SOLID-STATE ANAEROBIC CO-DIGESTION OF ORGANIC SUBSTRATES FOR BIOGAS PRODUCTION

Thesis

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

UMA S.

(145027CV14F11)

DEPARTMENT OF CIVIL ENGINEERING

NATIONAL INSTITUTE OF TECHNOLOGY KARNATAKA

SURATHKAL, MANGALORE-575025

JULY 2019

SOLID-STATE ANAEROBIC CO-DIGESTION OF ORGANIC SUBSTRATES FOR BIOGAS PRODUCTION

Thesis

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

UMA S.

(145027CV14F11)

Under the Guidance of

Dr. ARUN KUMAR THALLA

Dr. C. P. DEVATHA

DEPARTMENT OF CIVIL ENGINEERING

NATIONAL INSTITUTE OF TECHNOLOGY KARNATAKA

SURATHKAL, MANGALORE-575025

JULY 2019

D E C L A R A T I O N

I hereby declare that the Research Thesis entitled **"Solid-State Anaerobic Co-digestion of Organic Substrates for Biogas Production"** Which is being submitted to the **National Institute of Technology Karnataka, Surathkal** in partial fulfilment of the requirements for the award of the **Degree of Doctor of Philosophy** in **Civil Engineering** is a bonafide report of the research work carried out by me. The material contained in this Research Thesis has not been submitted to any University or Institution for the award of any degree.

Uma S.
145027CV14F11
Department of Civil Engineering

Place: NITK-Surathkal

Date:

C E R T I F I C A T E

This is to certify that the Research Thesis entitled **"Solid-State Anaerobic Co-digestion of Organic Substrates for Biogas Production"** submitted by **Uma S. (145027CV14F11)** as the record of the research work carried out by her, is accepted as the Research Thesis submission in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy.

Dr. Arun kumar Thalla & Dr. C P Devatha

Research Guides

(Signature with Date and Seal)

Prof. K Swaminathan

Chairman - DRPC

(Signature with Date and Seal)

ACKNOWLEDGEMENT

It is an immense pleasure in expressing my heartfelt gratitude to various people who helped and motivated me to pursue my Ph.D.

I express my sincere gratitude, and am thankful to my esteemed Supervisors **Dr. Arun Kumar Thalla** and **Dr. C P Devatha** Faculty, Department of Civil Engineering for their valuable guidance, suggestions, meticulous efforts and motivation, which played an inspiring role throughout the research work to fulfill the criteria.

I owe my gratitude to my Professors **Dr. K Swaminathan, Dr. Varghese George, Dr. Katta Venkatramma** and **Dr. Venkat Reddy,** Head of Department, Civil Engineering, NITK Surathkal for extending the facilities and wonderful support at every stage of research work in the Department. I also extend my sincere gratitude towards **Dr. Sai Dutta,** Academic Dean, NITK Surathkal for the support rendered throughout the work.

I would like to express my sincere thanks to the Doctoral Research Programme Committee members **Dr. Arun Isloo**r, Professor, Department of Chemistry and **Dr. Subhash C. Yaragal**, Professor, Department of Civil Engineering, NITK for their valuable advice and support throughout my research work.

I like to express my sincere thanks to the Doctoral Thesis Assessment Committee **Dr. Nagendrappa H**, Assistant Professor, Department of Electrical and Electronics Engineering and **Dr. A.U. Ravishankar**, Professor, Department of Civil Engineering, NITK for their valuable suggestions and advices to improves the quality of research.

I like to thank the Ministry of Human Resources Development, Government of India for the financial assistance for my research work from 2014 to 2019.

I express my heartfelt gratitude to all the **faculty and staff members**, Department of Civil Engineering, NITK Surathkal for their continuous encouragement and support inspired my work.

I thank **Mr. Manohar K. Shanbhogue** Senior Technical Assistant for his advice and cooperation in the laboratory experiments. I would like to thank **Mr. Dheeraj** for his help in the laboratory.

Special thanks to my lovable father (L) S**hri. M. Sakthivel (Retd.), Forest Ranger officer, Government of TamilNadu** for the continuous inspiration, enthusiasm in my research journey.

Heartfelt thanks to my Sister **Miss. Beaulah Sakthivel (a) Girisakthi and my mother Pushpam Sakthivel** for their continuous support, patience, sacrifice in providing favourable environment for the completion of my work.

I take this opportunity to personally appreciate and thanks to **Adhirashree V., Satish Kumar Kolluru, Teema Thomas and Sarath Chandra Pragada** for their fruitful encouragement and enlightening discussions.

My heartiest thanks to **Mr. Anup Denzil Veigas** for his timely support and suggestions to complete my research thesis.

I like to express my heartfelt gratitude to all my **friends and colleagues** for their unremitting encouragement, help and support. I am grateful to my **family** members whose endless encouragement and supports was a source of inspiration for this work.

I thank the god almighty, for his blessings upon me all through my life. Once again, I thank one and all who have helped me directly or indirectly in the completion of my project on time.

NITK Surathkal **Uma Sakthivel**

ABSTRACT

Solid waste management is an important problem in the developing countries due to the rapid quantity of waste generation as they urbanize. As the demand increases for bioenergy, biofuels produced from waste biomass replicates as a supplementary energy resource to satisfy the requirements. Most of these generated wastes consist of biodegradable organic matter, which could be utilized as a source for biofuel generation. These biodegradable wastes are highly opted by suitable treatment method, which is known by anaerobic biodegradation. This study investigated the performance of organic waste digestion in laboratory-scale for biogas production. Also, it focuses on the effects of process parameters such as pH, alkalinity and volatile acids on biogas yield performance by batch and semi-continuous digestion. Food waste and switchgrass is used as the feedstock in the present study, which is collected from NITK campus.

The objective (1) of this study aimed to investigate the effects of pretreatment of switchgrass on biogas production. Switchgrass is used as a feedstock, which is subjected to physical and chemical pretreatment for batch digestion at mesophilic condition. Batch experimental results from raw switchgrass yields 248 mL CH_4/g VS at mesophilic condition. The biomethane potential of pretreated SG is 53%, 52% and 12% higher for alkali, organosolv and thermal pretreatments respectively, and 44% and 20% lower at acid and liquid hot water pretreatments in comparison to raw SG yield. Highest biomethane yield confirms the enhanced biodegradability of switchgrass by alkaline and organosolv pretreatments.

The objective (2) aimed at co-digesting the food waste (FW) and switchgrass (SG) by batch and semi-continuous mode for biogas production. The performance of batch co-digestion is determined with FW and SG as a feedstock with different mix ratio (0:1; 1:1; 0:1 FW: SG) at mesophilic and thermophilic temperatures. Semi-continuous digestion is conducted by varying the loading from 4-8 g/L with mix ratios (100:0, 12:88, 25:75, 50:50 and 0:100 FW: SG) at mesophilic conditions. The process parameters (pH, alkalinity and volatile fatty acids) are monitored frequently for their interactive effects on biogas production by batch and semi-continuous digestion.

