing organic solid waste with animal manure or poultry litter.

2.7.7 Organic Loading rate

The relationship between mass of the feedstock injected in a unit mass of reactor capacity per day is referred to as organic loading rate (OLR). The OLR is a significant control aspect in operating anaerobic reactors. Overfeeding anaerobic systems can result in generation and buildup of harmful substances like fatty acids in the reactor slurry that reduces biogas yield under batch methods (Vandevivere, 1999); in continuous systems, overloading has resulted in failure in a number of biogas producing plants (RISE-AT, 1998). On the other hand, underfeeding the plant has led

to diminished gas production, which is uneconomical (Kossmann et al., 2007). Where retention time and the digester volume have been defined, organic loading rate can be used to determine food to microbe (F/M) ratio. For an efficient process, the ratio should be observed to avoid detrimental effect on safety and output of anaerobic digesters. Therefore, F/M ratio need to be matched with the minimum guaranteed retention time (MGRT), for example, by adjustment of feeding frequency. However, while considering the hygienic requirements of the digestate, the MGRT of sludge from the reactor should a factor to be considered (Farrell, et al., 1988).

2.7.8 Mixing/Agitation

The considered reason for mixing and agitation of substrate in a reactor is to mix new feedstock with the old digestate that already contains essential microbes that can stimulate and promote anaerobic digestion in the reactor (Mueller, 2007). Agitation is also considered important in elimination of scum development and elimination of temperature fluctuations within the reactor. However, when the mixing is excessively done, it can instead distort the activity of the microbes and interfere with the biomethanation process. Therefore, gentle mixing is the most commendable. The choice of mixing apparatus and intensity applied is contingent upon the design of reactor and solids loading therein (Shefali, 2012).

Although it is believed that agitation in anaerobic digestion is essential to assist in dispersal of substrate and improve its contact with microbes, Banister & Pretorius (1998) found no significant impact of vigorous mixing where primary sludge was used for volatile fatty acids (VFA) production. Additionally, a study by Perot et al., (1988) using mixed feedstock of organic component of municipal solid waste, primary and

secondary sludge reported no positive impact from continuous stirring on anaerobic digestion process and that it was detrimental at higher organic loading rates.

2.7.9 Total Solid Concentration

Anaerobic digestion systems are commonly classified based on solid concentrations as: low solid systems (LS) where total solids (TS) does not exceed 10%, medium solid systems (MS) with 15-20% TS, and high solid system (HS) where TS falls between 22-40%. Increasing the TS loading requires smaller digester volume because of less water requirements (Verma 2002).

2.8 Steps in Anaerobic Digestion of Substrates

Anaerobic digestion for organic material entails numerous biochemical steps; mainly, hydrolysis, acidogenesis, acetogenesis and methanogenesis (Batstone, 2002). The final output from anaerobic digestion process are biogas and sludge. Different microorganisms are involved in each of the biodegradation steps; namely, fermentative hydrolytic and acidogenic bacteria in hydrolysis and acidogenesis, syntrophic and acetogenic bacteria in the acidogenesis and acetogenesis (Breure and van Andel, 1984), and methanogenic archaea microorganisms in methanogenesis (Angelidaki, 2011). The hydrolysis process is commonly known as the extracellular step because it take place outside the cell through secreted enzymes. The three subsequent stages of acidogenesis, acetogenesis and methanogenesis are categorized as intra-cellular steps (Batstone, 2002). Figure 2-1 summarizes the details of anaerobic digestion process from hydrolysis to methanogenesis. The processes are elaborated in the subsections that follow.

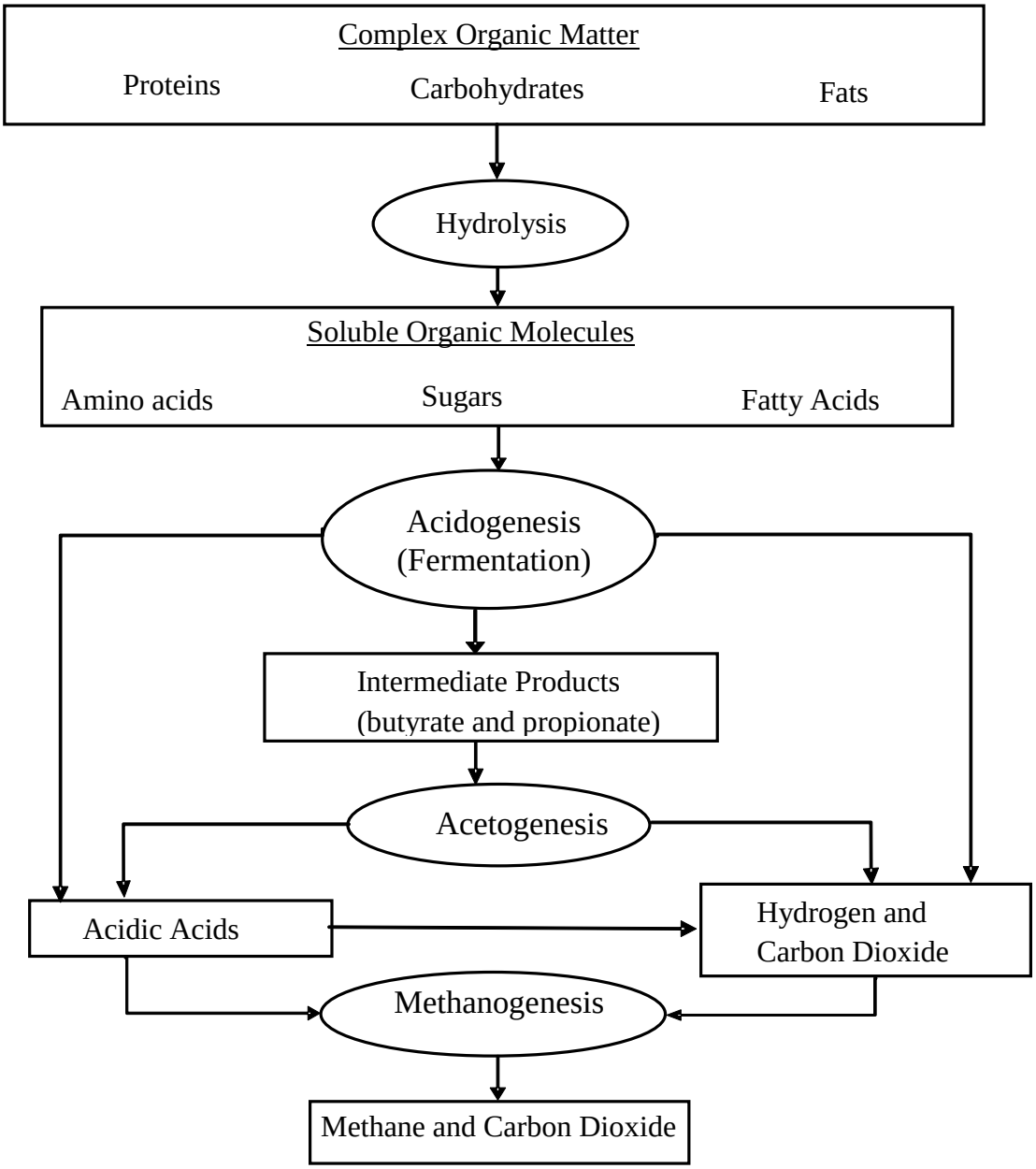

Fig. 2-1. Anaerobic digestion process for complex organic matter

2.8.1. Hydrolysis/Liquefaction

Hydrolysis is the initial step of the biogas generation processes where extracellular enzymes, like proteases, celluloses, lipases and amylases released by the bacteria hydrolyze long chain polymers and other complex organic matter. The process, which is also termed liquefaction, is of importance to the anaerobic digestion accessible by the fermentative microorganisms. The extent of accessibility of the polymers through hydrolysis is useful in determining the subsequent digestion steps. Hydrolysis process entails degradation of complex insoluble organic material into their primary constituents thereby stimulating their mobility via microbial cell membrane (Madigan et al., 2003). During this process, proteins get converted into amino acids by proteases that are secreted by proteolytic microbes while celluloses and/or xylanases, generated from cellulytic and xylanolytic microbes, hydrolyze cellulose and xylose into glucose and xylem, respectively. Subsequently, lipids are converted into long-chain fatty acids and glycerol by lipases produced by lipolytic microbes (Salminen and Rintala, 2002).

Hydrolysis is a gradual process that is often the rate limiting step. The limitation of the process can sometimes be overcome by the use of selected chemical reagents which under industrial operations shortens the digestion period and enhance methane yield (Shefali Verma, 2002). In the hydrolysis/ liquefaction process, biomass reacts with water to form monomers, carbon dioxide and hydrogen gas (e.g. Equation 2-1).

$$\text{Biomass} + H_2O \rightarrow \text{Monomers} + H_2 \text{ ---------- (2-1)}$$

Developing a balance for the population of microorganisms is important because it can help the microorganisms cooperate to increase cellulose degradation. Besides, decrease and increase of propionate and acetic acids in volatile fatty acids (VFA), respectively is performed by S.*cerevisiae* (Kumar et al, 1994; Newbold et al, 1998).

Such action increases acetogenesis following the VFA formation and further increases the resulting acetic acid (Chaucheyras et al, 1995). Acetic acid is a significant source of methane, hence, methane generation relates directly to acetic acid production.

2.8.2. Acidogenesis (fermentation)

During acidogenesis stage, products of hydrolysis including dissolved sugars, amino acids and long-chain fatty acids are converted to acetate, hydrogen and carbon dioxide through the action of facultative and anaerobic bacteria or by anaerobic oxidizers. The products of hydrolysis are converted to volatile fatty acids (VFAs) like propionic, butyric and acetic acid, ketones, alcohols and lactic acid through a similar action of anaerobic bacteria (Gujer and Zehnder, 1983), Equation 2-2. Additionally, the organic compounds are converted to ketones (acetone, glycerol) and alcohols (methanol, ethanol) as illustrated in Equation 2-3.

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \text{ ---------- (2-2)}$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2COH + 2CO_2 \text{ ---------- (2-3)}$$

Acidogenesis is an exothermic process that is usually the quickest of all anaerobic digestion steps. In an efficiently working anaerobic system, approximately 70-80% of the hydrolysis products are translated to hydrogen, carbon dioxide and acetate that is readily accessible by methanogenic bacteria. However, the deficit of 20-30% is converted to other intermediary products including alcohols and volatile fatty acids (VFAs) (Gujer and Zehnder, 1983; Schink, 1997; Ahring, 2003; Angelidaki et al., 2007). Anaerobic digestion often release toxic ammonia and hydrogen sulphide produced from amino acids; such products can be detrimental to anaerobic digestion process (Salminen and Rintala, 2002).

2.8.3. Acetogenesis

Acetogenesis is the digestion step where acetate is synthesized from CO_2 reduction while enhancing consumption of organic acids. In this process, some fermented substances like VFAs, alcohols, and aromatic fatty acids are converted to hydrogen and acetate through the action of obligate hydrogen producing bacteria (Lea et al., 2017). At this stage, hydrogen-producing acetogens oxidize the acids to acetate. The oxidation reaction produces electrons which are transferred to protons (H+) to produce H_2 or bicarbonate and produce formate (Westermann, 1996; Stams and Plugge, 2009). The oxidation processes is normally endothermic and cannot occur where H_2 partial pressure is high. Therefore, when protons are used as electron acceptors, fermentative bacteria attain maximum energy with concurrent H_2 production.

Acetogenic bacteria are responsible for formation of acetate, which can either be consumed straightaway by aceticlastic methanogens such as *Methanosarcina spp*. and *Methanosaeta spp*. or broken down by syntrophic collaboration of bacteria known as syntrophic acetate oxidizers and hydrogen consuming methanogenic archaea. The latter requires activation of acetate to acetyl-CoA split by a CO-dehydrogenase/acetyl-CoA synthase enzyme complex to a methyl and a carbonyl residue, which is oxidized independently by clearly defined pathways (e.g. Hattori, 2008).

Acetogenesis is facilitated by diverse microbes including *syntrophobacter wolinii*, a propionate decomposer and *sytrophomonos wolfei*, a butyrate decomposer. Other applicable acid formers include *clostridium spp*. *peptococcus anerobus*, *lactobacillus*, and *actinomyces* (Kangle et al., 2012). An examples of acetogenesis reaction is shown in equations 2-4 and 2-5:

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad \text{(2-4)}$$

$C_6H_{12}O_6 + H_2O \rightarrow 2CH_3COO + 2CO_2 + 4H_2$---------- (2-5)

2.8.4. Methanogenesis

Methanogenesis represents the ultimate step of anaerobic digestion where methanogens mineralize products of fermentation to methane. Various methane-forming archaea work in complementary manner for efficient biomethanation. The methanogenic archaea such as *methanococcus, methanosarcina, methanobacterium* and *methanobacillus* are the most common microbes in the process. This step is categorized into: (1) aceticlastic methanogens belonging to the genera *Methanosarcina* and *Methanosaera*, which degrade acetate (Equation 2-6), (2) hydrogen-utilizing methanogens (Equation 2-7), of which an array of genera exist (Madigan, et al., 2003). *Methanothrix spp*. also known as *methanosaeta and methanosarcina spp*. are considered the most essential species in AD both as H_2, and CO_2 acetate consumers. Accordingly, depending on the properties of substrate subjected to the methanogens, methanogenesis can be categorized into two groups (Bitton, 2005):

1. Hydrogenotrophic methanogenesis in which CO_2 and H_2 are converted to methane and water (Equation 2-6).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \text{ ---------- (2-6)}$$

2. Acetotrophic or aceticlastic methanogenesis in which methane is generated by converting acetate to methane and CO_2 (Equation 2-7)

$$CH_3COOH \rightarrow CH_4 + CO_2 \text{ ---------- (2-7)}$$

Up to 70% of methane in biogas is generated from acetotrophic reaction of conversion of acetate to CH_4 and CO_2 (Smith and Gosselin, 1979), while the balance of 30% is generated when carbon dioxide is reduced by hydrogen using hydrogenotrophic methanogens (Hashimoto et al., 1981).

2.9 Microbial Communinty in Anaerobic Digestion

Microbial communities involved in the AD process are largely dependent on substrate composition and reactor design as well as operating conditions (Manyi-Loh et al., 2013). Sequence analyses of bacteria domains in anaerobic digestion of sludge by Rivière et al. (2009) revealed four dominant phyla; namely, Chloroflexi, Proteobacteria, Bacteroidetes, Firmicutes and that the Archaea community that are responsible for methanogenesis were affiliated with *Methanosarcinales*, and *Methanomicrobiales* groups. The Proteobacteria are Gram-negative bacteria while Firmicutes are a low-G+C group, mostly Gram-positive consisting of Bacilli and Clostridia classes. Heeg et al. (2014), associated the higher efficiency anaerobic digestion in the thermophilic environment to abundance of Firmicutes and Methanosarcina sp. In anaerobic digestion of lignocellulosic residues of palm oil mill and wheat straw, bacterial community included *Ruminococcus sp., Thiomargarita sp., Clostridium sp., Anaerobacter sp., Bacillus sp., Sporobacterium sp.* (Heeg et al., 2014; Suksong et al., 2016).

2.10 Methods of Microbial Communinty Analysis

There are several methods for quantification of microbial communities (e.g. Raskin et al., 1994; Akarsubasi et al., 2005; Stainberg and Regan, 2009). The 16S rRNA and its genes is the most frequently used biomarkers for the determination of methanogenic populations in environments (Narihiro et al., 2009).

2.10.1 FISH Technique /Method

FISH based on 16S rRNA is commonly applicable in detecting specific groups of microbes and quantification of their populations of interest in environments by direct counting under a microscope (Amann et al., 1995). The method can also be used to visualize the spatial distribution of microbial population of interest in biofilms, such

as those of methanogens in sludge granules in methanogenic wastewater treatment systems (Sekiguchi et al., 1999). Oligonucleotide probes for in situ hybridization are basically similar to those developed and used for membrane hybridization of methanogen 16S rRNAs or reverse primers for PCR amplification of methanogen 16S rRNA genes (Raskin, et al., 1994).

2.10.2 qPCR Technique /Method

Quantitative PCR of 16S rRNA gene and mcrA has also been used to quantify the abundance of methanogens in recent years. TaqMan-based qPCR probes/primer sets are available for each of the orders Methanomicrobiales, Methanosarcinales, Methanobacteriales and Methanococcales, as well as the families Methanosaetaceae and Methanosarcinaceae (Yu et al., 2005). Quantitative PCR method provides sensitive, quantitative and interesting data with dynamic range of quantification (Zhang and Fang, 2006). qPCR may therefore be used for quantitative monitoring of methanogen taxa in complex microbial communities (Ottesen et al., 2006). The challenge with using qPCR is that it is PCR based and their data can be suspect because of biases involved in DNA extraction and primer/probe mismatches.

2.10.3 RNase H Technique /Method

RNase H method is a simple and rapid quantification method which has recently been developed to overcome the complexity challenges of membrane hybridization and qPCR (Uyeno et al., 2004). This method is based on the sequence-specific cleavage of 16S rRNA with ribonuclease H (RNase H) and oligonucleotide (scissor) probes (Narihiro et al., 2009). The targeted rRNAs are cleaved at the hybridization site in a sequence-dependent manner and are consequently cut into two fragments while the non-targeted rRNAs remain intact under the same conditions (Uyeno et al., 2004). To

detect cleaved rRNAs, the resulting RNA fragment patterns can be resolved by gel electrophoresis using RNA-staining dyes.

2.10.4. Other Techniques /Methods and Future Perspectives

A number of methods under DNA microarray platform, like PhyloChip, ANAEROCHIP and GeoChip which have been developed recently are becoming an important tool for parallel detection of different community members of microbes in ecosystems (Franke-Whittle et al., 2009; Wang et al., 2009).

Recent advances in analytical chemistry, such as isotope ratio mass spectrometry (Penning et al., 2006; Vavilin et al., 2008) and secondary ion mass spectrometry (Orphan et al., 2001), hold great promise for the highly sensitive determination of targeted microbes.

2.11 Biogas Composition and Characteristics

2.11.1 Composition of Biogas

Knowledge of the components of biogas can assist in achieving more efficient and effective processing and utilization of biogas which essentially comprises of methane (CH_4) and carbon dioxide (CO_2), with small proportions of hydrogen sulphide (H_2S) and ammonia (NH_3). Although raw biogas is similar to natural gas, it contains certain undesirable physicochemical characteristics that can restrict its processing and utilization as renewable energy (Bothi, 2007). Some trace elements commonly found in biogas include carbon monoxide (CO), hydrogen (H_2), oxygen (O_2) nitrogen (N_2), and saturated or halogenated carbohydrates. Often, raw biogas can contain dust particles and siloxanes and can at times be saturated with water vapor (Zicari, 2003).

The composition of biogas is differs from natural gas depending on the source of raw material (Rasi, 2009). Table 2.3 compares the characteristics of biogas generated from dairy manure waste through anaerobic digestion process. The characteristics are comparable to those of natural gas. The energy potential for biogas of 21.48M J/m^3 is approximately two thirds that of refined natural gas of 35.76 MJ/m^3, which is caused by the lower CH_4 concentration in biogas and the significant CO_2 therein (Bothi, 2007, Schomaker et al., 2000). Additionally, the relatively minute concentrations of trace gases complicates processing and utilization (Bothi, 2007). Despite these setbacks, biogas contains significant proportion of methane that is usable as renewable energy in diverse applications (Prakash, 2011). Biogas is regarded as source of environmentally friendly energy because it does not release carbon monoxide during combustion. Additionally, biogas releases approximately half the weight of CO_2 compared to traditional fossil fuels. These dual advantages makes biogas an environmentally safe energy worth exploiting (Bothi, 2007).

Table 2-3. Comparison of constituents of natural gas and biogas (Monnet, 2003).

