BIOGAS PRODUCTION BY CO-DIGESTION OF PAPERBOARD WASTE AND ORGANIC KITCHEN RESIDUES

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CERTIFICATE

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DECLARATION

I hereby declare that this research work titled as **"Biogas Production by Co-Digestion of Paperboard Waste and Organic Kitchen Residues"** is the outcome of my own efforts and has not been published anywhere else before. The material quoted in the text has been properly referred and acknowledged.

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Dedicated

To

Abbu, Ammi and Nauman

For their endless love, support and encouragement

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Per cent
BMP	Biological Methane Potential
C/N	Carbon - to - Nitrogen Ratio
COD	Chemical Oxygen Demand
FAS	Ferrous Ammonium Sulfate
g VS/l	Gram volatile solids per liter
HRT	Hydraulic Retention Time
MC	Moisture Content
ml CH_4/g VS	Milliliter Methane per gram of Volatile Solids
MSW	Municipal Solid Waste
OKR	Organic Kitchen Residues
OLR	Organic Loading Rate
PBW	Paperboard Waste
PCAT	Pakistan Council of Appropriate Technology
PCRET	Pakistan Council of Renewable Energy Technologies
SRT	Solids Retention Time
TA	Total Alkalinity
TKN	Total Kjeldhal Nitrogen
TOC	Total Organic Carbon
TS	Total Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids

ABSTRACT

Pakistan is critically leading towards amplified air, water and soil pollution caused due to open dumping of municipal solid waste. Organic fraction of biodegradable wastes may be converted into renewable bioenergy by the process of anaerobic digestion. Biological conversion of wastes may be one solution to many environmental problems and bioenergy is a virtuous, greenhouse friendly substitute for fossil fuels and way forward to sustainable development. Organic content of municipal solid waste generated in Pakistan comprises of 53-58 per cent kitchen waste and 6-8 per cent paper waste. Presence of 50-56 per cent cellulose content in paperboard waste makes it a potential feedstock for anaerobic digestion but the major limitation is the presence of lignin: 17-23 per cent , that hinders and elongates the hydrolytic phase of anaerobic digestion, therefore, leads to lesser biogas production. Pretreatment of lignocellulosic substrates prior to digestion may enhance the biogas yield and quality by minimizing the time required for the initial hydrolysis of the feedstock. The study was aimed to investigate the synergistic effect of anaerobic co-digestion of paperboard waste with organic kitchen residues at mesophilic temperature of 35±2°C and pretreatment of paperboard waste with alkali (NaOH), hydrothermal and ultrasonic on the rate of hydrolysis and biogas production. Paperboard waste undergoes 76, 68 and 42 per cent delignification for alkali, hydrothermal and ultrasonic pretreatments, respectively. Whereas the biogas yields increased up to: Alkali (70 per cent) > hydrothermal (61 per cent) > ultrasonication (45 per cent) as compared to the control (untreated paperboard waste only), with methane content ranged between 43.6-68 per cent . Organic loading rate for stable digestion process was optimized at 5 g VS/l, which resulted in 25 and 33.9 per cent more biogas production as compared to escalated loading rates of 10 and 15 g VS/l, respectively

Chapter 1

INTRODUCTION

1.1 BACKGROUND

Increased environmental pollution is caused due to upsurge in population growth, urbanization and industrialization. One of the majorly faced problem is management of municipal solid waste (MSW), when discharged in uncontrolled and large amounts it may cause detrimental effect on soil, water and air pollution (Chen et al., 2010). According to recent estimates of world's total MSW generation, approximately 1300 MT/year and is likely to increase up to 2200 MT/year, by 2025 with almost 46 per cent organic content (Al-Seadi et al., 2013). Several factors contribute in making MSW management more difficult including lack of resources, expertise, awareness and inadequate legislation (Fourie, 2006). Due to economical constrains, more than 90per cent of municipal waste generated in Asian countries is disposed in improper and non-engineered facilities (Al-Khatib et al., 2010). Land dumping is considered as the most feasible option for managing the masses of garbage generating but due to significant environmental impacts like ground water and soil pollution the regulations are becoming narrow and suitable options are under consideration (Browne & Murphy, 2013). However, these problems stipulate an environment friendly and sustainable solution. Fortunately, this organic waste may be treated by two major biological conversion methods: aerobic decomposition-converting the waste into usable compost and anaerobic digestion: biological breakdown of organic wastes into renewable and cleaner bioenergy.

A burnable gas is generated during the process of anaerobic digestion which is a mixture of methane, carbon dioxide and numerous low- molecular weight intermediates, and less energy is required to run the process as compared to aerobic decomposition. Bioenergy is considered as the fourth largest energy renewable resource and a good greenhouse neutral auxiliary for fossil fuels (Mao et al., 2015). Organic wastes like manure, organic content of municipal waste, agricultural, industrial and institutional residues are valuable due to their ability to be converted into renewable energy-biogas (Figure 1.1). Biomethane production may be one solution to many environmental problems and it is considered as a scorching topic of research in many

developing as well as developed countries which are shifting towards renewable energy options (Chen et al., 2010; Campuzano et al., 2016; Walter et al., 2016).

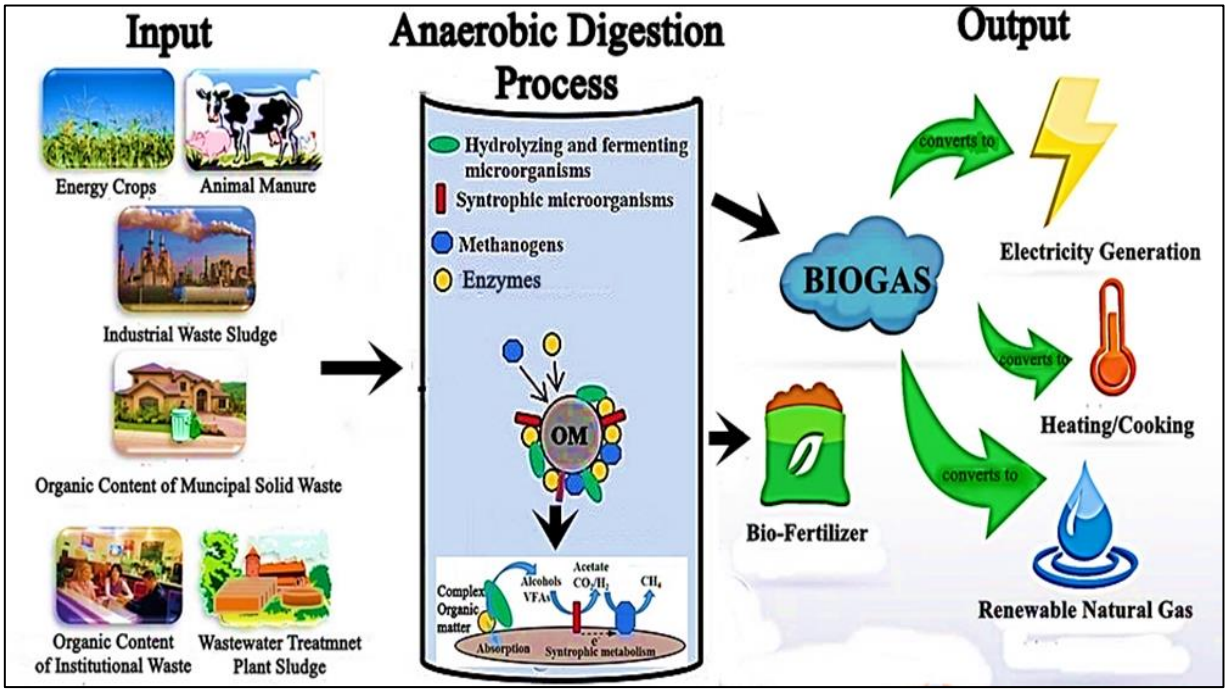

Figure 1.1: Illustration of organic wastes conversion to biogas

1.1.1 Biogas Industry in Pakistan: History and current scenario

Biogas technology was first introduced and developed in Sindh (1959) with farmyard manure as a feedstock. Government gave attention biogas as an alternate energy source in 1974 at domestic level and 21 biogas plants were installed by Pakistan Council for Appropriate Technology (PCAT). These plants were based on Chinese "fixed dome" design and failed due to cracks in their structures which led to the leakage of gas. Again in 1986, a project of 4000 Indian designed biogas plants installation was initiated. This program was completed in three phases: 1) 100 demo units by government; 2) 50 per cent subsidy was provided by the government and 3) only technical support was provided by the government. In the 3rd phase, program failed due discontinued funding's from the government, high cost of technology, lack of expertise, inadequate demonstration and political constraints (Ghaffar, 1995). After the failure of three-phased project another initiative was taken in 2000 named as Biogas Support Program (BSP), by government of Pakistan and 1200 household units were installed with a further plan installing up to 10,000 units. Similarly, another program was started by Rural

Support Programs Network (RSPN) in 2009, with an aim to provide people with different sizes of biogas plants to select as per their needs and convenience. Almost 70 units has been installed under this program, also subsidy of 7500 is given to the clients for initial installation (RSPN-Annual report 2008-2009). Afterward, RSPN developed a four-year project proposal of installation of 14,000 units in central Punjab and submitted to embassy of kingdom of the Netherlands (EKN). The project was approved by EKN and started in 2009 and ended on December, 2014 (RSPN-Annual report 2015). Alternate energy development board (AEDB) and Pakistan Council for Renewable Energy Technologies (PCRET) are also working actively to make biogas an alternate and cheap energy source (Amjid et al., 2011).

1.1.2 Feedstock for Biogas Production in Pakistan

Organic fraction of municipal solid waste (OFMSW), agricultural wastes including crops residues, cattle and poultry waste, all are readily generated in Pakistan and may be used as potential substrates for biogas production (Asif et al., 2009).

Table 1.1 MSW and its organic content generated in metropolitan cities of Pakistan

Cities	**Population** (Millions)	**MSW generation** (Ton)	**Organic content** (Ton)
Karachi	11.62	1378	716
Lahore	6.29	953	639
Islamabad	0.74	225	216
Rawalpindi	1.77	320	144
Multan	1.45	325	211
Hyderabad	1.39	374	206
Faisalabad	2.5	296	136
Peshawar	1.24	149	67
Gujranwala	1.44	128	51
Quetta	0.73	100	37
Total	29.18	4248	2423

(Source: Raheem et al., 2016)

Municipal solid waste (MSW) is composed of organic and inorganic contents and major proportion comes from household, hospitals, institutional, industrial and commercial areas and generated more in urban areas as compared to rural area depending upon the socio-economic behavior of populations, seasons and degree of development. Total solid waste generated in major cities of Pakistan is approximately 3,601,221 tones/year with 2.61 per cent increase due to rapid population growth and generation rates ranging from 2.83-6.13 kg/c/day (Pak-EPA, 2005). By, 2014 solid waste generation stretched up to 71,000 tones/day in the major metropolitan cities (Ilyas et a., 2017). Maximum organic content in MSW for biogas production is available in Karachi followed by Lahore, Islamabad, Multan and Hyderabad (Table1.1). Like other developing countries Pakistan is facing difficulties in proper handling of enormous quantities of MSW generated with easily biodegradable organic content of varying quantities, ranging from 42.2-80.1 per cent for food waste and 0.97-10.6 per cent for paper and cardboard. Conversion of these feedstocks into bio-energy products like biogas and ethanol is economically, socially and environmentally viable option (Kamran et al., 2015).

1.1.3 Environmental, Economic and Social Impacts of Biogas Industry

Biogas, categorized under bioenergy resource, may be beneficial in many ways (Shaukat et al., 2016).

- Agricultural residues which are mostly disposed off by open burning, may cause air pollution that leads to health hazards. Using these residues as feedstock for biogas production may be an effective solution.
- Increasing population demands higher production, hence the quality of agricultural land is also deteriorating. Anaerobically digested slurry may be utilized as a biofertilizer, reducing the input costs in agricultural sector and minimizing the negative environmental impacts of synthetic fertilizers.
- Using organic municipal solid waste (OMSW) as a feedstock may minimize the overall costs of transportation and constructing a landfill for waste management and methane escaping to the atmosphere from landfills and open dumping sites may be reduced.
- Biogas may also be used as a substitute to coal in power generation industry and the increase in global warming caused due to burning of fossil fuels may be abridged.

- ❖ The ammonia content of the substrate is lost up to 50 per cent in aerobic digestion where as it remained conserved in anaerobic digestion and co-digestion may further enhance the nutritional value of the slurry.
- ❖ Establishment of biogas industry may initiate new jobs opportunities, which is an additional social benefit for the communities.

1.2 SIGNIFICANCE OF THE STUDY

Inadequate waste management in Pakistan, from collection to disposal is a health and environmental hazard. Other than installation of proper waste disposal facilities, converting waste into energy is another economically feasible and sustainable solution for developing country like Pakistan (Khan et al., 2012). Majority of population of Pakistan may easily be shifted to biogas energy because of huge substrate availability for an anaerobic digestion at domestic levels as per needs of urban and rural populations. Researchers around the world are working on the process improvements for improved and efficient bio- energy production. Among the 4 stages of anaerobic digestion (AD), hydrolysis is considered to be a rate-limiting step. Hence, the performance of anaerobic digestion may enhance by several pretreatments (physical, thermal or chemical). Moreover, different substrates may be co-digested to enhance the AD process, pH, increase the nutrient balance and biomass profile of the digesters, and to get much optimum carbon-nitrogen ratios (Zheng et al.,2014). Therefore, this study was designed to investigate the biogas potential of paperboard waste, synergistic effects of pretreatments and effect of addition of organic kitchen residues as co-substrate.

1.3 RESEARCH OBJECTIVES

This study has two objectives:

1. Investigating the effect of several pretreatments on lignin removal from paperboard waste.
2. Evaluating the performance of pretreated paperboard waste when co-digested with organic kitchen residues for biomethane production.

Chapter 2

LITERATURE REVIEW

This chapter comprehends the main aspects of biogas production with reference to the latest and relevant literature. The background information will be used to interpret the results in the subsequent chapter.

2.1 ANAEROBIC DIGESTION

Anaerobic digestion (AD) is a naturally occurring process for biological conversion of organic fraction of solid, semi-solid and liquid wastes into methane, carbon dioxide, inorganic nutrients and digested compos by microbial decomposition. Major naturally existing methanogenic ecosystems are (Figure 2.1): (a) lacustrine and marine sediments, marshes, swamps, rice paddies, sludge and digesters; (b) ruminants and intestinal tracts of mostly all living organisms; (c) Hot springs: where methanogenesis occurs only from geochemical hydrogen formed as part of the geological process (Arsova, 2010).

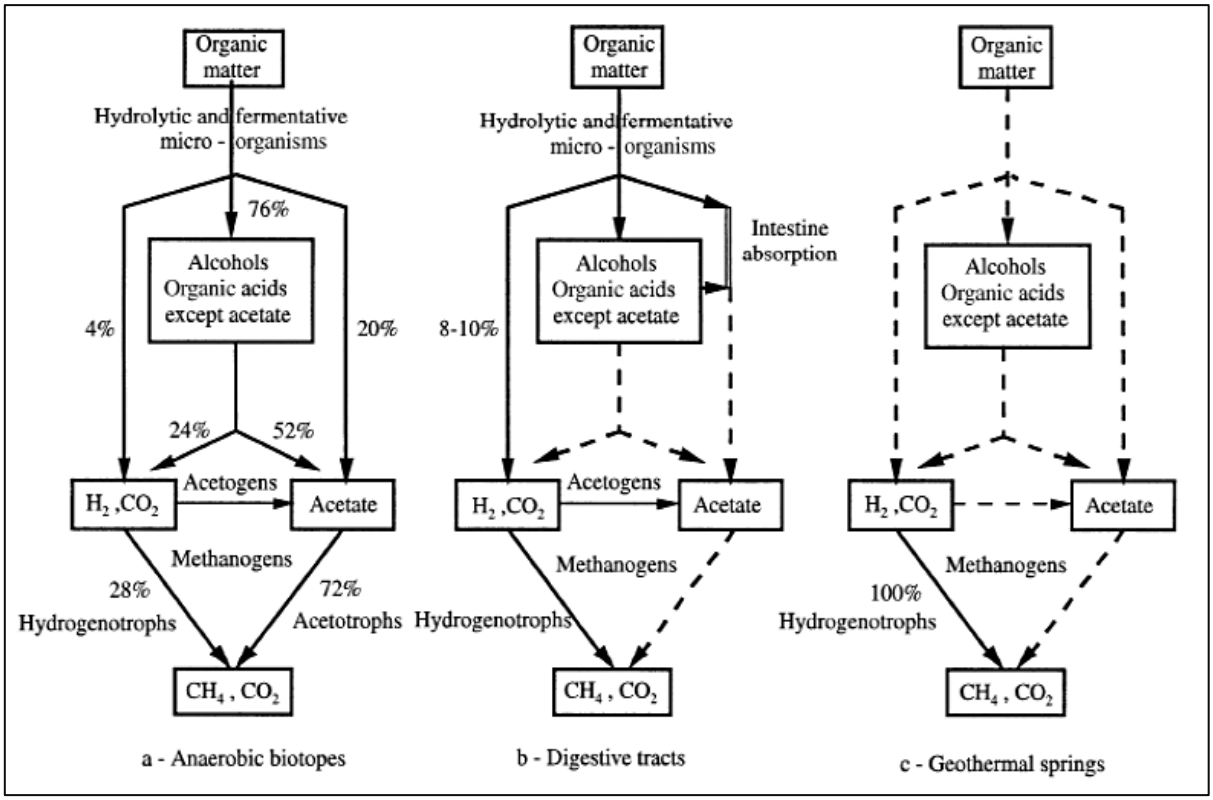

Figure 2.1: Naturally occurring bio-methanogenesis in various ecosystems

AD requires an oxygen- free environment and occurs over a chain of reactions held in four major phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis and for each step, different group of microorganisms are responsible for bio-chemical conversion reactions (Figure 2.2), most dominant are primary fermenting bacteria /fermentative bacteria, anaerobic oxidizing bacteria and methanogenic archaea (Hahnke et al., 2015). Generally, three reactions occur during the whole AD process: hydrolysis, acid formation and conversion of acids into methane (Arsova,2010).

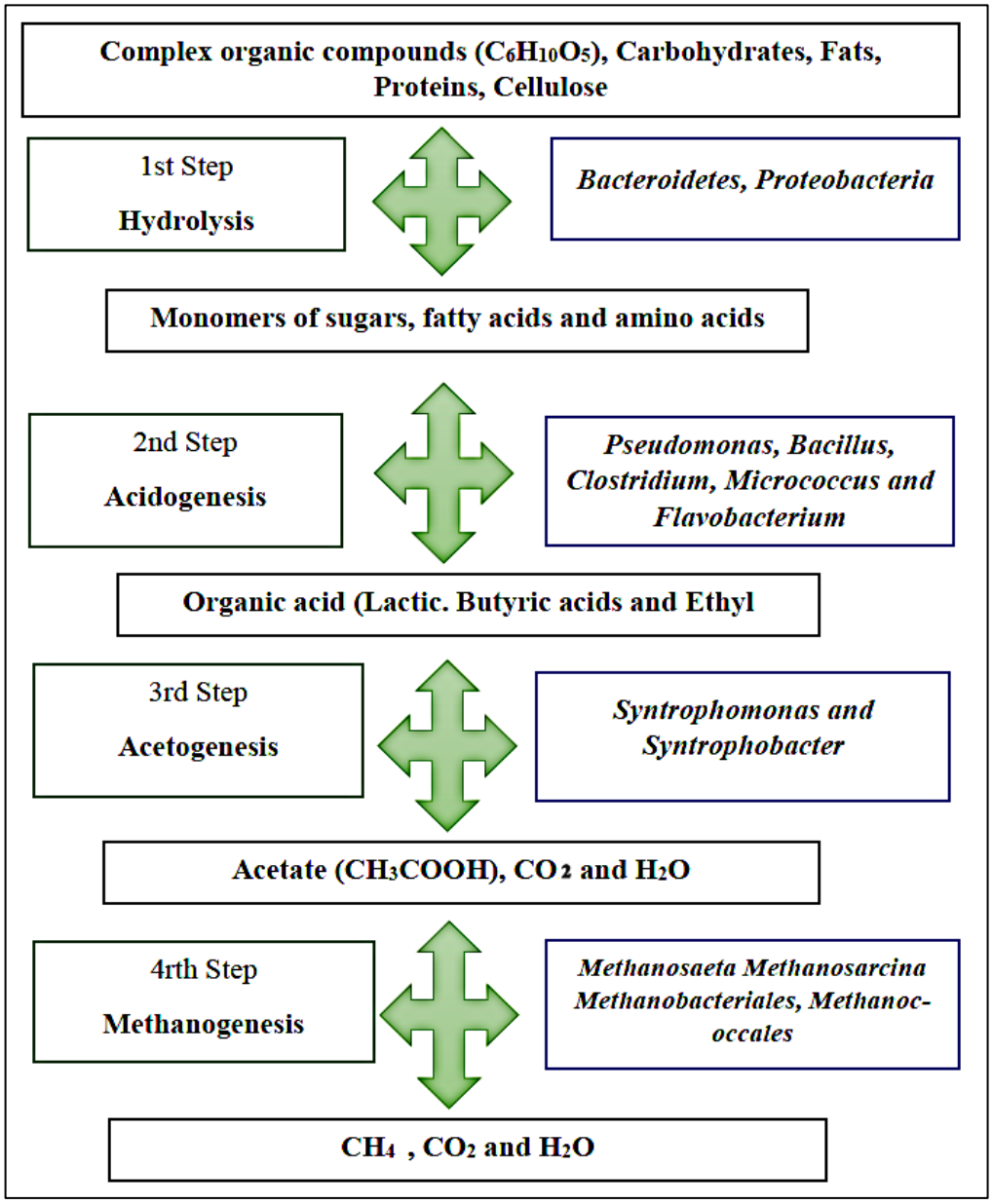

Figure 2.2: Schematic diagram of steps and microbiology involved in anaerobic digestion

Humans have adopted bio-methanogenesis for rapid and controlled decomposition of organic wastes and biomass conversion to methane, carbon dioxide and stabilized digested residue. In the generalized scheme of the anaerobic digestion, the substrates are collected, processed (cutting/shredding) and fed to the reactor along with active inoculums of methanogenic microorganisms. Methane is a significant greenhouse gas and AD has higher control over the methane production and contributes in lowering the carbon footprint of organic waste management and fugitive emissions are lower than the CH_4 release in cases of landfilling and aerobic composting of solid waste (Shafiei et al. 2013). Therefore, AD has farfetched applications in energy, environmental and agricultural sectors, and has been used as a helpful technique for handling municipal, industrial, solid and semi-solid wastes since last century. Methane produced via anaerobic digestion is contemplated as a clean, safe and environmentally friendly fuel.

2.2 METHANE PRODUCTION MECHANISM

Each microbial group (Figure 2.2) contributes in undergoing different phases of digestion from hydrolysis of polymer substrates, fermentation of sugars and amino acids, anaerobic oxidation and hydrogenotrophic methanogenesis to the production of final product-biomethane (Tsavkelova et al., 2012). Each step is discussed in detail below:

2.2.1 Hydrolysis

Anaerobic digestion starts with the hydrolysis-breakdown of complex organic wastes. Hydrolytic bacteria mainly belong to the anaerobes of genera Bacteroidetes and Proteobacteria and use their extracellular enzymes (amylases, proteases, lipases) to break down the complex organic structures like proteins, carbohydrates, cellulose and other polysaccharides into simpler compounds (Bryant 1979; Ren et al., 2016). When the feedstock is easy to hydrolyzed by the bacteria, this stage is quickly completed, but in case of complex polysaccharides and lignocellulosic materials, this stage is elongated due to harder initial degradation of feedstock. Therefore, pretreatment of substrates is required. Once this polymerized organic waste is hydrolyzed its then converted into simpler and soluble monomers and dimers like amino acids, sugars and fatty acids (Figure 2.2) by fermentative bacteria (Ofoefule et al., 2010).