The highest methane yield is observed with 1:1 FW: SG as 267 mL/g VS at mesophilic (32-day retention time) and 234 mL/g VS at thermophilic (18-day retention time) condition during batch digestion. Methane yield has a positive response on co-digestion and confirmed by digestion performance index (DPI). Results reveal that co-digestion at 1:1 ratio yields an enhanced performance with both FW and SG in mesophilic as well as thermophilic condition. This study confirms that the presence of slow and fast biodegradable organic matters has an equal contribution to methane yield. A t-VFA/Alk ratio maintains the consistency between acidification and methanation phase. The t-VFA/alk ratio is 0.2 to 0.9 for mesophilic and 0.3-1.5 for thermophilic condition. The release of volatile acids at shorter retention time is observed with thermophilic owing to, faster hydrolysis than at mesophilic conditions.

The maximum biogas yield is 628 mL/g VS for 4 g /L loading for semi-continuous mode. The methane content obtained is around 65% that shows the stable performance at varying ratios of FW and SG. Average value of methane yield is 320 mL CH_4/g VS which is estimated about 32,000 m^3 that produces the energy of 320, 000 kW-h. Results well agreed to implement the combined heat and power system, as electrical and thermal efficiencies by 35% and 50% are widespread across many countries for the energy conversion.

Keywords

Methane yield, biodegradability, digestion performance index, mesophilic, food waste, thermophilic, switchgrass

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA- Acetic Acid

AD- Anaerobic Digestion

AT - 1% H_2SO_4 switchgrass

Alk. - Alkalinity

ALT- 1% NaOH Switchgrass

APHA- American Public Health Association

ATA- Anaerobic Toxicity Assay

BA- Butyric Acid

BMP- Biomethane Potential Assay

CHP- Combined Heat and Power

COD- Chemical Oxygen Demand

C/N- Carbon-to-Nitrogen Ratio

DM- Dry Matter

DP- Degree of Polymerization

DPI- Digestion Performance Index

EC- Electrical Conductivity

EPA- Environmental Protection Agency

FAO- Food and Agricultural Organization

FAS- Ferrous Ammonium Sulphate

F/E- Food- to-Micro-organism ratio

FID- Flame Ionization Detector

FTIR- Fourier Transform Infrared Spectroscopy

FW- Food Waste

f- final

GC- Gas Chromatography

GP- Gas Potential

HRT- Hydraulic Retention Time

i-BA- iso-Butyric Acid

i- initial

i-VA- iso-Valeric Acid

LCFA- Long Chain Fatty Acids

LHW- Liquid hot water SG

M- Mesophilic

MSW- Municipal solid waste

NA- Not Applicable

ND- Not Detectable

OFMSW- Organic fractions of municipal solid waste

OG- Organosolv SG

OLR- Organic Loading Rate

PA- Propionic Acid

SEM- Scanning Electron Microscopy

SG- Switchgrass

S/I- Substrate-to-Inoculum

SMY- Specific Methane Yield

SS-AD- Solid State Anaerobic Digestion

t- total

T- Thermophilic

TA- Total Alkalinity

TAN- Total Ammonia Nitrogen

TKN- Total Kjeldahl Nitrogen

TS- Total Solids

TT- Thermal treated SG

UASB- Upflow Anaerobic Sludge Blanket

UT- Untreated/Raw switchgrass

VFA- Volatile Fatty Acids

VS- Volatile solids

w/v- Weight-to-Volume

CHAPTER 1

INTRODUCTION

1.1 Background and Motivation

India is the second highly populated country with the population of 1.35 billion contributing 17.5 percent of the total world population (7.7 billion) according to world population clock. The population of India is increasing with an annual growth rate of 1.19 percent and is estimated to be 1.53 billion population by the year 2030. India generates 62 million tonnes of waste (mixed waste containing recyclable and non-recyclable materials) with an average annual growth rate of 4% according to Press Information Bureau. The generated waste can be divided into three different categories such as organic (biodegradable waste), dry (recyclable waste) and biomedical (sanitary and hazardous waste) respectively. Almost 50 percent of the total waste is organic (recyclable and hazardous), this quantity of waste is increasing year by year as India becomes more urbanized. Rapid industrialization and population explosion generate thousands of tons, of waste on a daily basis in India. The quantity of waste will increase significantly in the near future as the country, will attain the status of an industrialized nation. The limited availability of resources is in the form of fossil fuels. The crude oil requirements of the country and the increase in the emission of greenhouse gas, led the researcher to focus on alternative renewable sources that helps to fulfill the human requirements. A recent economic improvement in India has led to increased waste generation rate, along with increased energy demands. The country's growing energy deficit and increased focus on developing alternative sources of energy has led India to innovate on renewable energy policies.

Anaerobic digestion is an appropriate technology for organic waste treatment in a smarter way, in order to minimize the environmental problem, which maintains the overall balance in the environment. Anaerobic digestion refers to anaerobic decomposition of organic matter into the state of gasification, mineralization and liquefaction. Generally, the process is biological process involving waste conversion and stabilization. The end-products are methane (CH_4), carbon dioxide (CO_2) and stable organic residue. This technique is applicable for the stabilization of solid waste, which proceeds to disposal. In addition to the stabilization of solid waste, this has been receiving attention in recent years with slow biodegradable (lignocellulosic) co-digesting with fast biodegradable (food waste) into methane production. Various advantages are cited for the anaerobic digestion process like high degree of waste stabilization, less residual organic waste quantity and nutrient requirements. Anaerobic process of waste treatment potentially helps in the energy requirements of the society. In the focus of methane that serves in the domestic purpose in appliances and combustion engines, that becomes an alternative fuel resource.

Now-a-days, Researchers focus on utilizing high biodegradable waste for biogas production which offers high productivity in comparison with fossil fuels (Capson-Tojo et al. 2017; Torrijos et al. 2008). Food waste (FW) offers numerous advantages for biogas production. Figure 1.1 shows the food waste composition in India. Food waste (both precooked and leftover) is a biodegradable waste discharged from households, food processing industries and hospitals. Approximately, About 70 million ton (40%) of food is being wasted every year which has been valued around 92,000 crores (Goswami 2018). The amount of FW has been projected to increase in the coming decades due to economic and population growth. It has been reported that the annual amount of FW could rise from 280 to 420 million tonnes from 2005 to 2025 (Paritosh et al. 2017). Apart from food and land resource wastages, the carbon footprint of food waste is estimated to contribute to the greenhouse gas (GHG) by accumulating 3.3 billion tonnes of carbon dioxide into the atmosphere per year (Mane et al. 2015). Conventionally, this food waste is dumped in the open area, which causes environmental damage. Current system of disposal on food waste is ending up in landfills that leads to public health issues and impacts the environment. Open landfills, liberates heat and methane resulting in global warming. Rainwater percolates through landfills that produce leachate, which contaminates groundwater and natural sources. Appropriate methods are required for managing these food wastes. Anaerobic digestion can be an option to strengthen world's energy security by employing food waste to generate biogas, while addressing the problems of waste management (Li et al. 2017; Yong et al. 2015). Food waste consists of carbohydrates, protein, lipids and traces of inorganic compounds. The composition varies according to the type of food waste and its constituents. Food waste consisting of rice and vegetables is abundant in carbohydrates, lipids and proteins.