Constituent	***Unit***	***Natural Gas***	***Biogas***
Methane (CH_4)	%	91	55-70
Ethane (C_2H_6)	%	5.1	0
Propane (C_3H_8)	%	1.8	0
Butane (C_4H_{10})	%	0.9	0
Pentane (C_5H_{12})	%	0.3	0
Carbon Dioxide (CO_2)	%	0.61	30-45
Nitrogen (N_2)	%	0.32	0-2
Volatile Organic Compounds (VOC)	%	0	0
Hydrogen (H_2)	%	0	0

Hydrogen Sulfide (H_2S)	ppm	~1	>500
Ammonia (NH_3)	ppm	0	~100
Carbon Monoxide (CO)	ppm	0	0
Water Dew Point	ºC	<-5	Saturated
Heating Value	BTU/SCF	1031	~600

2.11.2 Chemical Characteristics of Biogas

2.11.2.1 Methane Gas

Methane (CH_4) gas is the major component in natural gas. Decomposed plant and animals remains detained under earth surface are transformed into fossil energy products like oil, coal and natural gas through intense pressure and heat. Under anaerobic conditions, methanogenic bacteria convert organic matter into CH_4. Methane is both odorless and colorless. When mixed with air, the explosive limits of methane is 5-15%. The anaerobic digestion process can result in methane in yields between 50 and 60% in biogas produced from dairy manure wastes (Pellerin et al., 1987).

2.11.2.2. Carbon Dioxide

Large concentrations of CO_2 in biogas indicated its poor quality characterized by lower energy value that can hinder it from some energy applications (Moestedt et al., 2015). Scott and Minott (2003) reported that moderately high CO_2 concentrations in biogas has the potential to replenish essential carbonate electrolyte in molten carbonate fuel cells. Conversely, high levels of CO_2 can acidify diesel generators, prompting the need for removal where large-volume biogas is utilized in commercial natural gas pipeline streams (Bothi 2007 and Sri Rahayu, 2015). Cleaning up CO_2 and other trace

components from biogas can be expensive particularly where generation is on small scale.

2.11.2.3 Trace Components

Trace components in biogas generated from dairy manure, for example, are usually less than 2% (Bothi, 2007; Newman, 2012). The most common trace elements in biogas comprise ammonia, hydrogen sulfide (H_2S), and water vapor (Manyi - Loh et al., 2003, Zicari, 2003 and Bothi, 2007). Most trace components are undesirable in biogas and should be removed based on the energy use (Karlsson, 2014). For example, when water vapor combines with acidic trace proportions such as H_2S and CO_2, it can become hazardous and highly corrosive (Bothi, 2007). Hydrogen sulfide is indeed the major contaminant in biogas; it is not only poisonous but also corrosive and can cause serious damage to equipment, machines and instrumentation. During combustion, H_2S is released as sulfur dioxide which is a major atmospheric pollutant (Bothi, 2007 and Ertem, 2011).

2.12 Biogas Measurement Techniques

Available techniques for biogas quantification include volumetric gas measurement, manometry, liquid displacement and gas chromatography. The following subsections describe the measurement methods.

2.12.1 Volumetric and Manometry Gas Measurement

Biogas measurement can be carried out either by manometric method where volume is held constant while increase in pressure is measured, or by volumetric method where the pressure is held constant while biogas volume is measured (Jonas Bonn, 2008).

Numerous methods such as volume displacement devices, lubricated syringes, low pressure switch meters, pressure manometers or transducers and manometer assisted syringes have been employed to quantify biogas (Rozzi and Remigi, 2004). Research needs have prompted the development of different types of displacement gas measurement devices (Moletta and Albagnac, 1982, Matta-Alvarez et al., 1986; Liu et al., 2004; Smith and Stockle, 2008). They are guided by the general principle of pressure difference between inlet and outlet of the meter inducing recurrent filling and release of a definite gas volume in automated displacement gas meters. This concept employs a system of opening and closing of a two-way solenoid valve sensor that releases accumulated gas and resets the entire system. The total gas volume is the product of the number of filling or emptying, as registered through a check system and the preset volume of the chamber.

Manometric transducers with numerous arrangement are the most applicable method for determining the volume of gas produced. Solenoid valve is triggered on receiving the electric signal prompted by cautious increment of headspace gas pressure at a set value (Guwy, 2004).

2.12.2 Liquid Displacement Gas Measurement

Liquid displacement technique is the most common laboratory volumetric gas measurement method. In the method, the gas is collected over a selected colored liquid, which it displaces as it builds up (Figure 2-4). The method can be affected by the fluctuation of atmospheric pressure and room temperature, which can be sources of errors. Therefore, application of corrective factor determined by a record of variations in atmospheric pressure and temperature is required.

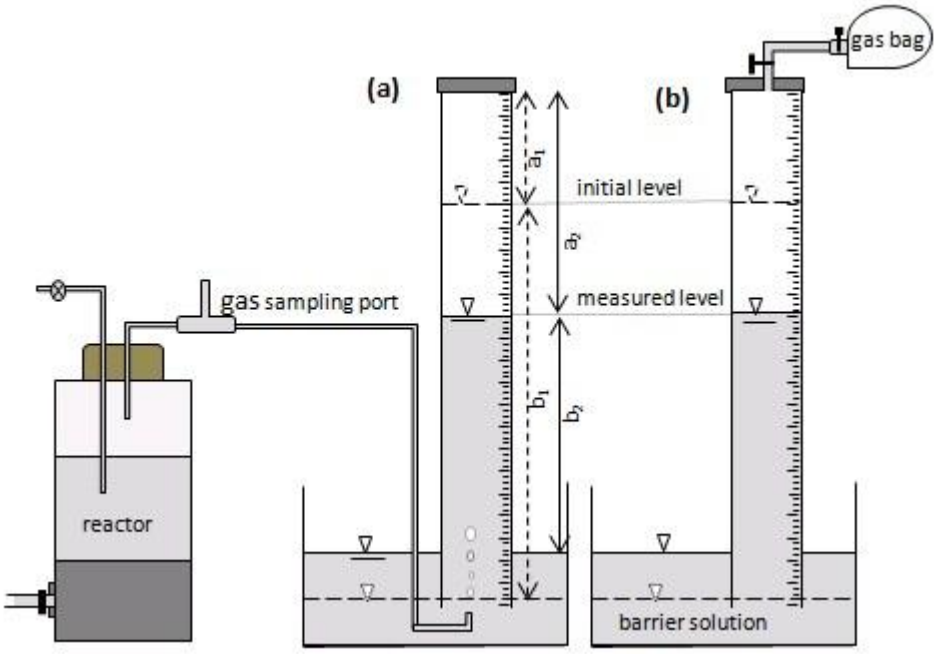

Figure 2-2: Types of liquid displacement gas measurement a) Direct method b) Indirect method (modified from Walker et al., 2009).

The selected sealing liquid determines the accuracy of automatically operated displacement instruments. Table 2-4 summarizes types of typical barrier solutions.

Table 2-4. Summary of various barrier solutions and their composition

Barrier Solution	Reference	Composition
NaCl/ acid	Walker et al. (2009)	Saturated NaCl solution, pH 2
Acidified water	Muller et al. (2004)	Water, pH not less than 2
NaCl/ acid	Kolb et al. (2006)	200 g NaCl +1 L distilled water + 5 g citric acid
Orsat confining solution	Apex Instruments (2000)	100 g of (Na_2SO_4) + 500 ml distilled water + 20 ml concentrated sulfuric acid

2.12.3 Gas Chromatography

Gas Chromatography (GC) has been used widely because of its advantages that include sensitivity, high resolution, speed, and quantitative results. The method is ideal in quantification of gas that is exposed to liquid phase (Kolb and Ettre, 2006). Calibration standards for CO_2 and CH_4 are first prepared and run through the GC before the actual samples. Although flame ionization detector (FID) is the most applicable method of gas detection for GC, thermal conductivity detector (TCD) is commonly used in sensing light hydrocarbons and compounds that are less responsive to FID. The TCD is however weak compared to FID (10-5-10-6 g/s, linear range: 103-104). FID is more sensitive to organic molecules (10-12 g/s, linear range: 106–107). Where measurements are performed for small quantities of hydrocarbons, FID analysis is more applicable because of its large signals response and greater precision (Poole, 2003).

2.12.4 Headspace Biogas Analysis with GC (HS-GC)

Headspace analysis involves determination of characteristics of volatile compounds related to a liquid or solid material devoid of direct contact with the analyte matrix. The basic principle involves testing the analyte from the vapor phase above a liquid or solid sample in a sealed vial or container. The analyte is carefully but successfully released to the inlet of a gas chromatograph for analysis. The thermodynamic equilibrium in the system guides the solubility and transfer of the analyte in liquid phase (Kolb and Ettre, 2006). The consistency of temperature and pressure is the most important factor due to a possible influence in the balance of gas concentration. Significant variation in temperature from that of the calibration gas can result in GC gas measurement errors leading to unreliable results (Kim and Daniels, 1869). Temperature changes/fluctuations also has a direct impact to the anaerobic

microbiology. GC can be used to establish the optimum methane prospect for substrates and their degradation rate. For biogas, temperatures of 35°C and to a lesser extent 20, 55 and 70°C is applicable during such determination. However, standard methane, adopted in comparison is constituted in laboratory room temperature of 20 to 23°C.

2.13 Synthesis of Literature Review

The literature reviewed in this Chapter has demonstrated that water hyacinth possesses the general requirements of a biomass plant for biogas generation. However, knowledge of its chemical, structure and nutrient composition is important in determining conversion of its biogas. Plant biomass is lignocellulose in nature, a property that can slow anaerobic digestion and lower biogas output. Co-digestion with a suitable substrate has potential to improve digestion of the biomass. The reviewed literature has identified ruminal slaughterhouse waste as possible co-substrate for water hyacinth digestion. However, most of available characterization data is on compounded slaughterhouse waste. Therefore, it is necessary to characterize ruminal slaughterhouse waste to understand its complementary role in co-digestion with water hyacinth. Additionally, there is need to understand the impact of various vital operating conditions such as temperature, pH, retention time and mix proportions of co-substrates on biogas yield. Similarly, to understand the synergy in co-digestion of the two substrates, there is need to establish the dynamics of the microbial communities that are key players in the digestion.

CHAPTER 3: METHODOLOGY

3.1 Overview of methodology

Figure 3-1 summarizes the methods used in this study. The methods were grouped into characterization of the substrates, co-digestion of the substrates for biogas production, biogas analysis and analysis of microbial community.

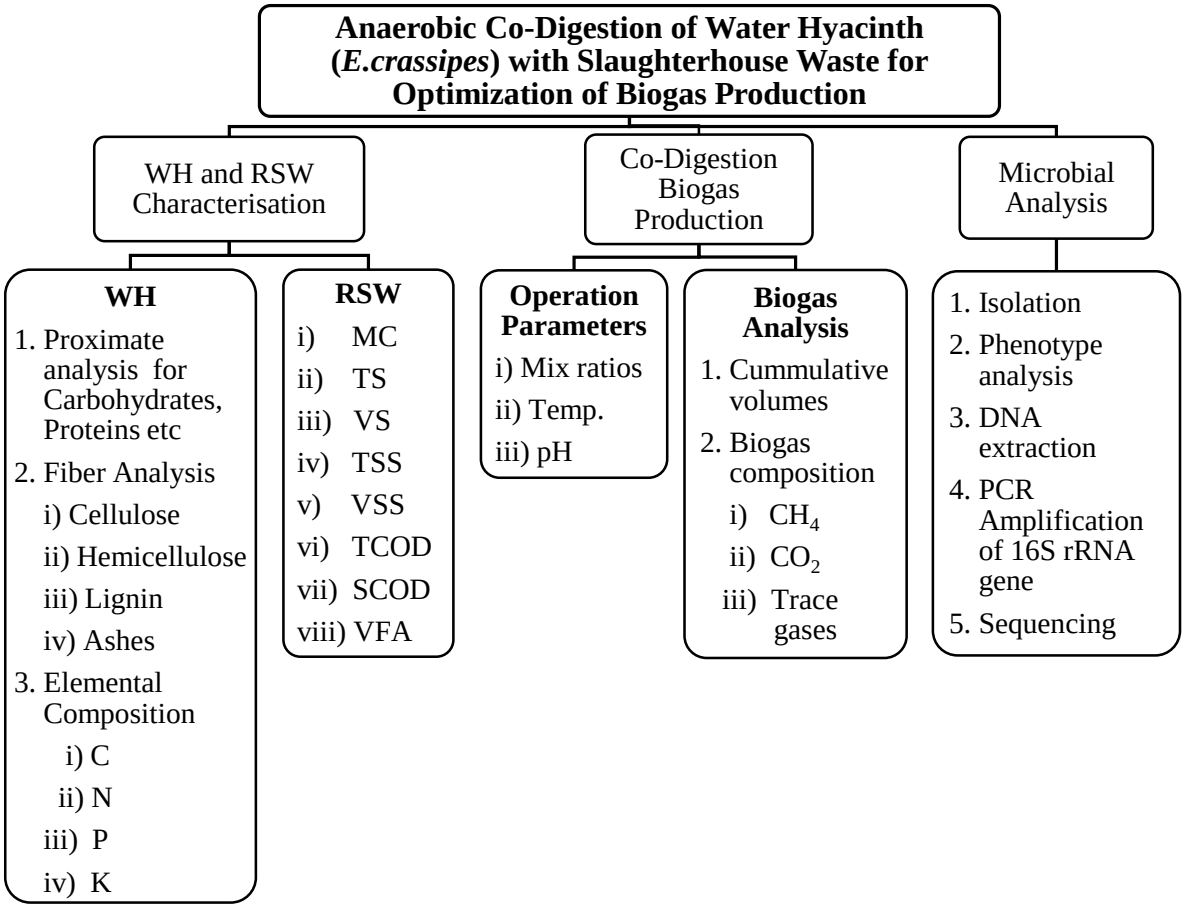

Figure 3-1. Structure of methodology

3.2 Characterization of Substrates

3.2.1 Overview of Methods

This part of the study characterized water hyacinth (WH) from Lake Victoria and ruminal slaughterhouse waste (RSW) from a slaughterhouse in Nairobi as substrates

for biogas productions. Tests carried out were proximate, elemental, fiber and biochemical analyses.

3.2.2 Sampling and Sample Preparation

Water hyacinth was collected from the shores of Winam Gulf, Lake Victoria, near Kisumu City, at coordinates -0° 53' 9.71" S, 34°45'2.44" E (Figure 3-2a). Fresh, healthy and mature plants were manually sampled about 50m from the shore on 2nd August 2017 at about 9am. The samples were packed in sampling bags before being transported the same day to the University of Nairobi's Environmental Engineering Laboratory in preparation for the experiment. Fresh and whole water hyacinth were cut to smaller pieces of approximately 2 to 2.5 cm then exposed to dry in the sun for a duration of seven days. Before the drying process, approximately 50 g of the freshly cut water hyacinth was sampled for determination of moisture content in raw and sun-dried states. The dried material was crushed to finer particles by a large mortar and pestle. Samples were placed in plastic bags, vacuum-sealed and refrigerated at 4°C awaiting characterization and use in biogas reactors.

Fresh ruminal slaughterhouse waste was collected from Dagoretti Slaughterhouse, located in western part of Nairobi at coordinates 1°17'3.71"S, 36°41'1.98"E (Figure 3-2b). The ruminal contents were manually sampled from the slaughterhouse waste yard on 6th August 2017 at about 8 am during the slaughtering process. The samples were packed in sampling five liter buckets and immediately transported to the University of Nairobi's Environmental Engineering Laboratory where they were kept at 4°C awaiting processing and analysis. The samples were homogenized and exposed to dry in the sun for another three days before further drying in an oven at 60°C for six hours (Tao et al., 2016). Moisture content was determined from weight difference before and

after drying. The oven-dried material were crushed to fine powder in a grinding mill and placed in plastic bags, vacuum-sealed and refrigerated at 4°C awaiting characterization and use in biogas reactors.

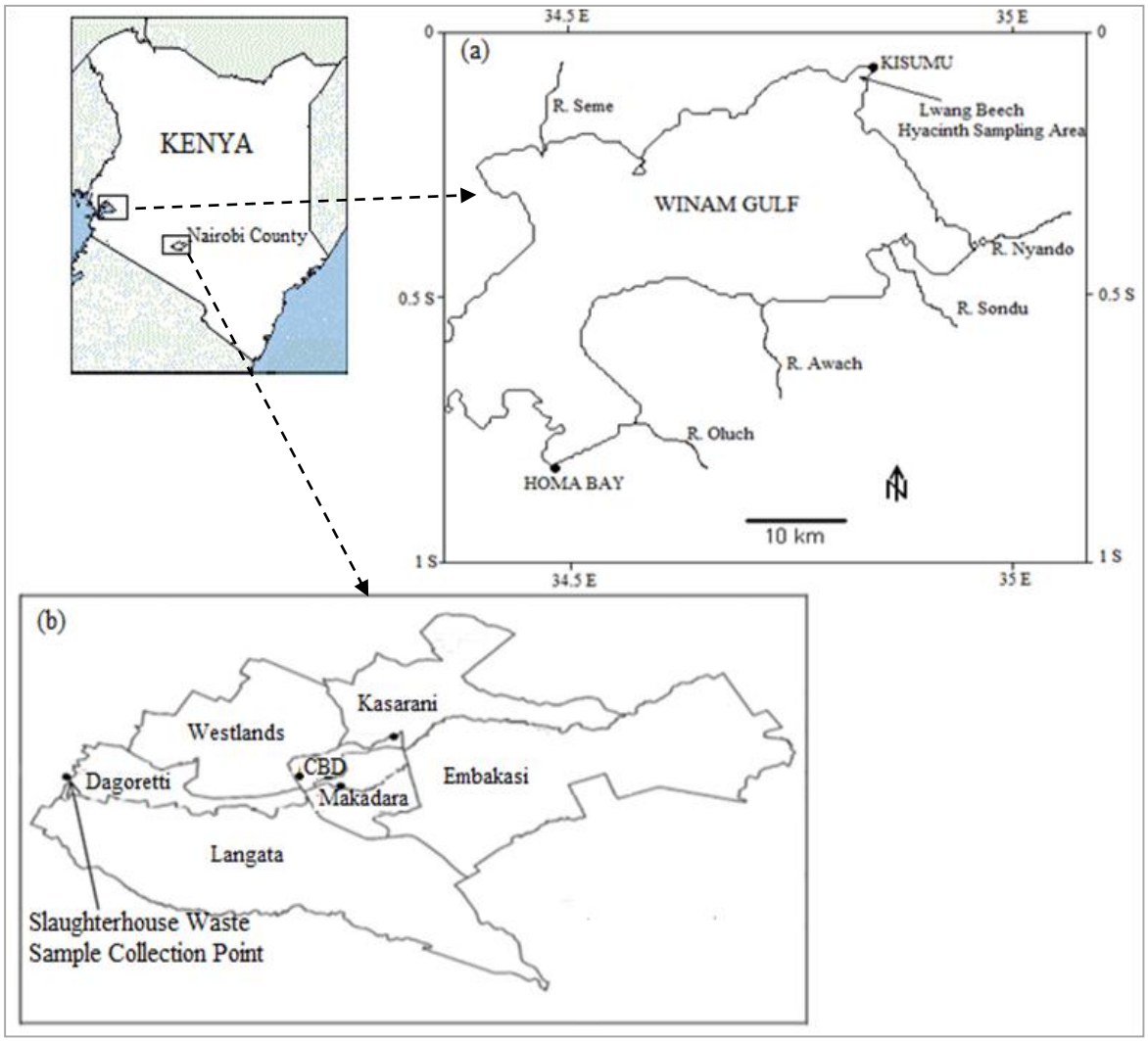

Fig. 3-2. *Map showing location of (a) Water Hyacinth Sampling Point in Winam Gulf, Kisumu (b) Slaughter house Waste sampling Point, Dagoretti - Nairobi*

3.2.3 Elemental Analysis

Approximately 3 g of the oven dried water hyacinth sample was digested for three hours with 20 mL acid mixture prepared from selenium powder, lithium sulfate, hydrogen peroxide and sulfuric acid. The digestion was carried out at 360°C until the solution and the residue appeared colorless. The contents were then adulterated using

distilled water to a final volume of 50 mL (Okalebo et al., 2012). The digest was then used in analyzing total Kjeldahl nitrogen, phosphorous, and potassium.