The rate at which the feedstock is being hydrolyzed also depends upon the size of the feedstock, initial pH, production of the enzymes, diffusion and adsorption of these enzymes on the surface

of waste particles. Following reaction shows how the complex organic waste is broken down into simpler structures (Themelis and Verma, 2004).

$$C_6H_{10}O_4 + 2H_2O \longrightarrow C_6H_{12}O_6 + 2H_2 \quad (2.1)$$

2.2.2 Acidogenesis

Water soluble monomers produced during hydrolysis and other chemical substances that are already present in the feedstock are utilized by the next group of microbes in the process of acidogenesis which converts these compounds into short - chain organic acids (formic, carbonic, butyric, acetic, propionic), alcohols (methanol and ethanol), carbon dioxide, hydrogen gas and volatile fatty acids (Ntaikou et al., 2010). Most of the times, acidogenesis is held as a two-directional process as: 1) Hydrogenation and 2) Dehydrogenation. As a result of catabolism, CO_2 and H_2 are produced, which may be directly used by the methanogens for methane production. While other acidogenesis by-products must undergo acetogenisis before consumed by the methanogenic microbes (Tsavkelova et al., 2012). Due to increase in amount of acids in the reactor the pH also falls around 5 - 6 during this stage. Obligatory anaerobes genera that are mainly involved in acidogenesis includes: *Pseudomonas, Bacillus, Clostridium, Micrococcus* and *Flavobacterium* (Ali et al., 2014). Usually the following reactions occurs during acidogenesis: 1) conversion of glucose into ethanol 2) conversion of glucose into propionate (Ostrem & Themelis, 2004).

$$C_6H_{12}O_6 \longleftrightarrow CH_3CH_2OH + 2CO_2 \quad (2.2)$$

$$C_6H_{12}O_6 \longleftrightarrow 2CH_3CH_2\,COOH + 2H_2O \quad (2.3)$$

2.3.3 Acetogenesis

Acetogens, that mainly belongs to the genera *Syntrophomonas* and *Syntrophobacter* are responsible for the conversion of acidogenesis by-products like alcohol, volatile fatty acids (VFA's), amino acids and aromatic compounds into acetate and hydrogen which then may be utilized by the methanobacters (Figure 2.2) for conversion to methane (Li et al., 2011). Excessive hydrogen production at this step may cause inhibition of the bacterial activities due to drastic drop in pH and increased toxicity in the reactors. This effect is minimized by the methanogens that are already present in the reactors and linked by a symbiotic association with the acetogens. Excessive hydrogen produced by the acetogens is consumed by the methanogens

making the reactors environment more favorable and energetic for other anaerobes and 70 per cent of methane is accrued during this phase of digestion (De Bok et al., 2005). This step mainly involves the alteration of propionate, glucose, ethanol and bicarbonate into acetate (Ostrem & Themelis,2004).

$$2HCO_3^- + 4H_2 + H^+ \longleftrightarrow CH_3COO^- + 4H_2O \quad (2.4)$$

$$CH_3CH_2OH + 2H_2O \longleftrightarrow CH_3COO^- + 2H_2 + H^+ \quad (2.5)$$

$$C_6H_{12}O_6 + 2H_2O \longleftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2.6)$$

$$2CH_3CH_2COO^- + 3H2O \longleftrightarrow CH_3COO^- + H+ HCO_3^- + 3H_2 \quad (2.7)$$

2.3.4 Methanogenesis

The last and the most important step of the whole anaerobic mechanism is methanogenesis. The by-products generated during the previous phases are converted into methane by obligate anaerobes like acetoclastic methanogens which are responsible for up to 60 per cent CH_4 production. Remaining 30 per cent CH_4 is generated as a result of Hydrogenotrophic and autotrophic methanogens activity of reducing CO_2 and H_2 (Ziganshin et al., 2011).

$$CH_3COOH \longleftrightarrow CH_4 + CO_2 \quad (2.8)$$

$$CH_3OH + H_2 \longleftrightarrow CH_4 + H_2O \quad (2.9)$$

$$CO_2 + 4H_2 \longleftrightarrow CH_4 + 2H_2O \quad (2.10)$$

Various studies showed that major methane producing groups that are found in mesophilic anaerobic systems are: *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales* and *Methanosarcinales*. Whereas, methane production by consuming acetate is performed mainly by the genera *Methanosaeta* and *Methanosarcina* (Demirel et al., 2008). Methanogenic communities are highly sensitive to drop in temperature, pH, alkalinity and excessive volatile fatty acids (VFA's) accumulation in the reactor. Temperature within the digester is also required to be kept within the optimum range of mesophilic or thermophilic methanogens for efficient contribution in biogas production (Table 2.1).

Table 2.1: Optimal conditions for methanogens stable activity

Conditions	Optimum	Marginal
pH	6.8- 8.2	6.5
Alkalinity (mg/L CaCO3)	≥ 1500-3000	1000-1500
Volatile fatty acids (mg/L CaCO3)	500-1000	≤ 2000
Temperature: Mesophilic range	30-35°C	27-30°C; 35-40°C

(Source: Chan et al., 2009)

2.3 PRODUCTS OF ANAEROBIC DIGESTION

Biochemical reactions involved in anaerobic digestion convert the organic waste into Biogas and the remains of feedstock after digestion may be utilized as nutrient rich bio-fertilizer. Composition and usage of these products are discussed in detail below.

2.3.1 Biogas

Key product of AD process commonly named as biogas, is a colorless and odorless gas that is 20 per cent lighter than air and burns with a blue flame similar to LPG gas (Vij, 2011). Methane (CH_4) and carbon dioxide (CO_2) are usually present in 3:1 with scarce traces of nitrogen (N_2), oxygen (O_2) and hydrogen sulfide (H_2S). Quality and quantity of biogas depends upon the substrate being used for digestion, the operating parameters of the reactor and also the microbial communities present in the reactor effect the composition of biogas (Carrere et al., 2010). Smaller AD facilities are able to generate enough gas for fulfilling cooking and heating needs at domestic levels, whereas larger plants may produce biogas larger quantities, which is further purified by scrubbing off the impurities (CO_2 and H_2S) and may be directly used as a substituent for natural gas or in electricity generation (Ostrem & Themelis, 2004). Combustion properties of biogas (Table 2.1), indicates that its deflagration speed is lower than liquified petroleum and natural gas which is due to excessive CO_2 content (Diaz et al., 2008). Biogas with 50 per cent or more methane content may be upgraded to further enhance the oxidation reaction kinetics (Cacua et al., 2011).

Table 2.2: Combustion characteristics of biogas

Parameters	Values
Relative Density	0.94-1 kg/m^3
Molecular weight	27.20 g/mol
Energy content	6-6.5 kWh/m^3
Low calorific Value	20.35 MJ/m^3 biogas
High calorific value	22.64 MJ/m^3 biogas
Minimum energy ignition	464.98 kJ

2.3.2 Digestate

Digestate is produced as a result of breakdown of organic matter by anaerobic microbes. Generally, it is obtained in semi-solid and liquid forms for wet-AD and solid form when originated from dry-AD. Slurry residues recovered from the digester contains greater nutritional qualities as compared to undigested waste and further digested by aerobic bacteria to reduce overall volume of the fertilizer. Also, ammonia that is present in the slurry may be converted into other forms (Nitrification process: Ammonia > nitrites > nitrates), by ammonia-oxidizing bacteria to further enhance its quality (Abbas et al., 2015; Wang et al., 2017). During biogas production, substrates that are mixed and co-digested together undergoes chain of biochemical process and degraded by the bacterial consortium, in result nitrogen (N), phosphorus (P) and potassium (K) which are present in complex form in organic wastes are converted to easily available forms for plants (Drosg et al., 2015). NPK are vital macronutrients for better plant growth and enhanced flower and fruit yield. Lower total solids (TS) and C/N ratio due to lesser total organic carbon after the completion of digestion, higher nitrogen content and pH values as compared to undigested slurries, digestate may be utilized as a potential bio-fertilizer prior to digestion without any further processing (Tambone et al., 2010). Higher C/N ratios (exceeding from 27:1) of organic wastes leads to N-immobilization when directly fed to the plants, whereas these organic materials are when anaerobically digested the lesser C/N ratio helps in better N-mineralization promoting plants growth and soil fertility (Fouda, 2011). Along with promoting the plant growth the nutrient rich bio-fertilizer also acts as soil conditioner which increases overall soils nutrient content and organic matter, whereas the mineral fertilizers

only cater the plants nutritional needs and in the long run soil quality deteriorates (Figure 2.3). Anaerobically digested solids may be further used as feedstock for ethanol production, while the sludge which resembles compost in appearance may be used a potential bio-fertilizer (Yue et al., 2010).

2.4 OPTIMUM CONDITIONS FOR ANAEROBIC DIGESTION

Efficiency of anaerobic process may be altered by several factors such as quality of substrates and inoculum, nutrient content, moisture content, pH, organic loading rate, hydraulic retention time, C/N ratio and process temperature. These factors are required to be optimized to attain the maximum biogas production and manage the waste more effectively. These parameters and their optimal operational ranges are discussed in detail below:

2.4.1 Feedstock and Nutrient Availability

Biological organic materials including: industrial waste, organic fraction of municipal waste, sewage sludge, domestic waste, lignocellulosic residues, slaughter house waste, animal manure, agricultural scums and energy crops all are the potential substrates for biogas production (Agrahari and Tiwari, 2014). Initial total solids content effect the overall performance of the anaerobic system, hence TS should be determined along with volatile solids (VS), carbon and nitrogen content (C/N), moisture content (MC), chemical oxygen demand (COD) prior to be used as a feedstock (Angelidaki et al., 2009).

Macro nutrients: carbon, nitrogen, hydrogen, phosphorus and potassium and micro nutrients iron, copper, cobalt, nickel, selenium and zinc, are required by the microorganisms for their adequate growth (Kayhanian and Rich, 1995). Anaerobic microbes consume chemical compounds present in the substrate such as carbohydrates, proteins and fats as an energy source which is when oxidized the electrons and protons are transferred through intermediates and at the end also to the electron receptor: CO_2, energy is produced through this pathway and then consumed by the bacteria. Due to this reason, quality and quantity of the feedstock directly influence the biogas yields e.g. when fats are digested more methane is produced as compared to carbohydrates and proteins (Teghammar, 2013). Hence, availability of sufficient organic material plays a vital role in anaerobic digestion. Adequate amount of carbon and nitrogen are required by the bacterial cells; carbon is utilized in the synthesis of biomass and used as an energy source, while nitrogen is used in production of nucleic acids of DNA. Optimum carbon

to nitrogen ratio (C/N) lies between 25-30: 1 for a robust degradation of waste into biogas by the bacteria (Wang et al., 2014). Feedstocks with higher nitrogen contents may be rapidly utilized by the bacteria and results in lower biogas production. Similarly, if the carbon content is higher in the feedstock with traces of nitrogen, this will cause inhibition due to excessive ammonia accumulation in the reactor (Amani et al., 2010). While using lignocellulosic waste, determination of its main components like cellulose, hemicellulose and lignin is important. Lignin content contributes in the inhibition and elongation of the hydrolysis phase of anaerobic digestion (Chandra et al., 2012). Physical pretreatment like shredding, grinding and cutting may make it easier for the bacteria to decompose harder feedstocks such as agricultural waste and paper residues (Teghammar, 2013).

2.4.2 Inoculum

Inoculum is the source of bacteria provided to the reactor. Major sources containing anaerobes includes: cattle excrement-cow dung (as anaerobic digestion naturally occurs in the intestinal tracts of ruminants: cows and buffaloes), digested manure from biogas reactors, sludge from membrane bio-reactors (MBR) plants. Good quality inoculum may have advantageous effects on the overall digestion process, as the microbes present are already used to work in anaerobic conditions with different substrates (Forster et al.,2007).

It should be used fresh and mixing different inoculums may also enhance the symbiotic relation between microbial consortium. It is necessary to degas the inoculum for 2-5 days, before adding it to the reactors or a blank should be separately run to minus the biogas produced only from the organic matter present in the inoculum. For degassing the same temperature range e.g. mesophilic or thermophilic, should be provided to the inoculum on which the anaerobic digestion assay of the substrate will be carried out (Angelidaki et al., 2009). Amount of inoculum to be used is based on the amount of substrate in the digester and it is termed as Substrate to inoculum ratio (S: I). This ratio should be selected vigilantly for a stable process, as inoculum in very high or low quantity may negatively distress the biogas production (Eskicioglu et al., 2011; Teghammar, 2013).

2.4.3 Particle Size

Size reduction is an important parameter and plays a dynamic role to enhance the biogas production as several studies focused on the effect of particle size showed exponential increase

in the biogas yields, were subjected to cutting and grinding as the particle size is an important aspect to be considered for anaerobic digestion (Palmowski et al., 2000). Larger particles may inhibit the easy movement of microbes and may cause clogging on inlet/outlets of the digesters. Size reduction may have positive impacts on the biogas production as more surface area is available to the microbes which influence the nutrient availability and rapid decomposition of the waste as the microbial growth rate increases (Angelidaki et al., 2009). Size reduction is required prior to the subsequent pretreatment to enhance its effect, it has been observed in past studies that methane yields were improved with combining chemical and biological pretreatments with physical treatments: chipping, grinding and milling, as they result in decreased crystallinity (Johnson & Elander, 2008).

Anaerobic digestion of milled food waste (MFW) showed 28 per cent increase in methane production, but the excessive size reduction resulted in higher VFA production and accumulation in the reactors which also disturbs the stability of the process and decreased methane yield was observed (Izumi et al., 2010). Similarly, Zhang & Banks. (2013) reported that it is not always necessary that smaller size always leads to better biogas production, it also contributes in inhibition of the process, hence the size should be selected carefully depended on the nature of substrate and type and mode (wet/dry) of digestion.

2.4.4 Moisture Content (MC)

Waning in moisture content may negatively influence the VS and carbohydrates removal and hydrogenotrophic and acetoclastic methanogenic populations (Fujishima et al., 2000). Therefore, reactors moisture content must be kept within optimal range of 60-80 per cent in contrast with 20-30 per cent solid content. This moisture acts as a transfer medium for microbes and also adequate water is required for the reactions taking place (Dhanya et al., 2009).

Mixing and agitation of the digester's contents is important to upsurge the interaction between organic matter and microbes so that microbes may have easy access to more surface areas resulting in shorter HRT and enhanced degradation. Optimum dilution of feedstock with water is required to provide a suitable medium with a TS of 7-10 per cent as too diluted slurry will settle down in the bottom and very thick solid particles would be a hurdle for microbes and gas passage (Azhar and Baig, 2011).

2.4.5 pH

pH is defined as "the hydrogen ions concentration in an aqueous solution" and it is one of the most important parameter that influence the whole process of anaerobic digestion. Microbial communities involved in each phase has their own optimum range for pH, based on their enzymatic activity. Hydrolytic and acidogenic bacteria works under pH ranged from 5.5 to 6.5. For overall process, the optimum range lies between 5-8.5 (Demirel et al., 2008). Specifically, methanogens are pH sensitive and become active in inert conditions (Table 2.1). Few exceptions include *Methanosarcina barkeri* and *Methanosarcina acetivorans*, which are acetate decomposers and isolated from acidic environments with pH as low as 5. While methylotrophic and hydrogenotrophic methanogens works in strongly alkaline conditions (Boone et al., 1993).

During acidogenesis, the pH of the system declined as up to 5 as a result of increased production of organic acids. As the digestion proceeds, these acids are converted into acetate in the next step of acetogenesis , meanwhile the pH within reactor rises due to increased NH_4 concentration. If the feedstock poses good buffering capacity, afterward acidogenesis the pH remains in the optimum range for methanogenesis (Tsavkelova et al., 2012). Stable pH indicates the system equilibrium and performance, whereas instability and fluctuation in pH indicates the system failure due to acid accumulation. If the pH does not increase up to 8 as the prolific methanogens produce ammonia obstructing further acidogenesis, this will have a lethal effect on methanogens and the digestion inhibits before the production of biomethane (Jayaraj et al., 2014). Maintaining pH according to the undergoing phase from acidogenesis to methanogenesis is very important and if the feedstock does not have enough buffering capacity (acidic feedstocks such as food waste), it is required to add a buffering agent like sodium hydroxide or calcium carbonate to neutralize the acids and increase the bicarbonate alkalinity as high as required for the survival of methanogens (Ostrem & Themelis, 2004).

2.4.6 Temperature

Anaerobic digestion may be performed under various temperature ranges and the microbial communities involved are classified on the basis of these temperature ranges. Psychrophilic range is between 12-18°C, mesophilic range is 28-40°C and thermophilic range is 45-65°C. Mesophilic and thermophilic obligatory anaerobes produce more biogas and are more active as compared to psychrophilic microbes (Silva et al., 2007; Bah et al., 2014; Ali et al., 2016). While thermophilic digestion caters higher loading rates with maximum waste degradation and

pathogen removal from the substrate but requires higher energy input, longer time for system startup and is more sensitive to toxins and temperature fluctuations of the reactor. On the other hand, mesophilic range require less energy, its more stable and less sensitive to environmental changes with easier startup but longer retention times (Ostrem & Themelis, 2004; Mondal et al., 2016). Temperature directly impact the biogas production as the microbial species work efficiently in a system running within their optimum range as the growth surges exponentially and more organic matter is being consumed by the increased microbial population, resulting in improved biogas production (Moset et al., 2015).

2.4.7 Organic Loading Rate (OLR)

Organic loading rate is the amount of substrate added per reactor volume of the digester and the retention time. It determines the biological conversion capacity of an anaerobic system and also the viable quantity of volatile solids that may be added as an input to the digester. Initially overloading of organic content may inhibit the process as the constraining elements (VFA's) produced and accumulated excessively (Sahito et al., 2016). Loading rate depends upon the availability and quality of feedstock and inoculum, digester (design, capacity, working volume) and retention time of the whole process (Azhar and Baig, 2011). For thermophilic digestion the ORL ranges between 4-5 kg VS/m ^{3}/day, while for mesophilic systems the range lies between 2-3 kg VS/m ^{3}/day (Teghammar, 2013).

2.4.8 Hydraulic Retention Time (HRT)

Hydraulic retention time or hydraulic residence time (HRT) is defined as the average time taken by all the organic matter in the digester to be utilized by the bacteria and converted into digestate. Normally not all the material is degraded and the mass of feedstock or mixed substrates is always higher than the mass of residues as a part of input slurry has been gasified (Sahito et al., 2016). HRT depends upon the type of feed, microbial activity and other parameters those effect the digestion stability. In anaerobic systems, HRT is most often as 10-30 days or longer than that influenced by the degradation dynamics of the substrates. Slowly degrading materials require to be kept in the digesters for longer time periods than the easily degraded materials. Also for higher OLR, longer retention time is required to reach maximum biogas production and substrate utilization (Teghammar, 2013). Longer the retention time with optimum reactors conditions, more organic matter is being consumed by the bacteria. However, the rate of reaction will decrease with the passage of time and less biogas will be produced as

the retention time reach to an end (Arsova, 2010). Moisture content also effects the HRT as for dry-digestion it ranges between 14-30 days and for wet-digestion results in a shorter retention time as 5-10 days with higher biogas production rate per reactor volume but overall lower degradation (Ostrem & Themelis, 2004).

2.5 APPROACHES FOR IMPROVED BIOGAS PRODUCTION

With increase in demand for an alternate energy resource, it is also essential to find out ways to increase the bio-energy production.

2.5.1 Anaerobic Co-Digestion

Co-digestion may enhance the overall performance of anaerobic system by increasing the production rate of biogas as well as its quality. It involves treating several wastes which also provides one solution for management of different wastes (Hagelqvist & Granstrom, 2016). It was initially pragmatic in Denmark in 1980 where animal manure was mixed and co-digested with several types of organic wastes. Animal manure as co-substrate is source of extra nutrients for the bacteria, neutralizes the pH and increase the buffering capacity within the reactor and also its moisture content may dilute the concentrated inhibitory compounds which may be inhibitory (Neshat et al., 2017). While using acidic wastes like food waste as a feedstock, another substrate or inoculum with higher buffering capacity is required to reduce the limitation of using fruit and vegetable waste due to its low pH (Montusiewicz et al., 2008).

Better volatile solids removal may also be achieved by co-digestion as it increases the availability of mixed nutrients for the bacteria and also accelerates the breakdown of micro and macro nutrients by bio-simulation and due to the availability of diverse consortia of microbial organisms which may breakdown several types of wastes (Li et al., 2009). Co-digestion helps in balancing the carbon: nitrogen, which is one of the most important process parameter for a stable AD process. Mixing organic substrates may keep the C/N in desired range that is particularly respite between 20-30. Higher biogas yields via co-digestion is mainly a result of synergistic effect of microbial consortiums working on the organic loads in the digesters (Yen & Brune, 2007). At industrial scale, several types of complimentary industrial residues may also be mixed and digested together for better energy production and management of wastes using one facility. Providing extra economical as well as environmental benefits (Fountoulakis et al., 2009). Thus, co-digestion is the most commonly selected option over mono-digestion

because of its increased benefits like, 1) Dilution of the toxic components of substrates 2) Balancing of higher C/N ratios 3) Better buffering capacity within the digester and pH adjustment 4) Increased biogas and methane yields 5) Mixing several substrates also produces high quality digested sludge in larger amounts and with improved nutritional contents (Mata-Alvarez et al., 2014; Patil & Deshmukh, 2015).

2.5.2 Pretreatments of Lignocellulosic Materials

Lignocellulosic wastes are generated in large quantities mainly from agricultural, municipal, institutional, commercial and industrial sectors as crops residues, pulp and paper industry sewage sludge and as several types of paper waste of all types. It is mainly made up of three natural polymers: cellulose and hemicellulose making the carbohydrate portion after the removal of third component that is lignin (Zheng et al., 2014; Kamali et al., 2016). Cellulose which is the major component present in lignocellulosic materials, is made up of cellobiose units packed as a linear polysaccharide polymer connected by β-1, 4 glycosidic linkages. These polymeric chains are amalgamated together by hydrogen bonds in microfibril bundles (Figure 2.3) which are attached together by hemicellulose, amorphous sugars, pectin and covered by lignin (Adeeyo et al., 2015). Due to variability in the orientation, cellulose consists of amorphous and crystalline regions. Higher crystallinity index designates difficult biodegradation potential of the material (Taherzadeh & Karimi, 2008). Hemicellulose is a heteropolymer which majorly contains pentoses: xylose and arabinose; hexoses: mannose, galactose, and rhamnose (Figure 2.3) and acids in small quantities: glucuronic acid, methyl glucuronic acid and galacturonic acid. Structurally its short chained, less complicated than cellulose and easily hydrolyzed into monomeric sugars (Persson et al., 2006). Although degree of hydrolysis of hemicelluloses is influenced by pH, temperature and moisture content (Stamatelatou et al., 2012). Lignin is the hardest and most complexed aromatic heteropolymer built with phenylpropane units: coniferyl alcohol and sinapyl alcohol with hydroxyl, methoxyl, and carbonyl functional group in long chained three-dimensional structure (Figure 2.5) that is chemically linked with cellulose and hemicellulose with ether, ester or glycosidic bonds (Palmqvist & Hahn, 2000; Teghammar, 2013). Presence of lignin in higher quantity contributes as a barrier in bioconversion of lignocellulosic wastes making the substrate difficult to be degraded by bacterial enzymes, (Stamatelatou et al., 2012). Due to its hydrophobic and inert nature, higher temperatures (180°C) and neutral pH ranges are required for lignin dissolution (Grabber, 2005).