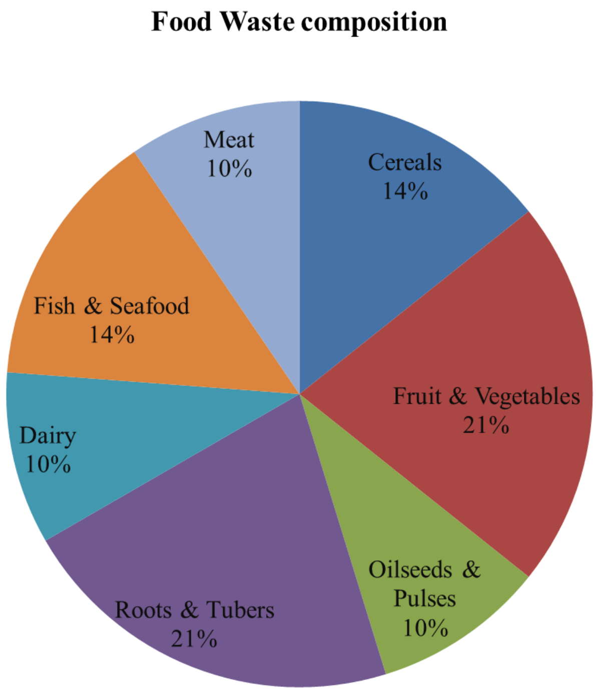


Figure 1. 1 Food waste composition

Food waste used as substrate mainly depends upon its chemical composition for biodegradability. Various researchers have investigated the potential of food waste as a substrate for biomethane potential. Browne and Murphy (2013) investigated biomethane potential from source segregated food waste that produced the methane yield between 467 and 530 L CH_4 per kg volatile solids added. Banks et al. (2011) used food waste as substrate and converted it to biogas yield about 643 m^3 per ton volatile solids added with the methane content of 62%. Food waste as a substrate has the potential to provide biogas yield and methane, which depends on the origin of wastes.

The major constraints faced with biodegradable waste are rapid acidification, pH variation and volatile acid accumulation that inverse the methanogenic activity. Additionally, it faces challenges with slow start-up, microbial accumulation, and rapid acidification with higher loading. Low C/N ratio causes less degradation and latent inhibition of methanogenesis (Madsen and Rasmussen 1996). The anaerobic co-digestion of organic waste has several advantages like bacterial diversity increases due to varieties of organic waste and stabilize a digester, dilute the inhibitory compounds (Nielfa et al. 2015). The biodegradable waste used as co-substrates at different combinations get the profit with better value-added products. Co-digestion has certain advantages; it is indistinct with some substrate combinations. Therefore, it is reasonable to examine the effects of mixed waste used for the anaerobic digestion with the intention to convert into biogas and biomethane potential. Besides researchers are focusing on co-digestion of mixed waste and biological sludge with different mixture proportions. Yong et al. (2015) investigated the biomethane potential of food waste and straw with the mix ratio 5:1 yields 0.392 m^3/kg volatile solids added, which is increased by 40% compared with individual substrate digestion. Study conducted by Rahman et al. (2017) on co-digestion of poultry droppings (PD) with lignocellulosic co-substrates (LCS) namely wheat straw (WS) and meadow grass (MG) for different mix ratio to optimize the substrate composition and C:N ratio in order to enhanced biogas production. The results confirmed that the co-digestion of PD and LCS obtained the highest methane yield 330-340 L per kg VS with the mix ratio (70:30 PD: WS) and C: N ratio of 32.0 respectively. Biochemical methane is applicable to the types of substrates from the varieties of possibilities on highest methane potential.

India has high potential biomass about 500 metric tons available per year. About 17500 MW power can be generated by this available biomass and additional power of about 5000 MW can be produced by surplus biomass around 150 MT respectively (Kumar et al. 2015). Various biomasses such as agricultural residues (wheat straw, sugarcane bagasse, and corn stover), lignocellulosic biomass (Switchgrass), forest products (hard and soft wood) and organic waste from edible resources are the renewable sources for biogas production, especially in India. Lignocellulosic biomass consists of cellulose (40-50%), hemicellulose (25-35%) and lignin (15-20%) which vary quantitatively and qualitatively according to plant origin. The presence of lignin acts as protective barrier, which prevents cell wall destruction by microorganisms for biodegradation. The cellulose and hemicellulose are broken down into monomers (sugars), which is utilized by microorganism during biodegradation (Li et al. 2017). Even the compositional and structural features (i.e. lignin, crystalline structure of cellulose and its surface area) limits their degradation (Monlau et al. 2012). Therefore, to achieve hydrolysis as well as to improve the biogas production, pretreatment is mandatory. However, the recalcitrance nature of biomass hinders the efficiency of biochemical conversion during biogas production. Pretreatment is generally a process for disruption of naturally resistant structure of lignocellulose that limits hydrolysis of carbohydrates i.e. cellulose and hemicellulose. The suitable pretreatment method reduces the recalcitrance and makes the biochemical process efficient, eco-friendly and economically viable. The efficiency of treating lignocellulosic biomass is limited with cell wall encapsulated with cellulose, hemicellulose and lignin, which hinders the accessibility to cellulose and hemicellulose for microbes during decomposition. Recent research reveals that anaerobic digestion of lignocellulosic biomass improves the biogas production and faces the challenge towards experimental initialization, microbial accumulation, acidification at higher loading of lignin content that considerably decreases the biodegradability (Liew et al. 2011; Shi et al. 2013). Approaching pretreatment technique for lignocellulosic biomass supports the breaking of cell wall and access to cell wall matrix during digestion (Song et al. 2014).

The different pretreatment alters the physical structure and chemical composition of lignocellulosic biomass and improves the hydrolysis rate. Various pretreatment are physical, chemical biological method or the combinations were commonly adopted for lignocellulosic biomass. Researchers focused on pretreated waste biomass as feedstock, digested anaerobically for improved methane potential (Sambusiti et al. 2015; Shu et al. 2015). Liu et al. (2012) investigated the effects of thermal pretreatment on the physical and chemical properties of municipal biomass waste (MBW), kitchen waste (KW),vegetable fruit residue (VFR) and waste activated sludge (WAS) on methane potential. The results shows WAS achieved 35% methane potential increase and doubled the rate of methane production with thermal pretreatment. It has been noted by Gu et al. (2015), Calcium hydroxide pretreatment effectively remove lignin and increases the fermentable sugars from pretreated rice straw (RS). The highest biogas production with 8% and 10% calcium hydroxide pretreated RS achieved 565 mL/g VS and 575 mL/g VS respectively.

Hence, it is a mandate to explore additional energy from natural biomass and convert it to various bioenergy resources, either by the application of pretreatment of biomass or implementing co-digestion of organic substrates to satisfy the current requirement of bioenergy in modern India. This thesis deals with the treatment of organic wastes for the biogas production by biological means at a laboratory scale.