Kjeldahl nitrogen was obtained by titration method using hydrochloric acid (HCl) and computing Kjeldahl nitrogen (Riddellová, 2012). Potassium content of the biomass was determined by analyzing the digest on a flame photometer (Flame Photometer Protocol: P05-031A and Medical chemistry LOKT.00.009, 2012), while total phosphorous (TP) was determined by ascorbic acid method (New Delhi, 2000; Doolittle, 2014).

Fixed carbon was determined by first determining volatile solids which involved weighing 1 gm of the sample in a silica crucible with a porous silica cover. The cover was used to control oxidation. The sample was then heated for seven minutes at consistent temperature of 900°C inside a furnace. Upon heating, the crucible was allowed to cool before transferring its content to a desiccator. The silica crucible was re-weighed after 10 minutes of cooling. The difference in weight before and after heating gave the amount of volatile matter in the sample. The following formula was used in determination of fixed-carbon in the samples (Zhou, 2017):

Fixed Carbon (%) = 100% - Ash (% Dry Basis) - Volatile Matter (% Dry Basis)

Elemental analysis for Nitrogen (N), Potassium (K), Carbon (C) and Phosphorus (P) for ruminal slaughterhouse waste were carried out using the same procedures as those employed for the water hyacinth biomass.

3.2.4 Proximate Analysis of Dry Matter

Proximate analysis of water hyacinth dry matter was done to determine the contents of the cell, cell wall and the moisture content. The cell contents include crude protein,

crude fats, carbohydrates and minerals while the cell wall comprise of crude fibre and ash (Queiroz et al., 2008). Moisture content, protein, fat, crude fibre and carbohydrates concentrations and ash content were determined using AOAC methods specification 950.46 (AOAC, 1995). Tests were carried out in triplicate.

Measurement of moisture content of water hyacinth was carried out by weighing about 2 g of dry sample in a crucible and drying it in an oven at 105°C for 1 hour. The moisture content was computed using Equation 3-1.

$$\text{Moisture (\% M)} = ((W_1-W_2)/W_3) \times 100 \qquad (3\text{-}1)$$

Where; W_1= Weight of the crucible and the air-dried sample (g), W_2 = Weight of the crucible & oven dried sample (g), W_3 = Weight of the air-dried sample taken (g).

Crude protein in water hyacinth was determined using kjeldahl method (Riddellová, 2012). About 15 mL conc. sulfuric acid (H_2SO_4) with dissolved two copper catalyst tablets was used to hydrolyze 1 g of raw material in a heat block Kjeltec system 2020 digester, Tecator Inc., Herndon, VA, USA) at 420°C for two hours. Upon cooling, hydrolysates were diluted with distilled water, then neutralized and titrated. The total nitrogen component determined in the samples were multiplied by a conventional conversion factor of 6.25 (Mariotti et al., 2008) and species-specific conversion factors (Lourenço et al., 2002) to arrive at total protein.

Determination of crude fiber was carried out by gravimetric technique following chemical digestion and solubilization of other constituents available in the sample. Approximately 2 g of the sample (W) was extracted by boiling in 200 ml of 1.25% H_2SO_4 for a period of 30 min under reflux condenser. Slight vacuum fitted with Pyrex glass filter was used in filtration while acids were fully removed by washing the

residue in boiling water. The washed residue was subjected to 200 ml of boiling 1.25% NaOH and further boiled under reflux for about 30 min. Pyrex glass filter formerly used for the acid was again used as a filter media. Boiling water was used to rinse the residue followed by 1% HCL and further in boiling water to ensure complete rinsing of the acid from the residue. The residue was twice washed with alcohol and thrice with ether. The residue was then placed in porcelain dish and dried up in an oven at 105°C until a constant weight (W_1). A muffle furnace was used to incinerate the samples at 550°C for three hours before the dish was cooled in a desiccator before finally weighing again (W_2). Computations for crude fiber was carried out as shown in equation 3-2.

$$Crude\ fiber(\%) = \frac{(W1-W2)}{W} * 100 \qquad (3\text{-}2)$$

To determine the ash content in the water hyacinth, 5 g of air dried sample was placed in pre-weighed crucibles and the sample charred by flame to clear organic material. The sample was incinerated at 550°C in a muffle furnace to achieve a white ash. The residue was cooled in desiccators and weighed as ash. The percentage carbohydrates in water hyacinth was computed arithmetically by subtracting the sum of moisture, protein, fat, ash and crude fibre percentages from 100 (AOAC, 1995).

Proximate analysis to determine; crude fat, crude protein, ash, crude fiber, moisture and carbohydrates for ruminal slaughterhouse waste was carried out using the same procedures as those employed for water hyacinth biomass.

3.2.5 Fiber Content Determination

The composition of lignocellulosic substrates was determined using direct method (Moubasher et al., 1982). Air dried water hyacinth sample of approximately 5 g was

boiled in ethanol four times for 15 minutes, followed by thorough washing with distilled water and storage in an oven at 40°C for 12 hrs. The dry sample was weighed (A) before treatment with 24% KOH for 4 hrs at 25°C, followed by thorough washing with distilled water and drying at 80°C overnight and then taking its dry weight denoted as (B). The sample was further subjected to 72% H_2SO_4 for three hours to disintegrate the cellulose before refluxing with 5% H_2SO_4 for two hours. The sample was washed in distilled water to eliminate H_2SO_4 followed by drying at 80°C in an oven for 24 hours then taking the dry weight as (C). The lignocellulic substrates were calculated as follows:

Cellulose = B-C

Hemicellulose = A-B

Lignin = C

Fiber content determination for ruminal slaughterhouse waste was carried out using the same procedures as those employed for water hyacinth biomass.

3.2.6 Determination of Total Solids, Volatile Solids and Fixed Solids

To determine the fixed and volatile solids for ruminal slaughterhouse waste and water hyacinth biomass, about 5 g of dried sample was ignited in muffle furnace at 550°C for one hour. The sample was cooled in desiccator and weighed. The ignition was repeated for 30 min and the sample cooled and weighed until the weight change was less than 4%. The samples were analyzed in duplicate and the calculations for fixed and volatile solids were carried out as shown in equations 3-3, 3-4 and 3-5:

$$\%\ total\ solids = \frac{(A-B)}{C-B} * 100 \quad (3\text{-}3)$$

$$\%\ volatile\ solids = \frac{(A-D)}{A-B} * 100 \quad (3\text{-}4)$$

$$\% \quad fixed\ solids = \frac{(D-B)}{A-B} * 100 \qquad (3\text{-}5)$$

where:

A = weight of dried residue + dish, mg,

B = weight of dish, mg,

C = weight of wet sample + dish, mg, and

D = weight of residue + dish after ignition, mg.

3.2.7 Determination of COD, TCOD, SCOD and Volatile Fatty Acids

The chemical oxygen demand (COD) was performed by dichromate method (APHA, 1998). About 2 g of the dried samples (WH or RSW) were placed in culture tube and acidified potassium dichromate digestion solution was added and concentrated sulfuric acid was carefully passed through the vessel forming an acid layer below the sample-digestion solution layer.

Total COD (TCOD) was run on unfiltered sample while soluble COD (SCOD) were run on samples sieved through 0.45 mm filter media followed by analysis to eliminate biological hindrances. Deionized water was used to dilute TCOD samples in a ratio of 1:5 before analysis. 2 mL of the samples, both for TCOD and SCOD were transferred into a COD digestion reagent vial. Mixing of the content was ensured by inverting the vial severally for about one minute. The vial was set up in COD chamber at 150°C for 2 hrs. Cooling and testing of the samples were carried out by a spectrophotometer. The total and soluble COD values were recorded in mg/L.

Gas Chromatography (GC) was applied to determine Volatile Fatty Acids **(**VFAs). The samples were acidified with nitric acid and VFAs extricated to diethyl ether phase and transformed into methyl esters which were then analyzed using GC. External

standard methodology was employed to quantitatively analyze the samples using calibration standards of five acids: caproic, acetic, valeric, propionic, and butyric in concentrations ranging from 5 to 1000 mg/ml.

3.3 Anaerobic Co-Digestion of Water Hyacinth with Slaughterhouse Waste for Biogas Production.

3.3.1 General Overview of Anaerobic Co-digestion Tests

This section of the study investigated biogas generation in co-digestion of water hyacinth (WH) from Lake Victoria with ruminal slaughterhouse waste (RSW). The co-digestion was conducted in batch digesters while biogas output was measured by displacement method.

3.3.2 Experimental Set-Up

The experimental setup comprised three round bottom 1,000 mL flasks and a graduated measuring cylinder (Figure 3-3). All the flasks were fitted with tight fitting rubber cocks for airtightness. The first flask was used as the reactor for anaerobic digestion. The reactor was fitted with a thermometer and a pH meter (HI98103 checker pH tester from Hanna Instruments) for monitoring temperature and pH respectively. A balloon with a needle inserted into the reactor headspace was set up to sample gas for characterization. The second flask contained a scrubber solution for CO_2 and other minor gases comprising 1 molar sodium hydroxide alkaline solution, prepared from 40 g sodium hydroxide dissolved in one liter of water. Three drops of phenolphthalein indicator were added for monitoring pH variation in the solution. The scrubber solution was replaced when the pink/violet color of the indicator turned colorless which is associated with a drop in pH below 8.2. The third flask was for gas displacement of water to measure the volume of gas generated. Water in the displacement bottle was

charged with a few drops of methyl orange to make it easier to read the volume in the graduated cylinder. The bottle was kept covered with an aluminum foil to minimize loss of water by evaporation.

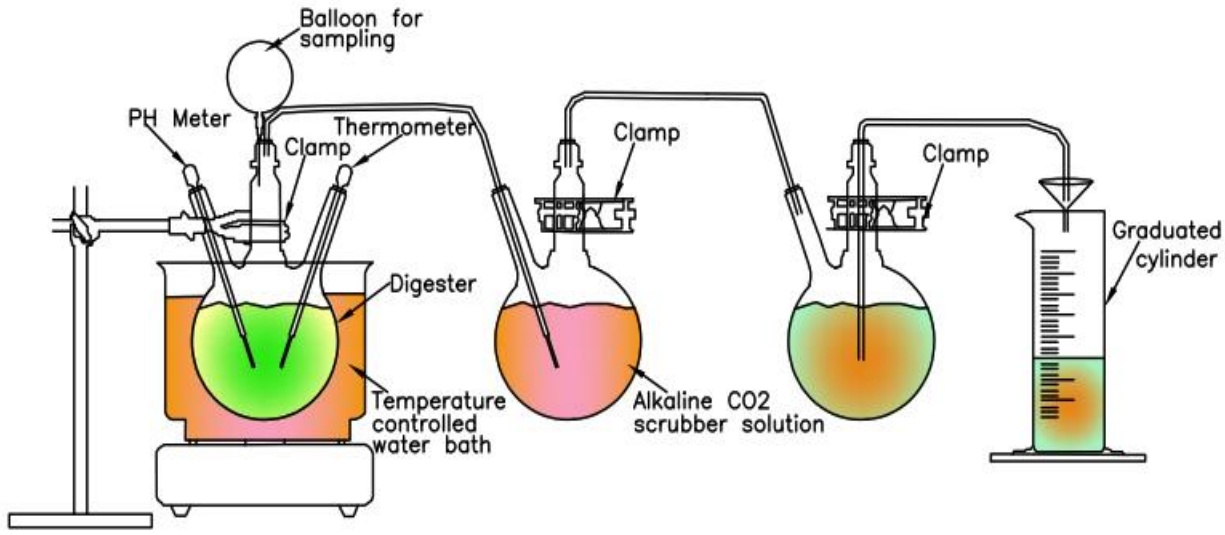

Figure 3-3. *Biogas production set up under water bath temperature control*

3.3.3 Materials and Instruments

This research was conducted using the following materials, tools and instruments: electronic weighing balance, electronic water bath, pH meter, 1-litre 3 neck round bottom flasks, 1-litre 2 neck round bottom flasks, mercury glass thermometer in the range of 0ºC to 100ºC, borosilicate desiccators, oven, silica glass crucibles, motars and pestles, balloons, 1000 ml graduated cylinders, tap water, rubber cork, 5 mm diameter clear connecting tubes and retort stands. Analytic grade sodium hydroxide and acetic acid were used as procured without any purification.

3.3.4 Anaerobic Digestion and Biogas Production

Substrate for bio-digestion were prepared by mixing 150 g of WH and RSW in different proportion with 500 mL of water in 1000 mL round bottom reactor flasks; a

total of eight reactor flasks labeled D1 to D8. The mix proportions used are illustrated in Table 3-1. The reactors were tightly sealed using rubber cocks and kept airtight to operate under anaerobic digestion mode for a duration of 60 days. The biogas generated was passed through a scrubber solution, as earlier explained. The volume of resultant methane gas was determined through water displacement method into a graduated measuring cylinder (Esposito et al., 2012). The cumulative volume of methane generated, pH and temperature were recorded daily at 9 am. Room temperature was also recorded throughout the test.

Gas for characterization was sampled in balloons through a needle in the headspace. Gas composition was determined in triplicate for each parameter, using a gas chromatograph fitted with flame indication detector (GCFID) (Sugumaran et al., 2014). The digesters were run at three different temperatures; namely, room temperature of about 24ºC, 32 and 37ºC.

Table 3-1. Mix Proportions of Dried Substrates

Digester	Water Hyacinth (g)	Slaughter-house waste (g)	Percent of co-substrate (%)
D1	150	Nil	0
D2	142.5	7.5	5
D3	135	15	10
D4	127.5	22.5	15
D5	120	30	20
D6	105	45	30
D7	75	75	50
D8	0	150	100

3.3.5 Biogas Characterization

The presence of methane defines the quality of biogas, whereby the more the methane, the better the biogas and vice versa. High methane content is therefore essential for maximum energy production. This study adopted gas chromatogram fitted with flame ionization detector commonly known as GC-FID for the analysis of biogas. The study used a PerkinElmer Collaboration (Clarus 680, USA) GC fitted with FID and capillary column (Elite-5, 30 m x 0.25 mm x 0.25 µL. The temperature for the column chamber, inlet chamber and the detector were set at 150, 200 and 250 ºC, respectively. Pure nitrogen, at a flow rate of 2.0 ml/min was adopted as the gas carrier. A split ratio of 20:1 was used to manage the quantity of biogas through the column to stop irregular peaks. Raw biogas volume of 500 µL was injected. Quantitative analysis were employed using the areas and the peak of the chromatograms to arrive at the percent composition of the biogas.

3.4. Dynamics of Microbial Communities in Co-Digestion of Water Hyacinth (*E. crassipes*) with Ruminal Slaughterhouse Waste in Batch Digesters

3.4.1. Overview of Microbial Analysis

This study investigated microbial communities in water hyacinth (WH) as a single substrate and in co-digestion with ruminal slaughterhouse waste (RSW). The dominant microbial communities at each of the digestion stages were studied by isolation, phenotype analysis, DNA extraction, PCR amplification of 16S rRNA gene and sequencing using basic local alignment search tool (BLAST) technique.

3.4.2 Isolation and Identification of Microbial Community

Isolation and identification of microbial community was carried out at the Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology (JKUAT). Microbial colonies were isolated by spread plate culture method and

identified using gram staining method. Approximately 1 g of the substrate collected from active digesters were weighed and transferred aseptically into a sterilized 250 mL conical flask with 90 mL of sterilized distilled water. The content was agitated gently at 150 rpm for 30 min to homogenize the samples and release the bacteria into solution. Simultaneously, nutrient agar media was prepared, autoclaved and placed into petri dish plates for inoculation. Serial dilution procedure was carried out up to 10^{-4}. The solutions were vortexed and 100 μL transferred onto plates and spread out uniformly. The inoculated plates were sealed for incubation at 37°C for 14 hrs. The organisms of interest were identified using colony morphology before being inoculated into sterile nutrient agar plates for detection of bacteria colonies. The plates were further incubated at 37°C for 12 hrs. Four distinct organisms were selected and streaked on to fresh plates. To avoid frequent culturing, pure bacterial colonies were preserved in glycerol awaiting DNA extraction and identification of different bacterial groups. The isolated colonies were prepared for DNA extraction and molecular identification of different bacterial groups.

3.4.3 DNA Extraction

Phenol chloroform procedures were used to extract DNA from the isolated bacteria colonies (Thikra, 2013). An overnight broth culture was centrifuged in a sterile tube at 6000 rpm for 5 min. The supernatant was disposed and the pellet re-suspended in 200 μL of TE (Tris EDTA) buffer and cells washed by vortex. The homogenate was centrifuged for 5 min at 6,000 rpm. The supernatant was disposed off and the pellet re-suspended in 200 μL of TE buffer. Approximately 20 μL of 10% SDS (Sodium dodecyl sulphate), 10 μL of lysozyme (20 μg/mL) and 10 μL RNase were added and mixed gently before incubation at 37°C for one hour. Approximately 10 μL proteinase

K was added and stirred gently and then incubated at 55°C for 1 hr. DNA extraction was conducted by adding the same volume of phenol/chloroform/isoamyl alcohol of ratio 25:24:1 to the sample. The samples were stirred gently by inversion then centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to a clean tube and a similar volume of chloroform/isoamyl (24:1) added, stirred gently and centrifuged. The DNA extraction with chloroform/isoamyl was repeated twice. The supernatant was transferred into sterile tubes and its volume determined. 2.5 mL of absolute ethanol was then added before the contents were mixed well and incubated overnight at -20°C. The samples were centrifuged at 10,000 rpm for 10 min and the supernatant disposed. 100 µL of 70% ethanol was added to the pellets and incubated at room temperature for 15 min. The samples were again centrifuged at 13,000 rpm for 10 min and the supernatant discarded. The pellets were dried in an incubator at 37°C for 30 min before suspension in 100 µL of ultra-pure water. The DNA solution was kept at 4°C. Electrophoresis of DNA was carried on 1% agarose gel in TBE buffer and 0.1 µL of ethidium bromide added. The gel was visualized under UV.

3.4.4 PCR Amplification of 16S sRNA gene

Several methods are available for quantification of microbial communities (Raskin et al., 1994; Akarsubasi et al., 2005; Stainberg and Regan, 2009). This study used the amplification of 16S rRNA and its genes. This method has been widely adopted as suitable biomarkers in determination of microbial populations in various environments (Takashi and Yuji, 2011). Amplification of DNA was carried out at the Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology. Bacterial 16S rRNA genes of the pure isolates were amplified using PeQlab advanced Primus 96 Hamburg thermal cycler (Applied Bio systems). Universal primer pair 8F forward 5'-AG (A/G) GTTTGATCCTGGCT-3' and 1492R- reverse, 5'-

CGGCTACCTTGTTACGACTT-3’ were used (e.g. Lane, 1991). DNA solutions were amplified by the polymerase chain reaction (PCR). The first denaturation was at 95°C for 5 min, second at 95°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s; 35 cycles were performed. The product was stored at -20ºC. PCR amplification was confirmed using Gel electrophoresis; the Gel was examined under UV trans-illuminator. Screening for bacterial diversity was conducted by sequencing using Basic Local Alignment Search Tool (BLAST) technique.