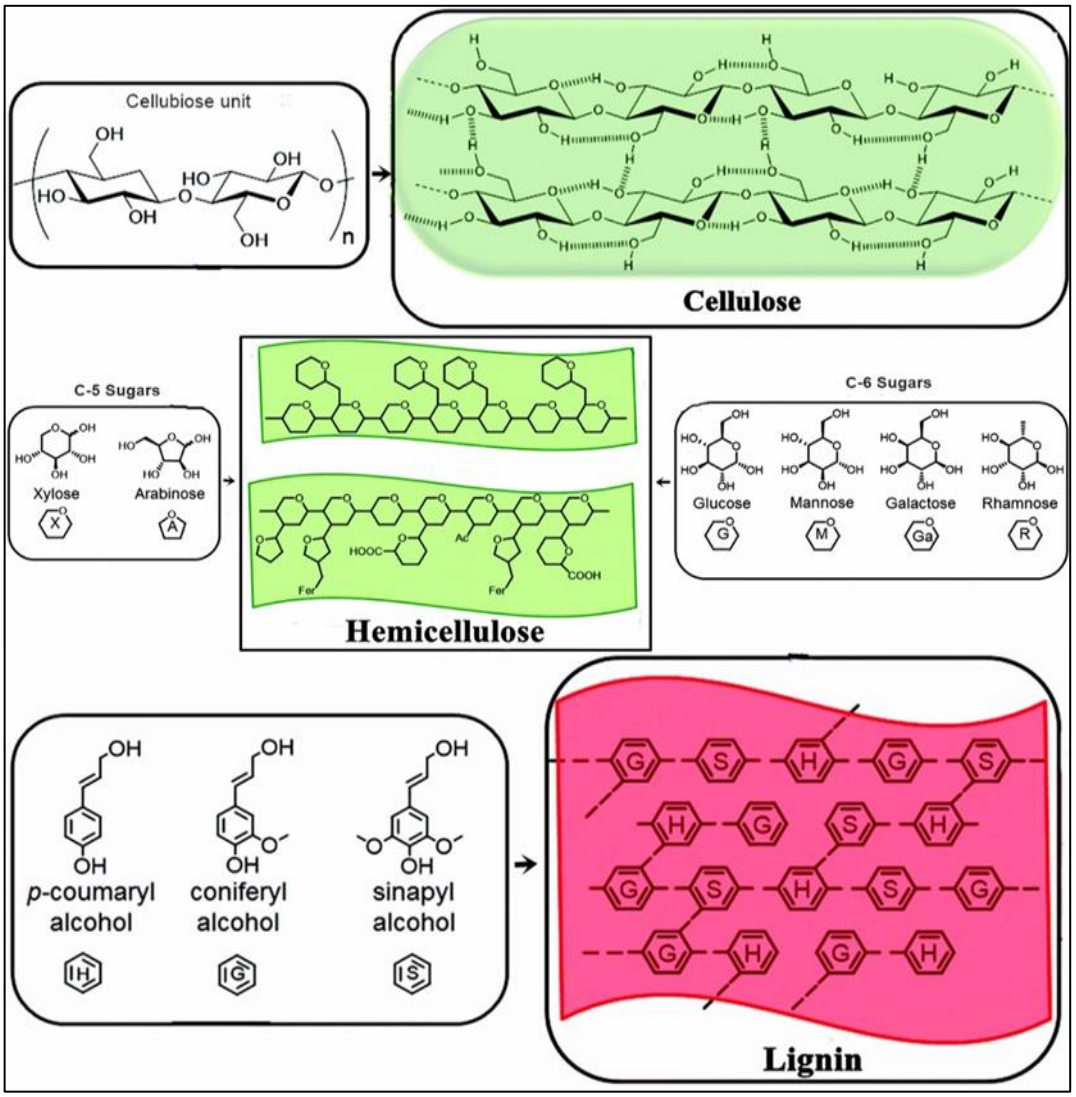

Figure 2.3: Structural orientation of cellulose, hemicellulose and lignin

Hence, hydrolysis is considered as the rate limiting step in anaerobic digestion and up to 50 per cent of such organic compounds remains in the primary state, reducing the efficiency of biogas production. Fermentable portion (cellulose and hemicellulose) is easily available to the bacteria after pretreatments for lignin solubility and removal (Parawira et al., 2008). Due to the complex and variable nature of lignocellulosic wastes the type of pretreatments should be selected according to the structural and compositional properties of the feedstock. Various pretreatments may be divided into groups of physical, chemical, thermal and biological methods (Table 2.3) which are available with a common goal of altering the surface area and lignin removal to shorten up the hydrolysis phase which may have a positive impact on overall biogas and methane yields (Qu et al., 2009; Lagerkvist et al., 2012). Potential lignocellulosic substrates for

anaerobic digestion may be pretreated to improve the biodegradation, solubilization and shorten the startup, which in result improve the biogas production and make all the organic material more easily available to the bacteria. Pretreatments may be mechanical, chemical, thermal or biological (Figure 2.4), all sharing the same goal of improving the convenience to bacterial enzymes by breaking the lignin bonding with cellulose and hemicellulose and affecting the degree of cellulose polymerization and reducing its crystallinity (Zheng et al., 2014).

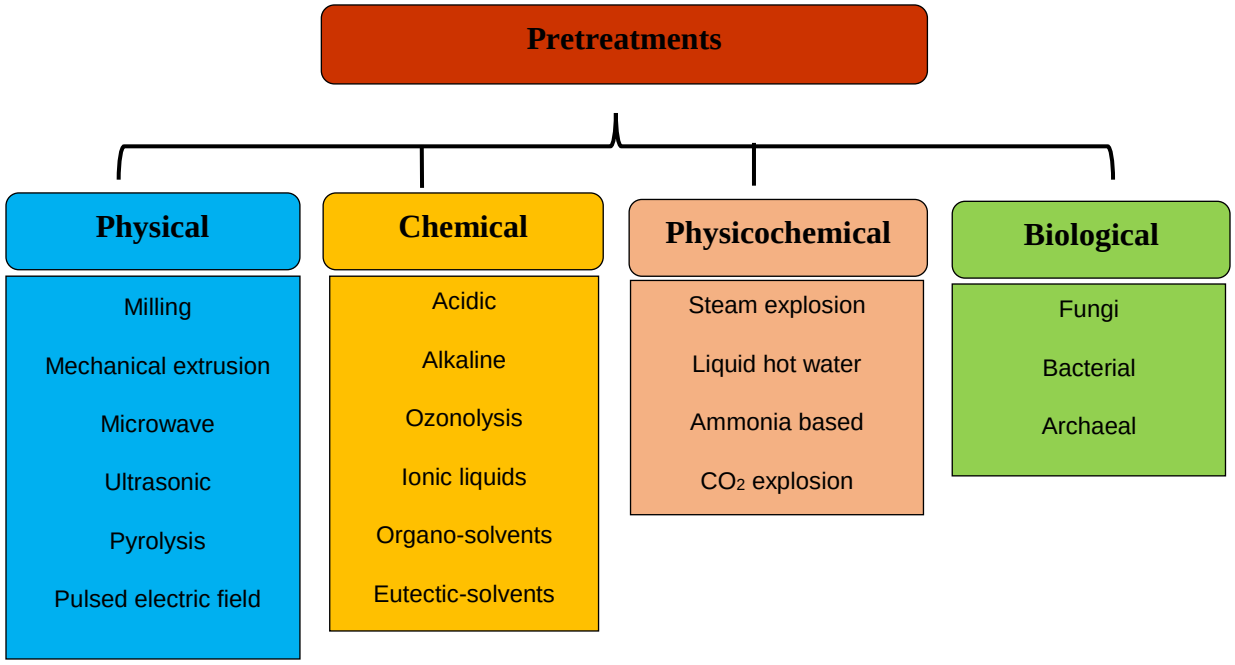

Figure 2.4: Overview of pretreatments for lignin removal

2.6 PREVIOUS STUDIES ON BIOGAS PRODUCTION FROM MSW

Feedstocks that contains macro-nutrients (carbohydrates, lipids, cellulose and hemicellulose) as their main constituents are all good substrates for bio-gasification (Das & Mondal, 2016). Due to their higher biological oxygen demand (BOD), chemical oxygen demand (COD), desired carbon and nitrogen contents, these are suitable to be used as promising feedstocks for anaerobic digestion (Labatut et al., 2011).

2.6.1 Organic Kitchen Waste (OKR)

Extensive research has been done in the past and still undergoing to investigate the different aspects and mechanisms of biogas production by using food waste. Zang et al. (2007) investigated the methane potential of food waste, mono-digested under thermophilic batch conditions. In digestion period of 28 days 0.435 l/g VS methane was produced with a 73 per cent total methane content in collected biogas samples and 81per cent volatile solids reduction. In a similar study, Alvarez et al. (2008) studied the effect of co-digesting several wastes including: slaughter house waste, animal manure and fruit and vegetable waste, under semi-continuous mesophilic conditions. Co-digestion resulted in better performance in terms of volatile solids reduction (65 per cent), methane production (0.3 m^3/kg VS) as compared to the mono- digestion of all wastes. Effect of co-substrates addition to fruit and vegetable waste under mesophilic conditions was investigated by Bouallagui et al. (2009) at varying organic loading rate (ORL) between 2.46-2.51 g VS/day. In a hydraulic retention time (HRT) of 10 days, biogas yield has been increased from 43 per cent to 51 per cent. Addition of co-substrates enhanced the C/N ratio within the digester which directly effect the biogas yields.

Lungkhimba et al. (2010) investigated the efficiency of a household compact biogas plant to manage the daily produced household by using it as feedstock. The setup was installed at laboratory and field scales. Results showed that average biogas production from a 1 m 3 was approximately 60 L, with a maximum methane content of 57.7 per cent. NPK analysis of the bio-slurry exhibited gradual increase in Nitrogen (N) and Phosphorus contents but they were remained below 1 per cent whereas Potassium (K) was increased up to 1.22 per cent. Similarly, Banks et al. (2011) determined the biodegradation efficiency of food waste during anaerobic digestion. Results showed that 90per cent of the feedstock (input) was converted to gaseous and digestate yields at the end of the process. Investigating the energy balance of the system showed that for every metric ton of input feedstock 405 kWh energy may be recovered. Methane content in the collected biogas samples was reported as 62 per cent.

In another study, Babaee & Shayegan. (2011) investigated the biogas production by using vegetable waste contained 9per cent total solids (TS) and 97 per cent volatile solids (VS). Anaerobic digestion was done as a dry semi-continuous process at three different organic loading rates (ORL) of 1.4, 2 and 2.75 kg VS/m^3/day for a time period of 25 days. Results

showed that increasing ORL resulted in decreased biogas production and the highest methane yield 64 per cent was obtained at a loading rate of 1.4 kg VS/m^3/day. Azhar and Baig. (2011) studied the biogas production potential of food waste (FW) to investigate the usage of anaerobic digestion as a waste stabilization and bioenergy production procedure. Pilot scale plant (1.2 m^3 capacity) was installed and food waste was co-digested with cow dung at thermophilic temperature (60-65°C). Results showed 0.04 m^3/kg FW with 60per cent methane content. The digested slurry analysis also proved it as a useful and good quality biofertilizer.Fernandez et al., (2013) compared the efficiency of anaerobic reactors fed with organic fraction of solid waste (OFMSW) under mesophilic (35°C) and thermophilic (55°C) ranges. Results exhibited that thermophilic temperatures may shortens the hydrolytic phase (T=8 days and M=14 days) and hence resulted in rapid consumption of organic matter by the bacteria. Whereas, mesophilic ranges may enhance the overall methane content in the biogas with a longer methanogenic phase (T=18 days and M=29 days).

Li et al. (2013) evaluated the biomethane potential, degradation rate and overall system stability of Kitchen waste (KW), poultry dropping (PD) and corn stover (CS) while co / mono- digested at mesophilic temperature (37°C) in batch mode. Volatile solids concentration of 3 g VS/l was kept constant at three different substrates to inoculum (S/I) ratios of 0.5, 1.5 and 3.0. Experimental results exposed that highest BMP and specific methane yields were attained from KW: 725 and 683 ml/g VS, followed by CS=470 and 214 ml/g VS, and PD=617 and 291 ml/g V, respectively. Correspondingly, biodegradation rate was also highest in KW (94 per cent) as compared to CS (45 per cent) and PD (47 per cent). KW produced better methane at S/I ratio of 1.5 while for CS and PD S/I ratios of 1.5 and 3.0 both were suitable. Synergistic effect of co-digestion was also observed in terms of better biogas yields, as compared to mono-digestion of substrates.

Likewise, Iqbal et al., 2014 investigated the biogas potential of kitchen waste when co-digested with cow dung and the effect of organic loading rate, temperature and pretreatment of kitchen waste with NaOH. In 1st phase substrates were subjected to mono and co-anaerobic digestion at room temperature (25~30°C) and 37°C (mesophilic range). Co-digestion under mesophilic range resulted in better degradation and biogas production as compared to room temperature. 2nd experimental phase was performed to check the effect of several organic loading rates (100-400 g/l) on digestion and the profligate degradation was achieved at 200 g/l ORL. During 3rd

phase, kitchen waste was firstly pretreated with three NaOH doses (1.0per cent, 1.5 per cent and 2.0per cent) and then degradation rate was inspected at 37°C and 200 g/l ORL. Extent of 1.5 per cent NaOH provided the best results and almost doubled the biogas production. Singh & Sankarlal. (2015) used kitchen waste as a substrate and cow dung as an inoculum and provided mesophilic temperature range to the digesters. Batch experiment was carried out for 20 days and biogas samples were collected to analyze the methane content. Results showed that kitchen waste is a good substrate and may produce biogas with upto 60 per cent methane content. Khairuddin et al,2016 investigated the effect of solid content on methane production by using household organic waste (HOW). Substrate was subjected to wet (<10 per cent TS) and dry (>15 per cent TS) anaerobic digestion. Results indicated that 15 per cent TS produced higher methane content of 63.7 l / kg VS as compared to 10 per cent TS that produced 29.8 l/kg VS. Hence it was concluded that 5 per cent increase in TS contributed 30-60 per cent raise in CH4 production.

Hobbs et al., 2017 performed lab scale experiments to determine the biomethane potential (BMP) of food waste at different food waste: inoculum ratios (F/I) of 0.42, 1.42 and 3.0 g COD/gVS. Results showed the 1.42 F/I ratio provided 90per cent CH_4-COD recovery which was the highest followed by 0.42 and 3.0 F/I ratios which provided 69 and 57per cent recovery, respectively. Furthermore, the results were interpreted by using Gompertz equation which gave lag times of 0, 3.6 and 30 days and methane production of 370,910 and 1950 ml for F/I ratios of 0.42, 1.42 and 3.0, respectively. Due to elongated lag phase at 3.0 F/I, 1.42 was considered as optimized F/I ratio, that gives the satisfactory results against all performance measures.

2.6.2 Paper Waste

Paper waste is one of the lignocellulosic materials as it is made from plants. It is mainly composed of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are biodegradable materials and may be utilized by the bacteria as energy source and in result bioenergy is produced. Major anaerobic bacterial species which are capable to degrade cellulose includes: *Bacterioides succinogenes*, *Clostridium lochhadii*, *Clostridium cellobioporus, Ruminococcus flavefaciens, Ruminococcus albus, Butyrivibrio fibrosolvens, Clostridium thermocellum, Clostridium stercorarium* and *Micromonospora bispora* (Elniski, 2017). Different aspects of using several types of paper waste as a feedstock are under investigation and not much work done up till now on this specific substrate regardless of its abundant

availability and biogas production potential. Few relevant and latest studies with findings are listed below:

Momoh & Nwaogazie, 2007 studied the effect of adding paper waste (PW) as a co-substrate to cow manure (CM) and water hyacinth (WH). Digesters were fed with constant quantity of cow manure and water hyacinth (5 g each) with varying concentrations of paper waste (4-20 g). Substrates were subjected to digestion at 29°C for 62 days. Maximum biogas production of 1.11 liters was obtained when 17.5 g of PW, 5 g CM and 5 g WH. Statistical analysis of data showed a parabolic relationship between increased biogas production as the amount of paper waste was increased in the reactors, with a goodness fit of 0.982. Similarly, Ofoefule et al. (2010) explored biogas potential of printing press paper waste when combined with cow dung (1:1), subjected to anaerobic digestion at mesophilic range for 45 days and solid to liquid ratio of 1:3. Mono digestion of paper waste resulted in cumulative gas yield of 6230 ± 0.07 ml/kg, while mixing both wastes together enhanced the cumulative gas yield up to 9340 ± 0.11 ml/kg.

Teghammar et al, 2012 used discarded paperboard tubes as a co-substrate to be used along with a nitrogen-rich mixture, called as buffer tank substrate (BTS) gathered from organic fraction of MSW, industrial bio-sludge, industrial wastewater sludge, slaughterhouse residues, industrial food waste, fish sludge and citrus waste. Prior to digestion paper tubes were subjected to thermochemical steam explosive pretreatments which were performed at 15-20 bar and 190°C for 10 min with 0-2 per cent NaOH was added. Batch mode anaerobic assay was performed at thermophilic temperature of 55°C for 50 days to check the effect of pretreatments on the biogas production. Results showed that addition of paper tubes waste had alleviating impact on overall system performance due to its high carbon content which provided the optimum C/N ratio for anaerobic digestion and methane yields also increased from 15-34 per cent. On the other hand, effect of increased concentration of NaOH also showed positive impact on methane yields and gave almost 50 per cent increase in methane concentration up to 403 N ml of CH_4/g VS was produced compared to untreated paper which gave maximum methane concentration of 268 N ml of CH_4/g VS.

Zhang et al (2012) co-digested food waste (FW) with cardboard (CB) in wet AD at a ratio 53:47 g VS /l, achieving effective methane production at a loading rate of 3 g VS/l $^{d-1}$ and proving that CB addition led to less accumulation of ammonia and VFA's. Aremu & Agarry.

(2013) investigated the effect of addition of corn cobs (CC) and paper waste (PW) as a co-substrate to poultry droppings (PD). Anaerobic fermentation was carried out under mesophilic temperature range (27-35°C) at a retention time of 30 days. Mono-digestion of poultry droppings resulted in cumulative average biogas volume of 3452 ml/g VS whereas the untreated co-substrates while added to the PD, enhanced the cumulative average biogas volume up to 4811 ml/g VS. When the CC and PW was subjected to pretreatment (milling and thermal hydrolysis) before digestion, it resulted in cumulative average biogas volume of 6454 ml/g VS. Hence it has been evidenced that co-digestion and pretreatment may make lignocellulosic waste a suitable feedstock for anaerobic digestion. Likewise, Elniski,2017 studied the effect of co-digestion of office paper waste (OPW) and cow manure (CM). Digesters were fed with varying total solids concentrations of OPW (0-5 per cent TS) and CM (1-6 per cent TS). During 20 days of digestion period, maximum biogas was generated in the reactor fed with 4 per cent OPW and 2 per cent CM, gave an optimum substrate to inoculum ratio of 2:1. Significantly higher quantity of biogas was produced at the above ratio as compared to the reactor which undergoes digestion without OPW (0 per cent OPW and 6 per cent CM).

Capson et al. (2017) inspected the efficiency of batch anaerobic co-digestion reactors treating food waste and cardboard. Results showed substrate to inoculum (S/I) as an important parameter. At lower S/I ratios (0.25ml/gVS^{-1}) higher methane was produced with *Methanosarcina* as essential archaea, as compared to the higher S/I ratios of 1 and 4 ml/gVS^{-1} due to which hydrogen and metabolites were produced which leads to lower substrate degradations. Li et al. (2018) evaluated the feasibility of co-digesting the carbon rich cardboard (CB) and office paper (OP) with nitrogen rich sheep dung (SD) for biomethane production. Results exhibited highest methane yields of 151.62 and 198.85 mL/gVS^{-1} were obtained during the co-digestions of SD with CB at 4:1 ratio (SDCB) and SD with OP at 2:3 ratio (SDOP), respectively.

2.7 SUMMARY OF LITERATURE REVIEW

Biological conversion of the organic portion of waste is an effective and environmentally sustainable way of waste disposal. Anaerobic digestion is widely used approach for stabilization and bioconversion of wastes into renewable biofuels like biogas. Four steps are involved in biogas production named as: hydrolysis, acidogenesis, acetogenesis and methanogenesis. During each phase several microbial consortia follows a series of metabolic pathways.

Biomethane may contains upto 50-70 per cent methane, depending upon the substrate being used. After the biogas production end product which is a nutrient rich slurry can be used as bio-fertilizer, minimizing the agricultural costs and environmental footprints of synthetic fertilizers. Easily degradable nature of kitchen waste makes it an ideal substrate for AD but its low pH leads to increased volatile fatty acids production disturbing the buffering capacity of the reactor and inhibiting the methanogenic activity. Similarly, paper waste has a high potential for biogas production due to higher cellulose proportion. But its lignin content contributes in the elongated hydrolysis phase that results in lesser biogas yields. Lignocellulosic substrates should be subjected to either physical, chemical or biological pretreatments prior to digestion to make cellulose easily available for the bacteria. Also Mixing of more than one substrates can adjust the higher C/N ratios and low buffering capacities.

Chapter 3

MATERIALS AND METHODS

This chapter includes details of lab based experimental setup and standard methods adopted for the analysis of substrates, biogas collection, biogas and digested slurry compositional analysis. Step wise adopted methodology is demonstrated below (Figure 3.1):

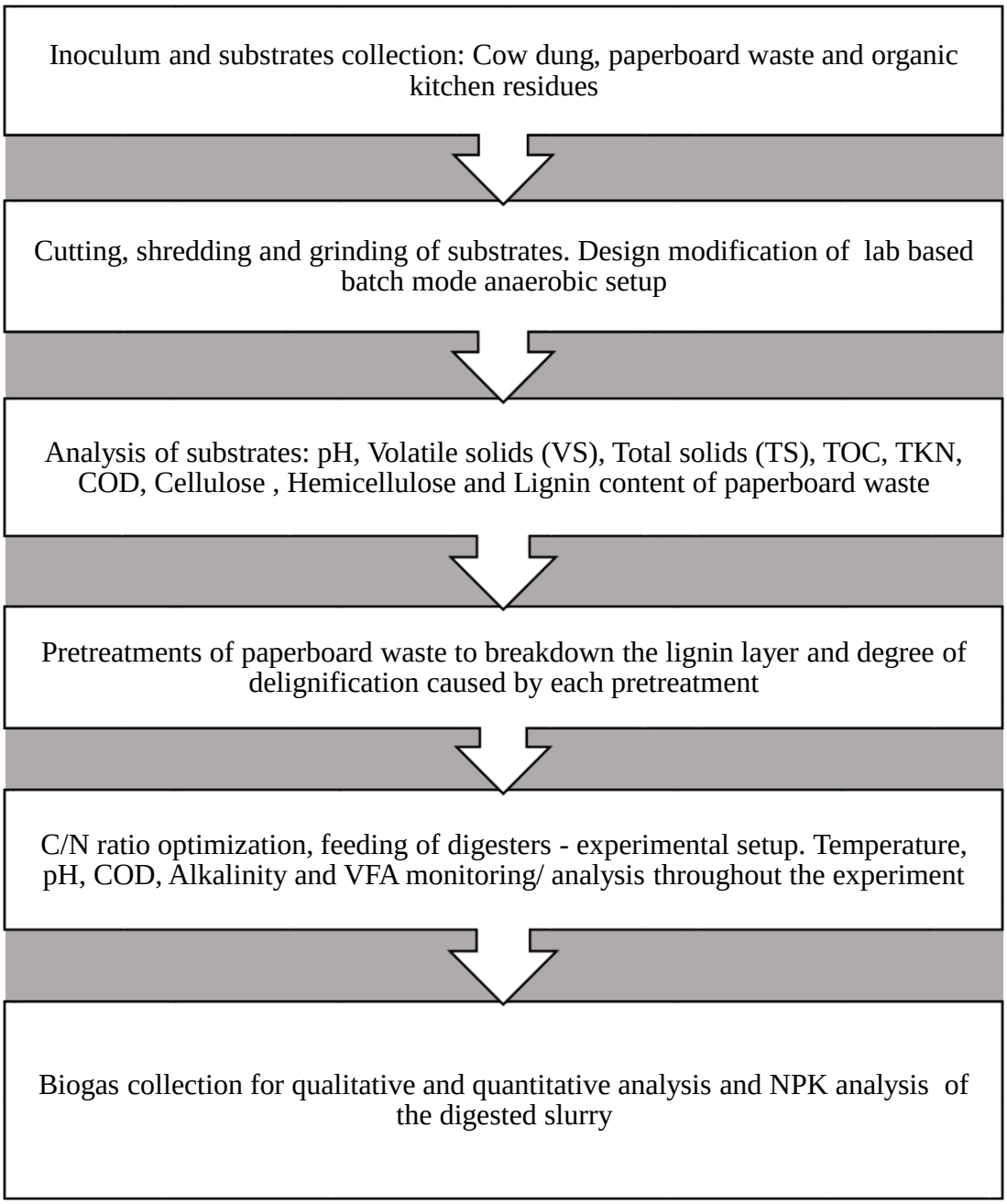

Figure 3.1: Adopted methodology for the experimental phase

3.1 COLLECTION AND PROCESSING OF SUBSTRATES

Substrates used in this study were all the redundant materials. Paperboard waste includes paper tubes which were segregated and collected from household waste and corrugated cardboard cartons were collected from IESE, NUST. Organic kitchen residues were collected from Concordia-1 and household waste. Cow dung was used as an inoculum and it was collected from local farm house.