1.2 Framework of the thesis

Thesis is organized into five chapters, which include Introduction, Literature Review, Materials and Methodology, Results and Discussion, Summary and Conclusion.

Chapter 1: Introduces the solid waste management, different treatment methods for handling the solid waste and their importance in bioenergy production.

Chapter 2: Details discussion on anaerobic digestion technology and various literature reviews on pretreatment, co-digestion techniques that are adopted in biogas production by various modes of treating the organic waste.

Chapter 3: Materials used for anaerobic digestion, biomass characteristics, experimental plan and methodology section is focuses on laboratory experiments by different modes of operation for biogas production.

Chapter 4: Results and discussions correlated for the process parameters, performance of biogas production by batch and semi-continuous digestion.

Chapter 5: Outlines the summary and conclusion of this present study and the scope for future study on solid waste management.

CHAPTER 2

LITERATURE REVIEW

2.1 General

This chapter focuses on brief overview about the fundamental aspects of anaerobic digestion, physical and chemical pretreatments for lignocellulosic biomass, co-digestion of heterogeneous waste as feedstock, and its operational parameters for biogas potential. Comparative evaluations of different types of digestions and its detailed outline about the earlier investigations on pretreatments, co-digestions of feedstock are discussed. Finally, research gaps identification and the objectives are presented based on the review of earlier investigations subsequently.

2.2 Fundamental aspects of anaerobic digestion

Anaerobic digestion is the biological process carried out in the absence of oxygen for the stabilization of organic wastes, which is converted to biogas and inorganic end products such as ammonia carbon dioxide etc. Anaerobic digestion is a unique ecosystem in which diverse groups of microorganisms catalyze the conversion of complex organic compounds into methane and carbon dioxide in a controlled condition. It involves into four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Figure 2.1 shows the biochemical pathway of anaerobic digestion (Bajpai 2017). This four-step process occurs through hydrolytic, acidogenic, acetogenic and methanogenic micro-organisms responsible for biogas production (Manyi-loh et al. 2013). In step one, the disintegration of organic matter, converts the complex organic compounds to simple compounds. The complex organic compounds (proteins, polysaccharides, lipids, and cellulose) are converted into simple soluble compounds like amino acid, sugar, fatty acid, and glycerol. In second stage, fermentative bacteria degrades the soluble compounds, results in the formation of mixed organic acid such as short-chain fatty acids, alcohol and other products. In stage three, these organic acids are converted into acetic acid and hydrogen during acetogenesis. Finally, methanogenesis produces methane and carbon dioxide at the final stage of digestion.

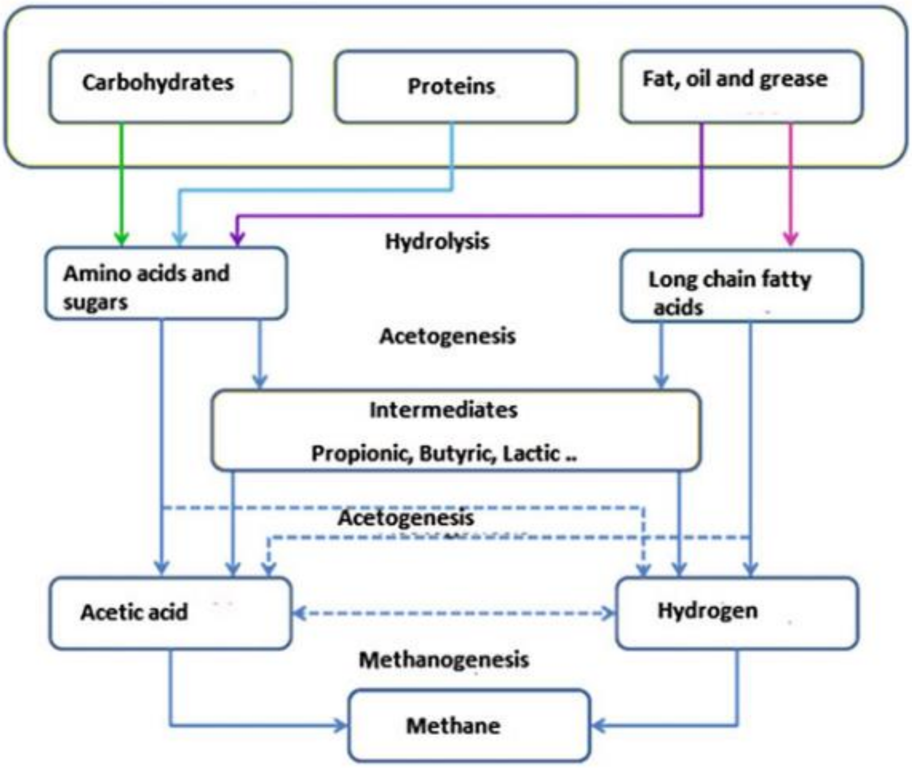


Figure 2.1 Pathway of anaerobic digestion (Bajpai 2017)

Anaerobic degradation of organic matter is a complicated microbial process consist of several independent consecutive and parallel reactions. The generalized equation for biochemical reactions for organic matter that is converted to methane and carbon dioxide is given as,

$$\text{Organic matter} \rightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S$$

<u>Stage 1</u> **Hydrolysis**

The hydrolysis is an extracellular process breakdown of complex organic matters (carbohydrates, proteins, lipids) into simple soluble compounds such as monosaccharides, amino acids and long chain fatty acids. The digestion of complex organic polymers directly by fermentative microorganisms occurs inside the cells wall, which is directly converted, to methane and carbon dioxide.

$$\text{Organic matter} + 2H_2O \rightarrow C_6H_{12}O_6 + H_2$$

Stage 2 **Acidogenesis**

The organic compound is converted to soluble intermediates (sugars, amino acids and volatile fatty acids) that are utilized by acidogenic microorganisms, further converted to carbon dioxide, water and hydrogen known as acidogenesis. The intermediate compound i.e. volatile fatty acids are produced in a faster rate which replicates the microorganisms occurs within 30 minutes duration.

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH \text{ (Acetic)} + 2CO_2+2H_2$$

$$C_6H_{12}O_6+ 2H_2 \leftrightarrow 2CH_3CH_2COOH \text{ (Propionic)} + 2H_2O$$

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2CH_2COOH \text{ (Butyric)} + 2H_2O + CO_2$$

Stage 3 **Acetogenesis**

Microorganism utilizes the volatile fatty acids namely acetic acid, propionic acid, butyric acid, and valeric acid for their survival during acetogenesis stage. Acetogenic and hydrogen utilizing microorganisms converts the metabolic products produced from 1st stage into acetate and hydrogen, which refers to a cyclic process. This process is takes place between the time span of 1.5 to 5-days. The thermodynamic reaction occurred with the group of microorganisms and their metabolic growth rate depends with carbon and energy sources.

$$CH_3CH_2COOH+ 2H_2O \leftrightarrow CH_3COOH+CO_2+3H_2$$

Stage 4 **Methanogenesis**

In last stage, acetate, hydrogen and carbon dioxide are converted into methane during methanogenesis (Smith and Almquist 2014). The process of methane production is classified into two types, which are aceticlastic methanogenesis and hydrogenotropic methanogenesis. Almost 60% of aceticlastic microorganism contributes to methane and 30% of hydrogenotropic microorganisms are responsible to convert as hydrogen and carbon dioxide. Further, those hydrogen and carbon dioxide are converted to methane. This process occurs between two to four days of digestion.