3.5 Data Analysis Methods

The results for biogas production were analyzed graphically using plotted line graphs where the trend and ultimate production were established within the retention time. pH data were also presented and analyzed graphically to determine the trends. Sequencing data for microbial community was analyzed using basic local alignment tool (BLAST) which uses probability technique to determine the most dominant organisms by similarity. The data for biogas characteristics were analyzed graphically using simple bar charts.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Characterization of Substrates

4.1.1 Proximate Analysis

Results of proximate analysis of water hyacinth (WH) and ruminal slaughterhouse (RSW) are presented in Table 4-1. Carbohydrates, ash, crude fiber and crude protein accounted for 28.2%, 25.7%, 15.6% and 10.3%, respectively in the WH. Comparatively, RSW had larger concentrations of carbohydrates, crude fiber and crude protein which accounted for 45.8%, 18.4% and 16.8%, respectively but had less ash content of (15.7%). The large concentration of carbohydrates and fiber in WH and RSW biomasses signify potential for biogas production with RSW having a greater potential than WH (e.g. Funda, 2011). However, conversion efficiency of such biomass characteristic largely depends on the operation conditions (Fayyaz et al., 2017).

Table 4-1: Proximate composition of crude protein, fiber, fat, ash and carbohydrate in water hyacinth (WH) and ruminal slaughterhouse waste (RSW)

	Parameter				
Biomass	Crude Protein (%)	Crude Fiber (%)	Crude Fat (%)	Ash (%)	Carbohydrates (%)
Water hyacinth	10.3 ± 0.03	15.6± 0.27	0.73± 0.02	25.7± 0.40	28.2 ± 0.02
Slaughterhouse waste	16.8± 0.29	18.4± 0.18	0.47± 0.35	15.7± 0.13	45.8 ± 0.08

Crude fat content was higher in WH, (0.73%) compared to RSW (0.47%). Crude fat has the potential for significant conversion to biogas but with longer retention time in

the reactor (Kris et al., 2016). However, concentrations of fats exceeding 3 g/L may inhibit methanogenic bacteria and hamper methane gas production (Rasit et al., 2015).

The smaller concentration of crude protein recorded for water hyacinth of 10.3% compared to 16.8% for RSW indicates less nitrogen concentration, and suggests larger C/N ratio in WH compared to RSW. High C/N ratio signifies nitrogen deficiency where carbon remains unutilized leading to reduced biomethanation.

Water hyacinth had approximately 10% more concentration of ash than RSW, which indicated that it had less biodegradable matter.

4.1.2 Crude Fiber Characteristics

The concentration of cellulose, hemicellulose and lignin in WH biomass were 33.1%, 23.2% and 9.9%, respectively and 35.3%, 16.8% and 25.5% in cellulose (Figure 4-1). Cellulose and hemicellulose (holocellulose) accounted for about 56% of WH biomass and 52% for RSW. The large concentration of holocellulose in the WH suggested a large potential for biogas production under anaerobic system (Odhner et al., 2012). The smaller concentration of lignin for WH of 10% compared to RSW 25.5% suggests that WH biomass may biodegrade faster than RSW. Moreover, the lignin concentration for WH is less than a third that of common wood of 25-36%, which implies that it is a better feedstock for biogas generation (Ayeni, et al., 2015).

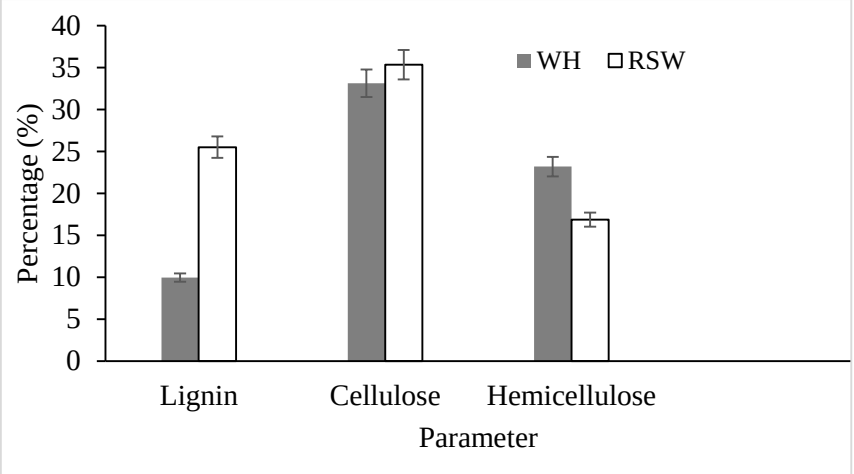

Figure 4-1. Crude fiber concentrations in WH and RSW

4.1.3 Elemental Characteristics

The results of elemental characteristics of water hyacinth (WH) and ruminal slaughterhouse waste (RSW) are summarized in Table 4-2. Elemental composition of substrates gives an indication of nutrients balance in anaerobic digestion.

Table 4-2: Elemental composition for water hyacinth and ruminal slaughterhouse waste

Biomass	**Parameter**					
	Nitrogen (N) (mg/L)	Carbon (C) (mg/L)	Potassium (K) (mg/L)	Phosphorus (P) (mg/L)	C/N Ratio	C/P Ratio
Water hyacinth	1,650± 60	15,480± 350	137± 0.03	50± 7	9.38:1	309:1
Slaughterhouse waste	1,390 ± 260	26,220± 600	7,476± 19.7	40± 1	18.8:1	655:1

The carbon to nitrogen ratio (C/N) for water WH substrate was 9.38 while that for RSW was 18.8. The C/N ratios for the two substrates fall within the recommended 8-20 range for optimal biogas production (Kossmann et al., 2007). However, the C/N

ratio for WH was near the lower limit, below which it can cause ammonia inhibition (Mata-Alvarez, 2000). Conversely, the C/N ratio for RSW was near the upper limit, above which it can cause deficiency of nitrogen and result in lower gas production (Mata-Alvarez, 2000). Managing C/N ratio to an optimum range of 8-20 can be achievable by co-digestion of biomass of diverse characteristics (e.g. Monnet, 2003).

Potassium concentration in water hyacinth and slaughterhouse waste was 137 and 7,476 mg/L respectively. Potassium is optimal in the range 200-400 mg/L, moderately inhibitory at 2,500-4,500 mg/L and extremely inhibitory at above 12,000 mg/L (Gregor and Victor, 2012). Consequently, potassium concentration in water hyacinth was below the optimal range while that in ruminal slaughterhouse waste was in inhibitory range. Mixing the two substrates would balance the potassium levels to the optimal range thereby stimulating biomethanation.

The phosphorus concentrations were 3% and 2.8% of nitrogen for WH and RSW, respectively, and 0.32 % and 0.15% of carbon for WH and RSW. Consequently, the concentrations for both substrates were below the requirement for optimal performance of 15% nitrogen (Britz et al., 2003) and 0.7% carbon (Piyarat et al. 2014). Alternatively, carbon to phosphorus (C/P) ratios for WH and RSW were 309:1 and 655:1 respectively. A C/P ratio of about 100:1 is suitable for the hydrolysis and acidogenesis step of biogas generation while that of about 150:1 is suitable for methanogenesis (Gregor and Victor, 2012). On average, C/P ratio of 150:1 would be suitable for optimal performance. Therefore, both WH and RSW showed P deficiency with RSW having greater deficiency. Co-digestion of the two substrates may not remove P deficiency but it would reduce the greater deficiency in RSW.

4.1.4 Biochemical Characteristics

Table 4-3 shows the mean ruminal slaughterhouse waste and water hyacinth biochemical characteristics. The concentration of total solids (TS) for slaughterhouse waste and water hyacinth were 244.1 and 1.9 g/kg respectively. Organic matter (VS) accounted for 72% and 57% TSS in RSW and WH, respectively whereas fixed solids (FS) and ash accounted for 16% and 43% of TSS. TCOD concentrations in RSW and WH were 304 g/L and 1.3 g/L respectively, indicating high and low organic matter in the substrates. Ruminal slaughterhouse waste had 98.7 g/kg VSS compared to 1 g/kg for water hyacinth, suggesting high organic content and microbes in the substrate, largely attributed to the rumen content. The VSS is an indication of prescence of microorganism (Onofre et al., 2013). Because rumen microorganisms are anaerobic, this portion of the substrate can act as inoculum in the reactors leading to early digestion and reduced retention times (Emine et al., 2018). The significant content of microbes also signifies more biogas potential (Shikilkar et al., 2017).

Table 4-3. Total solids (TS), volatile solids (VS), total suspended solids TSS), volatile suspended solids (VSS); total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) concentrations, volatile fatty acids (VFA), and pH for RSW and WH biomass

Biomass	**Parameter**							
	TS (g/Kg)	VS (g/Kg)	TSS (g/Kg)	VSS (g/Kg)	TCOD (g/L)	SCOD (g/L)	VFA (g/L)	pH
RSW	244.1±8.4	174.6 ±3.6	147.3 ±1.5	98.7 ±2.8	304.0 ±0.0	272.0 ±9.2	117.6 ±13.5	7.1 ±0.09
WH	1.9 ±0.06	1.1 ±0.15	1.8±0.17	1.0±0.84	1.3±0.1	0.7 ± 0.1	N/A	6.4±0.1

4.2 Co-digestion of Water hyacinth with Ruminal Slaughterhouse Waste

This section highlights results of biogas generation in anaerobic digestion of WH and RSW separately and in co-digestion at various mix proportions. The results cover variation in digesters pH with residence time and temperature for various substrates mix proportion. The Section further presents variations of the quality of biogas produced with WH and RSW mix proportions.

4.2.1 Variations of pH with duration of Co-Digestion

The pH of bio digestion reactor contents varied with retention time and mix proportion of the co-substrates WH and RSW first at 24 ˚C for various mix proportions followed by 30% RSW at 24, 32 and 37 ˚C as shown in Figure 4-2. RSW proportion of less than 15% had pH drop below 6.5, after day 7 up to day 25 (Figure 4-2a). The pH however was always above 6.8 for the RSW proportions of 20 -100%. Varying the RSW proportion from 15 to 20% resulted in greatest increase in the hydrolysis pH from about 6.2 to 6.8 during hydrolysis dominated period (day 7 to day 20). The reduction in duration of hydrolysis step with increasing RSW proportion, from 33 days for 5% RSW to 25 days for 50%, correlates with observation of previous studies (e.g. Feng et al., 2009) and maybe an indication of prolonged acidogenesis step that affected the methanogenesis step.

Anaerobic digestion of WH and RSW at different temperatures; namely 24, 32 and 37°C for 30% RSW showed varied changes in pH with time (Figure 4-2b). After the seventh day, when pH was similar for all the reactor temperatures, there was a clear pattern of greater increase of pH with temperature, which was attributed to increased formation of ammonium ions with the higher temperature.

The various steps of anaerobic digestion take place at different pH and, therefore, the pH of the digesting substrates can give an indication of the dominant digestion step and its duration. Generally the first digestion step, hydrolysis of lipids and protein to volatile fatty acids and amino acids, resulted in a drop in pH. The onset of acetogenesis step resulted in rise in pH, which may be attributed to production of CO_2 and NH_3 and the associated CO_3HNH_4 (e.g. Malakahmad et al., 2012). Further rise in pH occurred in the predominantly methanogenesis step because of diminished hydrolysis of volatile fatty acid and continued production of CO_3HNH_4.

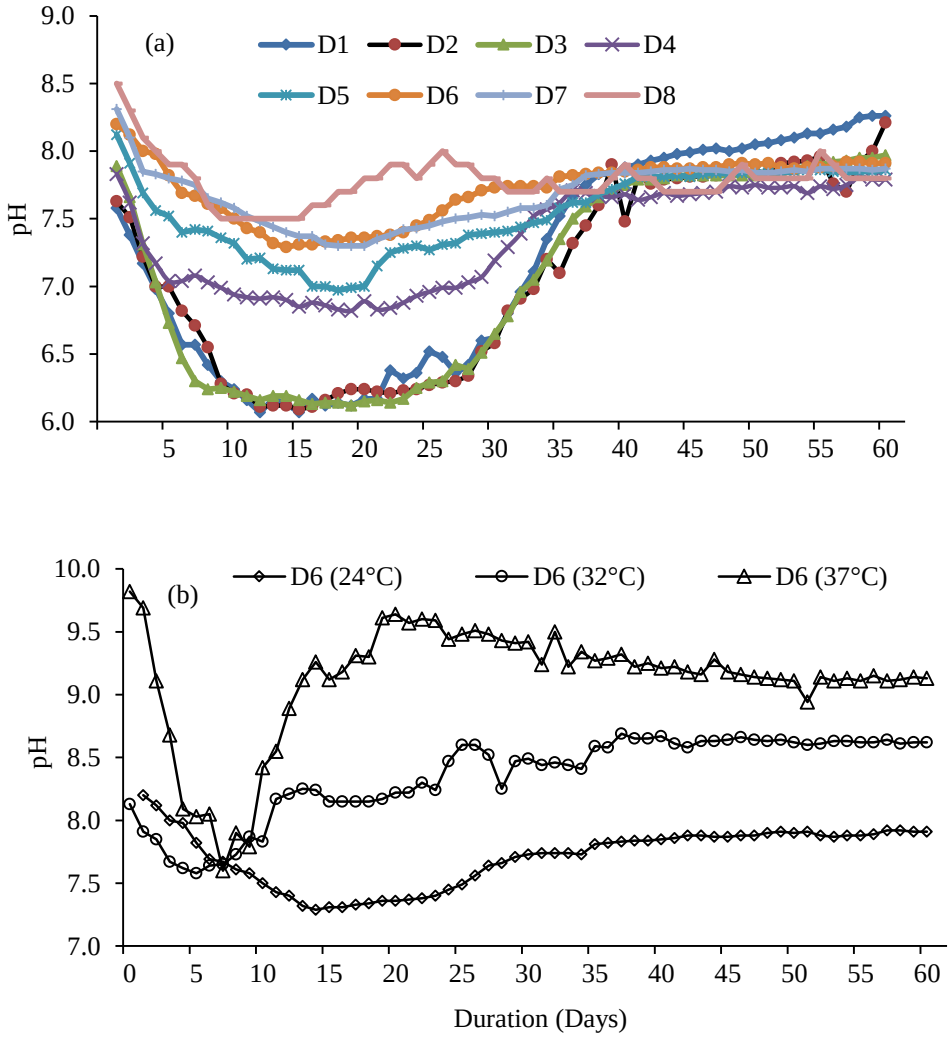

Figure 4-2. pH variations for (a) Ruminal Slaughterhouse Waste substrate proportions (D1=0%, D2=5%, D3=10%, D4=15%, D5=20%, D6=30%, D7=50%, D8=100%) at 24°C; and (b) 30% RSW mix proportion at 24, 32 and 37°C

4.2.2 Biogas Production for Various Substrates Mix Proportion

Variations in cumulative biogas production over 60 day residence time for various proportions of WH and RSW in reactors operated at room temperature are presented in Figure 4-3. Water hyacinth alone produced 8.0 liters of methane (CH_4) per kg of substrate, which was 45% of the 17.8 L CH_4/kg observed for RSW. Co-digestion of WH with 30% RSW achieved 79% of the yield for 100% RSW suggesting synergy in co-digestion. During the first seven days, all digesters exhibited initial rapid biogas production, which may be attributed to high volatile solids originally present in WH biomass. Subsequently, there was a lag in biogas production for all the digesters except for the 50% and 100% RSW. The lag maybe attributed to production of volatile organic acids during hydrolysis and acidogenesis that reduced pH and inhibited methanogenic organisms (Lukitawesa et al., 2020). The lag lasted 20 to 25 days for 0 to 15% RSW mixture and 0 to 10 days for 20 to 100% RSW mixture. Resumption of biogas generation was linked to consumption of volatile acids and reformation of bicarbonate buffer during methane formation (Karlsson et al., 2014). However, the 50 and 100% RSW showed less defined lag in biogas production, which could be related to activity of microorganisms in the volatile solids.

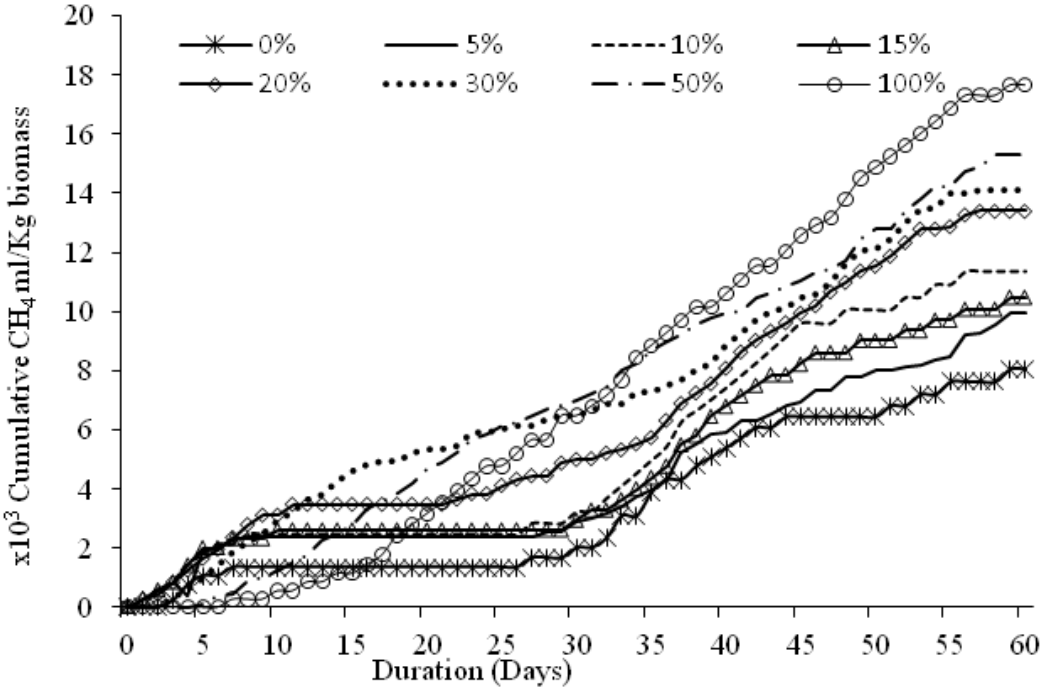

Figure 4-3. Biogas production for various mix proportions at 24 °C

The 30% RSW co-digestion exhibited the most stable biogas production throughout the retention period. The result was attributed to complementing of process parameters. Inclusion of RSW in the digestion may have collated some process parameters by reducing ammonia toxicity caused by depressed C/N ratio, reducing the C/P ratio that was in inhibitory range, and increasing buffer capacity. Achieving balanced conditions requires careful selection of co-digestion substrates to overcome the low pH lag period and achieve a stable anaerobic digestion process (Kugelman and Chin, 1971).

4.2.3 Impact of Temperature on Biomethanation

Co-digestion of WH and RSW at 30% RSW proportion at temperatures of 24, 32 and 37ºC resulted in marked improvement of biomethanation rate from 0.23 at 24 ºC to 0.75 and 0.96 $x10^3$ ml-kg/day at 32 and 37ºC respectively. Increasing the temperature from 24 to 32 ºC increased methane cumulative yield from 14 to 40 L/kg, a 186%

increase, but increasing it to 37ºC only yielded a further 30% to 52 L/kg (Figure 4-4). Consequently, there may be no merit in further increase of operating temperature from 32 to 37 ºC unless the benefit of the gas production and reduced capital cost exceeds the extra cost of energy.