3.1.1 Processing of Organic Kitchen Residues (OKR)

Composition of organic kitchen waste (1 kg), comprises peels and remains of: spinach, onion, tomato, potato, cabbage, cauliflower, pea pods, banana and apple 100 grams each along with cooked pasta and rice 50 grams each. The waste was cut and then grinded by using a household kitchen grinder (GEEPAS-GSB-2013), sieved to make a uniform mixture of particle size ≤ 5 mm. Grinded mixture was then poured in plastic bottles and refrigerated for future use.

3.1.2 Processing of Paperboard Waste (PBW)

Corrugated paper board and paper tubes, both types of PBW were grinded by using a heavy-duty grinder (SKU: GF422-A), passed through 5 mm sieves, then mixed in equal quantities (1:1) and stored in zip lock bags to be used in future for pretreatments and anaerobic digestion

3.2 PRETREATMENTS OF PAPERBOARD WASTE

Paperboard waste was subjected to three pretreatments including: hydrothermal, alkaline treatment with NaOH and ultrasonic. Hydrothermal pretreatment was done in accordance with the method explained by Anna et al. (2010), while alkaline treatment and ultrasonic treatment were performed according to the method described by Andrea et al. (2016).

3.2.1 Hydrothermal Pretreatment

Effect of different temperatures (150-200 °C) with respect to time (10, 20, 30 and 40 minutes) was investigated. 5 grams of grinded and sieved (5mm) substrate was mixed with 100 ml distilled waste in a 250-ml conical flask, covered with aluminum foil and then placed in an oven (Memmert- VO-400) After the completion of treatments, the substrate was dried at 60 °C and stored in plastic zip lock bags for lignin determination.

3.2.2 Alkaline Pretreatment with NaOH

5 grams of substrate was exposed to NaOH at different concentrations (2, 4, 6, 8 and 10 per cent w/v; 100 ml distilled water), for 24 hours at room temperature. After 24 hours, the NaOH containing supernatant was carefully removed and the substrate was washed with distilled water to bring its pH to normal. The substrate was then oven dried at 60°C and stored in zip lock bags for lignin determination.

3.2.3 Ultrasonication

For ultrasonic pretreatment of paperboard waste, 250 ml glass beaker was used and 5 grams of substrate was mixed in 50 ml distilled water. The beakers were then covered with aluminum foil and placed in a sonicator (JINWOO-1505) at 40 kHz for time ranging from 10-90 minutes.

3.3 LIGNIN CONTENT ANALYSIS

- 1 g of paperboard waste was weighed and transferred to a 500-ml conical flask. 10 ml of 75 per cent v/v H_2SO_4 solution was then added, mixed and the solution was placed at 20° C in a water bath for 4 hours.
- After the first step was done, 560 ml of distilled water was added to it and was left to reflux in a boiling water bath (99 ± 1 temperature), again for 4 hours.
- Once completed, the residue is filtered by using a pre-weighed filter paper and washed with 500 ml of distilled water. Finally, the filter cake was dried at 105°C for 4 hours and weighed. The residue is taken as the lignin fraction of the biomass. Following equation was used to calculate the lignin content:

$$\% \text{ ADL} = \text{Wt (i)} - \text{Wt (f)} \qquad (3.1)$$

Where,

Wt (i): Initial weight of the filter cake before drying - weight of filter paper

Wt (f): Final weight of the filter paper after drying - weight of filter paper

3.4 PARTICLE SIZE ANALYSIS (PSA)

After the pretreatments samples were analyzed for lignin removal. Samples which undergoes maximum delignification for all treatments were then washed with distilled water and the filtrate was subjected to particle size analysis to check the size of particles present after the

pretreatment as compared to the control (untreated PBW). Samples were examined in replicates by using laser scattering particle size distribution analyzer (Horiba - LA300).

3.5 SCANNING ELECTRON MICROSCOPY (SEM)

Similarly, samples with most effective delignification were subjected to electron microscopy to examine the surface and topographic changes occurred due to pretreatments as compared to the control. Dried and finely grinded samples were placed in the electron microscope (JESMAY) at angular range of 40-50° and at varying magnification of 100-10 x stereoscopic observations were performed to get the best image depicting the changes in macro and micro roughness of the surface depth of the field.

3.6 PHYSICOCHEMICAL ANALYSIS

Physicochemical tests of substrates and inoculum were performed as per standard methods (APHA et al., 2017 and ASTM 2017-98).

3.6.1 Total Solids (TS)

Gravimetric method was used to determine the total solids separately in each substrate, and collectively before and after the co- digestion. Total solids is a term referred to the residues left in the dishes after 24 hours of drying at a constant temperature of 105°C, may be calculated by putting the values in the following formula:

$$\text{Total solids (\%)} = \frac{(A - B) \times 100}{C\text{-}B} \qquad (3.2)$$

Where,

A = weight of dried residue + dish after 24 hours at 105°C (g)

B = weight of dish (g)

C = sample size (g)

3.6.2 Volatile Solids (VS)

The pre-dried sample used for determination of total solids was weighed (A) and ignited at 550° C in a muffle furnace for 30 minutes. The vessel is then transferred to a desiccator for half an hour. Then the vessel is weighed (D) and the acquired values were used to calculate the volatile solids in the sample by using the following formula:

$$\text{Volatile solids} = \frac{(A - D) \times 100}{A - B} \quad (3.3)$$

Where,

A = weight of dried residue + dish after 24 hours at 105°C (g)

B = weight of dish (g)

C = sample size (g)

D= weight of dish after ignition at 550°C (g)

3.6.3 Total Organic Carbon (TOC)

Organic carbon was determined by using the standard method of organic matter determination in domestic and industrial wastes/soils (Walkley-Black 1984). Following equation was used to determine the OC in the sample:

$$\%\ \text{Total Organic Carbon} = 1.334 \times \%\ \text{Oxidizable Organic Carbon} \quad (3.4)$$

3.6.4 Total Kjeldhal Nitrogen (TKN)

Nitrogen determination was performed as according to the Kjeldhal method (AOAC- 1998). Following equation was used to calculate TN of the sample:

$$\%\ N = \frac{(S - B) \times N \times 1.470}{\text{Sample size (g)}} \quad (3.5)$$

Where,

S = volume of H_2SO_4 used for sample (ml).

B = volume of H_2SO_4 used for blank (ml).

N= Normality of H_2SO_4.

3.6.5 Carbon-to-Nitrogen Ratio (C/N)

C/N for all digesters was calculated from the TOC and TKN values on the basis of total solids of the substrates, by using the following equation:

$$C/N = \frac{\text{Total amount of Carbon in a sample}}{\text{Total amount of Nitrogen in a sample}} \quad (3.6)$$

3.6.6 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) was determined by using closed reflux titrimetric method. Following equation was used for calculating the COD of each sample:

$$\text{COD (mg/l)} = \frac{(A-B)\times 8000\times M}{\text{Sample Size (ml)}} \quad (3.7)$$

Where,

A = Volume of FAS used for titration of blank (ml)

B = Volume of FAS used for titration of sample (ml)

M = Molarity of FAS

3.6.7 Alkalinity

- 3 ml sample from each digester was collected and its pH was measured by using a (WTW-720) pH meter.
- Samples with pH above 6.5 were titrated with 0.02 N H_2SO_4 and the pH was adjusted to 6.5, then it was further titrated with H_2SO_4 until the pH measured as low as 3.
- Volume of acid consumed was noted and alkalinity of the digesters were calculated by using the following equation.

$$\text{Alkalinity (mg/l)} = \frac{\text{Volume of acid used} \times \text{Normality of acid} \times 50{,}000}{\text{Sample size (ml)}} \quad (3.8)$$

3.6.8 Volatile Fatty Acids (VFA's)

- For VFA quantification, the samples used for alkalinity determination were heated on the hot plate by placing the beakers on temperature around 80-100 °C.
- Then, the samples were cooled down at room temperature and titrated against 0.02 N NaOH, to bring back the pH up to 6.5.
- Volume of NaOH was noted and following equation was used to measure the VFA concentration in digesters.

$$\text{VFA (mg/l)} = \frac{\text{Volume of base consumed} \times \text{Normality of base} \times 50,000}{\text{Sample size (ml)}} \quad (3.9)$$

3.7 DESIGN MODIFICATION OF REACTORS

Digesters were made of PET plastic with a total volume of 1600 ml, wall thickness of 0.95 cm, height of 26 cm, diameter of 11.5 cm (6 cm wide on the top). The design of digesters was further modified, so that the samples may be taken to monitor the temperature, pH, alkalinity and VFA concentrations within the reactors during the experimental run.

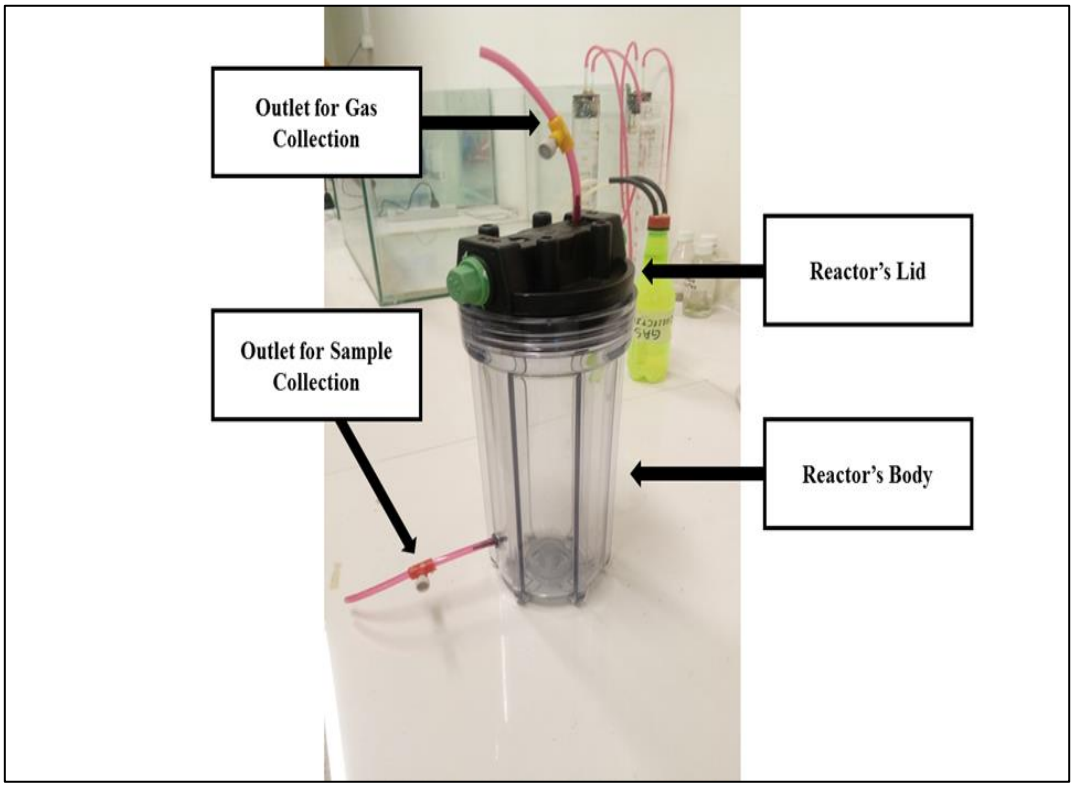

Figure 3.2: Illustration of modified reactor's design

The digesters contained two outlets: one at the top of the reactors cap and another hole was drilled at a height of 8 cm from the bottom of digesters (Figure: 3.2). Pipes having diameter of 0.5 cm were glued and fixed into those holes and control valves were used to tightly close the outlet pipes to stop leakage from the reactors and to avoid the entrance of oxygen for providing pure anaerobic environment to the microbes.

3.8 DIGESTERS FEEDING AND BATCH SETUP

A water tank was initially filled with 60 liters of distilled water and an electric water heating rod attached to a temperature controller was placed to bring the temperature upto 35 ± 1.A thermometer was also placed in the water tank check the temperature of water (Figure 3.3).

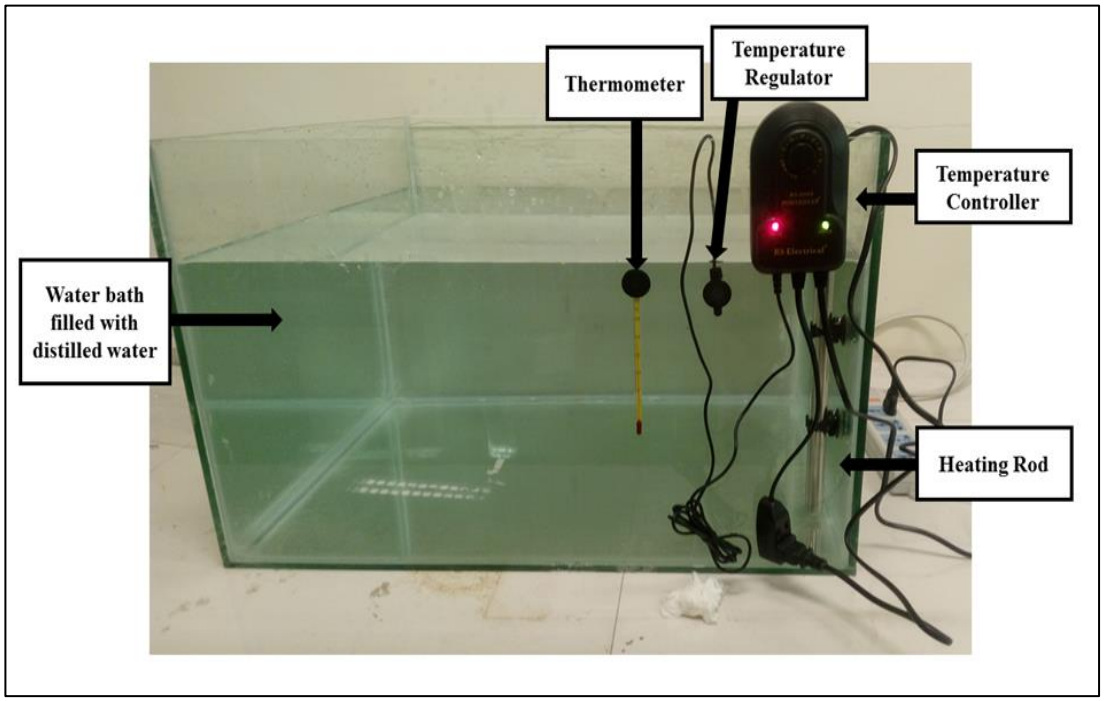

Figure 3.3: Water bath with connected temperature controller

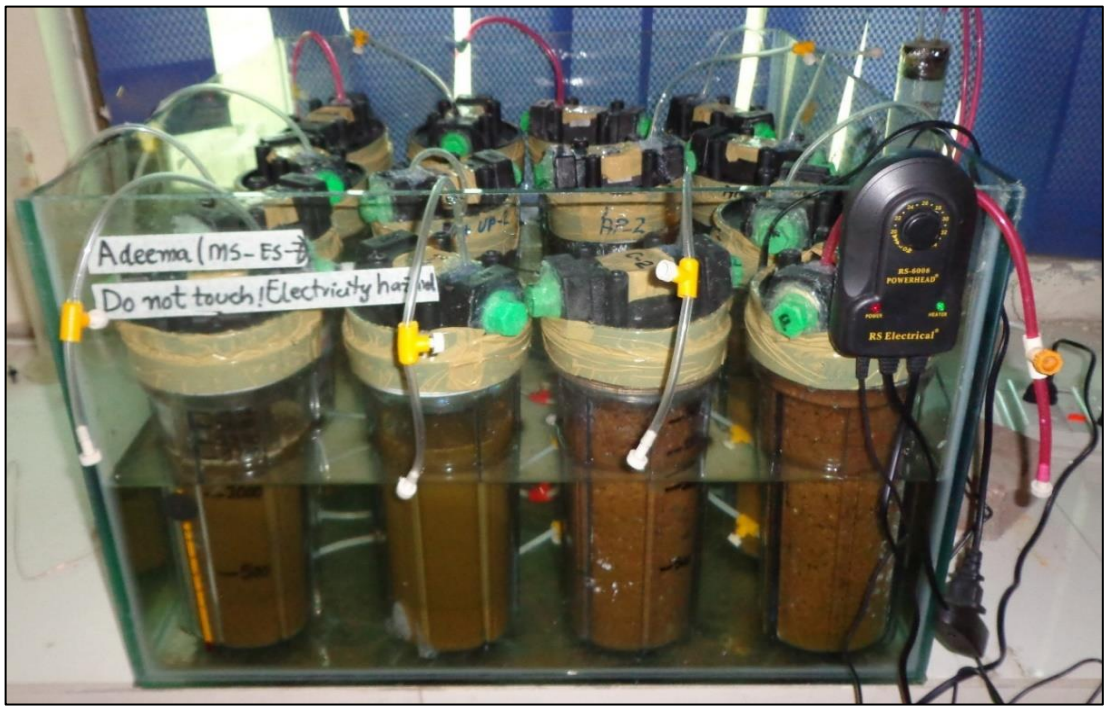

Figure 3.4: Pictorial depiction of the batch mode setup for anaerobic digestion

Digesters were filled with the substrates, inoculum and distilled water calculated on basis of total solids and loading rate. For the 1st AD run, OLR was kept constant at 5 g VS/l and 19 per cent solids (247 g solids: 1053 ml). Whereas for the 2nd run loading rate was increased to 10 g VS/l with 28 per cent solids (364 g: 936 ml) and 15 g VS/l with 42 per cent solids (548 g: 752 ml). Due to higher carbon content in paperboard waste 1.24, 1.86 and 2.50 g urea (containing 46 per cent Nitrogen) was added for 5,10 and 15 g loading rates, respectively, to bring the C/N ratio within the desired range. Feedstocks were mixed and initial pH was measured without adding any buffering agent. Reactors were then capped and shaken and the control valves were tightened to stop any gas escape. Digesters were then placed in the water bath (Figure 3.4) for 60 days (1st run) and 35 days (2nd run).

3.8.1 pH and Temperature Control

Throughout the run, temperature was maintained within the mesophilic range (36±1). Initially, at the time of feeding the digesters no buffering agent was added. Therefore, to monitor pH and observe the buffering capacity of substrates, twice a week samples were collected from the lower outlet of the reactors by using a syringe.

3.8.2 Biogas Measurement

The gas produced was measured by water displacement method on daily basis. For that, a volumetric cylinder of 500 ml was filled with water and placed upside down in a water tub filled up to 10 liters (Figure: 3.5). At a time one digester was taken out from the water bath and its outlet pipe emergent from the digesters lid was connected to another pipe with another end submerged into water poured in the cylinder. Once the control valve of the digester was opened, the water in the cylinder was replaced with the amount of gas produced in 24 hours.

3.8.3 Gas Collection in Sampling Bags

When the pH reached the suitable range for methanogenesis, multi layered aluminum foil bags were attached to the digesters by upper outlets used for daily biogas measurements. Bags were filled sufficiently with gas samples and then removed and sent for compositional analysis (Figure 3.6).

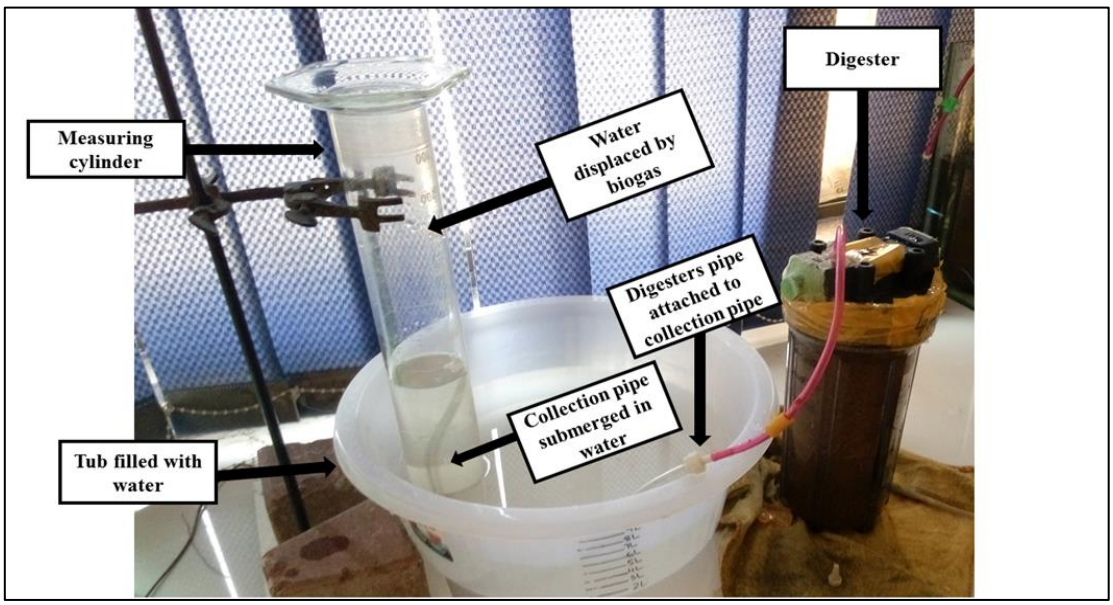

Figure 3.5: Illustration of biogas measurement through water displacement method

Figure 3.6: Illustration of biogas collection for compositional analysis

3.9 GAS COMPOSITIONAL ANALYSIS

Biogas produced in the 1st experimental run was analyzed by GC-MS (UOP 539), whereas the samples of 2nd run were analyzed by using a biogas analyzer (Geotech - 5000).

- Gas chromatography was performed with a molecular sieve column equipped with a thermal conductivity detector. The gas chromatograph was wrought at the oven temperature of 50°C, inlet temperature 125°C and detector temperature of 200°C.

Helium was used as the carrier gas and the reference flow and make up flow was 45.0 ml/min and 3.0 ml/min, respectively. Biogas sample volume was 0.1 ml injected into the chromatograph by using a syringe.

- Biogas analyzer was optimally calibrated before the analysis, then the sampling bag filled with biogas was attached to the outlet and pressed until the gas was fully released and a stable reading was obtained (Figure 3.7).

Figure 3.7: Biogas compositional analysis by using biogas analyzer

3.10 DIGESTED SLURRY ANALYSIS

The slurry produced after the anaerobic digestion was collected (250 ml) in plastic bottles and stored in the refrigerator (≤ 0 ° C) for further analysis (Figure 3.8).

- Nitrogen content was determined as TN by using the Kjeldhal method (AOAC-1998).
- Phosphorus was measured as total phosphate (TP) by digesting the sample with molybdovanadate and then analyzing by UV- visible spectrophotometer (PG – T6OU) at wavelength of 470 nm (APHA-2017) and values were then plotted against the graph obtained by using the P- standards (2,5,10,15,20 and 25 ml) to measure the P concentration in the samples.
- Similarly, Potassium was determined by digesting the samples with ammonium acetate and then analyzing at flame photometer (Spectrolab S20-4) at wavelength of 767 nm

(APHA-2017). Values obtained for each sample were then plotted and potassium concentration was calculated with reference to the calibration curve prepared by aspiring (20, 40, 60, 80 and 100 ppm) potassium standards.

Figure 3.8: Digested slurry collection and storage for analysis

3.11 STATISTICAL ANALYSIS AND KINETIC MODELLING

All experiments were performed in replicates and data was statistically analyzed by using Microsoft-excel 2016: analysis of variance (one-way ANOVA) was used to test the significance of results, and a confidence level of 0.05 was considered to be statistically significant. For kinetic modelling non-linear regression was applied by using SPSS 16.0 and following modified Gompertz kinetic model:

$$P*\exp(-\text{Exp}(((R*2.7183)/P)*(L-t)+1)) \quad (3.10)$$

Kinetic modeling was performed to compare the experimental methane yields and theoretical methane yields predicted by the model, to validate the results.