Aceticlastic Methanogens:

$$\text{Acetate}+ H_2 \rightarrow CO_2 + CH_4$$

$$2CH_3CH_2COOH + CO_2 \leftrightarrow 2CH_3COOH + CH_4$$

$$CH_3COOH + CO_2 \leftrightarrow CH_4 + 2CO_2$$

Around 60 to 70 percent of methane is produced from acetoclastic methanogens and other microorganisms grow slowly in three days at 35°C and are mostly sensitive in the ecosystem. Furthermore, sensitiveness of microorganism is prone to the inhibitors with organic acids. During the methanogenic phase, organic acids are produced from fermentation step in which acetate and H_2/CO_2 is further converts to methane and carbon dioxide by acetoclastic methanogens. The hydrogenotropic methanogens utilizes hydrogen reduces to CO_2 and further converts to methane.

Hydrogenotrophic Methanogens:

$$CH_3OH + H_2 \leftrightarrow CH_4 + H_2O$$

$$CO_2 + 4H_2 \leftrightarrow 2H_2O + CH_4$$

The hydrogenotropic methanogens produces methane from hydrogen and carbon dioxide grows faster than acetoclastic methanogens. There is a synergistic relation between hydrogen producers. At partial pressures of hydrogen, hydrogenation is more thermodynamically favourable than acetate producers thereby increase. The optimal pH is 7 for methanogens and acetoclastic methanogens is generally a rate limiting step. The growth rate improves the activity of microbial population causing the variation in process efficiency.

2.3 Process parameters on AD

Anaerobic digestion processes is the complicated process depending on the synergetic interaction between groups of microorganisms. Anaerobic processes depend on various environmental factors such as biodegradability, temperature, pH, buffering capacity, reactor designs etc. and operational factors such as feeding strategies, mixing, hydraulic and solid retention time and so on.

2.3.1 Biodegradability of substrates

The characteristics of substrate determine the efficient operation of digestion processes i.e. high biogas potential, biodegradability. In municipal solid waste, substrate characteristics may vary because of seasonal variation, collection methods etc.

Biodegradability and biogas potential of solid waste depends on elemental compositions and its main components such as lipids, proteins, carbohydrates, cellulose, hemicellulose and lignin content. Biodegradability are classified into two categories i.e. rate and ultimate degradation of substrate, which is based on its characteristics. Rate explains about the substrate utilization at steady state condition, which yields product recovery during fermentation and acetogenesis process of digestion. The ultimate degradation signifies the maximum biological process rate at which solid retention time equivalent to infinity. In a batch operation, ultimate degradation is equivalent to zero, which refers to stable digestion performance. Also, biodegradability of feedstock is determined by physico-chemical and biological characteristics interacting with each other. This process is limited with the complex organic compounds interacting with physico-chemical characteristics during enzymatic hydrolysis of biodegradation. Further, the possible inhibition or toxicity occurs during the second stage i.e. fermentation, which results in an imbalance between reactant and product leads to accumulation of volatile fatty acids as an end products. The system exhibits steady and optimal environmental and biological condition with the conditions such as (i) suitable microbial community, extracellular enzymatic assay (ii) toxicity assay and (iii) favourable operating parameters.

2.3.2 pH and alkalinity

The pH value is an important indicator for determining the performance and stability of anaerobic digester. All products of a metabolic stage are continuously breaking down and converted into next products without any interpretation of intermediary compounds such as volatile fatty acids, which causes a pH drop. pH is the reliable indicator to predict the process imbalance occurs between reactant and products. A pH is the amount of dissociated H^+ and OH^- ion that penetrates into the cellular membrane i.e. hydrogen ion in the system. The pH influences the function of extracellular enzymes, which influences the hydrolysis rate. The efficient transformation of organic matter achieved in the neutral pH during digestion process. The optimum pH value required for hydrolysis is around 5.5 and 6.5 is for acidogenesis respectively (Kim and Lee 2002). The optimum pH for methane-producing micro-organisms is in the pH range of 6.6 to 7.4, even though stable methane production occurs between 6.0 and 8.0 (Ward et al. 2008). From hydrolysis to methanogenesis requires different pH level is maintained between 6 to 8 during digestion (Cioabla et al. 2012). The pH value represents the role of acid/ base reactions in the system. The process is stable, acid-producing bacteria are less sensitive than methanogenic organisms when the pH at 4.5. The anaerobic digester operated below the pH at 4.5 or above 8.0 causes significant effects on methane production rate (Stamatelatou and Antonopoulou 2011). Methanogens are prone to sensitiveness to acids and possible deviations in their growth occur during digestion. Anaerobic transformation of organic matter achieved efficiently in the neutral pH. Additionally, pH variation causes adverse effects on product recovery, which depends on many factors and the type of microorganism in the process.

Alkalinity expresses the buffering capability of anaerobic system in the well-balance system. The alkalinity or buffering capacity of the system is the ability of the solution to resist massive changes in pH as acids and stabilize the system with optimum pH, which favour digestion. pH and alkalinity are the reliable indicators for predicting the process imbalance, which even occurs in the highly buffered system. Two factors are important are related to each other, that provides a suitable condition during anaerobic process. Alkalinity can be adjusted by using several chemicals such as bicarbonates of sodium and potassium, carbonates and hydroxides of calcium and so on. The pH value is slowly adjusted by adding these selected chemical to prevent the adverse impacts on the microorganisms during the process. Mostly methanogenic microorganism requires bicarbonate alkalinity, which provides desirable solubility and minimal adverse impacts (Gerardi 2003).

2.3.3 Temperature

Temperature is the most prominent factor to operate anaerobic digesters. In general, the rate of biochemical reactions is faster at high temperature. The reaction speed of biological processes depends on the faster growth rate of microorganisms and temperature. AD occurs in the wide range of temperatures from 5°C to 65°C based on microbial activity. Temperature profiles are classified as psychrophilic (below 20°C), mesophilic (between 25°C to 45°C), preferably 35°C and thermophilic (between 45°C to 65°C). Temperature has many effects on the process of biodegradation. Conventionally, AD systems are designed to operate in either mesophilic or thermophilic conditions. As the temperature increases, biochemical reaction take place at faster rate until the structure of cellular component that helps in protein, lipids etc. interprets the modification in cell wall. Also, it induces the partial pressure of hydrogen, growth rate and metabolic activity in the digester.

Psychrophilic digestion is preferable when biogas is used for the heating purpose. Recently, mesophilic is popular, however; thermophilic conditions are applied in large-scale centralized biogas systems (Vindis 2009). Sometimes, solids loading in the digester produces faster hydrolysis rate, produces volatile acids and thus reduces the system stability during thermophilic digestion. Biogas production and solid destruction should occur simultaneously improves the digestability performance in a faster rate with higher loading at thermophilic condition. Meanwhile, the digestion performance is improves by changing the temperature from 55°C to 35°C for obtaining higher biogas yield and for better system stability.