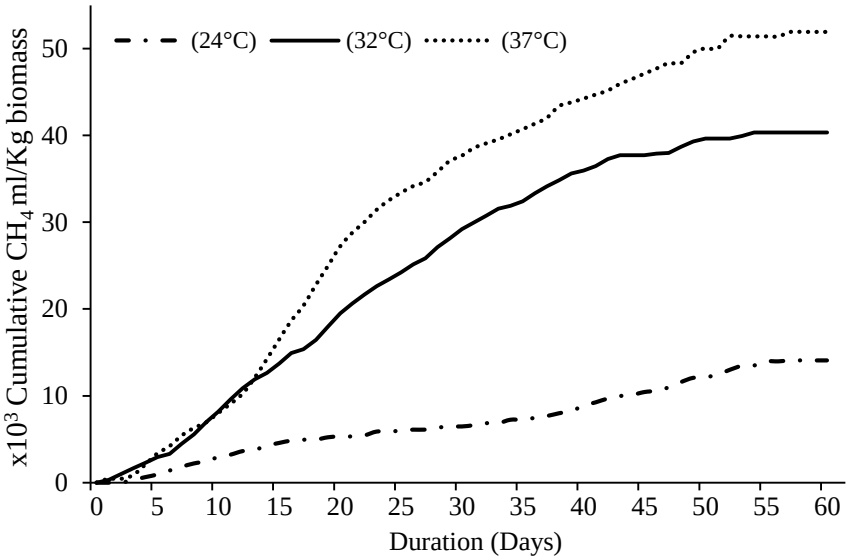

Figure 4-4. Cumulative methane gas production for 30% RSW at 24, 32 and 37°C operating conditions.

4.2.4 Biogas Characterization

The proportions of methane, CO_2 and trace gases in the biogas for various WH-RSW mix proportions are presented in Figure 4-5. The proportion of methane gas increased with increase in RSW in the substrate mix from 59% for WH alone (0% RSW) to a maximum value of 68% at 30% RSW and then decreased to 58% for RSW alone (100% RSW). The proportion of trace gases in the biogas was less 4% for all the WH-RSW mix proportions. The 30% RSW mix produced the highest biogas quality with 68% methane, 30% CO_2 and 2% trace gases. The results illustrate that co-digestion can enhance overall biogas output and methane proportion. Similar results were

achieved through co-digestion of biodiesel waste and glycerin with municipal waste sludge which resulted in 100% rise in biogas yield with 20% increase in methane yield (Anahita et al., 2018).

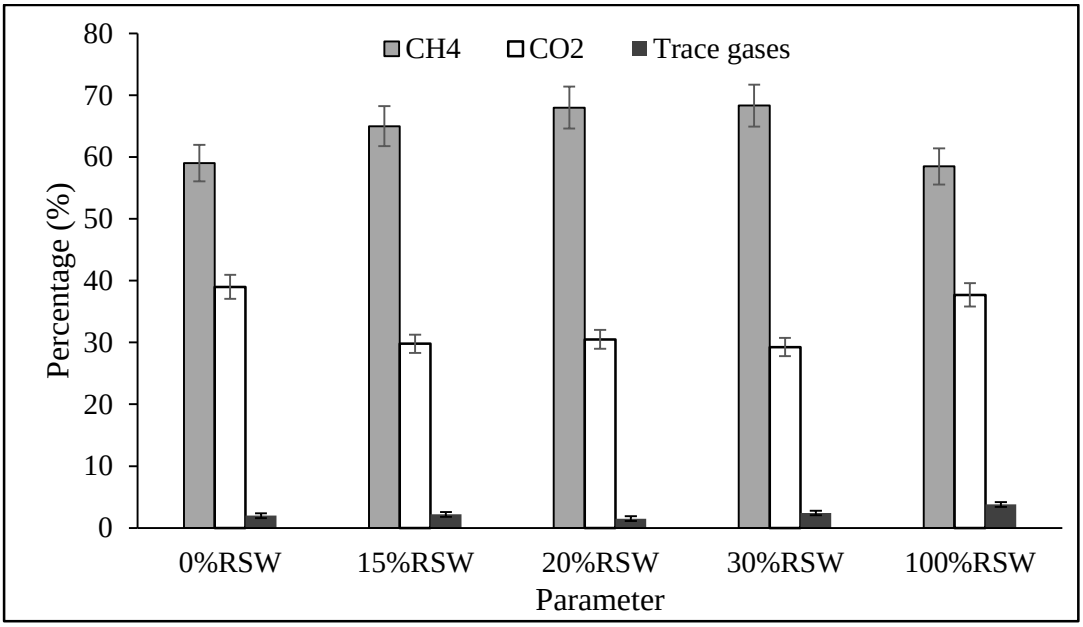

Figure 4-5. Composition of biogas for different WH-RSW co-digestion mix proportions at 24°C

4.3 Microbial Community Dynamics

4.3.1 Sampled Microbial Communities

Dynamics of microbial communities in co-digestion of WH with RSW were evaluated at 32˚C for RSW proportions of 0, 30 and 100%, digester operation days 5, 20 and 35 and the biomethanation profiles and associated pH shown in Figure 4-6. Day 5 samples characterized acclimatization of microbial community where fermentation process began and culturing of methane producing microbes occurred. In this stage, the three digestion configuration exhibited steady biogas production but declining pH. The Day 20 samples were characterized with stagnation in biogas production for both of the single substrates and depressed pH of down to 6.1 for RSW alone. The 30% RSW co-digestion experienced moderate drop in pH to 7.5 but unsteady biogas production. The

operating environment at 20 days was associated with acid formation that lowered the pH inhibiting methanogenic microorganisms and resulting in lag in biogas production. The third sampling at Day 35 was generally characterized by recovery of pH to the alkaline range and steady biogas production.

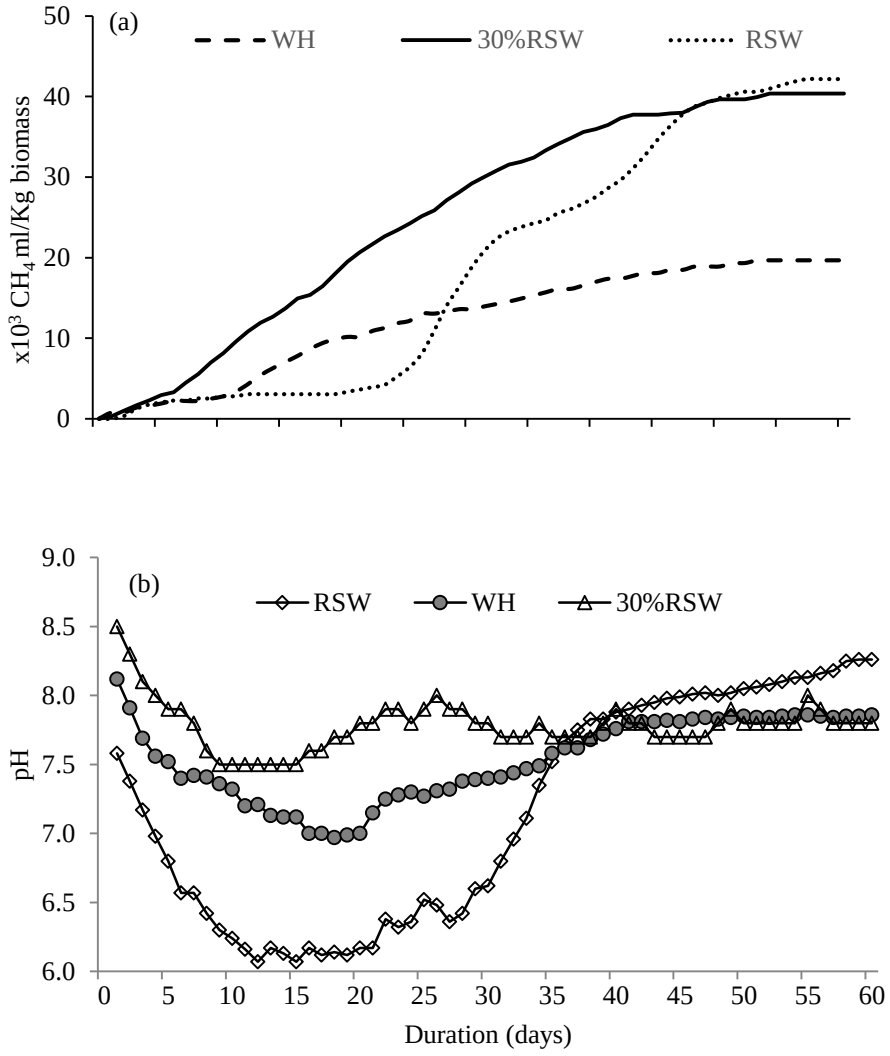

Figure. 4-6. (a) Cumulative Biogas production for WH, 30% RSW and RSW substrates at 32°C with sampling on day 5, 20 and 35. **b).** Associated pH variations

4.3.2 Morphological Characteristics of Bacteria Isolates

The morphology of microbial communities present in the digestion of WH, RSW separately and in co-digestion with 30% RSW were studied by isolation of colonies

from digester sludge collected at days 5, 20 and 35. Most colonies aggressively grew within two to three days of incubation (Plate 4-1). Morphologies of the isolates were diverse, spanning from circular to flat to filamentous. The colors also ranged from white to cream and bluish clear with elevations categorized as cocci, short rods, long rods, bacilli long and streptococcus (Table 4-4). About 77% of the isolates were Gram positive, mostly spore-forming, while 23% were Gram negative. The results indicated possible dominance of the largely Gram positive spore-forming Firmicutes phyla that comprises of the Bacilli and Clostridia classes. Overall, the morphological characteristics did not reveal definite distinction between the two substrates and their mixture or trend with retention times. Instead, morphological characteristics were distributed generally across substrates and retention times.

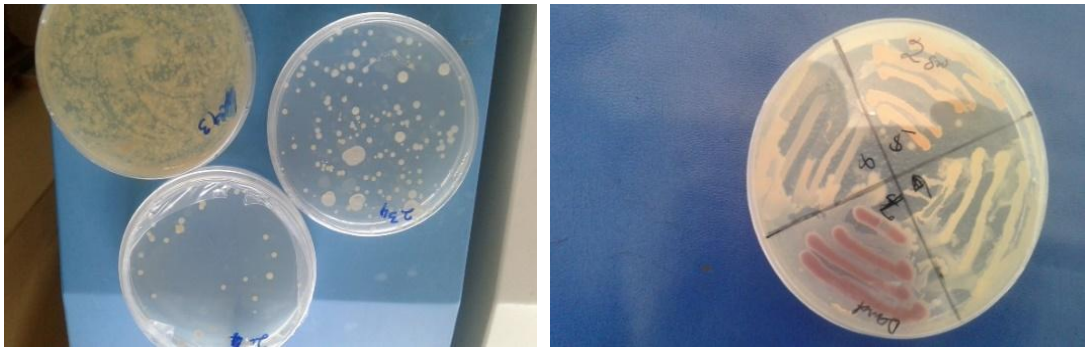

Plate 4-1. Images of colonies of bacteria isolates growing on culture media

Table 4-4. Morphological Characteristics of bacteria isolates for (a) WH, (b) 30%RSW and (c) 100% RSW substrates

Sample No	Sample Source	Day of Sampling	Org No	Gram Status	Mophology
1	WH	5	5	-	Cocci
2	RSW	5	6	+	Rods (non- spore formers)
3	WH	5	7	+	Rods (septate)
4	RSW	5	1	+	Rods terminal spore
5	WH	5	2	+	Bacilli long branching
6	WH	5	4	+	Rods round terminal spore
7	RSW	35	2	+	Rods short (small)
8	RSW	20	3	+	Spores with terminal central septate
9	RSW	20	6	(-) &(+)	Cocci & rods mix
10	RSW	20	5	-	Rods central spore
11	WH	20	8	+	Rods in chain & spores
12	WH	20	5	+	Rods in chain
13	WH	20	8	+	Streptococcus
14	RSW	20	1	+	Rods short
15	RSW	20	4	+	Rods long -terminal spore
16	RSW	20	6	+	Rods sub terminal spores
17	30%RSW	20	5	+	Rod terminal spores
18	30%RSW	20	10	+	Rods
19	RSW	20	3	-	rods
20	WH	35	1	+	Staphylococcus
21	WH	35	2	+	Streptococcus
22	30%RSW	35	3	+	Rods branched chains
23	30%RSW	35	1	-	cocci
24	30%RSW	35	2	+	Rods sub terminal spores
25	30%RSW	35	4	-	Rods non sporelating
26	WH	35	6	+	Rods round central spore
27	30%RSW	35	4	+	Rods in pairs sub terminal spore
28	RSW	35	1	+	Rod thin long
29	RSW	35	4	+	Rods in pairs sub terminal spore
30	WH	20	8	+	Rods in chain and spores

4.3.3 Diversity of Methanogenic Bacteria Isolates

Screening for bacterial diversity was carried out by Basic Local Alignment Search Tool (BLAST) for day 5, 20 and 35 that represented acclimatization, acidic stagnant and active biomethanation stages. All samples had clear DNA gel and PCR amplified images as visualized under UV and UV – trans-illuminator, respectively (Figures 4-7 and 4-8). Table 4-5 presents the most likely identity of the observed bacteria communities in the three reactors. Generally, diverse *Bacillus sp.* dominated the alkaline pH of acclimatization and active biomethanation phases. On the other hand, *Lysinibacillus* and *solibacillus sp.* dominated acidic and low alkaline pH, which were associated with low biogas yield.

At day 5, all the three reactors had alkaline pH of 7 to 8.1 (Figure 4-3a). The microbes observed in the WH reactor were *Alcaligenes faecalis*, *Bacillus sp. (thurigiensis, toyonensis* and *cereus). Alcaligenes faecalis*, is a Gram-negative bacterium of Proteobactria phylum known for its ability to aerobically desaturate fatty acids to mono-saturated fatty acids (Ghaneker and Nair, 1973). This bacterium may have used the initial oxygen in the reactor to degrade the fatty acids, which in concert with other microbes, contributed to the initial gas production noted in all three reactors.

Anaerobic digestion day 20 of anaerobic digestion was characterized by stagnant biomethanation for RSW in acidic pH but active biomethanation for co-digestion and WH substrates in alkaline environment. Microbes present in the RSW reactor with acidic pH were predominantly *Lysinibacillus sp. mangiferihumi, sphaericus,* and *fusiformis, and solibacillus* genus that are of the same class *bacilli* as *bacillus* in Firmicutes phylum. Both genus were also observed by Zainudin et al. (2014) in decomposition of ligneous oil palm empty fruit bunch. *Lysinibacillus sp*. are diverse

group of bacteria that inhabit various environments including farming soil and factory wastewater and grow under general pH range of 5.5–9.5 with optimum pH of 7.0–8.0 (Ahmed et al., 2007). The bacteria are Gram-positive, mesophilic and rod-shaped and typically facultative anaerobes (Todar, 2012). Low pH conditions have a negative impact on generation of bacillus species hindering anaerobic process (Ivanova et al., 2003). Some studies have indicated that benzoic and propionic acids as well as esters of p-hydroxybenzoic acid (parabens) can make bacillus dormant however, their vibrancy may be pH dependent (Wipat et al., 1999). The dominance of *Lysinibacillus and solibacillus* genera in acidic environment and the near absence of *Bacillus* genera indicate a shift in microbial community in the lag phase to adjust to the acidic pH. However, the acidic pH inhibited the action of methanogens leading to stagnation in biomethanation. Co-digestion avoided the drop in pH for both substrates, and therefore changed the microbial community, which allowed the *bacillus sp*. to continue with the biomethanation process in consort with methanogens.

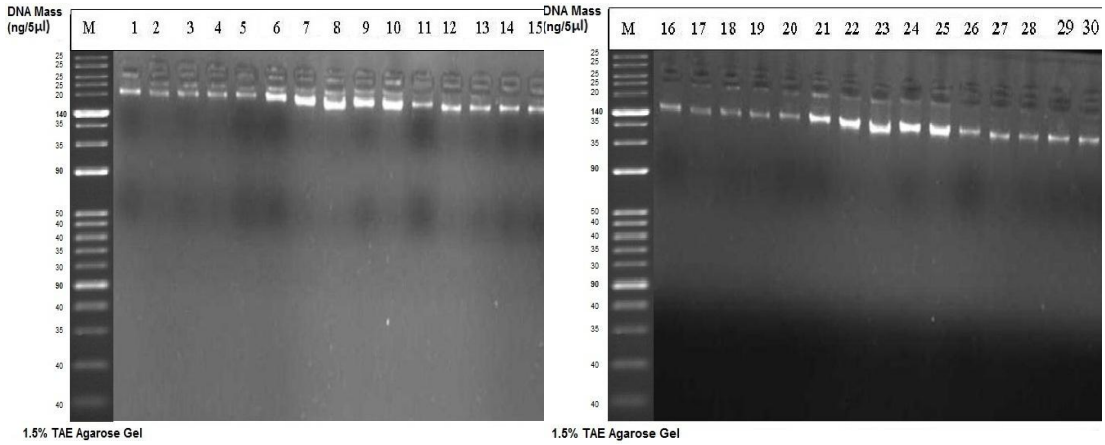

Figure 4-7). Geonomic DNA images for water hyacinth (WH), Ruminal slaughterhouse waste (RSW) and 30% RSW sludges. Lines; M =Hind III marker, 1, 3, 5 and 6 =WH day5, 2 and 4 = RSW day5, 11, 12, 13 and 30 = WH day20, 8, 9, 10, 14, 15 and 19 = RSW day20, 17 and 18 = 30%RSW day20, 20, 21 and 26 = WH day 35, 7, 28 and 29 = RSW day 35, 22, 23, 24, 25 and 27 = 30% RSW day35.

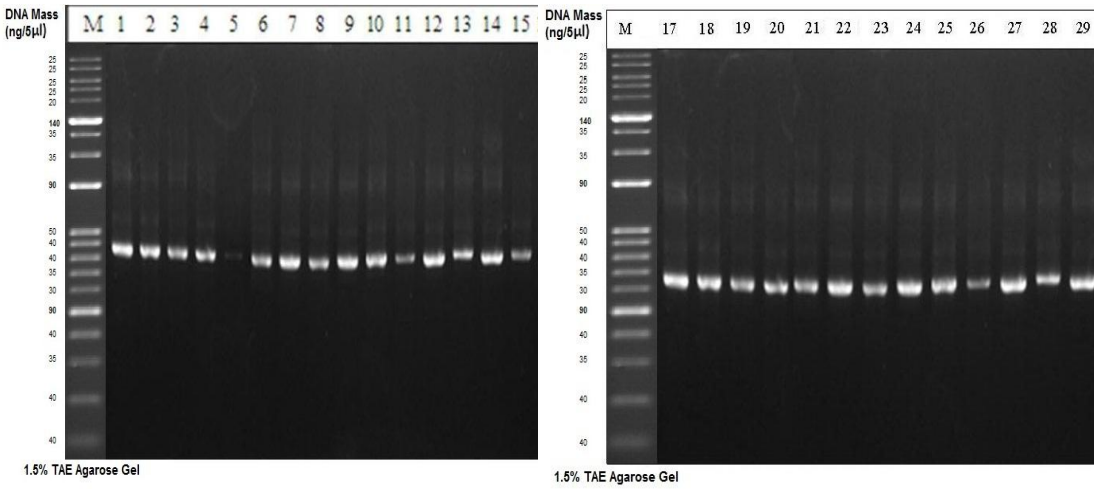

Figure 4-8). Agarose gel photos showing PCR amplification of DNA samples (1-30) extracted from WH, RSW and 30% RSW reactors with universal 16 rRNA primers. Lines; M =Hind III marker, 1, 3, 5 and 6 =WH day5, 2 and 4 = RSW day5, 11, 12, 13 and 30 = WH day20, 8, 9, 10, 14, 15 and 19 = RSW day20, 17 and 18 = 30%RSW day20, 20, 21 and 26 = WH day 35, 7, 28 and 29 = RSW day 35, 22, 23, 24, 25 and 27 = 30% RSW day35.

Table 4-5: Identity of reactor bacterium at various stages of anaerobic digestion.