Chapter 4

RESULTS AND DISCUSSION

This chapter includes the interpretation of data congregated during the experimental stage along with justifications of the results from the literature. Results will be discussed in three phases.

4.1 PHASE – I

Phase-I comprises the results of: lignocellulosic analysis of paperboard waste (PBW), size reduction of the substrates, effect of particle size on lignin removal from PBW, effect of hydrothermal, alkaline (NaOH) and ultrasonic pretreatment on the lignin reduction, particle size analysis (PSA) of the pretreated PBW washings and scanning electron microscopy (SEM) to check the effects of pretreatments on surface topography of PBW and physicochemical analysis of the substrates and inoculum.

4.1.1 Lignocellulosic Content of Paperboard Waste

Lignocellulosic content of corrugated cardboard and paper tubes are presented in Table 4.1. Total holocellulose (cellulose + hemicellulose) content was 73 per cent and 66per cent for corrugated cardboard and paper tubes, respectively. In previous studies, holocellulosic content range of paperboard waste is reported between 52-73 per cent and lignin content up to 18-20 per cent (Talebnia & Taherzadeh, 2012; Ioelovich, 2014). Lignocellulosic materials due to higher cellulose content, may be utilized as promising feedstock for bioenergy production (biogas and ethanol), but to make conversion more effective and efficient the lignin content must be removed by pretreating the waste (Yngvesson, 2011).

4.1.2 Size Reduction of Paperboard Waste and Organic Kitchen Residues

The results of grinding and sieving of paperboard waste (PBW) is shown in Table 4.2. Substrate were subjected to size reduction upto 5 and 2 mm. For 5 mm the grinding and sieving times were shorter - 50.5per cent and 44per cent, respectively as compared to the 2 -mm size because the substrate became fibrous and harder to pass through the small holes of the sieve, whereas it was easier to pass the grinded paperboard waste through the 5- mm sieve. Similarly, in case of organic kitchen residues (OKR) the grinding time was decreased up to 53 per cent for 5 mm,

whereas sieving time was reduced up to 67. 6 per cent, as compared to 2 mm grinding and sieving (Table 4.3).

Table 4.1: Lignocellulosic content of paperboard waste

Paperboard waste	**Cellulose** (per cent)	**Hemicellulose** (per cent)	**Lignin** (per cent)
Corrugated cardboard	58 ± 0.70	15 ± 0.52	20 ± 0.65
Paper-tubes	55 ± 0.35	11 ± 0.85	21 ± 0.32

Table 4.2: Grinding and sieving of paperboard waste

Paperboard waste size (mm)	**Grinding Time** (minutes)	**Sieving Time** (minutes)	**Weight attained** (g)
5	1.40	4.20	50
2	2.83	7.50	50

Table 4.3: Grinding and sieving of organic kitchen residues

Kitchen residues size (mm)	**Grinding Time** (minutes)	**Sieving Time** (seconds)	**Weight attained** (g)
5	1.10	34	50
2	2.34	1.05	50

4.1.3 Effect of Particle Size on Lignin Removal

Paperboard substrate that was grinded and sieved was subjected to lignin content determination to check the effect of size reduction on lignin removal. Results of 5 and 2 mm sized corrugated

cardboard and paper tubes were compared with their controls that ranged between sizes of 20-40 mm strips (Figure 4.1).

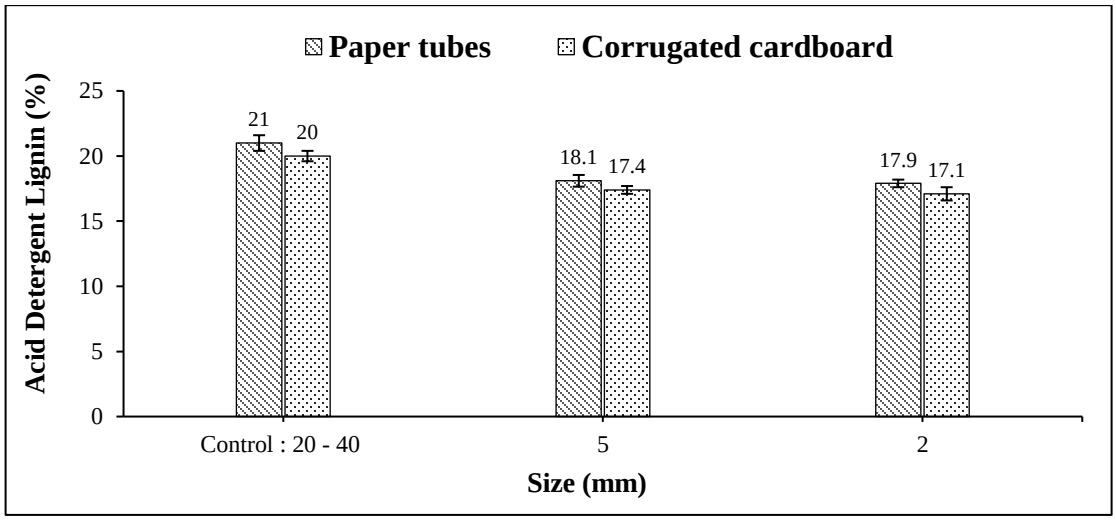

Figure 4.1: Effect of size reduction on lignin content of paperboard waste

Corrugated cardboard undergoes: 13 per cent delignification for 5 mm and 14.5 per cent for 2 mm size as compared to the control. Whereas, for paper tubes lignin removal was 13.8 per cent for 5 mm size and 14.7 per cent for 2 mm size. Data was statistically analyzed by one way-Anova and a non-significant difference between 2 mm and 5 mm sizes was found. Increased size also requires more effort and electricity in grinding and sieving hence 5 mm particle size was chosen as an optimized size to be used for anaerobic digestion and to provide an easy and uniform access of organic matter to the bacteria. Excessive particle size reduction (<1-3 mm) may lead to less polymer lignin removal and also inhibits methane production due to higher VFA production and drop in alkalinity inside the digester (Izumi et al., 2010).

4.1.4 Effect of Pretreatments on Delignification

After grinding the paperboard waste to 5 mm size, it was subjected to thermal, ultrasonic and chemical pretreatment. The selected pretreatments were chosen on the basis of studies done in the past and economic feasibility was also taken in consideration.

4.1.4.1 Hydrothermal pretreatment

Paperboard waste was subjected to hydrothermal pretreatment at varying temperature (150°C-200°C) and time (10-40 minutes) and effect on lignin removal was determined (Table 4.4) as

compared to the control (Figure 4.2). Results showed the highest lignin removal of 68.2per cent was achieved when PBW was subjected to 170 °C for 20 minutes and the lowest lignin removal of 27.8 per cent was observed at 200°C for 40 minutes concluding that with increased temperature and pretreatment time the lignin removal efficiency reduced up to 59.2 per cent.

Table 4.4: Effect of time and temperature on delignification

Time (minutes)	**150** (°C)	**160** (°C)	**170** (°C)	**180** (°C)	**190** (°C)	**200** (°C)
10	31.7	40.9	65.3	30.2	30	32.1
20	40.4	46.3	68.2	40.9	35.6	36
30	40.9	45.3	56.5	36.5	35.1	29.2
40	37	38.5	52.1	30.7	32.1	27.8

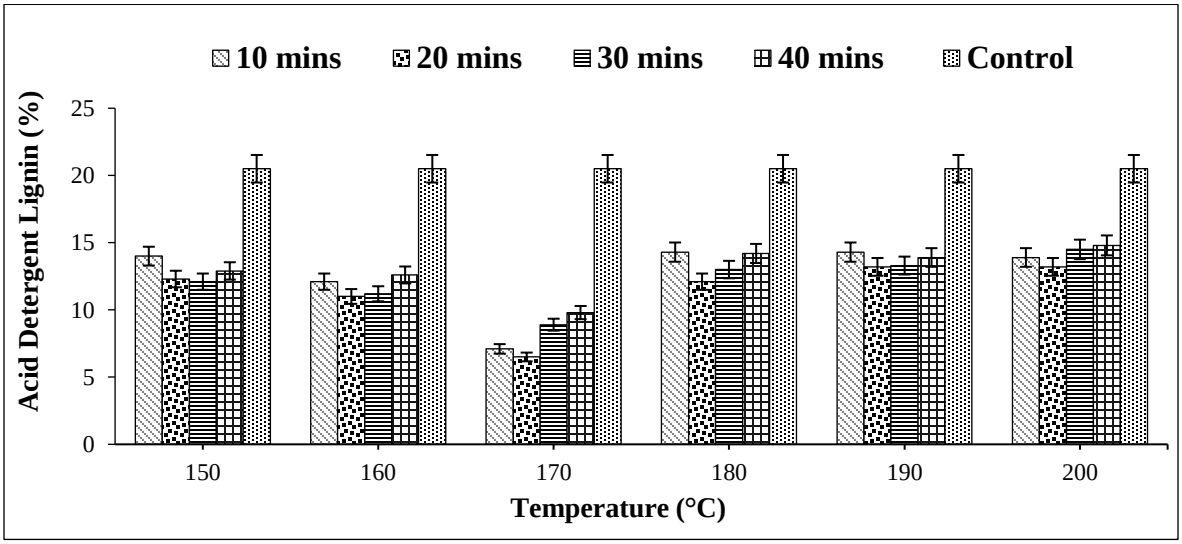

Figure 4.2: Effect of hydrothermal pretreatment as compared to the control

During thermal pretreatments, temperature should not exceed the desired range of lignin solubilization and transition, otherwise the lignin may be amalgamated into larger bodies. Solubilization temperature range for lignin is reported between 150-180°C (Bobleter, 1994; Garrote et al., 1999; Mosier et al., 2005; Bauer et al., 2014). It has been reported in past that temperatures higher than 180°C may lead the removed lignin droplets to re-occupy the surface area and cover the cellulose. Hence, these results are in accordance with the fact that higher temperatures may lead to redistribution and redepositing of lignin (Dien et al., 2006; Donohoe

et al.,2008; Li et al., 2014; Fan et al., 2016).Higher temperatures (≥ 200°C) may also impact the efficiency of the substrate to be used for anaerobic digestion by promoting the production of enzymatic inhibitors like; furfurals and hydroxymethylfurfural (HMF), vanillin and methane content may decrease due to the higher production of furan derivatives and pseudo-lignin formation (Laser et al., 2002).

4.1.4.2 Alkaline pretreatment with NaOH

The effect of different Sodium hydroxide (NaOH) concentrations on lignin is summarized in table 4.5. At the highest concentration of 10per cent the lignin removal significantly increased up to 76.1per cent as compared to the control: untreated PBW and 10.3per cent more delignification occurred as compared to the hydrothermal pretreatment (Table 4.4).

Table 4.5: Effect of NaOH concentration on per cent lignin removal

NaOH Concentration (%)	**Lignin removal** (%)
2	37.5
4	50.2
6	47.3
8	55.1
10	76.1

Low concentrations of alkali (≤ 2 per cent) are suitable for materials with low lignin content (< 10 per cent) but at higher concentration it may further increase the porosity by breaking the linkages between lignin and cellulose and it is found to be more effective than other chemicals: H_2O_2 or H_2SO_4 (Silverstein et al., 2007). Because of the higher lignin content in paperboard waste: 20.5 per cent (Table 4.1), the concentration of NaOH was increased up to 10 per cent (w/v) and the results proved that higher concentration removed lignin more efficiently than other pretreatments and as compared to the control (Figure 4.3). Lignin is mostly soluble in water at alkaline pH, which may be the reason behind the most effective lignin removal when the substrate is treated with alkali (Beisl et al., 2017). Treatment with dilute NaOH may be effective in removing the lignin from the substrate by breaking the ester bonding between

lignin, hemicellulose and cellulose with less fragmentation so that the droplets of lignin may be fully removed, providing increased surface area, porosity and biodegradability (Zhao et al., 2008;Gaspar et al., 2007). Strong alkali concentrations may lead to better dissolution and solubilization of dissolved polysaccharides, which has a positive effect on cellulose degradation, but thermochemical (alkali + heat) may cause inhibitory effect to methanogenic microorganisms (Hendriks & Zeeman, 2009).

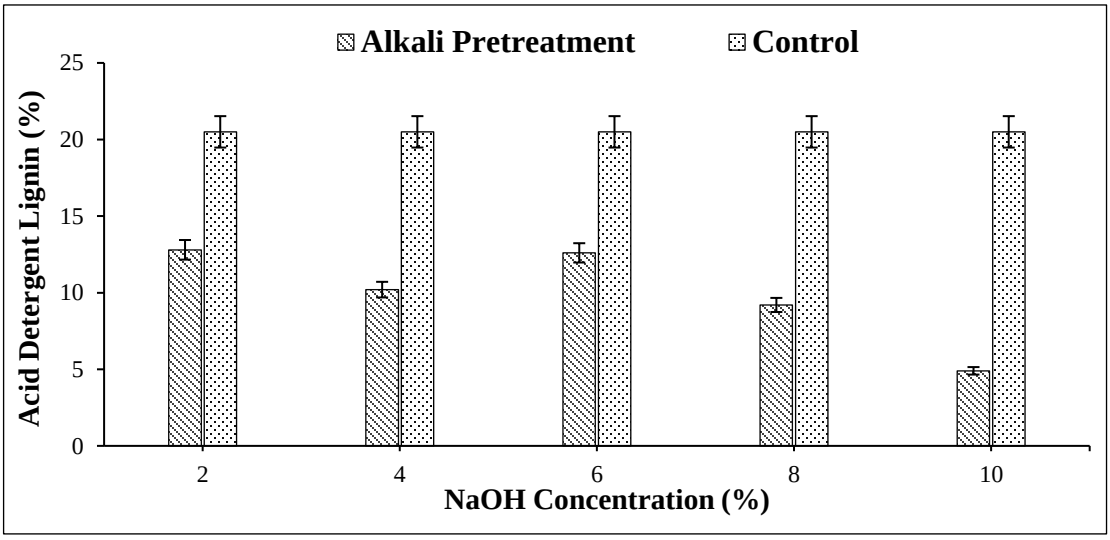

Figure 4.3: Effect of alkali pretreatment as compared to the control

4.1.4.3 Ultrasonication

Ultrasonic pretreatment was proved to be the least effective pretreatment method for the lignin removal form the paperboard waste, with highest lignin removal: 42.1 per cent (Table 4.6), was achieved when the substrate was subjected to sonication for 90 minutes. Alkali pretreatment undergoes 44 per cent while hydrothermal pretreatment resulted in 38per cent more delignification as compared to sonication. As compared to the control, the lignin removal by ultrasonic pretreatment ranged between 22.4-42.1 per cent (Figure 4.4), showing that up to some extent, the sonic waves did break the linkages between lignocellulosic components (lignin, cellulose and hemicellulose) and hence hydrolysis may be accelerated producing more VFA's which ultimately may be converted to biogas (Rodriguez et al., 2017). PBW was mixed with water prior to the pretreatment because the sound transmission in the water-solid interface is better than the air-solid medium, which may positively influence the delignification.

Table 4.6: Effect of ultrasonication time on per cent lignin removal

Sonication time (minutes)	**Lignin removal** (%)
10	22.4
30	32.6
50	29.2
70	32.6
90	42.1

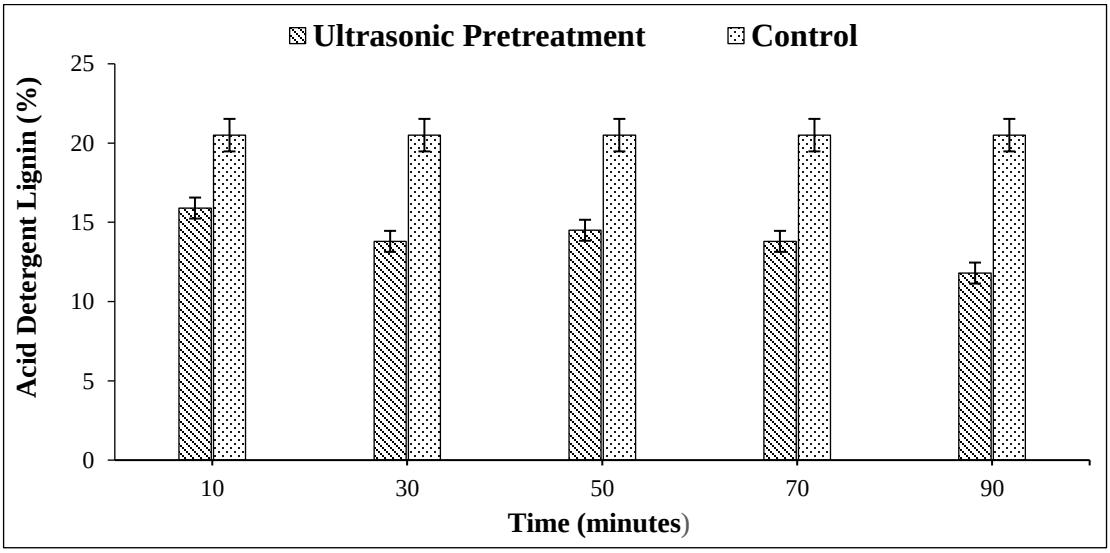

Figure 4.4: Effect of ultrasonication as compared to the control

4.1.5 Particle Size Analysis (PSA) of Paperboard Waste

Results of PSA are presented in Table 4.6. Longest particle size of 7.42 µm was observed for alkali pretreatment washings followed by hydrothermal: 6.29 µm, ultrasonic pretreatment: 3.33 and control: 1.45 (Table 4.7). Presence of 80.4per cent larger particles in the PBW washings after alkali pretreatment as compared to the control may be related to the most efficient pretreatment method for lignin removal from PBW. Previous studies showed that lignin has highly branched and elongated structure with a diameter of 100 -223 nm and with a typical length of upto 11 - 18.6 µm, due to attached polymers of phenylpropane units (Ten et al., 2013; Beisl et al., 2017). Hence variations in the particle sizes of washings obtained from different pretreatments indicates the removal of lignin. It has been also reported that cellulose is shorter

than lignin with 0.148 µm length and 0.196 µm diameter and the larger particle found in the samples indicates that pretreatments does not lead to significant cellulose losses (Wulandar et al., 2013).

Table 4.7: Comparison of particle size of pretreated paperboard with control

Pretreatment (PBW)	**Particle size** (µm)
Alkali (NaOH)	7.42 ± 1.06
Hydrothermal	6.29 ± 2.13
Ultrasonication	3.33 ± 1.37
Control (untreated)	1.45 ± 0.91

4.1.6 Scanning Electron Microscopy (SEM) of Pretreated Samples

Scanning electron microscopy is a tool used to visually determine the micro and macro roughness of the any surface to understand the surface topography by looking onto the stereoscopic images. Hence, SEM was conducted to check the effect of pretreatments on the surface morphology of the paperboard waste and results are shown in (Figure 4.5). Alkaline treated (10per cent NaOH) samples subjected to SEM shown that the structure was opened up and parallel strips indicates the exposure of cellulose bundles which are mostly present as a microfibril cluster embedded in hemicellulose and lignin (Reza et al., 2015). Samples which were hydrothermally pretreated (170°C for 20 minutes) showed dismantled and uneven surfaces which were separated from each other and the topography was significantly different from the control. SEM results of ultrasonically treated samples showed less morphological changes but the structure was opened up and segregated more as compared to the control which was visible as a closely packed compact structure. Pretreatments of material for lignin removal may significantly open the surface morphology and makes the surface more porous and irregular as compared to untreated samples. In results the surface area and total pore area also increased making it easier for the bacteria to access the organic content. Higher chemical concentrations (≥ 4 per cent NaOH) may effectively separate the discrete fibers while at low concentrations

visibility of unattached and hollow bundles clearly indicates that increased chemical concentration and delignification are directly proportional to each other (Rezende et al., 2011; Wang et al., 2016).

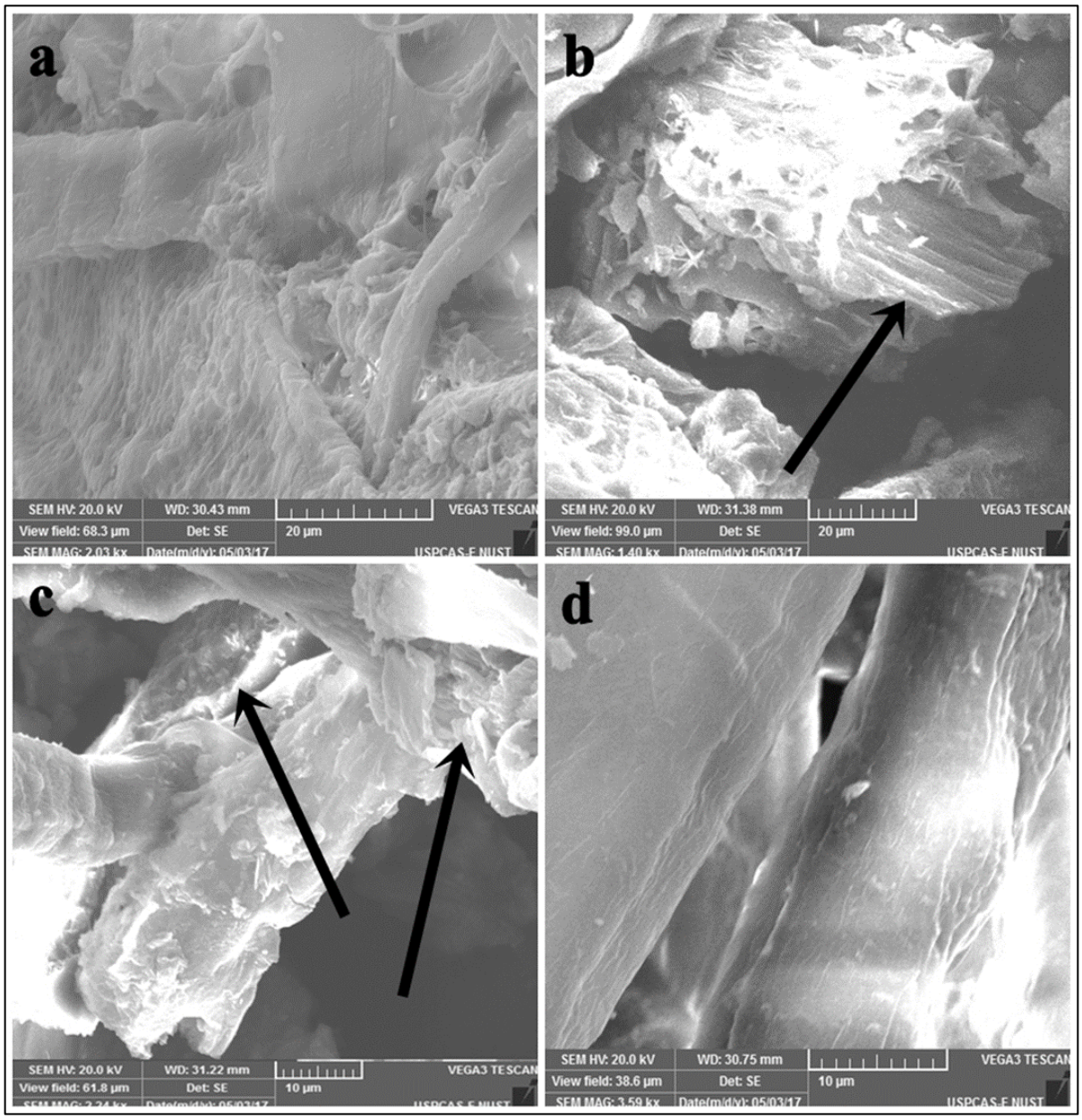

Figure 4.5: Surface images of untreated and treated paperboard waste: (a) Surface topography of control / untreated sample; (b) Alkaline treated paperboard waste: arrow is showing the exposed cellulose cluster; (c) Hydrothermally treated paperboard waste: arrows showing uneven surface topography and opened-up structure; (d) Ultrasonically treated paperboard waste: separation of strands occurred but the surface is smoother indicating less lignin removal

4.1.7 Physicochemical Characteristics of Substrates and Inoculum

Physicochemical characteristics of substrates and inoculum are given in (Table 4.8). Results showed that OKR and PBW both are promising substrates for anaerobic digestion with compatible characteristics. Food waste is one of the most suitable feedstock for anaerobic digestion due to its biodegradable nature, high and diverse nutrients availability, high volatile solid (VS) content and due to more than 80per cent moisture content and 10-20per cent TS makes it an ideal co-substrate to be used with paperboard waste that lacks moisture and have higher TS content (Lin et al., 2011; Li et al.,2013; Tanimu et al.,2014).