Recent technology employs a different temperature profile for the multi-stage processes (Ariunbaatar et al. 2014). From overall suggestion for temperature, the stable operating temperature is mandatory for digestion under anaerobic condition, in which temperature variation is susceptible to biogas production. Thermophilic digestion requires less retention time compared to the mesophilic condition, which is owing to the catalytic activity of thermophiles during pilot scale study. Thermophiles provides added benefits in terms of low contamination in pilot scale (Böske et al. 2015).

2.3.4 Solids content of the substrates

Solids content is predominant parameters for biological process, which is classified as total and volatile solids. It is further sub-categorized as low solids, medium solids and high solids respectively. AD consist of low solids contains less than 10% total solids (TS); medium solids is about 15% to 20% TS and high solids ranges from 22% to 40%. Total solids content represents the mass percentage of dry solids present in the organic matter/sample. Solids determination is the process in which heat transfer between the surrounding environment and solid surface causes moisture to evaporate. Water trapped in the solids microstructure migrates to the surface and evaporates due to the temperature gradient within the solids. Volatile solid is the percentage of solids that are volatile in nature. An increase in total solids decreases the reactor volume simultaneously during the process of digestion. Solids content is directly relates with the performance of digestion.

Determing the solids content is cost-effective, easy and rapid for the samples, which are organic in nature. If samples containing inorganic salts (presence of bicarbonates) leads to loss of weight due to the release of carbon dioxide when exposed to high temperature around 100°C. If municipal solid waste is used as a substrate, shows the loss in volatile organic compounds such as fatty acid and alcohols during the fermentation process (Li et al. 2011). Total solids (TS) remains constant, which is used to standardize the other operational parameters namely volatile solids, biogas and methane yield from the substrates. It has been reported by various researchers that total and solids is determined by oven drying method and the duration of drying varies until to obtain its constant weight (Shi et al. 2014; Teater et al. 2011). Similarly, for volatile acid are determined by heating at 550°C and varies with the duration of heating by researchers (Motte et al. 2013; Smith and Almquist 2014).

2.3.5 Volatile fatty acids

Volatile fatty acids (VFA) are defined as C_2-C_7 monocarboxylic aliphatic acids. VFAs are important intermediates in the metabolic pathway of anaerobic digestion. The deterioration occurs with VFA build-up, which is produced by acidogenic, and acetogenic bacteria reflect on acid producers as well as consumers. The VFA concentrations were high, causing lowering of pH in the system.

Possibly, VFA present at higher concentration provides microbial stress and decreases the pH, which can leads to the failure of the digestion process. The intermediate compounds were acetic, propionic, butyric, valeric and i-form of butyric and valeric acid, hexanoic acid. Among these, acetic and butyric acids are the major compounds produced during digestion, which indicate the performance of digestion process. The concentration of individual volatile acid especially acetic acid, propionic acid and butyric acid are the best control parameters. Total volatile acids can be measured by titrimetric method and the VFA composition measured by gas chromatography method (Buyukkamaci and Filibeli 2004). The propionic acid to acetic acid ratio is an indicator to assess the process imbalance in the digester. If propionic acid to acetic acid ratio is greater than 1.4 shows the imbalance in the process of digestion. Likewise, the concentration of volatile acids has an effect on methanogenic activity. Wang et al. (2009) reported that, acetic acid and butyric acid concentration at 2400 and 1800 mg/L has a sign of null inhibition in methanogenic activity. Whereas propionic acid produce 900 mg/L show the positive sign of inhibition on methanogens (Franke-Whittle et al. 2014).

2.3.6 Organic loading rate

Organic loading rate (OLR) is defined as the capacity of digestion system to convert the biological component or represent the feed of organic materials expressed in terms of volatile solids or chemical oxygen demand to the volume expressed as m^3 of bio-digester per day (kg VS/m^3.d). The biogas production depends on the loading rate in the digester (Ahn et al. 2010). The biogas production decreases and inversely proportional to the loading condition, possible accumulation of inhibitory substance during digestion. Loading rates is the important parameter for the continuous systems, possibly failure occurs with overloading the system. Nartker et al. (2014) optimizes the loading rate from 25 to 60% for glycerol at mesophilic condition yields 82 to 280% improved specific biogas production. Liu et al. (2017) reported the food waste used as feedstock and revealed the desirable organic loading rate yields highest biogas production at thermophilic condition. So, it is necessary to monitor the reactor performance at steady state condition on which the loading rates leads to failure.

2.3.7 Mixing

Mixing is an important concern about the anaerobic digester based on the following categories: Continuous interaction between substrate and microbial population; (2) prevents the formation of surface layers as well as sedimentation in the system; (3) eliminating thermal stratification and sustaining uniform temperature in the entire digester; (4) prevents the formation of dead spots, which reduces effective volume of digester. The overall purpose of mixing the digester is to maintain the uniformity in the digestion process, which is accomplished by manual and mechanical system (Stroot Peter G. et al. 2001). The mixing allows it to blend the fresh materials (substrate) with the digestate contain microbes. Slow mixing is preferable for the digestion that depends on reactor type and solids content present in the digester. Inadequate mixing has a negative impact on the performance of the digester and its periodic tested provides efficient performance. Lerdrattranataywee and Kaosol (2015) reports the effects of mixing on multiple substrates in a continuous system with a mixing time of 12 and 24 hours per day produces significant methane as 441 mL/day, which confirms as the best performance.

2.3.8 Solids and hydraulic retention time

The most important factor for the anaerobic digester is that microorganisms are allowed to give sufficient time to reproduce and metabolize volatile solids. The solids retention time is the average time that the solids held in the digester. It can be operationally explained as follows: 'SRT in days is equivalent to the mass of the solids in the digester (kg) divided by the mass of solids withdrawn per day (kg/day)'. Hydraulic retention time (HRT) refers to the average time required for the organic matter digested during biodegradation. HRT is calculated based on feeding rate or removal rate for the system if considering "without recycle". For such systems, SRT and HRT are equal. The reactions in the anaerobic system are directly related to SRT (or HRT). The extent of reactions is increases with the increase in SRT, similarly for the decreased extent of reactions. SRT is the important variable; it controls directly the major steps of digestion processes, which affects the time to metabolize the volatile solids. The microbial activity occurs in a minimum SRT for each reaction, which controls the digester performance. Commonly, Retention time required between 15 to 40 days for the mesophilic which is lesser for a thermophilic condition around 15 days (Kim et al. 2006). The degradation rate is directly proportional to retention time. The higher degradation rate occurs with lower retention time (Liu et al. 2016). The performance of activated sludge is analyzed with different retention time and loading rate yields least biogas with respect to the solids and hydraulic retention time increases from 8 to 35 days (Bolzonella et al. 2005). Hashimoto and Hruska (1982) studied the effects of HRT on methane production for cattle waste at mesophilic and thermophilic temperatures in which results yields highest methane achieved as 6.11 m^3 CH_4/d for four days HRT at thermophilic (55°C) conditions.