Sample ID	Sampling Day	Source	Percent identity/ similarity	Likely microorganism (s)
1_8F	5	WH		*ND*
3_8F	5	WH	98.82%	*Alcaligenes faecalis*
5_8F	5	WH	99.61%	*Bacillus thurigiensis , Bacillus cereus*
6_8F	5	WH		*ND*
2_8F	5	RSW		*ND*
4_8F	5	RSW	100%	*Bacillus sp(toyonensis, cereus, thurigiensis)*
30_8F	20	WH		*ND*
18_8F	20	30%RSW	97.38%	*Bacillus aerophilus, Bacillus pumilus*
17_8F	20	30%RSW	100%	*Lysinibacillus mangiferihumi, Lysinibacillus fusiformis*
9_8F	20	RSW	98.99%	*Lysinibacillus mangiferihumi, Lysinibacillus fusiformis, Bacillus sp.*
10_8F	20	RSW	99.27%	*Lysinibacillus fusiformis*
14_8F	20	RSW		*ND*
15_8F	20	RSW		*ND*
16_8F	20	RSW	98.48%	*Lysinibacillus sphaericus*
19_8F	20	RSW		*ND*
26_8F	35	WH	98.97%	*Bacillus sp. , Bacillus aquimaris*
21_8F	35	WH	99.38%	*Lysinibacillus mangiferihumi, Bacillus sp.*

22_8F	35	30%RSW		*ND*
23_8F	35	30%RSW	99.68%	*Alcaligenes faecalis*
24_8F	35	30%RSW	99.87%	*Bacillus cereus, Bacillus thuringiensis*
25_8F	35	30%RSW	100%	*Bacillus licheniformis, Bacillus glycinifermentans*
27_8F	35	30%RSW	99.85%	*Lysinibacillus mangiferihumi, Lysinibacillus fusiformis*
28_8F	35	RSW	90.71%	*Bacillus licheniformis*
29_8F	35	RSW	99.81%	*Bacillus sp. , Bacillus pumilus*

ND means No Detection.

In the WH and RSW reactors, biomethanation resumed after 7 and 20 days of stagnation. Taconi et al. (2008) found that the methanogens can operate in acidic environment provided they had sufficiently long retention time to acclimatize. However, this study observed emergence of *Lysinibacillus* and *Solibacillus* species. The observation of *Solibacillus sp* at acidic pH of 6.0 - 7.0 confirmed reports by Sielaff et al. (2017) who found a strain of the genus growing at a pH range of 6.0 to 10. Similar *Solibacillus sp* utilization of volatile acids in the RSW reactor may have been responsible for removal of acidity and recovery of biomethanation process.

On Day 35, all the reactors had alkaline pH of about 7.4 and depicted active biomethanation (Figure 4-3a). The reactors had diverse microbial community dominated by *Bacillus sp.* that included, *Bacillus aerophilus, Bacillus pumilus, Bacillus cereus, Bacillus thuringiensis, Bacillus licheniformis, Bacillus glycinifermentans, Bacillus aquimaris, and Staphylococcus xylosus* and also some *Lysinibacillus*. These species are known to grow in alkaline environment; for example, *Bacillus licheniformis* is cultured in alkaline conditions to obtain protease for use in biological laundry detergent that has an optimum pH of around 9 and 10. (Alya et al., 2008). Similarly, *Bacillus fusiformis* and *Bacillus sphaericus* have been found to dominate a pH between 7.0 - 8.0 (Ahmed et al., 2007). *Bacillus* genera are known to

play a significant role in biogas generation (e.g. Horváth et al., 2016). Rabah et al., (2010) observed *Bacillus megaterium, Bacillus licheniformis, and Bacillus pumilus* in biogas production using slaughterhouse waste as the inoculum. The outcome of this study also concur with that of Onwuliri et al., (2016) where microbial isolates responsible for biogas production from cattle manure included *Bacillus licheniformis, Escherichia coli and Clostridium sp.*

Single substrate digestion of WH exhibited a lag phase of up to seven days and a lower biogas yield of 45% that of RSW. Although, RSW performed better in biogas yield, it had a long stagnation phase of up to 20 days that was associated with acidic reactor pH. This study identified gram positive syntrophic phyla, firmicutes, particularly the *Bacilli* class as the key players in the digestion process at the acidic pH associated with stagnation in biomethanation. While phenotype analysis did not reveal distinctive pattern with the three biomethanation phases of acclimatization, acidic stagnation and active biomethanation phylogenetic identification observed a clear shift in microbial community from predominantly *bacillus genus* to *lysinibacillus* and *solibacillus* during stagnation stage and back to *bacillus* genus in active biomethanation.

The microbial community varied with the pH conditions from diverse *Bacillus* species in the initial alkaline condition to predominantly *lysinibacillus sp*. and *Solibacillus sp.* in acidic pH. The tolerance of *Bacillus* bacteria allowed syntrophic reactions to proceed neutralizing acidity and allowing active methanogenesis. After acclimatization of the methanogens (e.g. Taconi et al., 2008), their activity resulted in consumption of fatty acids leading to increase in pH to the alkaline zone where diverse microbial community dominated by *Bacillus Sp*. emerged leading to active biomethanation.

The synergistic role of WH in anaerobic digestion appear to be maintaining the microbial community, which helps in avoiding low pH lag phase. The resulting operating environment allowed the minor substrate, RSW, to play complementing role in co-digestion throughout the retention time resulting in consistent biogas generation.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

This study characterized water hyacinth and ruminal slaughterhouse waste, investigated synergy in co-digestion of the two substrates in biogas generation and dynamics of microbial community associated with anaerobic digestion. The study concludes the following:

1. Water hyacinth Fiber and proximate characteristics of 33.1 and 23.2% of cellulose and hemicellulose, respectively and lesser lignin concentration of 9.9 % that is desirable in biomass for biogas production indicated that it is a viable substrate for biogas production

2. Co-digestion of WH with RSW can potentially balance the C/N ratio of WH of 9.4 that tended towards ammonia toxicity, with that of RSW of 18.8 that tended towards nitrogen deficiency to near the middle of the 8-20 optimal range for biogas generation; similarly, co-digestion has potential to balance the WH concentration of potassium of 137 mg/,L which was deficient with that of RSW of 7,476 mg/L that was in the inhibitory range to within or near the 200-400 mg/L optimal range for biogas production. .

3. Co-digestion of WH with RSW 5 to 30% RSW mix proportions increased biogas production by between 25 and 75% to up to 14.1 L/Kg biomass, and improved biogas quality by increasing concentration of methane gas by 9% from 59 to 68%.

4. Varying temperature from 24 to 32ºC had a significant positive impact in the biogas yield by 186% for co-digestion of WH with RSW biomass at 30% RSW but less increase of 32% from 32 to 37ºC.

5. Microbial community varied from *Bacillus* genus that thrived at the initial alkaline pH to acid tolerant *Lysinibacillus* and *Solibacillus* species with drop in pH to acidic range 7.0-6.0 that inhibited methanogenesis before re-emergence of diverse Bacillus species on restoration of alkaline conditions; co-digestion of WH and RSW avoided the acidic conditions and maintained active biomethanation Bacillus species throughout the retention time, increasing biogas yield.

This study recommends that:

1. Future studies evaluate biogas production outcome between 24 and 32˚C.
2. Future studies should investigate methanogenic archaea in digestion of WH and RSW separately and in co-digestion.

REFERENCES

Adney, W. S., Rivard, J. C., Shing, M. & Himmel, M. E. (1991). ***Anaerobic digestion of lignocellulosic biomass and wastes. Celluloses and related enzymes.*** Appl. Bio- chem. Biotechnol., 30, 165-183.

Afazelia H. Jafaria A., Rafieea S. Nosrat M., (2014). ***An investigation of biogas production potential from livestock and slaughterhouse wastes.*** Renewable and Sustainable Energy Reviews Volume 34, June 2014, Pages 380-386.

Agbo, B. E., Asikong, B. E., Epoke, J., Antai, E. E., and Eja, M. E., (2013), ***Four Potentials of biogas yield from cow dung-CD,*** European Journal of Experimental Biology, 3, (3):273-282

Ahmed I, Akira Yokota, Atsushi Yamazoe and Toru Fujiwara, (2007). ***Proposal of Lysinibacillus boronitolerans gen. nov. sp. nov., and transfer of Bacillus fusiformis to Lysinibacillus fusiformis comb. nov. and Bacillus sphaericus to Lysinibacillus sphaericus comb. nov***. International Journal of Systematic and Evolutionary Microbiology, 57, 1117–1125

Ahring B.K. (2003). ***Perspectives for Anaerobic Digestion. In: Ahring B.K. et al. (eds) Biomethanation I.*** Advances in Biochemical Engineering/ Biotechnology, vol 81. Springer, Berlin, Heidelberg

Ahring BK, Westermann P (1983). ***Toxicity of heavy metals to thermophilic anaerobic digestion.*** Eur J Appl Microbiol Biotechnol 17:365–370

Ahring, B. K., Ibrahim, A. A., and Mladenovska, Z., (2001). ***Effect of temperature increase from 55 to 65°C on performance and microbial population***

dynamics of an anaerobic reactor treating cattle manure, Water Research, vol. 35, no. 10, pp. 2446–2452.

Akarsubasi AT, Ince O, Kirdar B, Oz NA, Orhon D, Curtis TP, Head IM, Ince BK. (2005). ***Effect of wastewater composition on archaeal population diversity,*** Water Res. 39:1576–1584.

Akuzawa, M., Hori, T., Haruta, S., Ueno, Y., Ishii, M., Igarashi, Y., (2011). ***Distinctive responses of metabolically active microbiota to acidification in a thermophilic anaerobic digester***. Microbial Ecol. 61, 595–605.

Alemdar A, Sain M., (2008). ***Isolation and characterization of nanofibers from agricultural residues – wheat straw and soy hulls.*** Bioresour Technol 99 (6):1664 – 71.

Ali, M., Saleem, M., Khan, Z., & Watson, I. A. (2019). ***The use of crop residues for biofuel production.*** *Biomass, Biopolymer-Based Materials, and Bioenergy, 369–395.*

Alya Sellami-Kamoun, Anissa Haddar, Nedra El-Hadj Ali and Moncef Nasri (2008). ***Stability of thermostable alkaline protease from Bacillus licheniformis RP1 in commercial solid laundry detergent formulations.*** Microbiological Research 163(3):299-306

Alzate S., Restrepo-Cuestas B., and Jaramillo-Duque Á. (2019). ***Municipal Solid Waste as a Source of Electric Power Generation in Colombia: A Techno-Economic Evaluation under Different Scenarios.*** Resources 2019, 8, 51.

Amani T., Nosrati M., Mousavi S. M. and Kermanshahi R. K., (2011). ***Study of syntrophic anaerobic digestion of volatile fatty acids using enriched cultures at mesophilic conditions,*** Int. J. Environ. Sci. Tech., 8 (1), 83 -9 6

Amann, R.I., Ludwig, W., and Schleifer, K.H. (1995). ***Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation.*** Microbiol Rev 59: 143–169.

Anahita Rabii, Saad Aldin, Yaser Dahman and Elsayed Elbeshbishy (2018). ***A Review on Anaerobic Co-Digestion with a Focus on the Microbial Populations and the Effect of Multi-Stage Digester Configuration*** Energies, 12, 1106

Angelidaki I., Karakashev D., Batstone D. J., Plugge C. M., Stams A. J. M., (2011). ***Methods in Enzymology Methods in Methane Metabolism***. Part A, C. R. a. S. Amy, Ed. Academic Press, pp. 327-351.

Angelidaki, I., Sorensen, A. and Schmidt, J., (2007). ***Notes for the course 12133***. Environmental engineering.

Anjanabha, B. and Pawan, K., (2010). ***Water Hyacinth as a Potential biofuel Crop.*** Electronic Journal of Environmental, Agricultural and Food Chemistry 9 (1). 112-122

AOAC (Association of Official Analytical Chemists), 1995. ***Official methods of analysis,*** 16th Ed. Association of official analytical chemists. Washington DC, USA.

Apex Instruments, (2000). ***Orsat Analyzer Model VSC-33 Operator's Manual.*** [Online]. Available: www.intecconinc.com

APHA (1998). ***Standard Methods for the Examination of Water and Wastewater.*** 20th Edition, American Public Health Association, American Water Works Association and Water Environmental Federation, Washington DC.

Asikong, B. E., Epoke, J., Agbo, B. E., Antai, E.E., and Eja, M. E., (2013). ***Effects of starter culture on biogas production from combination of poultry droppings and cow dung in Cross River State, Nigeria.*** Journal of Microbiology and Biotechnology Research accessed on (*http://scholarsresearchlibrary.com/archive.html)*

Aurora, S.P., (1983). ***Microbial Digestion in Ruminants,*** Indian Council of Agricultural Research, New Delhi.

Ayeni A. O., Banerjee S., Omoleye J. A., Hymore F. K., Giri B. S., Deskmukh S. C., Pandey R. A. and Mudliar S. N., (2013). ***Optimization of pretreatment conditions using full factorial design and enzymatic convertibility of shea tree sawdust***, Biomass and Bioenergy. 48, 130-138.

Azmi N. S., Khairul F Md. Y., (2014).***Wastewater Treatment of Palm Oil Mill Effluent (POME) by Ultrafiltration Membrane Separation Technique Coupled with Adsorption Treatment as Pre-treatment.*** Agriculture and Agricultural Science Procedia Volume 2, 2014, Pages 257-264

Bajpai P., (2017). ***Anaerobic Technology in Pulp and Paper Industry,*** Springer Briefs in Applied Sciences and Technology, DOI 10.1007/978-981-10-4130-3_2

Bandaw T., Herago T. (2017). ***Review on Abattoir Waste Management.*** Global Veterinaria 19 (2): ISSN 1992-6197, 517-524

Banister, S.S. and Pretorius, W.A., (1998). ***Optimization of primary sludge acidogenic fermentation for biological nutrient removal.*** Water SA 24(1), 35-41.

Barton JR, Issaias I, Stentiford EI (2008) ***Carbon making the right choice for waste management in developing countries.*** Waste Manage 28:69–76

Batstone, D.J., Keller, J., Angelidaki, I.., Kalyuzhnyi, S., Pavlostathis, S.G., Rozzi, A.., Sanders, W.,Siegrist, H. and Vavilin, V. (IWA Task Group on Modelling of Anaerobic Digestion Processes) (2002). ***Anaerobic Digestion Model No. 1 (ADM1).*** IWA Publishing, London.

Bauer, D.H., E.T. Conrad, and D.G. Sherman. (1979). ***Evaluation of On-Site Wastewater Treatment and Disposal Options.*** U.S. Environmental Protection Center, Boulder, CO.

Bazrafshan E, Kord Mostafapour F, Farzadkia M, Ownagh KA, Mahvi AH (2012). ***Slaughterhouse Wastewater Treatment by Combined Chemical Coagulation and Electrocoagulation Process***. PLoS ONE 7(6): e40108.

Bernabé-Santiago, R., Ávila-Calderón, L. E. A., and Rutiaga-Quiñones, J. G. (2013). ***Componentes químicos de la madera de cinco especies de pino del municipio de Morelia, Michoacán.*** Madera y Bosques 19(2), 21-35.

Berndes G, Hoogwijk M, van den Broek R, (2003). ***The contribution of biomass in the future global energy supply: a review of 17 studies***, Biomass and Bioenergy 25/1 1-28

Bilgen S, Keles S, Kaygusuz A, Sari A, Kaygusuz K,. (2008). ***Global warming and renewable energy sources for sustainable development: a case study in Turkey.*** Renewable and Sustainable Energy Reviews 12/372-396

Bitton, G., (2005). ***Overview of co-digestion study. Wastewater microbiology.*** John Wiley and sons, 3:1-13.

Bothi K. L., (2007). ***Characterization of biogas from anaerobically digested dairy waste for energy use.*** MSc Thesis Presented to the Faculty of the Graduate School of Cornell University

Bouzas, A., Gabaldon, C., Marzal, J., Penya-Roja, M. and Seco, A., (2002). ***Fermentation of municipal primary sludge: Effect of SRT and Solids P.concentration on volatile fatty acid production.*** Environ. Technol., 23, 863-875.

Breure A.M. and van Andel J.G., (1984). ***Hydrolysis and acidogenic fermentation of a protein, gelatin, in an anaerobic continuous culture.*** Applied Microbiology and Biotechnology 20(1):40-45

Britz T.J. Noeth C. Lategan P.M. (2003) ***Nitrogen and phosphate requirements for the anaerobic digestion of a petrochemical effluent.*** Water Research Volume 22, Issue 2, February 1988, Pages 163-169

Budiyano, I. N., Widiasa, J. and Sunarso, S., (2010). ***The kinetic of biogas production rate from cattle manure in batch mode.*** International Journal of Chemical and Biomolecular Engineering, 3(3), 39-44.

Bunsen R., Roscoe H.E. (1857). ***Gasometry: comprising the leading physical and chemical properties of gases***, translated by Henry Roscoe. London: Walton and Maberly.

Bustillo-Lecompte C.F., Mehrvar M., (2015). ***Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: a review on trends and advances.*** Journal of Environmental Management. 161: 287–302.

Callaghan FJ, Wase DAJ, Thayanity K, Forster CF (2002) ***Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure***. Biomass Bioenergy 22:71–77

Callaghan FJ, Wase DAJ, Thayanity K, Forster CF (2002) ***Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure***. Biomass Bioenergy 22:71–77

Callaghan J.F, Wase A. J. D, Thayanithy, K., Forster F.C., (1999). ***Co-digestion of Waste Organic Solids: Batch Studies.*** Bioresource Technology, Vol. 67, 117-122 DO - 10.1016/S0960-8524(98)00108-4

Carrere H., C. Dumas, A. Battimelli, D.J. Batstone, J.P. Delgenès, J.P. Steyer, I. Ferrer (2010) ***Pretreatment methods to improve sludge anaerobic degradability:*** A review, Journal of Hazardous Materials 183 (2010) 1–15

Castillo, R.T., P.L. Luengo, and J.M. Alvarez (1995). ***Temperature effect on anaerobic of bedding manure in a one phase system at different inoculums concentration***. Agriculture, Ecosystems and Environment, 54:55-66

Chaucheyras, F., Fonty, G., Bertin, G., and P. Gouet. (1995). ***In Vitro H2 Utilization by a Ruminal Acetogenic Bacterium Cultivated Alone or in Association with an Archaea Methanogen Is Stimulated by a Probiotic Strain of Saccharomyces cerevisiae.*** Applied and Environmental Microbiology, Sept. Vol.61 No.9: 3466– 3467.

Chen Y, Cheng JJ, Creamer KS. (2008). ***Inhibition of anaerobic process,*** a review. Bioresour Technol 99:4044–4064

Chen, C.L., Wu, J.H., Tseng, I.C., Liang, T.M., and Liu, W.T. (2009). ***Characterization of active microbes in a full-scale anaerobic fluidized bed reactor treating phenolic wastewater.*** Microbes Environ 24: 144–153.

Choong Y, Y. , Ismail N., Abdullah A. Z. and Yhaya M. F., (2016). ***Impacts of trace element supplementation on the performance of anaerobic digestion process:*** A critical review Bioresource Technology 209

Cu, T. T., Nguyen, T. X., Triolo, J. M., Pedersen, L., Le, V. D., Le, P. D., & Sommer, S. G. (2015). ***Biogas production from vietnamese animal manure, plant residues and organic waste: influence of biomass composition on methane yield***. Asian-Australasian journal of animal sciences, 28(2), 280–289. doi:10.5713/ajas.14.0312

Cuetos J., Gomez X., Otero M., Moran A (2010) ***Anaerobic digestion and co-digestion of slaughterhouse waste (SHW): influence of heat and pressure pre-treatment in biogas yield***. Waste Manage 30:1780–1789

Demirel, B. and Scherer, P., (2008). ***The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review,***

Reviews in Environmental Science and Biotechnology, vol. 7, no. 2, pp. 173–190.