Table 4.8: Physicochemical characteristics of substrates and inoculum

Parameter	Organic Kitchen Residues	Paperboard waste	Cow dung
pH	4.8 ± 0.26	8.3 ± 0.16	7.7 ± 0.02
Moisture Content (%)	90.7 ± 0.17	4.8 ± 0.1	81.75 ± 0.47
TS (%)	10.5 ± 1.16	95.2 ± 0.21	18.25 ± 0.30
VS (% TS)	96.4 ± 0.32	71.3 ± 1.05	86.30 ± 0.55
TKN (wt %)	2.07 ± 0.46	0.3 ± 0.03	2.8 ± 0.13
TOC (wt %)	34.01 ± 1.41	42.1 ± 0.26	30.1 ± 1.60
C: N ratio	16: 1	140 :1	10 :1
COD (mg/l)	24,608 ± 53.2	3,377.2 ± 33.2	21,677 ± 44.4

Volatile solids were higher in both substrates: 96.4 per cent for OKR and 71.3 per cent for PBW. VS is the major portion that is converted into biogas, hence indicating the potential of these substrates for AD. Higher carbon content in PBW leads to much higher C/N ratio of about 140:1, which may be reduced up to the optimum range by adjusting the mixing ratio of nitrogen rich substrates (OKR) because the microbial communities utilizes 25-30 parts more

carbon than nitrogen (Zhang et al., 2012). Similarly, the lower pH of kitchen waste: 4.8 is another hurdle while using it as a feedstock for anaerobic digestion because the pH sensitive methanogens does not survive in acidic environment, higher pH of PBW: 8.3 and inoculum: 7.7 (Ofoefule et al., 2010; Tanimu et al., 2014) may increase the overall alkalinity of the reactor and helps in removing this hindrance to use OKR and maintaining the ideal buffering capacity for methanogens. The higher COD value of kitchen waste: 24,608 mg/l and for PBW: 3,377 mg/l indicates the abundance of organic pollutants. Generally, anaerobic digestion of kitchen waste is a complex process, associated with the accumulation of ammonia and excessive volatile fatty acids, leads to inefficient system performances and process failure. Therefore, a suitable co-substrate for kitchen waste must have a high C/N ratio, high TS content and provide enough buffering capacity to avoid sudden pH drops (Capson et al., 2017). Paperboard waste fulfills all these requirements, with negligible nitrogen contents, having higher pH and buffering capacity, TS content and being slowly biodegradable. In addition, PBW is a particularly convenient co-substrate for kitchen waste in urban areas, where OKR and PBW are usually the main components of MSW (Zhang et al.,2012).

4.2 PHASE – II

Phase – II includes results of the effect of pretreatments and co-digestion on the biogas production at organic loading rate (OLR) of 5 g VS/l for 60 days and overall process stability of anaerobic digestion and quality of the digested slurry.

4.2.1 Effect of Pretreatments on Biogas Production

Comparative results of daily biogas production of pretreated sample and control is shown in Figure 4.6. Highest biogas production was observed for alkaline treated (AP) waste when co-digested with organic kitchen residues. During 60 days of digestion the total 26,890 ml biogas was produced (average value of replicates). Maximum gas was produced from day 20-30. Alkaline pretreatment and co-digestion instigated 70 per cent increase in biogas production as compared to the control (untreated paperboard waste and cow dung) which produced 8093.5 ml total biogas. Noticeable quantity of biogas was produced from co-digestion of alkali treated PBW and OKR from the very first day, indicated the shortening of the hydrolysis phase. PBW subjected to hydrothermal treatment when co-digested with OKR, the combination (HP) resulted in the production of 20, 786 ml/g VS biogas, which is 61per cent higher than the control but 12per cent lower than alkali treated PBW. Highest yield was measured between day 22-31.

The startup trend was similar to the AP, which is an indication of shorter hydrolysis phase. Bougrier et al.,2008 concluded in their study that thermal treatment (up to 190°C) may allow the increased biogas production due to a shorter hydrolysis phase and rapid initial biodegradability of the substrate. Ultrasonic pretreatment (UP) led to lesser biogas production with a total yield of 14,880 ml /g VS. The less degree of delignification was somehow compensated by co-digestion and 42per cent more gas was produced as compared to the control.

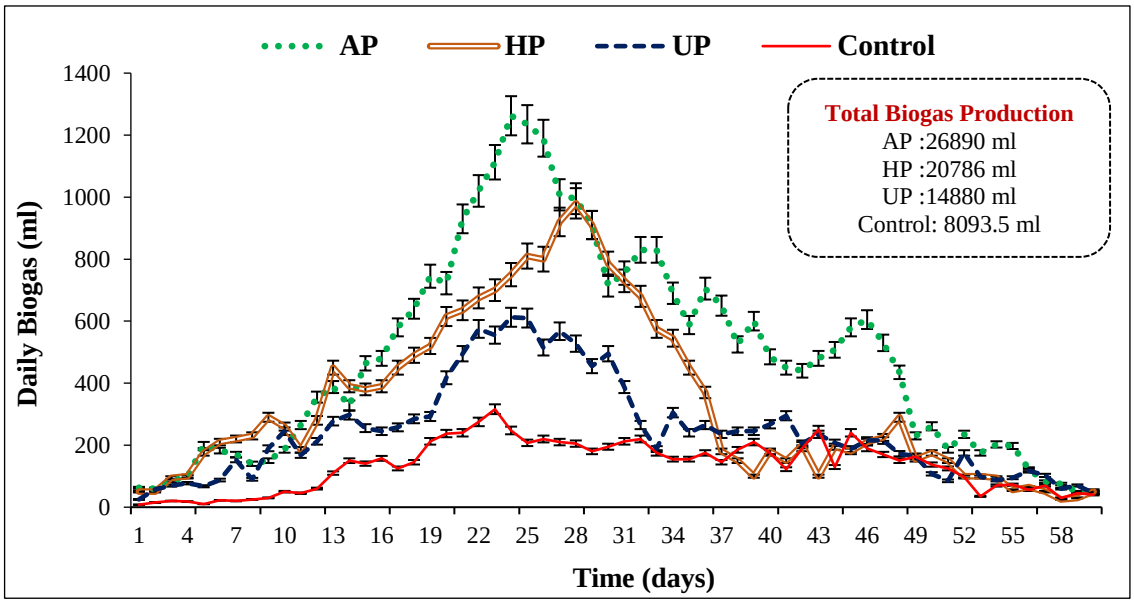

Figure 4.6: Comparison of daily biogas production of pretreated samples/control

Effect of pretreatments on daily biogas yields during 60 days of digestion are shown in Figure 4.7. biogas yields followed the similar trends of daily biogas production (figure 4.6). Highest yield of 105.4 N ml/g VS was measured for alkali treated paper board waste followed by HP: with 85.06 N ml/g VS, UP: 60.23 N ml/g VS and control (untreated PBW): 20.6 N ml/g VS. Teghammar et al. (2012) also reported the daily biogas yield of untreated paperboard around 18-22 Nml/g VS which increased to 41 Nml/g VS after pretreatment. Whereas, for food waste the highest reported daily biogas yield ranged between 80-85 N ml/g VS (Lin et al., 2011). Similarly, variations due to pretreatments and co-digestion in the cumulative biogas yield are elaborated in Figure 4.8. Highest cumulative biogas yield (at standard temperature and pressure-STP) was noticed for AP:1061.2 N ml/g VS followed by HP: 842 N ml/g VS, UP: 592.7 N ml/g VS and control: 353.8 N ml/g VS. Cumulative yield of kitchen waste is reported around 498-

796 N ml/g VS, whereas for paperboard waste the range is reported between 250-484 N ml/g VS with 34per cent increase due to pretreatments (Teghammar et al.,2012; Capson et al.,2017).

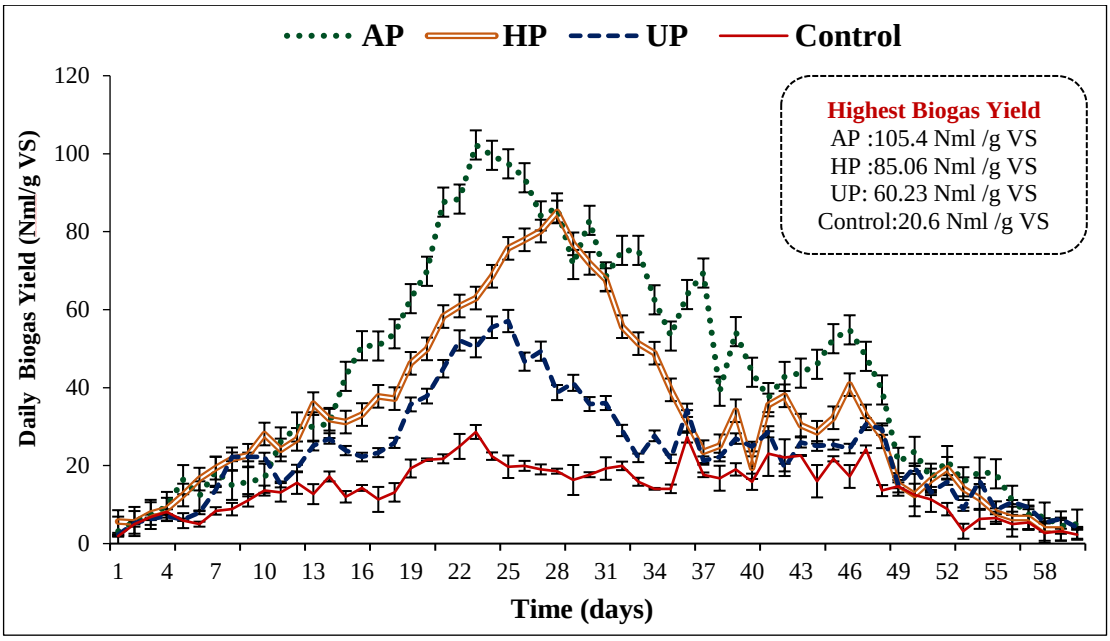

Figure 4.7: Comparison of daily biogas yield of pretreated samples and control

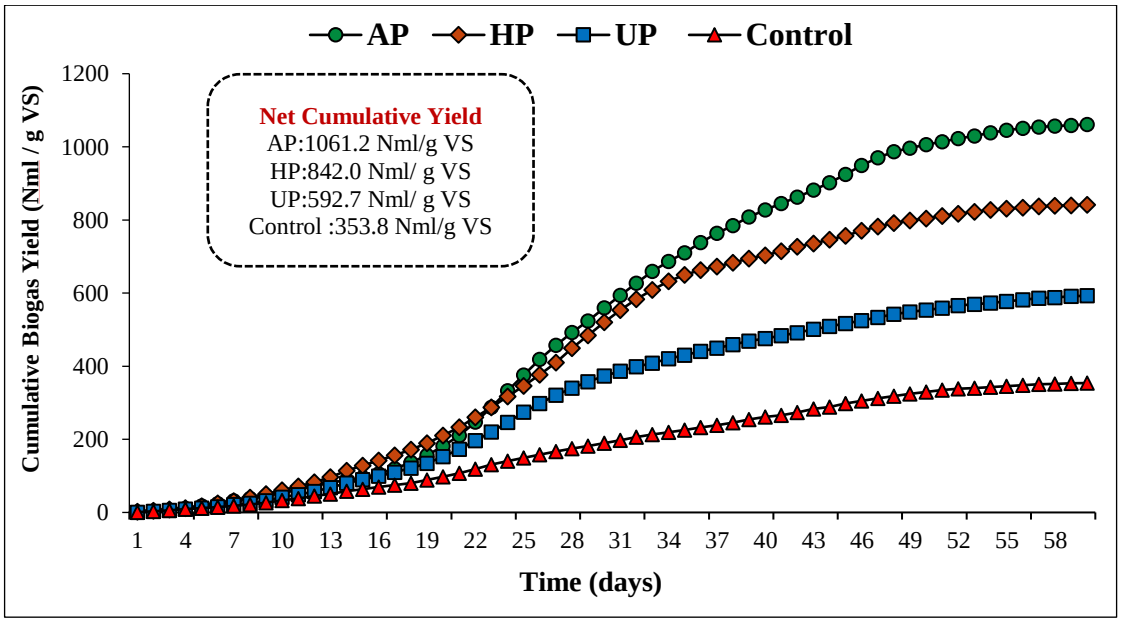

Figure 4.8: Comparison of cumulative biogas yield of pretreated samples/control

4.2.2 Comparison of Pretreatments by Kinetic Parameters

Kinetic parameters of cumulative methane curves and the prediction of final productions were fitted to the modified Gompertz equation and the results are presented in Table 4.9. These parameters include the lag time (λ), the biogas production potential (EMY), and the maximum cumulative biogas production rate (R_{max}). The correlation coefficient (R^2) of 0.992-0.997 was achieved for pretreatments and control. Variations in trends of the maximum methane production potential and the maximum methane rate (R_{max}) were found to be similar: AP > HP > UP > control. Shorter lag phase was also observed for the pretreatments that undergoes maximum delignification.

Table 4.9: Modified Gompertz model parameters for various pretreatment/control

Condition	TMY (N ml/g VS)	EMY (N ml/g VS)	Difference (%)	R_{max} (N ml/g VS/d)	λ (day)	R^2
AP	1028.34	1061.20	3.1	38.93	10.04	0.996
HP	822.11	842.09	2.4	30.97	11.4	0.995
UP	574.39	592.75	3.1	20.02	12.7	0.997
Control	334.14	353.89	5.7	10.05	15.2	0.992

4.2.3 Effect of Pretreatments on Biogas Composition

Pretreatments also possess positive impacts on the compositional analysis of the biogas and the results are shown in Figure 4.9.

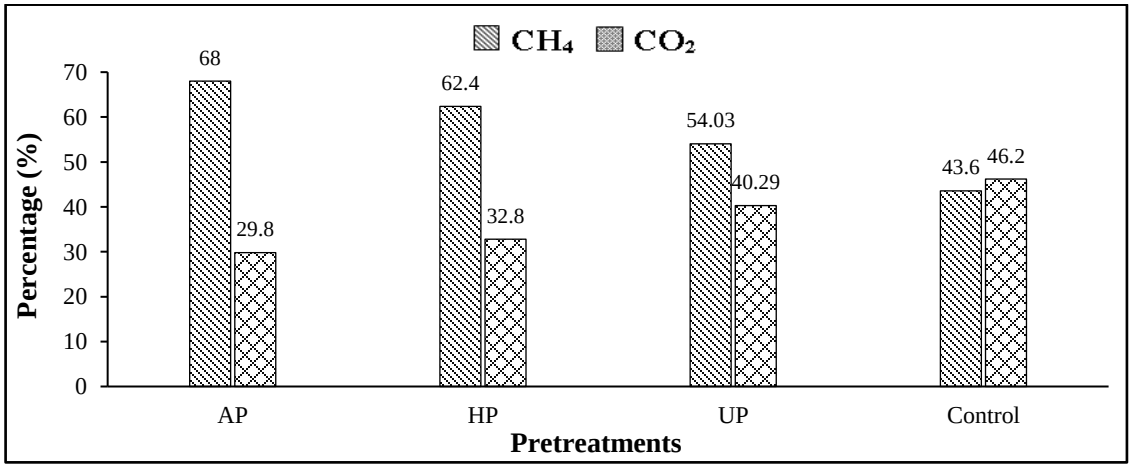

Figure 4.9: Comparison of CH_4 and CO_2 content of pretreated samples and control

Highest methane content was detected as 68 per cent, with a total methane yield of 18, 346.4 ml CH4 for AP, followed by HP with 62.4 per cent methane content and total yield of 12,970 ml CH4, UP: with 54.03 per cent methane and total yield of 8,039 ml CH_4 and control: with 43.6 per cent methane content and yield of 3,528.7 ml CH4. Methane content of biogas produced from food waste only in reported between 52-63.8p er cent, depending on the composition of the waste (Voegeli et al., 2009; Zhu et al., 2009; Lungkhimba et al., 2010). Whereas, methane content of paperboard waste only is reported as the 40-50per cent (Li et al., 2018). Compared with the control the increased methane content in in all pretreated and co-digested digesters indicates that both co-digestion and treatments significantly enhanced the quantity and quality of the biogas.

4.2.4 pH and Buffering Capacity

pH, alkalinity and VFA's analysis were conducted twice a week to monitor the changes in pH and buffering capacity within the reactors. The overall system performance in terms of pH remained within the desired range for anaerobic digestion. Above three parameters are reported and discussed separately to give a better understanding.

4.2.4.1 pH

Overall trend for variations in pH is shown in Figure 4.9. Initial pH of all reactors was measured at the beginning before closing the lids. Despite the low pH of food waste there was no drop in the initial pH values which was the synergistic effect of the comparatively neutral/alkaline values of paperboard waste and cow dung and there was no extra buffering agent added. At the end of the first week the pH starting to drop which was an indication of the start of acidogenesis. The pH values increased within the next 15 days and afterward no drop in pH was observed till the end of the experimental run (Figure 4.9). It has been observed that paperboard waste provided extra buffering capacity and the pH of control (untreated PBW only) remained more stable than all the other reactors. This is an additional benefit of using paperboard waste with an acidic nature kitchen waste. Alkaline pretreated samples also showed stable and slightly higher pH than other two treatments. Although the paperboard subjected to alkaline pretreatment was washed (up to 6-7) times to bring the pH from 12 to normal pH of the substrate that was measured around 8 (Table 4.1).

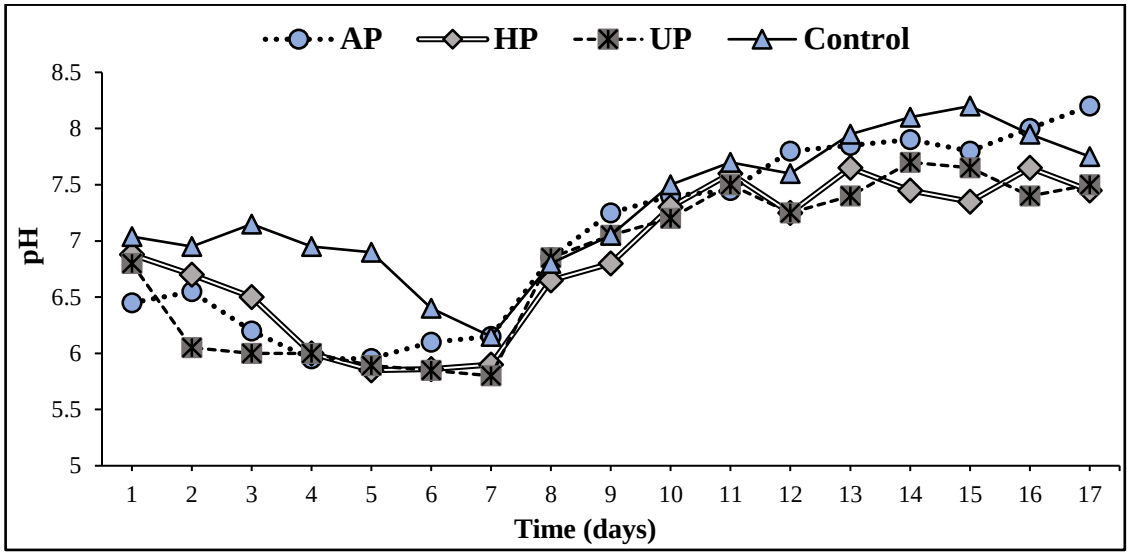

Figure 4.10: Comparison of pH of pretreated samples and control

4.2.4.2 Alkalinity

Variations in alkalinity of all reactors over the experimental run are shown in Figure 4.11. Alkalinity of the control (PBW+CD) remained higher throughout the run.

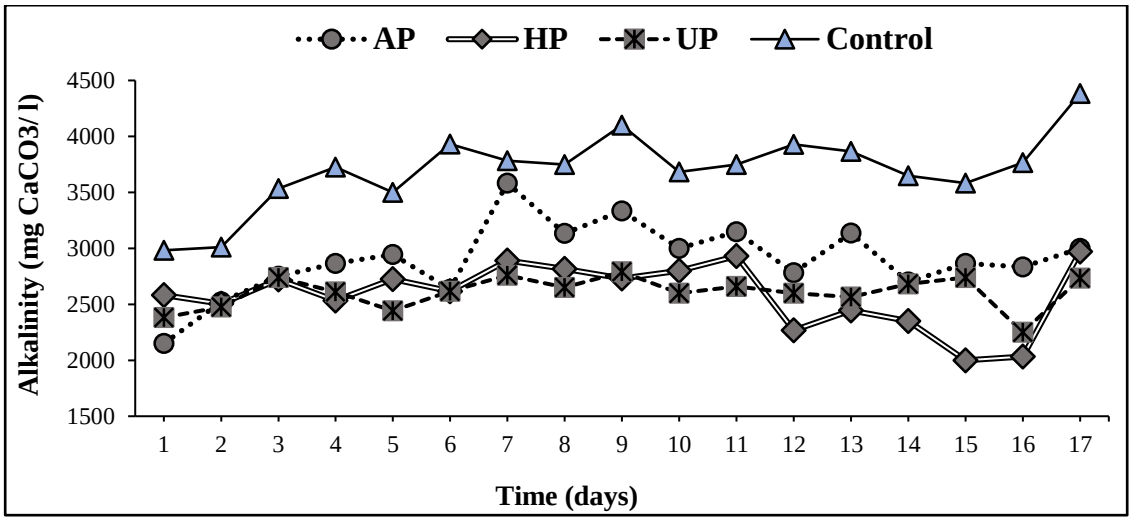

Figure 4.11: Comparison of alkalinity of pretreated samples and control

Results of other reactors fed with pretreated PBW (AP, HP and UP), were also remained in the optimum range of alkalinity that is the most important requirement for a stable anaerobic digestion process (1500-4000, Chan et al.,2009). Digesters with alkali treated PWB+OKR have shown much higher alkalinity as compared to other two proving that along with lignin removal,

alkali treatment may also effect the other properties: like increase in volatile solids has been observed after the treatment, making it more ideal for the digestion.

4.2.4.3 Volatile fatty acids (VFA's)

Differences in volatile fatty acids of all reactors are shown in Figure 4.12. Higher VFA's during the start up from alkali treated reactors indicates the shorter hydrolysis step, also the decrease in the VFA concentration at the end of 2nd week may be related to the successful conversion of fatty acids into organic acids which were further transformed into methane by acetogenic and methanogenic communities. Similar trends were observed for HP and UP.

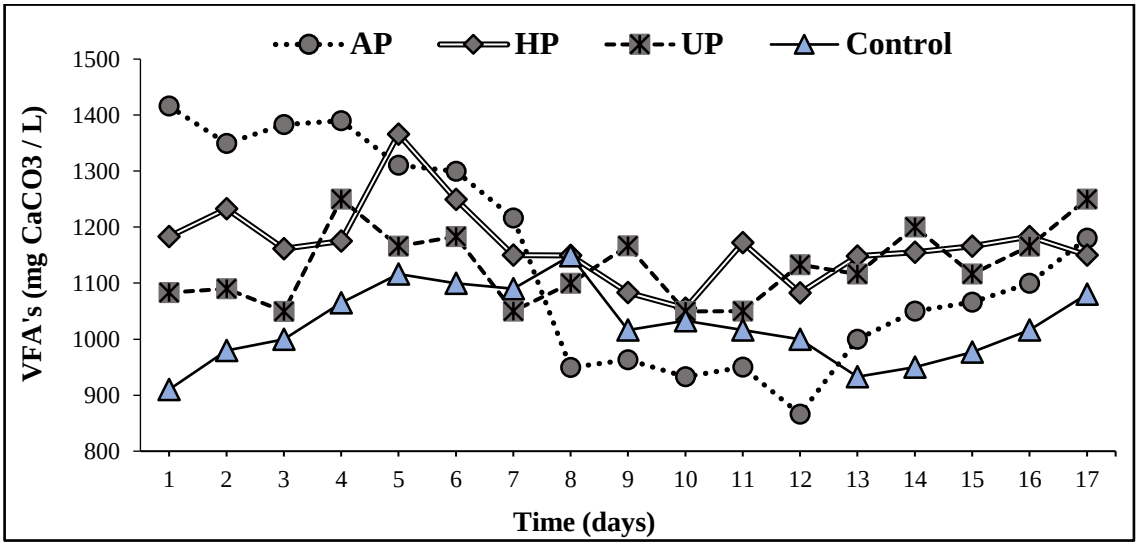

Figure 4.12: Comparison of VFA's of pretreated samples and control

While in control (untreated PBW only) the VFA's were measured lesser at the beginning as compared to the other reactors, proved that reactors undergo an extended hydrolysis phase. VFA's and alkalinity are inversely proportional to each other and for a stable digestion process the VFA/alkalinity ratio should be ≤ than 4. Also, the rapid increase in VFA's in the beginning shows the speed and extent of hydrolysis occurring within the digesters as in this phase the complex organic substrates are converted into simpler sugar monomers and volatile fatty acids. Higher VFA production means enhanced and higher biogas yield but the excessive VFA's accumulation in the reactor may lead to process inhibition (Chen et al., 2014). The rate of VFA's production should be in equilibrium with rate of VFA's conversion to organic acids by the anaerobic bacteria in the 2nd step (acidogenesis) of anaerobic digestion. If VFA's are

accumulated in higher concentrations, the environment within the digester becomes acidic inhibiting the activity of methanogenic bacteria which are very sensitive to drop in pH. The result will be less methane content in the biogas (Burhanuddin , 2010).