2.3.9 Carbon to nitrogen ratio

The carbon to nitrogen ratio is the relationship between the carbon and nitrogen content of organic materials. The C/N ratio balance is optimum for the methanogens which is continuously producing biogas in a stabilize condition. The low C/N ratio produces ammonia and increases the pH content that is accumulating in the system causes toxic to methanogenic bacteria. The C/N ratio for the higher biogas yield is in the range 20-30 (optimum is 25) (Weiland 2001). Feedstock consists of carbon sources, which support the different groups of microbes during anaerobic digestion. The optimum C/N ratio were selected based on the type of feedstock and varies with the feedstock characteristics. Nitrogen is the essential source for the protein synthesis and used in the form of inorganic nutrient required for the microbial survival during anaerobic digestion. The nitrogen available in the form of nitrate and nitrite, converted to nitrogen gas. The presence of nitrogen in the organic waste is mostly proteins, which convert to ammonium during the process of digestion. Nitrogen presents in an ammonium form, stabilizes the pH when digestion takes place. The higher concentration of ammonia produces inhibition that affects the biological process. It inhibits methanogens at a 100mM concentration approximately (Khalid et al. 2011). Minor changes in ammonia nitrogen have least effect on total biogas production while at higher changes inhibited the biogas around 50% respectively. Methanogenic activity decreased by 10% with ammonia concentration 3750 mg NH_4-N/L while 50% inhibited with 5500 mg NH_4-N/L and around zero with 6000 mg NH_4-N/L respectively (Rajagopal et al. 2013). Zeshan et al. (2012) reports the C/N ratio at 30 for biodegradable feedstock has 30% less inhibition compared to C/N ratio at 27 respectively.

2.3.10 Substrate composites

The organic fractions consist of carbohydrates, proteins, lipids and inorganic fractions present in substrate composites. Depends on the degree of complexity and the structural identity, it may varies from simple to complex organic forms (Sluiter et al. 2011).

2.3.10.1 Carbohydrates

A carbohydrate is an important parameter for the organic waste conversion to produce biogas. Carbohydrates comprise of an assorted group of molecules available readily in the degradable sugars and lignin bind biomaterials. Among these factors, biodegradable characteristics are classified based on glycosidic linkage, intermolecular attraction, the linkage between size, linkage and the affinity of molecules in the medium and so on (Monlau et al. 2013). Carbohydrates are classified into two groups: structural and non-structural compounds. Structural compound is witnessed in lignocellulosic materials and non-structural compound are rich in starch.

Lignocellulosic materials are the most common energy crop utilized for biogas production along with the application of animal manure, usage of energy crops (switchgrass) or crop residues (Corn Stover) to convert as bioenergy. Lignocellulosic matrix mainly composed of three polymers i.e. cellulose, hemicellulose and lignin which are strongly inter-related and chemically bonded by non-covalent forces and covalent cross-linkages (Perez et al. 2002). Mostly, cellulose and hemicellulose are made up of 65-75% lignocellulosic structure, while the other contains lignin as 25-35%. In addition to, the cell wall matrix contains biopolymers, proteins prove the additional complexity and provides a barrier to degradation (Klinke et al. 2002).

Cellulose is a complex polymer insoluble in water consists of D-glucose linkages. All the structural celluloses are combined in some degree to make bondage with lignin and hemicellulose in a cell wall structure (Chanakya, H N, Ramachandra and Vijayachamundeeswari 2006). The cellulose degradability mainly depends upon the degree of delignification. Cellulose molecules are strongly connected by inter- and intramolecular hydrogen bonding and the van der Waals forces result in micro-fibrils formation. The presence of micro-fibrils scatters by disorder in the crystalline domain and the amorphous region which is digested by enzymes in crystalline compound (Mosier et al. 2005).

Hemicellulose is a complex structural carbohydrate composed of different polymers, named as pentose, hexose and other forms of sugars. The xylan is present as a prevailing compound as it arrives from agricultural plankton (Kumar et al. 2009b). It has low molecular weight than cellulose and short lateral chains in linkages consist of various sugars with hydrolysable polymers (Hendriks and Zeeman 2009). Micro-fibrils occupied in-between cellulose and hemicellulose degrades effectively by the cellulolytic bacteria.

Lignin consists of a highly branched polymer, a hydrophobic poly-phenolic aromatic compound present in the cell wall matrix (Harmsen et al. 2013). Lignin acts as a protective barrier to the cell wall that provides resistance to biodegradation. It establishes a significant limiting factor to lignocellulosic biodegradability with the conventional digestion system. Lignin has a tight bond which is associated with hemicellulose that shields the cellulose and produces a physical barrier for hydrolytic enzymes (Lu et al. 2011). Hemicellulose biodegradation directly related to cellulose and inversely proportional to lignification. Upto date, few studies focused on the biodegradability of lignocellulosic biomass which consist of highest lignin value (Kumar P, Barrett D M 2009). Lignin provides recalcitrant to the biomass in the anaerobic environment especially for rumen micro-organisms (Castillo-Gonzalez et al. 2014).

Starch is a biopolymers, which consist of amylase and amylopectin Starch is the non-structural carbohydrates primarily consist of partially polysaccharides dissolved in water and used for the energy storage. And some starch is insoluble and protective barrier to degradation which remains bioavailable (Ghimire et al. 2015). Starch is present in most of the organic wastes such as municipal and food wastes, primarily from grains named as corn, wheat, and tapioca (Alibardi and Cossu 2016).

2.3.10.2 Protein

Proteins composed of amino- acids covalently linked with a peptide bond formed from a group of amino acids. The chemical structure is one of the key factors determining the hydrolysis and its biodegradability. Proteins made up of chemical structure based on two groups: fibrous and globular proteins. Fibrous proteins are made with polypeptide chains arranged in sheets, while globular protein with polypeptide chain folded in a spherical shape (Sant'Anna and Souza 2012). Some of the fibrous proteins are keratin, collagen insoluble and tough to hydrolyze (Boe et al. 2010). Globular proteins are namely casein, albumin that is readily hydrolysable and soluble in nature. Extracellular enzymes converted into polypeptides and amino acids hydrolyze proteins. The carbohydrate fermenting bacteria degrades protein and amino acids prone to provide sufficient energy for the microbial survival. Proteolytic bacteria resolve protein degradation and the processes in anaerobic digesters to yield energy. Proteins are present in most of the food processing wastes and dead biomass subjected to which is subjected to complex substrate digestion.