Dias T, Fragoso R, Duarte E., (2014). ***Anaerobic co-digestion of dairy cattle manure and pear waste.*** Bioresour Technol 164:420–423. doi: 10.1016/j.biortech.2014.04.110

Doolittle P (2014). ***Ascorbic acid method for phosphorus determination.*** University of Wisconsin-Madison, pssemrad@wisc.edu

Edstrom M., Nordberg A., Thyselius L. (2003). ***Anaerobic treatment of animal by-products from slaughterhouses at laboratory and pilot scale.*** Appl Biochem Biotechnol 109:127–138

Elefsiniotis, P. and Oldham, W.K., (1994). ***Effect of HRT on acidogenic digestion of primary sludge.*** J. environ. eng., 120(3), 645-660.

Emine G. O., O. Ince, B. Ince, H. Harms and Sabine K., (2018). ***Comparison of Rumen and Manure Microbiomes and Implications for the Inoculation of Anaerobic Digesters*** microorganisms 2018, 6, 15.

Ertem F. C., (2011). ***Improving biogas production by anaerobic digestion of different substrates - calculation of potential energy outcomes,*** Master thesis in Applied Environmental Science Halmstad University

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., Pirozzi, F., (2012). ***Anaeriobic co-digestion of organic wastes.*** Rev Environ Sci Biotechnol DOI 10.1007/s11157-012-9277-8.

FAO statistical database, Food and Agricultural Organization, (2013). http://faostat3.fao. Org/home/index .html# download.

Farrell, J. B., Erlap, A. E., Rickabaugh, J., David, F., Hayes, S., (1988). ***Influence of feeding procedure on microbial reductions and performance of anaerobic digestion.*** Journal WPCF, *60 (5), 635-642.*

Fayyaz A. S., Qaisar M., M. M. Shah, A. Pervez, and S. A. Asad., (2017). ***Microbial Ecology of Anaerobic Digesters: The Key Players of Anaerobiosis,*** Scientific World Journal. 3852369

Feng, H., Hu, L., Mahmood, Q., Fang, C., Qiu, C., &Shen, D. (2009). ***Effects of temperature and feed strength on a carrier anaerobic baffled reactor treating dilute wastewater.*** Desalination, 239(1), 111-121.

Ferrer I, Palatsi J, Campos E, Flotats X., (2010). ***Mesophilic and thermophillic anaerobic biodegradability of water hyacinth pre-treated at 80°C.*** Waste Manag 30(10):1763–1767

Fileto-Pérez, H. A., Rutiaga-Quiñones, O. M., Sytsma, M. D., Lorne, I. M, Luo, W, Pankow, J. F., and Rutiaga-Quiñones, J. G. (2015). ***GC/MS analysis of some extractives from Eichhornia crassipes,*** BioResources 10(4).

Food and agriculture organization of the United Nations (2004). ***Good practices for the meat industry.*** Animal production and health manual

Franke-Whittle, I.H., Goberna, M., Pfister, V., and Insam, H. (2009). ***Design and development of the ANAEROCHIP microarray for investigation of methanogenic communities.*** J Microbiol Methods 79: 279–288

Funda C. E., (2011). ***Improving Biogas Production by Anaerobic Digestion of Different Substrates - Calculation of Potential Energy Outcomes,*** Halmstad University Master Thesis in Applied Environmental Science.

Ganguly, A., Chatterjee, P. K., and Dey, A. (2012). ***Studies on ethanol production from water hyacinth – A review,*** Renewable and Sustainable Energy Reviews 16, 966-972.

Ghaneker, A.S., and Nair, P.M. (1973). ***Evidence for the Existence of an Aerobic Pathway for Synthesis of Monounsaturated Fatty Acids by Alcaligenes faecalis***, J. Bacteriol. 114, 618–624.

Gichuki, J. R. Omondi, P. Boera, T. Okorut, A. S. Matano., T. Jembe, and A. Ofulla, (2012). ***Water Hyacinth Eichhornia crassipes (Mart.) Solms-Laubach Dynamics and Succession in the Nyanza Gulf of Lake Victoria (East Africa): Implications forWater Quality and Biodiversity Conservation.*** The Scientific World Journal. Volume 2012, Article ID 106429, 10 pages doi:10.1100/2012/106429

Golbaz S., Farzadkia M., Vanani A. & Emamjomeh M. (2017).***Livestock slaughterhouses waste management in urban environment,*** Int. J. Hum. Capi t al Urban Manage., 2(2): 163-1

Gomez, X., Cuetos, M. J., Cara, J., Moran, A and Garcia, A. I., (2006). ***Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes conditions for mixing and evaluation of organic loading rate,*** Renewable Energy, 31, 2017.

Gopal, B. (1987). ***Aquatic Plant Studies 1. Water Hyacinth***. Elsevier Publishing, New York, New York, USA.

Gregor D. Zupančič and Viktor Grilc., (2012). ***Anaerobic Treatment and Biogas Production from Organic Waste,*** Management of Organic Waste, Dr. Sunil Kumar (Ed.), ISBN: 978-953-307-925-7, InTech, Available from: http://www.intechopen.com/books/management-of-organic-waste/anaerobic-treatment-and-biogas-productionfrom-organic-wastes

Güereña, D, H. Neufeldt, J. Berazneva, and S. Duby (2015). ***Water hyacinth control in Lake Victoria: Transforming an ecological catastrophe into economic, social, and environmental benefits,*** Elsevier, 3:59-69

Gujer, W. and Zehnder, A. B., (1983). ***Conversion process in anaerobic digestion.*** Wat. Sci. Tech. 15, 127–167

Guwy A.J., (2004). ***Equipment used for testing anaerobic biodegradability and activity.*** Reviews in Environmental Science and Bio/Technology, Vol. 3, pp. 131–139.

Haryati, T. (2006). ***Biogas: Livestock wastes as alternative energy source.*** Livestock Research Agency, Bogor, Indonesia.

Hashimoto, A., Chen, Y. and Varel, V., (1981). ***Theoretical aspects of methane production: state of-the-art in livestock waste: a renewable resource.*** Proceedings of the fourth international symposium on livestock wastes. ASAE publication, Amarillo: 86-95.

Hattori, A. (2008). ***Syntrophic acetate-oxidizing microbes in methanogenic environments.*** Microbes Environ.23 (2), 118–127

Heeg K., Pohl M., Sontag M., Mumme J., Klocke M and Netmann E., (2014). ***Microbial communities involved in biogas production from wheat straw as the sole substrate within a two-phase solid-state anaerobic digestion.*** Systematic and Applied Microbiology 37(8)

Horváth, I. S., Tabatabaei, M., Karimi, K., & Kumar, R. (2016). ***Recent updates on biogas production-areview.*** Biofuel Reserch Journal, 10, 394– 402 http://doi.org/10.18331/BRJ2016.3.2.4

Ivanova, Natalia et al. (2003). ***Genome sequence of Bacillus cereus and comparative analysis with Bacillus anthracis.*** Nature, Vol. 423: 87-91.

Jagadish H Patil, Prajwal H Sanil, Abitha and Chaitra D., (2012). ***Study on biomethanation of water hyacinth using primary sludge as inoculum.*** Journal of Chemical and Pharmaceutical Research, 2012, 4(4):2255-2260

Jayaraj S., Deepanraj B., Velmurugan S., (2014). ***Study on the effect of pH on biogas production from food waste by anaerobic digestion.*** 9th Annual Green Energy Conference in Tianjin China (IGEC-IX)

Jeyaseelan, S. and Matsuo, T. (1995). ***Effects of phase separation in anaerobic digestion on different substrates.*** Water Science and Technology, 31 (9), 153-162.

Jonas Bonn (2008). ***Improved techniques for sampling and sample introduction in gas chromatography***. Royal Institute of Technology.

Julien, M. H., Griffiths, M. W. and Wright, A. D., (1999). ***Biological control of water hyacinth.*** *ACIAR Monograph No. 60.* 87 pp.

Kangle, K., Kore, S., Kulkarni, G., (2012). ***Recent Trends in Anaerobic Co-digestion.*** A review. Universal Journal of Environmental Research and Technology, volume 2, issue 4:210-219.

Karki Amrit B., (2009). ***Biogas as renewable energy from organic waste in Nepal***: Theory and Development. BSP-Nepal.

Karlsson Anna, Annika Björn, Sepehr Shakeri Yekta,Bo H. Svensson (2014). ***Improvement of the Biogas Production Process.*** Biogas Research Center (BRC) Report

Kim B.K, and Daniels L., (1869). ***Unexpected errors in gas chromatographic analysis of methane production by thermophilic bacteria***, Appleed and Environmental Microbiology, Vol. 57.

Kim, J., Kim, W., Lee, C., (2013). ***Absolute dominance of hydrogenotrophic methanogens in full-scale anaerobic sewage sludge digesters***. J. Environ. Sci. 25, 2272–2280.

Kiros H., Jianpeng Z., Dongxue L., ChangLiu X., (2017): ***Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives.*** Renewable and Sustainable Energy Reviews

Kolb B., L. S. Ettre, (2006). ***Static headspace gas chromatography:*** Theory and Practice. Wiley interscience.

Kossmann, W., Ponitz, U., Habermehl, S., Hoerz, T., Kramer, P., Klingler, B., Kellner, C., Wittur, T., Lopotek, F., Krieg, A., Euler, H., (2007). ***Biogas digest.*** Information and Advisory Service on Appropriate Technology (ISAT) 1, 1-46.

Kris T. D., S. Y. Westman, R. Millati, M. N. Cahyanto, M. J. Taherzadeh, and C. Niklasson., (2016). ***Inhibitory Effect of Long-Chain Fatty Acids on Biogas Production and the Protective Effect of Membrane Bioreactor.*** Biomed Res Int. 2016; 2016: 7263974. Published online 2016 Sep 8. doi: 10.1155/2016/7263974

Kriticos DJ, Brunel S (2016). ***Assessing and Managing the Current and Future Pest Risk from Water Hyacinth, (Eichhornia crassipes), an Invasive Aquatic Plant Threatening the Environment and Water Security***. PLoS ONE 11(8): e0120054

Kugelman, I. J. & Chin, K. K. (1971). ***Toxicity Synergism and Antagonism in Anaerobic Waste Treatment Processes. ed. R. F. Gould.*** Advances in Chemistry Series 105, American Chemical Society, Washington, DC, pp. 55-90.

Kumar A. K. and Sharma S., (2017). ***Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review,*** Bioresour Bioprocess. 2017; 4(1): 7. Published online 2017 Jan 18. doi: 10.1186/s40643-017-0137-9

Kumar L, and Mohan M.R., (2005). ***Biofuels: the key to India's sustainable energy needs,*** Proceedings of the RISO International Energy Conference 423-438.

Kumar, A., Singh, L. K., and Ghosh, S. (2009). ***Bioconversion of lignocellulosic fraction of water-hyacinth (Eichhornia crassipes) hemicellulose acid hydrolysate to ethanol by Pichia stipites,*** Bioresource Technology 100(13), 3293-3297.

Kumar, U., Sareen, V. K., and Singh, S. (1994). ***Effects of Saccharomyces cerevisiae culture supplement on ruminal metabolism in buffalo calves given a high concentrate diet***. Anim. 59: 209.

Lane, D. J. (1991). ***16S/23S rRNA sequencing, p. 115-147. In E. Stackebrandt and M. Good fellow (ed.),*** Nucleic acid techniques in bacterial systematics. John Wiley & Sons, New York, NY.

Lara-Serrano, J. S., Rutiaga-Quiñones, O. M., López-Miranda, J., Fileto-Pérez, H. A., Pedraza-Bucio, F. E., Rico-Cerda, J. L., and Rutiaga-Quiñones, J. G. (2016). ***Physicochemical characterization of water hyacinth (Eichhornia crassipes (Mart.) Solms),*** BioRes. 11(3), 7214-7223.

Lazor M., Hutnan M., Sedlacek S., Koles´arov´a N., and Spalkova V., (2010) ***Anaerobic co-digestion of poultry manure and waste kitchen oil***. In 37th International conference of SSCHE May, pages 24–28.

Lea Cabrol, Antonella Marone, Estela Tapia-Venegas, Jean-Philippe Steyer, Gonzalo Ruiz-Filippi and Eric Trably, (2017). ***Microbial ecology of fermentative hydrogen producing bioprocesses: useful insights for driving the ecosystem function,*** *FEMS Microbiology Reviews*, Volume 41, Issue 2, Pages 158–181

Lee C., Kim J., Hang K., OFlaherty V., Hwang S., (2009). ***Quantitative nalysis of methanogenic community dynamics in three anaerobic digesters treating different waste waters.*** Water Res. 43, 157-165.

Leenstra, F.R.; Vellinga, Th.V; Neijenhuis, F.; Buisonje, F.E. de (2014). ***Manure: a valuable resource.*** Livestock Research, - 35 p. (http://edepot.wur.nl/294017)

Li J, Jha AK, He J, Ban Q, Chang S, Wang P., (2011). ***Assessment of the effects of dry anaerobic co-digestion of cow dung with waste water sludge on biogas yield and biodegradability.*** Int J Phys Sci 6:3679–3688

Liu J, Olsson G, and Mattiasson B. (2004). ***A volumetric meter for monitoring of low gas flow rate from laboratory-scale biogas reactors. Sensors and Actuators B:*** Chemical 97:369–372.

Lourenço, S.O, Barbarino, E, De-Paula, J.C, Pereira, L.O.d.S, Lanfer Marquez, U.M (2002). ***Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds.*** Phycol. Res. **2002**, 50, 233–241.

Lucian A. L. and J. R., Orlando, (2009). ***The Nanoscience and Technology of Renewable Biomaterials*** *Department of Forest Biomaterials, North Carolina State University, USA*

Lukitawesa, Regina J. Patinvoh, Ria Millati, Ilona Sárvári-Horváth & Mohammad J. Taherzadeh (2020) ***Factors influencing volatile fatty acids production from food wastes via anaerobic digestion,*** Bioengineered, 11:1, 39-52, DOI: 10.1080/21655979.2019.1703544

Madigan, T. M., Martinko, J. M. and Parker, J., (2003). ***Brock Biology of Microorganisms"*** (10th ed). Pearson Education, Inc., Upper Saddle River, New Jersey.

Mailu A.M., (2001). ***Preliminary Assessment of the Social, Economic and Environmental Impacts of Water Hyacinth in the Lake Victoria Basin and the Status of Control.*** Proceedings of the 2nd Meeting of Global Working

Group for the Biological and Integrated Control of Water Hyacinth, Oct. 9-12, Beijing, China, Canberra, Australia, pp: 130-139.

Malakahmad, A., Zain, S. M., and Basri, N. E. A. (2012). ***Biomethanation of kitchen waste and sewage sludge in anaerobic baffled reactor.*** Paper presented at the Humanities, Science and Engineering Research (SHUSER), 2012 IEEE Symposium on.

Manyi-Loh C. E., Mamphweli S. N., Meyer E. L., Okoh A. I., G. Makaka, and Simon M. (2013). ***Microbial Anaerobic Digestion (Bio-Digesters) as an Approach to the Decontamination of Animal Wastes in Pollution Control and the Generation of Renewable Energy.*** Int J Environ Res Public Health. Sep; 10(9): 4390–4417.

Mariotti, F, Tome, D, Mirand, P.P (2008). ***Converting nitrogen into protein—Beyond 6.25 and Jones' factors.*** Crit. Rev. Food Sci. **2008**, 48, 177–184.

Mata-Alvarez J., Mtz-Viturtia A. and R. Torres, (1986). ***A simple device to measure biogas production in laboratory scale digesters.*** Biotechnology Letters, Vol.8, pp. 719–720.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M. & Astals, S., (2014). ***A critical review on anaerobic co-digestion achievements between 2010 and 2013***. Renewable and Sustainable Energy Reviews, 36(0), pp. 412-427.

Mata-Alvarez, J., Mace, S. and Llabres, P., (2000). ***Anaerobic digestion of organic solid wastes***: an overview of research achievements and perspectives. Bioresource technology, 74:3-16.

McInerney, M. J., Bryant, M. P. and Stafford, D. A., (1980). ***Metabolic stages and energetics of microbial anaerobic digestion.*** In Stafford D. A., Wheatley B. I., Hudges D. E.(eds), Anaerobic digestion, Applied Science, London, 91-98.

Medical chemistry LOKT.00.009 (2012). ***Determination of sodium and potassium by flame photometry*** available on http://tera.chem.ut.ee/~koit/arstpr/nak_en.pdf.

Medina-Herrera, M., Rodríguez-García, A., Montoya-Herrera, L., Cárdenas-Mijangos, J.,Godínez-Mora-Tovar, L.A., Bustos-Bustos E., Rodríguez-Valadez, F.J. and Manríquez-Rocha, J., (2014). ***Anaerobic Digestion of Slaughterhouse Solid Waste for the Optimization of Biogas Production*** Int. J. Environ. Res., 8(2):483-492, ISSN: 1735-6865

Meyer T. and Edwards E. A., (2014) Anaerobic digestion of pulp and paper mill wastewater and sludge. Water Research, Volume 68, 1 January 2015, Pages 849

Michael ~ulkoski (1958). ***Fuels and combustion fundamentals.*** Mineral Industries Extension Services College of Mineral Industries the Pennsylvania State University University Park, Pennsylvania

Michael N.N. Terry W.S. and Graig L.B., (1988). ***Anaerobic Contact Pretreatment of Slaughterhouse Wastewater.*** Proc. Ind Waste Conf. 1987, 42nd 647.

Mittal G. S. (2004). ***Characterization of the Effluent Wastewater from Abattoirs for Land Application,*** Food Reviews International, 20:3, 229-256,

Moestedt J., Malmborg J. and Nordell E., (2015). ***Determination of Methane and Carbon Dioxide Formation Rate Constants for Semi-Continuously Fed***

Anaerobic Digesters. Energies ISSN 1996-1073 www.mdpi.com/journal/energies

Moletta R., and Albagnac G., (1982). ***A gas meter for low rates of gas flow: Application to the methane fermentation,*** Biotechnology letters, Vol. 4, p.p319-322.

Monnet F., (2003). ***An introduction to Anaerobic Digection of organic Wastes,*** A report by Remade Scotland

Morales-Polo C., Cledera-Castro M. M. and Soria B. Y. M., (2018). ***Reviewing the Anaerobic Digestion of Food Waste: From Waste Generation and Anaerobic Process to Its Perspectives,*** Appl. Sci., 8, 1804.

Moubasher, M, Abdel-Hafez S, Abdel-Fattah M. and Mohanram A (1982). ***Direct estimation of cellulose, Hemicellulose and Lignin***. J.Agricu. Res.46.1467-1476.

Mueller, C. (2007). ***Anaerobic Digestion of Biodegradable Solid Waste in Low and Middle Income Countries.*** Swiss federal Institute of Aquatic Science (EAWAG), Department of Water and Sanitation in Developing Countries (SANDEC).

MÜller W.R., I. Frommert & JÖrg R., (2004). ***Standardized methods for anaerobic biodegradability testing.*** Reviews in Environmental Science and Bio/Technology, Vol 3, p.p 141–158.

Munack, A., (2002). ***Agriculture and the environment: new challenges for engineer.*** Agricultural Engineering International 4: 18

Myszograj S., Stadnik A., and Płuciennik-Koropczuk E., (2018).***The Influence of Trace Elements on Anaerobic Digestion Process,*** Civil and Environmental Engineering Reports 28(4):105-115

Narihiro, T., Terada, T., Ohashi, A. et al. (2009). ***Quantitative detection of culturable methanogenic archaea abundance in anaerobic treatment systems using the sequence-specific rRNA cleavage method.*** ISME J 3, 522–535

Ndimele P.E., Kumolu-Johnson C.A. and. Anetekhai M.A, (2011). ***The Invasive Aquatic Macrophyte, Water Hyacinth {Eichhornia crassipes (Mart.) Solm-Laubach: Pontedericeae}: Problems and Prospects.*** Research Journal of Environmental Sciences, 5: 509-520.