4.2.5 Total Solids (TS) and Volatile Solids (VS) Removal

Solids removal from the waste is one of the major applications of anaerobic digestion which is attributed to the conversion of volatile solids and waste stabilization by removal of total solid concentrations. TS removal also plays an important role in increasing the nutritional content of the digested slurry. Results of solids removal in terms of total and volatile solids are shown in Figure 4.13. It has been observed that for pretreated PBW solid removal was higher, due to easy excess to the organic matter for the bacteria and hence waste was converted efficiently into useful products (biogas and digested slurry).

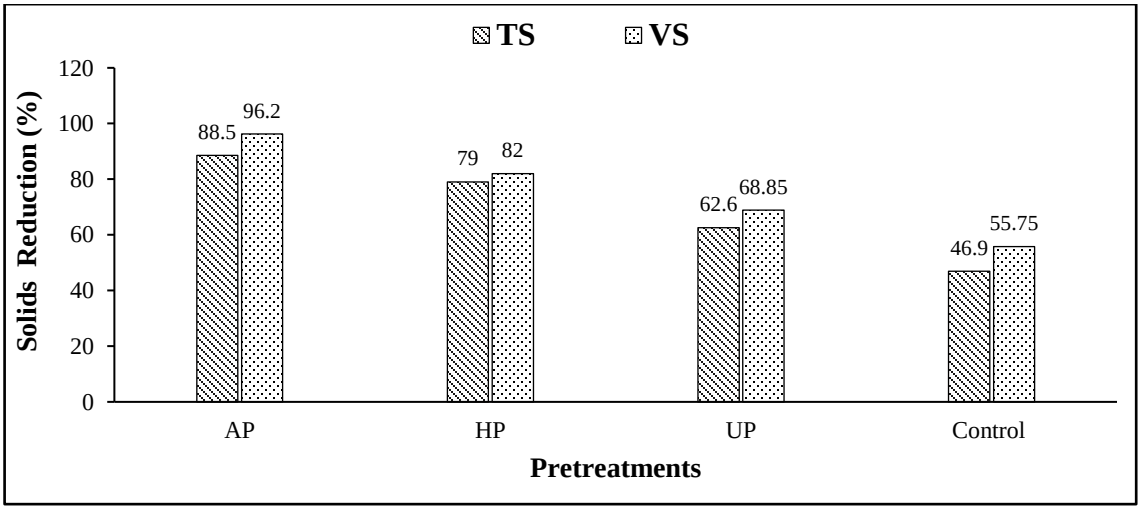

Figure 4.13: Comparison of solids removal of pretreated samples and control

Highest solid removal in terms of TS and VS has been observed in AP: 88.5 per cent TS and 96.2 per cent VS removal; followed by HP:79 per cent TS and 82 per cent VS removal, UP: 62.6 per cent TS and 68.85 per cent VS removal and control: 46.9 per cent TS and 55.75 per cent VS removal. The net TS and VS consumption for all the pretreated digesters were significantly greater than non-treated digesters (control) in accordance with the results shared by Park et al. (2010). Visual appearance of control after digestion was more on the solid side while the pretreated waste was converted into a homogenous slurry. These findings specify that anaerobic digestion is an effective way of handling the organic municipal solid waste. Also,

pretreatments and co-digesting PBW+OKR may increase the solids removal efficiency of the process. VS reduction for food waste has been reported between 70 - 92.2 per cent (Voegeli et al., 2009; Garcia et al., 2011).

4.2.6 Chemical Oxygen Demand (COD) Removal

Another important aspect for checking the system efficiency is that how well the organic matter is consumed by the bacteria. Chemical oxygen demand is the amount of oxygen consumed during the chemical degradation of the organic waste within the reactor. Comparison of results are presented in Figure 4.14. The highest removal was observed in AP with initial and final values of 53840 and 7825 mg/l, respectively that is 85.4per cent COD removal.

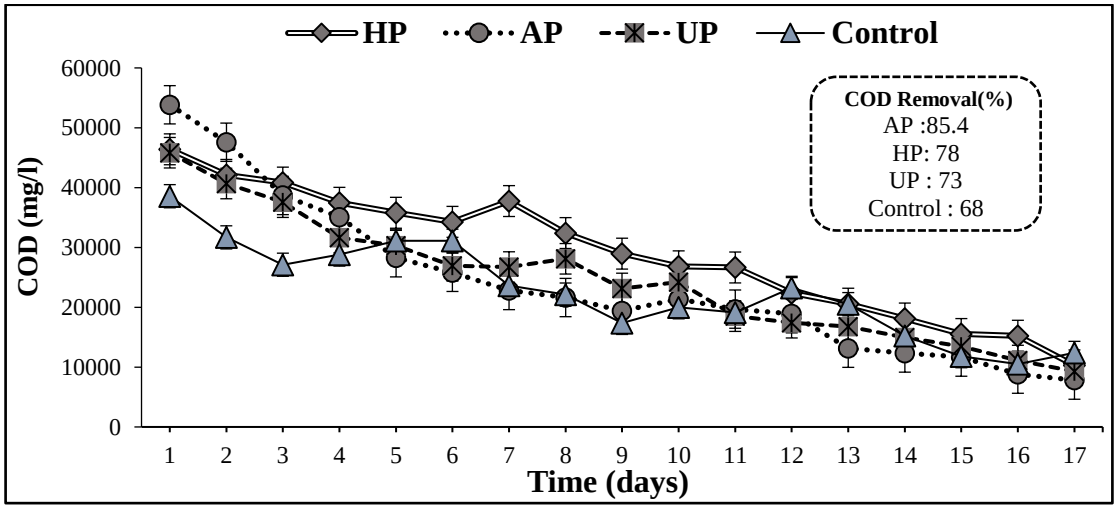

Figure 4.14: Comparison of COD removal of pretreated samples and control

Initial and final COD for HP was measured as 46400 and 10293 mg/l with a 78 per cent COD removal. UP undergoes 73 per cent COD removal with initial and final COD values of 45830 and 9280 mg/l. Control showed the lowest COD removal efficiency with initial and final values of 38560 and 12372.5 mg /l, respectively with a total COD removal was 68per cent. Initially low COD was due to mono-digestion of PBW and non-addition of OKR. Dawood et al, 2011 reported the COD removal efficiency when the substrate was mono-digested as 50.0 per cent at HRT of 72 days. The increased COD efficiency may be attributed as a synergistic effect of co-digestion. Elango et al. (2007) also reported 85 per cent of maximum COD reduction when municipal solid waste was co-digested (containing 52 per cent food waste and 3.5 per cent paper waste).

4.2.7 Post-digestion Analysis of Slurry

In this study the digestate was obtained as a thick slurry of much darker color as compared to the startup feed of the digesters with less smell. Results of NPK analysis slurry are shown in Figure 4.15, clearly indicates that co-digestion positively impacted the quality of slurry whereas the quality is also altered by pretreatments, due to which substrates were degraded up to full extent and hence the nutritional value increased. Feeding of substrates was kept constant for all the digesters (5 g VS / l), the difference in the quantity of NPK in the slurries may be correlated with pretreatments.

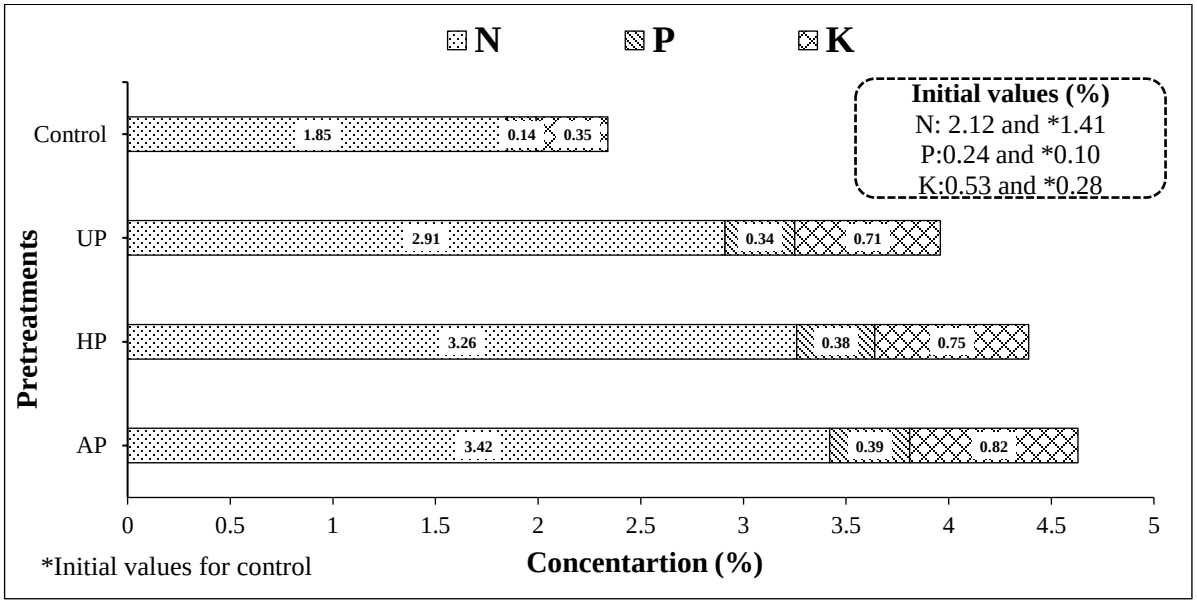

Figure 4.14: Comparison of NPK content of pretreated samples and control

Increase in N content was determined as: AP:46.9 per cent; HP:42.37 per cent; UP:31.4 per cent and control :26.9 per cent. Phosphorus content followed the similar trend and increased as AP: 47.6 per cent; HP: 45.1 per cent; UP:34.4 per cent and control:33.3 per cent. Whereas, potassium increased as: AP:42.9 per cent: HP:34.3 per cent; UP:29.2 per cent and control :24.5per cent. Increase in TN/TS content of digested food waste and kitchen waste was reported in the range of 16.1-34 per cent depending on the initial N content of the substrate (Fouda ,2011; Drosg et al.,2015). Maximum increase of 51.5 and 46.4 per cent in N and K, respectively, was quantified by Abbas et al.,2015. Whereas, Bachmann et al. (2016), reported 21 per cent more P in digestate as compared to undigested feedstock. Moller et al., 2008 also reported 1.7-3.54 times increase in the nutrient content of digested slurry as compared to the untreated feedstock.

Increase in nitrogen content after digestion is due to N-mineralization occurred within the digesters. The digestate is transformed into liquid fraction which contains dissolved ammonia nitrogen while the organic nitrogen is retained in the solid portion of the slurry, overall improving the fertilizer value.

4.3 PHASE – III

Pretreated paperboard waste resulted in highest biogas yield, methane content, total solids and COD removal was found to be alkali treated PBW in previous phase. Therefore, for the third phase the pretreatment was kept constant and PBW was co-digested with kitchen waste at three different loading rates of: 5, 10 and 15 g VS/l. During experimental phase – III, effect of varying loading rates was investigated and the results are discussed below:

4.3.1 Effect of Organic Loading Rate (OLR) on Biogas Production

The overall effect of increased OLR: 5,10 and 15 g VS/l on daily biogas production is shown in Figure 4.16. Increased organic loading rate may enhance the biogas quantity and quality. When more organic matter is available for microbes, under favorable conditions it may be converted to biogas (Fang et al., 2010). But for this specific study, at lowest loading rate of 5 g VS/l the system was more stable and total 13096.7 ml biogas was produced in 36 days, which was 25 per cent and 33.9 per cent greater than gas produced at 10 and 15 g VS/l, respectively. With increased loading rates, amount of OKR added to the digesters was also increased and resulted in excessive VFA's accumulation and therefore, overall digestion process was under stress conditions due to low pH and buffering capacity of the digesters. Although the system did not collapse but the rate of biogas production and its quality was deteriorated with increased loading rates in accordance to the trends reported by Babaee and Shayegan, 2011; Noor, 2017 shared comparable results when the anaerobic digestion efficiency with increasing OLR's was investigated. It has been concluded that at higher OLR excessive VFA accumulation resulted in decreased biogas production with a VFA/Alkalinity ratio way above the desired range of 0.3. All other parameters including biogas composition, buffering capacity, solids and COD removal are found to be strongly correlated with increased OLR's. Similarly, the daily biogas yield and net cumulative yields were also decreased with increase in loading rates as shown in Figures 4.17 and 4.18.

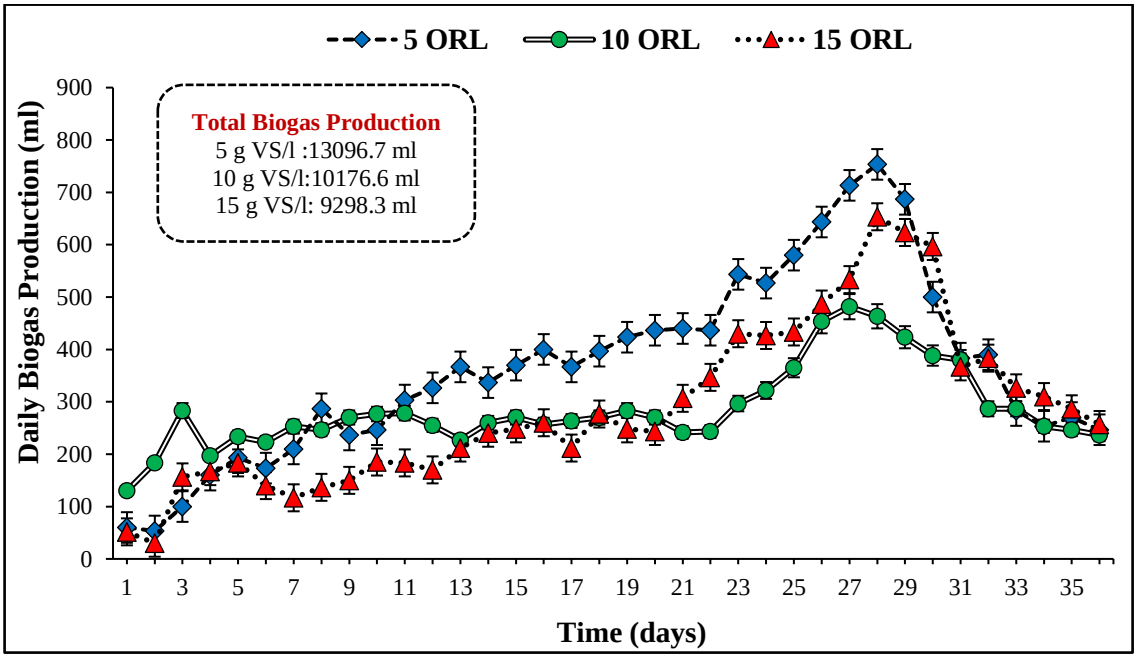

Figure 4.16: Effect of varying loading rates on daily biogas production

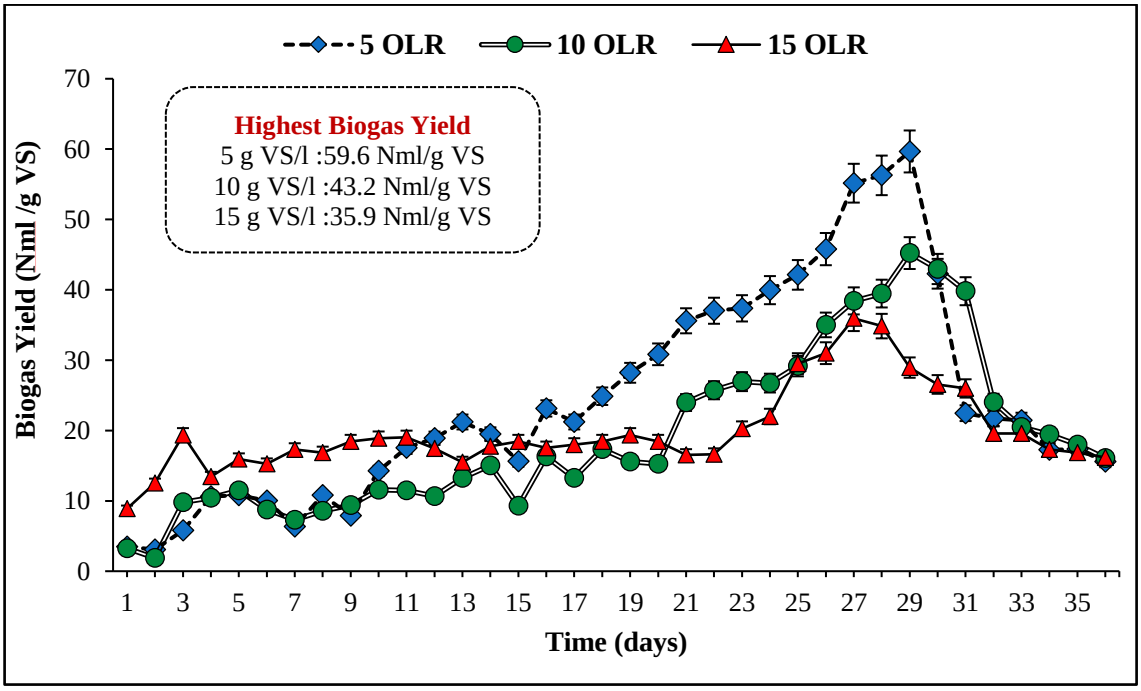

Figure 4.17: Effect of varying loading rates on daily biogas yield

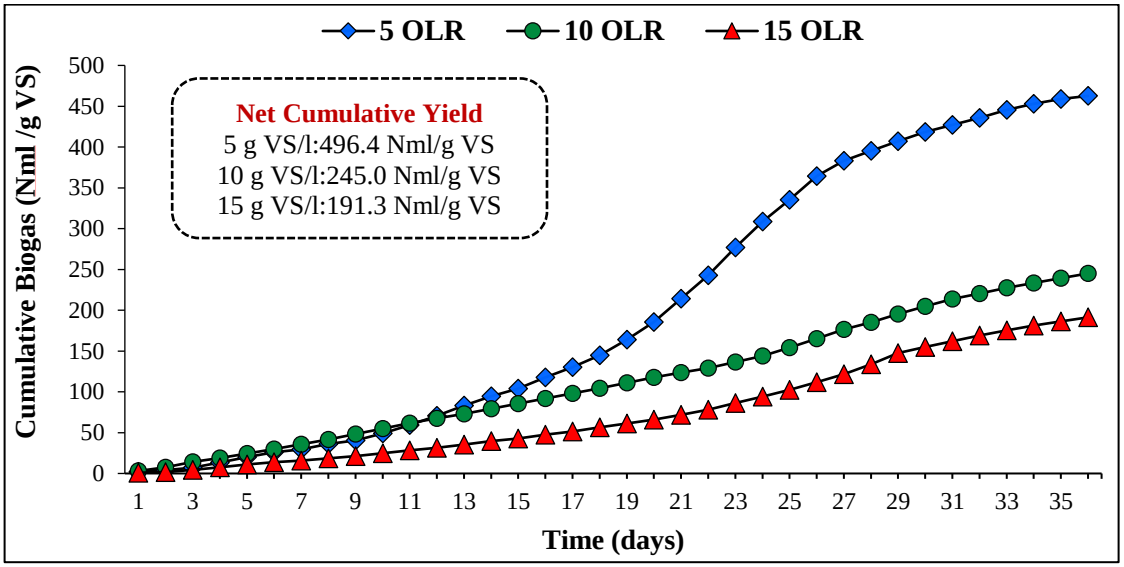

Figure 4.18: Comparison of varying loading rates on net cumulative biogas yield

4.3.2 Kinetic Study of the Effect of Varying Loading Rates

The fits of the modified Gompertz model to varying loading rates are numerically presented in Table 4.10. Parameters of the model include the lag time (λ), the biogas production potential (EMY), and the maximum cumulative biogas production rate (R^{max}), and the comparison of theoretical methane yield (TMY) which was predicted by the model and the experimental methane (EMY) are also shown with percentage difference.

Table 4.10: Variations in modified Gompertz model parameters with altering OLR

OLR (g VS/L)	**TMY** (N ml/g VS)	**EMY** (N ml/g VS)	**Difference** (%)	$\mathbf{R_{max}}$ (N ml/g VS)	**λ** (day)	R^2
5	449.59	462.64	2.8	26.5	11.5	0.979
10	236.18	245.08	3.6	8.85	5.05	0.970
15	186.68	191.34	2.6	8.22	10.47	0.959

Goodness of fit: R^2 ranged between 0.959-0.979. The system with 5 g VS/L, had the highest maximum biogas production rate (R_{max} and biogas production potential (EMY), followed by the reactors fed with increased loading rates of 10 and 15 g VS/l.

4.3.3 Effect of Organic Loading Rate (ORL) on Biogas Composition

Results of compositional analysis of varying loading rates are presented in Figure 4.19, revealed that the methane content of 5 OLR was 14.4per cent and 20 per cent higher than that of 10 and 15 OLR, respectively. It may be related to the stressful conditions for methanogens within the reactors as a consequence of increased OLR. Results from a quantitative PCR analysis detected the decline in methanogens at shorter retention time and increased OLR (Lee et al., 2011). Due to increased VFA generation and drop in pH for longer time span, acidogenic and acetogenic communities dominates the methanogens, results in increased CO_2 concentration and less CH_4 content (Babaee & Shayegan, 2011).

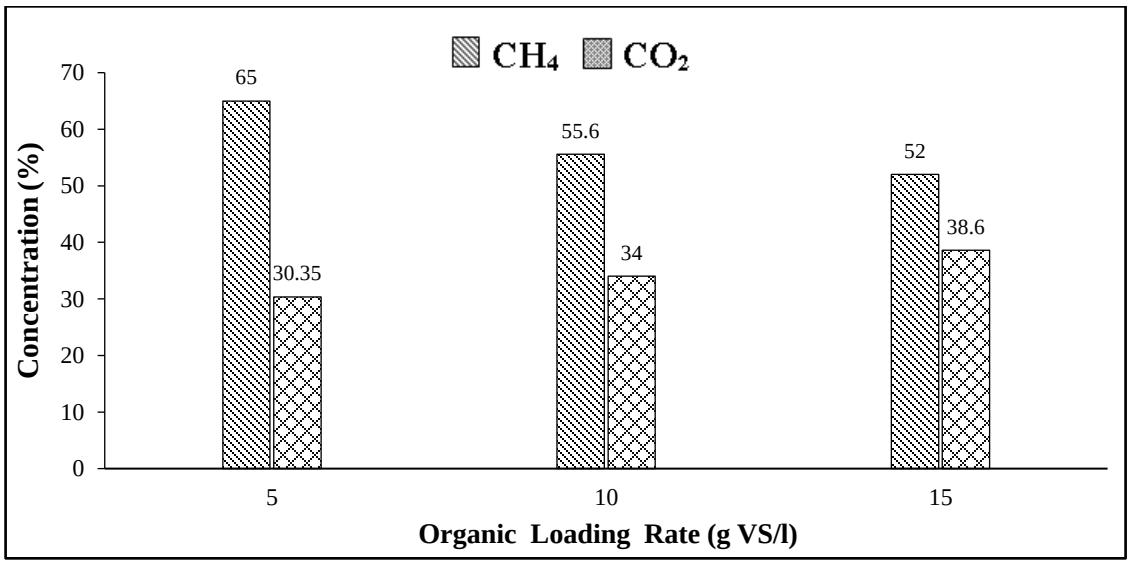

Figure 4.19: Effect of OLR on the biogas composition

4.3.4 pH and Buffering Capacity of the Reactors

A stable anaerobic process requires an optimum alkalinity, VFA's ratios and pH to establish a desired balance between acidogenesis and methanogenesis and for stable anaerobic digestion VFA production should be equal to VFA consumption (Cheong and Hansen, 2008). Under favorable conditions the amount of acid produced during acidogenic phase is buffered by the CO2 generated as by-product of organic degradation of the substrates, which is the major

contributor in alkalinity (Akuzawa et al., 2011). Results of the effect of varying loading rates on pH, alkalinity and volatile fatty acids are discussed in detail:

4.3.4.1 pH

Variations in the pH of digesters loaded with varying loading rates are shown in Figure 4.20. Reactors with escalated loading rates of 10 and 15 g VS/l have shown drastic and continuous drops in pH, lower than the desired range of methanogenic bacteria till the middle of 3rd week.