New Delhi (2000). ***How to measure Total Phosphorus: Ascorbic Acid Method,*** Hydrology Project Training Module File: 41 World Bank & Government of The Netherlands funded

Newbold, C. J., McIntosh, F.M., and Wallace, R.J. (1998). ***Changes in the microbial population of a rumen simulating fermenter in response to yeast culture.*** Can. J. Anim. Sci. 78: 241.

Newman Alan (2012). ***Dairy Manure Anaerobic Digesters*****.** Department of Ecology Report to the Legislature Publication no. 12-02-035

Njoka S.W., (2004). ***The biology and impact of Neochetina weevils on Water Hyacinth Eichhornia crassipes in Lake Victoria Basin, Kenya***. Ph.D Thesis, Moi University

Odhner P. B., Grontmij I. S. H., H. B.Kabir, H. Borås and A.S. Grontmij, **(**2012**).** ***Biogas from lignocellulosic biomass,*** Rapport SGC 247. 1102-7371. ISRN SGC-R-247-SE

Okalebo J. R., Gathua K. W., Woomer P. L. (20012). ***Laboratory Methods of Soil and Plant Analysis:*** A working manual the second edition TSBF-CIAT and SACRED Africa, Kenya

Onofre M. A., M. Teresa., C. Barragán and Francisco J. A. T., (2013). ***Microbial Biomass in Batch and Continuous System*** http://dx.doi.org/10.5772/55303

Onwuliri, F., Onyimba, I., & Nwaukwu, I., (2016). ***Generation of Biogas from Cow Dung. Bioremediation and Biodegradation***. http://doi.org/10.4172/2155-6199.S18-002

Orphan, V.J., House, C.H., Hinrichs, K.U., McKeegan, K.D., and DeLong, E.F. (2001). ***Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis.*** Science 293: 484–487.

Ottesen, E.A., Hong, J.W., Quake, S.R., and Leadbetter, J.R. (2006). ***Microfluidic digital PCR enables multigene analysis of individual environmental bacteria.*** Science 314: 1464–1467.

Pages-Díaz J. P., (2015). ***Biogas from Slaughterhouse Waste***. PhD Thesis, University of Borås, Allégatan 1, Borås, Sweden.

Palatsi, J., Laureni, M., Andrés, M.V., Flotats, X., Nielsen, H.B., Angelidaki, I., (2009). ***Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors.*** Bioresource Technology 100, 4588e4596

Patil J.H., Raj M.A., Gavimath C.C., Hooli V.R., (2011). ***A comparative study on anaerobic co-digestion of water hyacinth with poultry litter and cow dung.*** Int J Chem Sci Appl 2(2):148–155

Pellerin, R.A., Walker L.P., Heisler M.G., and Farmer G.S. (1987). ***Operation and Performance of Biogas-Fueled Cogeneration Systems.*** Energy in Agriculture, Vol. 6, 295-310, 7.

Penning, H., Claus, P., Casper, P., and Conrad, R. (2006). ***Carbon isotope fractionation during acetoclastic methanogenesis by Methanosaeta concilii in culture and a lake sediment.*** Appl Environ Microbiol 72: 5648–5652.

Perot, C., Sergent M., Richard, P., Phan Tan Luu, R. and Millot, N., (1988). ***The effects of pH, temperature and agitation speed on sludge anaerobic hydrolysis-acidification.*** Environ. technol. lett, Vol. 9, pp. 741-752.

Petrell RJ, Bagnall LO., (1991). ***Hydromechanical properties of water hyacinth mats***. Aquacult Eng 10:133–147

Peyruze, O. and Solmaz, A., (2009). ***Biogas production from municipal waste mixed with different portions of orange peel.*** MSc thesis, University of Boras, School of Engineering.

Piyarat B., A. Rerngnarong, S. Chaiprapat and C. Tongurai., (2014). ***Effect of nitrogen and phosphorus on the performance of acidogenic and methanogenic reactors for treatment of biodiesel wastewater,*** Songklanakarin J. Sci. Technol. 36 (6), 643-649.

Poole C.F., (2003). ***The essence of chromatography***, Elsevier Science B.V.

Prakash P., (2011). ***Biogas measurement techniques and the associated errors.*** MSc Thesis University of Jyväskylä

Promdee, K., Vitidsant, T., and Vanpetch, S. (2012). ***Comparative study of some physical and chemical properties of bio-oil from manila grass and water hyacinth transformed by pyrolysis process,*** International Journal of Chemical Engineering and Applications 3(1), 72-75.

Pycke, B.F., Etchebehere, C., Van de Caveye, P., Negroni, A., Verstraete, W., Boon, N., (2011). ***A time-course analysis of four full-scale anaerobic digesters in relation to the dynamics of change of their microbial communities.*** Water Sci. Technol. 63, 769–775.

Queiroz M. A., Fukushima R. S., Gomide C. A., Braga M. R., (2008). ***Substitution of crude cell wall for neutral detergent fibre in the equations of the Cornell Net Carbohydrate and Protein System that predict carbohydrate fractions: Application to sunflower (Helianthus annuus L.)*** animal 2(7):1087-92

Quevedo B., E. Giertsen, V. Zijnge, H. Lüthi-Schaller, B. Guggenheim, T. Thurnheer, and Gmür R. (2011). ***Phylogenetic group- and species-specific oligonucleotide probes for single-cell detection of lactic acid bacteria in oral biofilms.*** BMC Microbiol. v.11; PMC3032641, doi: 10.1186/1471-2180-11-14.

Rabah, A. ., Baki, A. ., Hassan, L. ., Musa, M., & Ibrahim, A., (2010). ***Production of biogas using abattoir waste at different retention time.*** Science World Journal, 5(4), 23–26.

Rao P.V. and Baral S.S., (2011). ***Experimental design of mixture for the anaerobic co-digestion of sewage sludge.*** Chem Eng J 172:977–986. doi: 10.1016/j.cej.2011.07.010

Rasi S., (2009). ***Biogas composition and upgrading to methane.*** Studies in biological and environmental science. University of Jyvaskyla

Rasit Nazaitulshila, Azni Idris, Razif Harun, Wan Azlina G., (2015). ***Effects of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludges: An overview.*** Renewable and Sustainable Energy Reviews 45(2015):351-358

Raskin L, Poulsen LK, Noguera DR, Rittmann BE, Stahl DA., **(**1994). ***Quantification of methanogenic groups in anaerobic biological reactors by oligonucleotide probe hybridization,*** Appl. Environ. Microbiol. 60: 1241–1248.

Raskin L., J. M. Stromley, B. E. Rittmann, D. A. Stahl, (1994). ***Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens.*** Appl. Environ. Microbiol., 60, 1232–1240.

Regional Information Service Centre for South East Asia on Appropriate Technology (RISE-AT) (1998). ***Review of current status of Anaerobic Digestion Technology for treatment of MSW***.

Riddellová Kateřina (2012). ***Determination of total nitrogen in food and crude protein calculation (Kjeldahl method)***, accessed on https://web.vscht.cz/~kohoutkj.

Rivière D., Virginie D., Pelletier E., Chaussonnerie S., Guermazi S., Weissenbach J., Li T., Camacho and Sghir A. (2009). ***Towards the definition of a core of***

microorganisms involved in anaerobic digestion of sludge. The ISME Journal (2009) 3,700 –714

Rodríguez-Abalde, A., Fernández, B., Silvestre, G. and Flotats, X. (2011). ***Effects of thermal pre-treatments on solid slaughterhouse waste methane potential***. Waste Management, 31, 1488-1493.

Rozzi A. and E. Remigi, (2004). ***Methods of assessing microbial activity and inhibition under anaerobic conditions: a literature review,*** Reviews in Environmental Science and Bio/Technology, Vol. 3, pp. 93–115.

Saini, J. K., Saini, R., & Tewari, L. (2015). ***Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments.*** 3 Biotech, 5(4), 337–353.

Salminen, E. and Rintala, J., (2002). ***Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: effect of hydraulic retention time and loading.*** Water research, 36:3175-3182.

Sarkar N, Ghosh SK, Bannerjee S and Aikat K. (2012). ***Bioethanol production from agricultural wastes: an overview.*** Renew Energy 37(1):19-27.

Schink, B., (1997). ***Energetics of syntrophic cooperation in methanogenic degradation.*** Microbiol. mol. biol. rev., 61, 262-280.

Schink, B., Ward, J. C. and Zeikus, J. G., (1981). ***Microbiology of wet wood: importance of pectin degradation and Clostridium species in living trees.*** Appl. Environ. Microbiol. 42, 526-532.

Schomaker, A., Boerboom A., Visser A., and Pfeifer A., (2000). ***Anaerobic Digestion of Agro-Industrial Wastes: Information Networks.*** Technical Summary on Gas Treatment, AD-NETT Project FAIR-CT96-2083 (DG12-SSMI)

Sedlak, R. ed. (1991). ***Phosphorus and Nitrogen Removal from Municipal Wastewater,*** Principles and Practice. 2nd ed. The Soap and Detergent Association. Lewis Publishers, New York, NY.

Sekiguchi, Y., Kamagata, Y., Nakamura, K., Ohashi, A., and Harada, H. (1999). ***Fluorescence in situ hybridization using 16S rRNA-targeted oligonucleotides reveals localization of methanogens and selected uncultured bacteria in mesophilic and thermophilic sludge granules.*** Appl Environ Microbiol 65: 1280–1288

Sharda Vidya and Lakshmi Girish (2014). ***Water hyacinth as a green manure for organic farming.*** International Journal of Research in Applied Natural and Social Sciences, ISBN (E): 2321 – 8851; ISSN (P): 2347-4580bVol 2 pg 65-72.

Shefali Verma. (2002). ***Anaerobic digestion of Biodegradable Organics in Municipal Solid Wastes.*** Department of Earth & Environmental Engineering, Fu Foundation School of Engineering & Applied Science Columbia University. Unpublished manuscript.

Shikilkar M. *A., Raviraj* K. M., Shubham U. M., Birudev B. S. and Dipak P. O., (2017). ***Purification of Biogas,*** International Journal of Advance Research, Ideas and Innovations in Technology.

Sielaff Aleksandra C. S., Kumar R. M., Pal D., Mayilraj S. and Venkateswaran K., (2017). ***Solibacillus kalamii sp. nov., isolated from a high-efficiency particulate arrestance filter system used in the International Space Station.*** Int J Syst Evol Microbiol; 67:896–901

Smith R.P. and Gosselin R.E., (1979). ***Hydrogen sulfide poisoning.*** Journal of Occupational Medicine, 21:93–97.

Smith S. A., Stöckle C. O., (2008). ***A biogas meter with adjustable resolution and minimal back-pressure.*** Bio resource Technology, Vol. 99, pp. 8537-8539.

Sotolu, A. O. (2012). ***Management and utilization of weed: Water hyacinth (Eichhornia crassipes) for improved aquatic resources.*** Journal of Fisheries and Aquatic Science 8(1), 1-8. DOI.10.3923/jfas.2012

Sri Rahayu A., Dhiah K., Hari Y.,Ira T., Shinta M. S., Rahardjo S., Hokermin V and Paramita (2015). *"**POME-to-Biogas**,"* Winrock International Journal

Stams, A. J. M., and Plugge, C. M. (2009). ***Electron transfer in syntrophic communities of anaerobic bacteria and archaea.*** Nat. Rev. Microbiol.7, 568–577.

Steinberg LM, Regan JM. **(**2009). ***mcrA-targeted real-time quantitative PCR method to examine methanogen communities.*** Appl. Environ. Microbiol. **75:**4435–4442.

Strong, P. J. and Gapes, D. J. (2012). ***Thermal and thermochemical pre-treatment of four waste residues and the effect on acetic acid production and methane synthesis***. Waste Management, 32, 1669-1677.

Subhabrata A. Amit G., Bhattacharya A., Dey A., Chatterjee P. K., (2013). ***Enzymatic hydrolysis of water hyacinth biomass for the production of ethanol: Optimization of driving parameters.*** Indian journal of experimental biology. 51. 556-66.

Sukong W., Kongjan P., Prasertsan P., Imai T and Sompong T., (2016). ***Optimization and microbial community analysis for production of biogas from solid waste residues of palm oil mill industry by solid-state anaerobic digestion.*** Bioresour Technol., 214:166-174.

Sylvina L. C, Romina P. C, Alicia S.G. P., Juan J. N., (2014). ***The Influence of Water Hyacinth Floating Meadows on Limnological Characteristics in Shallow Subtropical Waters.*** American Journal of Plant Sciences Vol.5 No.13 (2014), Article ID: 47181, 12 pages

Taconi Katherine A., Mark E. Zappi, W. Todd French, Lewis R. Brown (2008). ***Methanogenesis under acidic pH conditions in a semi-continuous reactor system.*** Bioresource Technology 99 8075–8081

Takashi Narihiro and Yuji Sekiguchi (2011). ***Oligonucleotide primers, probes and molecular methods for the environmental monitoring of methanogenic archaea.*** Microb Biotechnol. 4(5): 585-602).

Tao R., R. Zeng, Y. Xiao-Yan, S. Zhang and Z. H.,Yang., (2016). ***Water Hyacinth (Eichhornia crassipes) Biomass as a Biofuel Feedstock by Enzymatic Hydrolysis,*** BioResources 11(1), 2372-2380.

Tchobanoglous, G., and F.L. Burton. (1991). ***Wastewater Engineering: Treatment, Disposal,*** Reuse, 3rd ed. McGraw-Hill, Inc., New York, NY.

Tchobanoglous, G., Theisen, H., and Vigil, S., (1993). **Integrated Solid Waste Management**, chapter 9, McGraw-Hill, New York

Thikra A. Abed (2013).***Evaluation of methods for the extraction and purification of DNA of cultured Lactobacillus colony isolated from dairy products.*** JAMBR 1 (2013) 20-25

Todar, K., (2012). ***Staphylococcus aureus and Staphylococcal Disease.*** In Todar's Online Textbook of Bacteriology.

Tritt, W.P., Schchardt F., (1992). ***The Anaerobic Treatment of Slaughterhouse Wastewater in Fixed Bed Reactors.*** Bioresource Technology, 41, 201-207.

Tu W, Zhang L, Zhou Z, Liu X, Fu Z., (2011). ***The development of renewable energy in resource-rich region: A case in China.*** Renewable and Sustainable Energy Reviews 15/1 856-860

Tufaner F.and Avsar Y., (2016) ***"Effects of co-substrate on biogas production from cattle manure: a review"*** Int. J. Environ. Sci. Technol. (2016) 13:2303–2312 DOI 10.1007/s13762-016-1069-1

Uyeno, Y., Sekiguchi, Y., Sunaga, A., Yoshida, H., and Kamagata, Y. (2004). ***Sequence-specific cleavage of small-subunit (SSU) rRNA with oligonucleotides and RNase H: a rapid and simple approach to SSU rRNA-based quantitative detection of microorganisms.*** Appl Environ Microbiol 70: 3650–3663.

Vandevivere, P., Baere, LD., & Verstraete, W., (1999). ***Types of anaerobic digesters for solid wastes***. Accessed on; http://www.ees.adelaide.edu.au /biogas/pvdv

Vavilin, V.A., Qu, X., Mazeas, L., Lemunier, M., Duquennoi, C., He, P.J., and Bouchez, T. (2008). ***Methanosarcina as the dominant aceticlastic methanogens during mesophilic anaerobic digestion of putrescible waste.*** Antonie Van Leeuwenhoek 94: 593–605.

Verma S., (2002). ***Anaerobic digestion of biodegradable organics in municipal solid wastes,*** MSc thesis, accessed; http://www.seas.columbia.edu/earth/vermathesis.pdf

Von Sachs, J., Meyer, U., Rys, P. and Feitkenhauer, H. (2003). ***New approach to control the methanogenic reactor of a two-phase anaerobic digestion system***. Water Resource, 37, 973-982.

Walker M., Zhang Y., Heaven S., Banks C., (2009). ***Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes.*** Bioresource Technology, Vol. 100, p.p 6339-6346.

Walter R.H., Shermah R.M. and Downing D.L., (1974). ***Reduction in Oxygen demand of abattoir effluent by Precipitation with metal.*** J. Agric. Fd Chem. 22, 1097-1099.

Walter, L. J.; McAllister, T. A.; Yang, W. Z.; Beauchemin, K. A.; He, M.; McKinnon, J. J., (2012). ***Comparison of wheat or corn dried distiller's grains with solubles on rumen fermentation and nutrient digestibility by feedlot heifers***. J. Anim. Sci., 90, 1291-1300

Wang, F.P., Zhou, H.Y., Meng, J., Peng, X.T., Jiang, L.J., Sun, P., et al. (2009). ***GeoChip-based analysis of metabolic diversity of microbial communities at***

the Juan de Fuca Ridge hydrothermal vent. Proc Natl Acad Sci USA 106: 4840–4845.

Wei wu., (2010). ***Anaerobic Co-digestion of Biomass for Methane Production: Recent Research Achievements.*** Accessed on. http://citeseerx.ist.psu.edu/viewdoc/summarydoi=10.1.1.456.258

Weiers W., Fischer R. (1978). ***Definition of 'slaughter house waste'. In: The Disposal and Utilisation of Abattoir Waste in the European Communities***. Springer, Dordrecht

Westermann, P., (1996). ***Temperature regulation of anaerobic degradation of organic matter.*** World j. microbiol. biotechnol. 12, 497-503.

Williams, P.E.V., Tait,C.A.G., Innes, G.M., and Newbold, C.J. (1991). ***Effects of inclusion yeast culture (Saccharomyces cerevisiae plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers***. J.Anim. Sci. 69: 3016.

Wipat, Anil & Colin R. Hardwood. (1999). ***The Bacillus subtilis genome sequence: the molecular blueprint of a soil bacterium.*** FEMS Microbiology Ecology, Vol. 28: 1-9.

World Bank, (1996). ***Kenya, Tanzania, Uganda - Lake Victoria Environmental Management Project.*** World Development Sources, WDS 1996. Washington, DC: World Bank.

Yadviva, Santosh S., Sreekrishnan T. R., Kohli S., Rana V. (2004) ***Enhancement of biogas production from solid substrates using different techniques*** – a review. Journal of Bioresource Technology; 95:1–10.

Yu, Y., Lee, C., Kim, J., and Hwang, S. (2005). ***Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction.*** Biotechnol Bioeng 89: 670–679.

Zainudin M H M, Hassan M A, Shah U K M, Abdullah N, Tokura M, Yasueda H, Shirai Y, Sakai K, and Baharuddin S A (2014). ***Bacterial Community Structure and Biochemical Changes Associated With Composting of Lignocellulosic Oil Palm Empty Fruit Bunch,*** Bio Resources9 (1), 316-335.

Zhang, T. and Noike, T., (1994). ***Influence of retention time on reactor performance and bacterial trophic population in anaerobic digestion processes.*** Wat.Res. 28[1], 27-36.

Zhang, T., and Fang, H.H.P. (2006). ***Applications of real-time polymerase chain reaction for quantification of microorganisms in environmental samples.*** Appl Microbiol Biotechnol 70: 281–289.

Zhou H (2017). ***Combustible Solid Waste Thermochemical Conversion,*** Springer Theses, DOI 10. 1007/978-981-10-3827-3_2

Zicari S. M., (2003). ***Removal of hydrogen sulfide from biogas using cow-manure compost.*** MSc Thesis Presented to the Faculty of the Graduate School of Cornell University

Zinder, S. H., and Koch, M. (1984). ***Non-acetoclastic methanogenesis form acetate: Acetateoxidation by a thermophilic syntrophic coculture***. Arch. Microbiol.138, 263–273.

Zonta, T., Alves, M.M., Flotats, X., Palatsi, J., (2013). ***Modelling inhibitory effects of long chain fatty acids in the anaerobic digestion process.*** Water Research 47 (3), 1369e1380.