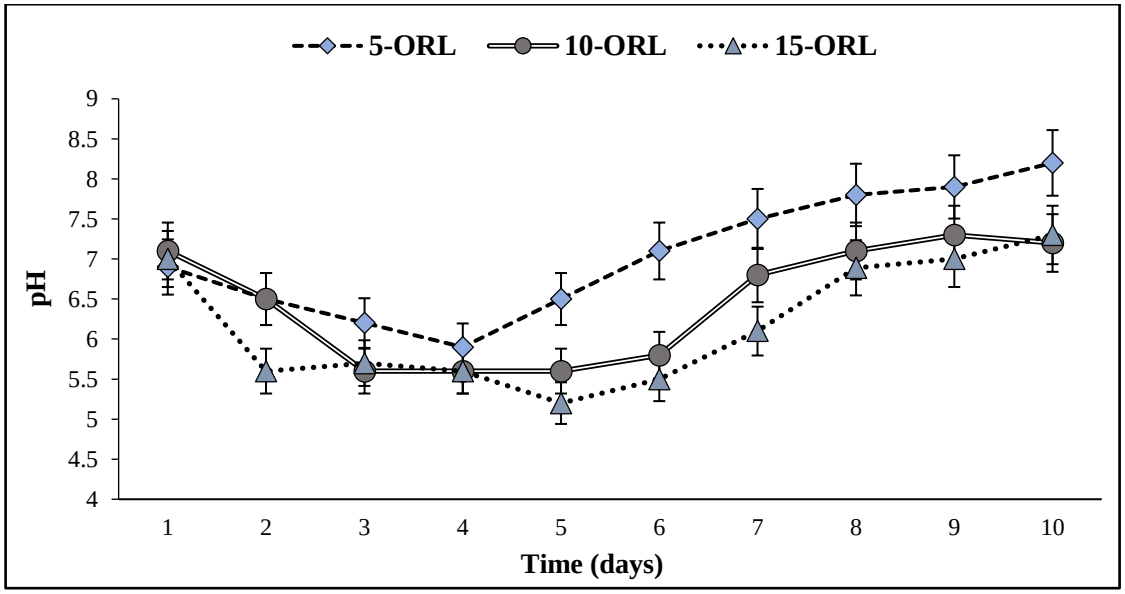

Figure 4.20: Effect of varying loading rates on the pH of reactors

Whereas, at loading rate of 5 g VS/l, a drop was observed from the middle of 1st week till the end of 2nd week and then the pH raised indicating the start of acetogenesis and methanogenesis. Reactors loaded with 5 g VS/l showed the pH values within the desired working range of 6.6-8.1 of methanogens for longer time span and hence the methane content was found higher in the collected biogas samples of these digesters. Continuous decline in pH at higher loading rates was probably due to the fast conversion of organic matter to fatty acids in a chemical reaction that utilized the alkalinity of the reactors during the initial two phases of anaerobic digestion (Nguyen et al.,2017). Once the alkalinity declined the pH value dropped leading to stressful conditions for the methanogens.

4.3.4.2 Alkalinity

Overall trend of the variations in alkalinity due to varying loading rates are shown in Figure 4.21. Difference in maximum increase in alkalinity of 15 g VS/l was measured as 16 per cent and 14.3 per cent more as compared to 5 and 10 g VS/l, respectively.

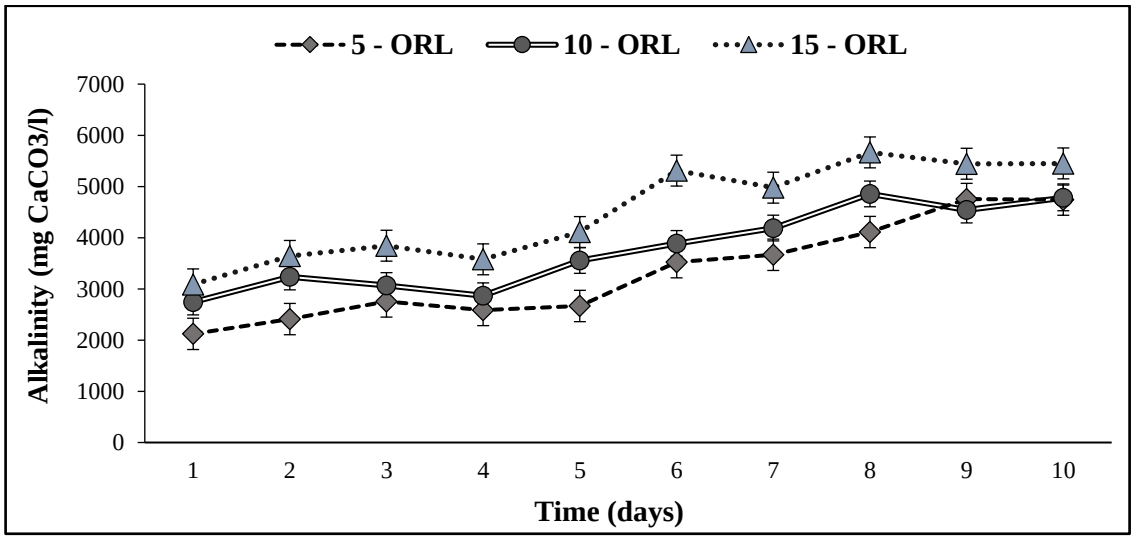

Figure 4.21: Effect of ORL on the alkalinity of reactors

With increase in overall loading rate the amount of PBW added to the reactors also increased which acted as buffering agent led to an increase in alkalinity throughout the experiment. However, alkalinity alone should not be considered as a stability indicator for and the overall system permanency is checked by the VFA/Alkalinity ratio which should be within 0.1-0.4 and above 0.4 means higher VFA's accumulation, instable digestion with less biogas yield and methane content (Barampouti et al., 2005; Sanchez et al., 2005). Similar trend of increase in OLR and alkalinity was reported (Noor, 2017) when loading rate was increased from 2 to 6 g VS/l.

4.3.4.3 Volatile fatty acids (VFA's)

Effect of adding higher organic content to the reactors on volatile fatty acids production is shown in Figure 4.22. At 5 g VS/l the PBW was able to provide enough buffering for a stable anaerobic process but increased loading rates were problematic. VFA/alkalinity ratio higher than 0.4 was hence noticed at 10 and 15 OLR's with an increase up to 0.7 which was way higher

than measured at 5 OLR. The rate of VFA production decreased from the end of 2nd week onwards that shows the adaptation of microbes to the increased loading rates and also during this time the alkalinity of reactors further increased but still the methanogenic communities were not able to convert all the organic acids to methane therefore lesser methane content was found from the biogas samples from higher OLR's (Wang et al., 2009). Excessive generation and accumulation of VFA's indicated an inversely proportional relationship between increase in OLR and VFA's production. Increased concentration of OKR leads to rapid degradation and excessive conversion of organic matter into fatty acids.

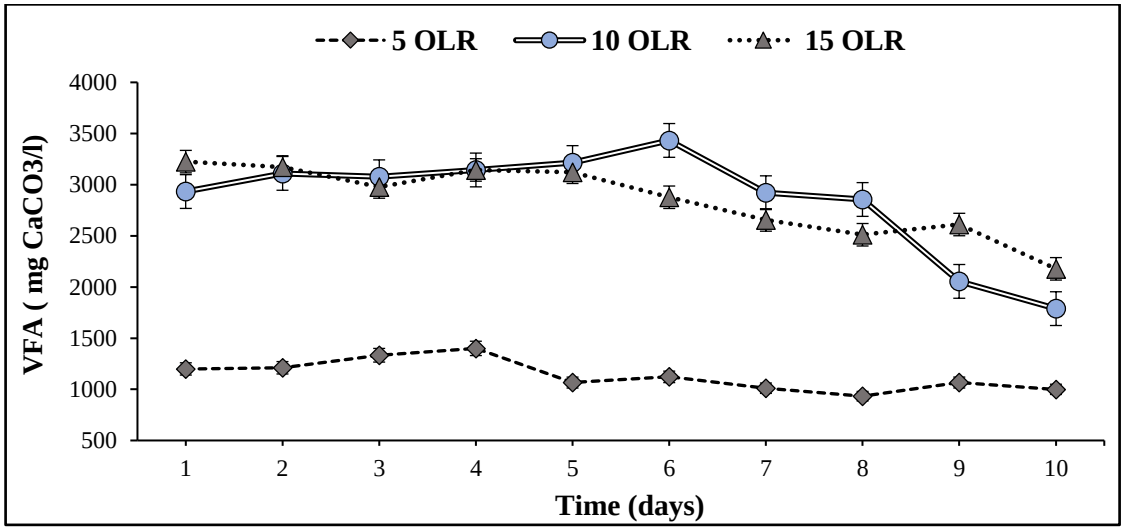

Figure 4.22: Effect of ORL on volatile fatty acids production

4.3.5 Total Solids (TS) and Volatile Solids (VS) Removal

Effect of varying loading rates on the solids removal is depicted in Figure 4.23. Total solids removal for all reactors ranged between 42-63.2 per cent, whereas, volatile solids removal ranged between 53.3-75.3 per cent. Results showed decline in solid removal with increased loading rates from 5-15 g VS/l. Less solids removal might be the results of imbalance occurred between the 4 phases of anaerobic digestion. Once these volatile fatty acids have been accumulated in the reactors, the acidogenic and acetogenic bacteria becomes more active whereas hydrolytic and methanogenic bacteria slower down. At reduced or shorter solids retention time (SRT), the solid removal efficiency decreased when the loading rates were increased, because more time is required by the bacteria for utilizing all the organic matter present as substrate (Mahmoud et al.,2003). When the OLR in increased bacteria start focusing

more on converting the already e accumulated excessive VFA's into organic acids instead of hydrolyzing and utilizing solids to full extent disturbing the overall biochemical pathways of volatile solids conversion to biogas and total solids degradation (Babaee and Shayegan ,2011; Noor, 2017).

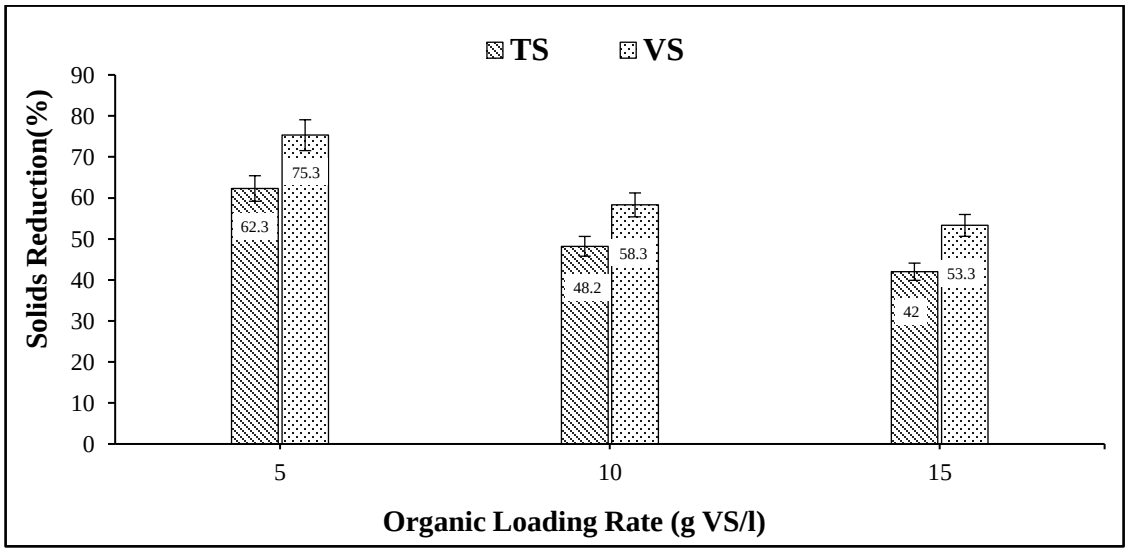

Figure 4.23: Effect of increased loading rates on solids removal

4.3.6 Chemical Oxygen Demand (COD) Removal

Trends of COD removal with respect to the increased loading rates are shown in Figure 4.24.

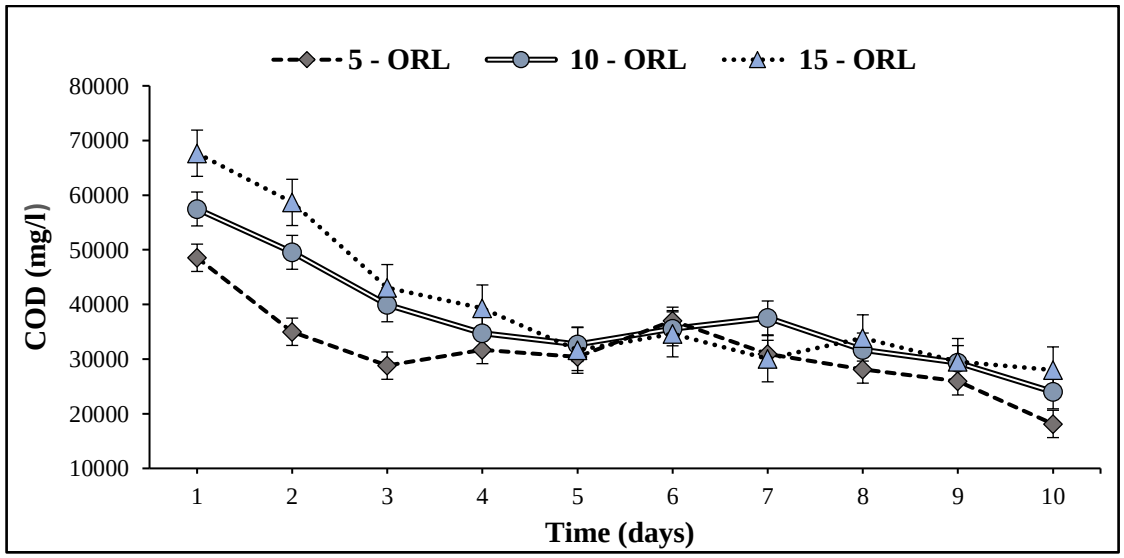

Figure 4.24: Effect of increased loading rates on COD removal

For 5 g VS/l, COD removal efficiency was 63 per cent followed by 10 and 15 g VS/l with COD removal efficiency of 58.2 per cent and 54.6 per cent, respectively. Decrease in COD removal with increasing OLR is due to initially weaker adaptation of microbes to the higher concentrations of organic matter in the reactors. Similar results were reported by Rubia at al., and Noor that increased OLR, COD also increased initially, which require longer retention times by the bacteria to significantly remove the organic pollutants present within the reactors and at VFA/TA ratio between 0.5-0.7, biogas production, COD and solids removal also decline.

4.3.7 NPK Analysis of Digested Slurry

Effects of increased loading rates on nutritional content of the digested slurry are shown in Figure 4.25. Amount of substrates added to the reactors was kept constant with varying loadings rates (5,10 and 15 g VS/l).

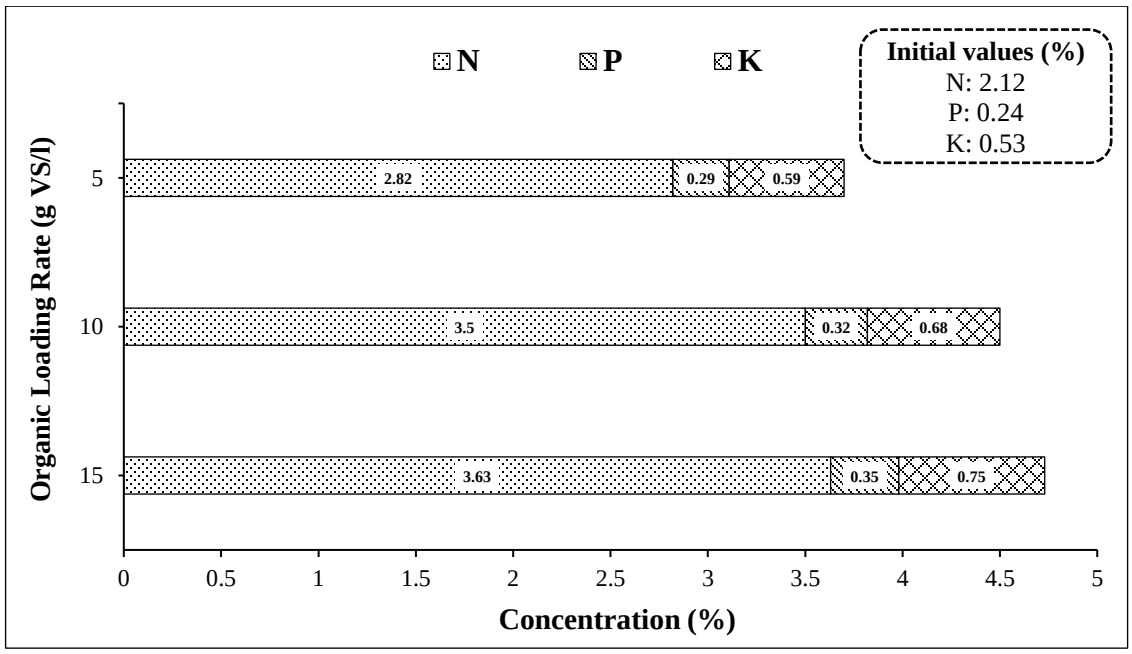

Figure 4.25: Effect of variations on loading rates quality of the digested slurry

Increase in N content was determined as: 5 OLR:28.3 per cent; 10 OLR:49.1 per cent and 15 OLR:52.3 per cent. Phosphorus content also increased at higher loading loads and measured as 5 OLR: 18.8 per cent; 10 OLR: 28.5 per cent and 15 OLR: 37.2 per cent. Whereas, potassium increased as: 5 OLR: 10.7 per cent: 10 OLR: 24.7 per cent and for 15 OLR: 34.3 per cent. Results indicates that due to increased loading rates feeding to the digesters, post digestion

slurry contains higher nutritive component. Khorsandi, & Nourbakhsh. (2007) found a strong correlation ($R^2 = 0.96$) between N-mineralization and N-content, means the material containing higher nitrogen content will be able to release more nitrogen when undergoes digestion. C/N ratio also plays vital role in N release. Substrates with low C: N release more nitrogen due to their high organic nitrogen content (Qian & Schoenau, 2002). Gunnarsson et al. (2010) compared the nutrient efficiency of the digested effluent and mineral fertilizers. 180 days pot experiment revealed that there was not significant difference between the nutrient availability and uptake by the plants and the digested slurry was a good substitute of synthetic fertilizers.

Chapter 5

CONCLUSIONS & RECOMMENDATIONS

This chapter will cover the final conclusions from all experimental phases and recommendations for future work that may be steered to improve the understanding of different experimental aspects and further optimization of the conditions.

5.1 CONCLUSIONS

Conclusions of all phases will be discussed separately:

5.1.1 Phase - I

- Particle size effected the lignin removal in following order: 2mm < 5 mm < control, but there was non-significant difference between 2 and 5 mm sizes.
- Trend for the most to least effective pretreatment was observed as: Alkaline (NaOH)> Hydrothermal > Ultrasonication.
- Particle size analysis results showed the presence of larger particles (7.24±1.06µm) in the washings of most effective pretreatment. Lignin has a large polymeric structure (length:10.5-18.6µm) and the sizes found in PSA is closer to the size of lignin reported in the literature.
- Results of scanning electron microscopy (SEM) were also concordant with pretreatment and PSA. Structure of PBW was opened up the most in the samples of superlative effective pretreatment.
- Characteristics of substrates:
- Organic kitchen residues: The feedstock have higher moisture content (90.7 ± 0.17); TOC(34.01 ± 1.41); TS(10.5 ± 1.16); VS(96.4 ± 0.32) and COD(24,608 ± 53.2) but the pH(4.8 ± 0.26) and C/N ratio(16:1) was lower than the optimum range of anaerobic digestion;
- Paperboard waste's physicochemical characteristics revealed that it has TOC (42.1 ± 0.26) higher than OKR, with a suitable pH(8.3 ± 0.16) and lower moisture content (4.8 ± 0.1) and nitrogen content(0.3 ± 0.03) , makes its C/N(140:1) ratio higher form the optimum range of AD. Therefore, both substrates were ideal to be co-digested, as both possess compatible characteristics.

5.1.2 Phase – II

- Overall trend of biogas production/yield was observed as: Alkaline (NaOH)> Hydrothermal > Ultrasonication > Control.
- Highest methane content of 68 per cent was detected in the biogas samples of PBW treated with 10per cent NaOH.
- Buffering capacity remained higher in the control (untreated paperboard waste only). The pH, VFA/TA ratio remained within optimum range (AP:0.24-0.39; HP:0.26 -0.41; UP: 0.33-0.40; C :0.24-0. 35)for methanogens due to higher initial pH values of both feedstock and the inoculum.
- COD and solids removal was altered by pretreatments. For alkaline pretreatment 88.5 per cent, 96.2 per cent and 85.4 per cent TS, VS and COD removal has been noticed.
- NPK analysis of digested slurries revealed that the nutritional content of co-digested wastes was significantly higher than control. The substrates were co-digested successfully at loading rate of 5 g VS/l, without facing any stressful conditions within in the reactors.

5.1.3 Phase – III

- Increase in OLR from 5 to 10 and 15 g VS/l led to significant decrease in pH and buffering capacity. Increased concentration of OKR resulted in excessive VFA's accumulation, disturbing the VFA/Alkalinity ratio (upto 0.7). Once the ratio deviates from the optimum range for stable digestion, acidogens and acetogens dominate the methanogenic communities.
- Highest biogas yield and methane content of 65 per cent was detected from the samples collected from 5 OLR digesters which was found comparable to the biogas samples collected in 1st run when the anaerobic digestion was conducted under similar conditions.
- Solids (TS: 62.3,48.2 and 42per cent for 5,10 and 15 OLR; VS:75.3,58.3,53.3 per cent for 5,10 and 15-OLR) and COD (5-OLR: 63.5 per cent ;10-OLR: 58.2 per cent;15-OLR: 54.6 per cent) was removed lesser at higher loading rates due to slower degradation of the organic matter.
- However, increase in feedstock concentration (5-OLR<10-OLR<15-OLR) led to the escalation of nutritional content of the digested slurry even at shorter retention times.

5.2 RECOMMENDATIONS

- Designing and optimization of a pilot scale anaerobic setup for household, institutional and commercial levels for minimization and stabilization for organic waste.
- Investigation of digested slurry as biofertilizer on growth pattern and yield of crops and on soil quality as compared to synthetic fertilizer.
- Comparison of biogas production by using organic content of municipal solid waste under mesophilic and thermophilic conditions.
- Optimization of parameters effecting the methane content of the biogas to further increase the methane content of biogas.
- Study the relation between pretreatments, improvements in biogas production and the cost estimation to get better understanding of input and output resources.

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