2.3.10.3 Lipids

Lipids consist of the mixture of fat, oil, grease accounted for 30% volatile solids in waste sludge, around 50% COD reduction, and biomethane production possibly occurs from lipid degradation. Simple lipid forms are triglycerides, or neutral lipids which composed of fatty acids in ester linkage with glycerol (Pena et al. 2012). Triglycerides contribute to solids from the food processing industry and comprise 65% from meat waste (Broughton et al. 2010). The lipid structure varies by its chain length, the degree of the bond, physical state (solid and liquid). Hydrolysis depends upon chemical characteristics as well as the specific surface area (Hult and Holmquist 1997). The long chain fatty acids and double bond slower the degradation rate due to the lower solubility in water and higher melting point (Sang et al. 2013). The fat rich foods are degraded during anaerobic digestion that affects the floating properties of the lipids. In addition, low surface area to volume ratio in the mixture improves the biological degradation. Inhibitions are also possible with the presence of long chain fatty acids (LCFA) accumulation during digestion (Lalman and Bagley 2004). Hydrolysis of triglycerides converts to glycerol and LCFA by ester groups of lipid. Glycerol is again fermented into different compounds named as VFA, alcohols and formic acid respectively. LCFA is degrades through β-oxidation and hydrogen-producing acetogenic bacteria to produce acetate and hydrogen (Jenkins et al. 2008), further, methanogens consume these acetate and hydrogen that converts to produce biomethane as end products.

2.4 Process outcome

Process outcome is estimated based on the products recovery in the form of biogas composition from organic waste. Biogas composed of methane (50% to 75%), carbon dioxide (25% to 50%), nitrogen (0% to 10%) and traces of other gases are the major products from the conversion of organic wastes. Table 2.1 shows the biogas yield and methane yield obtained from different solid organic wastes. The biogas yield is affected by many factors includes the composition and type of feedstock, microbial composition, temperature, moisture, bioreactor design. For instance, Bouallagui et al. (2009) found that biodegradability of MSW decrease with moisture content beyond 70% especially for fruit and vegetable waste due to the rapid acidification at lower pH in the bioreactor. Volatile fatty acid production inhibits the methanogenic bacterial activity, instead co-substrate addition enhance biogas production by up to 50% respectively.

Table 2.1 Biogas yield from solid organic waste

Feedstock	Biogas yield (L/kg VS)	Methane yield (L/kg VS)	Reference
Food waste	762	396	(Zhang et al. 2011)
Lignin-rich organic waste	-	200	(Jayasinghe et al. 2011)
Household waste	350	228	(Ferrer et al. 2011)
Maize silage & straw	579	312	(Mumme et al. 2010)
Rice straw	350	290	(Lei et al. 2010)
Fruit & vegetable wastes	850	530	(Forster-Carneiro et al. 2007)
Municipal solid waste	585	380	(Liu et al. 2002)

2.5 Different types of anaerobic digestion

Different types of digestion system used for the bioenergy recovery from solid wastes. They include batch, and continuous reactor, one stage and two stage reactors, solid-state digestion system. The process configuration is important for efficiency of methane production process. In batch digestion, waste is feed into the system and all the degradation steps are allowed to follow consequently. One stage digestion is the traditional process, all the reactions simultaneously takes place in single digester. All the polymeric compounds such as carbohydrates, protein and fat are converted to methane, ammonia and other gaseous products. Multistage digestion (two- stage) uses two different digesters for different phases between acidogenesis and methanogenesis. Both the digesters can utilize the process by batch or semi-continuous digestion. It can be further sub-classified as low and high rate digestion system based on organic loading rates. The presence of solids in the digester relatively had an impact on digester volume and their process. Low solids content consists of solids contain less than 10% and huge volume of water required for digestion, which depends on reactor volume and post-treatment technologies. During 1980's, low solids are used for anaerobic digestion and later trend changed to use high solids feedstock for digestion to improve the performance of digestion (Abbasi et al. 2012). In addition to multistage digestion, solid state anaerobic digestion has several advantages over liquid anaerobic digestion in terms of reactor volume configurations, feedstock quality and water requirements.

2.5.1 Single- and multi-stage digestion process

In the single stage process, both acidogenic and methanogenic phase occurred in the single reactor, depending on low or high solids respectively. Currently the trend shifted to wet or dry systems with prior importance to dry systems over the wet digestion systems. Dry systems uses the solids ranges 20-40% comparatively lesser than for wet digestion systems (Kothari et al. 2014).

In single stage process, single digester is required for hydrolysis, acetogenesis and methanogenesis reactions however in the multi-stage system require separate digester units for different stage to improve the digestion performance. In multistage anaerobic digestion process, two separate reactors are used. The multistage process undergoes different phases: hydrolysis and acidogenesis in one digester and other digesters phased with acetogenesis and methanogenesis. The hydrolysis and acidification occur in the first reactor whereas hydrolysis rate is limited with the presence of organic compounds such as carbohydrates and further optimized to methanogenesis. The methanogenic microorganisms prefer pH between the range of 6.5 to 7.5. While acidogenic microorganisms required low pH less than 5and organic acids diluted in this stage. Consequently, optimum condition is each phases and overall reaction rate is well maintained in the system. Mainly in multistage systems, intermediate reactions, reactor configuration, operating parameters are possible to maintain separately or in combinations. It has been reported that efficiency of operational processes depends on the digester configurations. Ariunbaatar et al. (2015) compared the efficiency of methane in a single-stage and two-stage system operating in a continuously stirred tank reactor, which shows the inhibitions with two-stage reactor compared with single-stage reactors. Luo et al. (2011) compared the single and two-stage digestion processes revealed the stable performance occurred at two-stage process when increasing the organic loading rate compared to single-stage digestion.

2.5.2 Batch and continuous digestion

The batch digestions are simple and strong in nature, has many advantages such as less maintenance, minimum energy loss, and less capital cost. However, less loading rate and waste accumulation at the bottom of the digester reduce the biogas yield and develop risk during unloading. Typically, total solids of feedstock is about 20 to 40% present in the batch system (Kothari et al. 2014). In a batch system, the fresh feedstock and inoculum is used for every batch digestions. Batch digestions are classified based on digester such as single stage, sequential and upflow anaerobic sludge reactors. Single stage batch digesters are operated in mesophilic and thermophilic conditions. Liu et al. (2017) studied the effect of food waste under mesophilic and thermophilic conditions showed the biogas yield of 33-49% highest in thermophilic than the mesophilic condition.

Continuous digestion is the continuous flow reaction occurs in the reactor by providing equilibrium conditions. All reactions occur in a steady state with consistent input to output balance between feedstock and biogas produced in the system. For instance, Microalgae used as a feedstock for anaerobic digestion to produce biomethane yields as 279 mL CH_4 gVS^{-1} for batch and 320 mL CH_4 g^{-1} VS in semi-continuous operation justifying the performance (Jard et al. 2012).

2.5.3 Solid state anaerobic digestion (SSAD)

Solid-state anaerobic digestion (SS-AD) is classified as the part of digestion systems used to convert various feedstocks into biogas. Based on solids content, digestion can be classified as wet (<15%), semi-dry (15-20%) and dry (20-40%) of total solids respectively. Wet digestion requires an equal or more considerable amount of water than biomass quantity. The presence of high water content in the form of slurry reduces the nutrient value per unit volume. In dry anaerobic digestion, this process has advantages with high productivity and waste management all over the world. The process is feasible for organic waste from industries to recover energy and reduces environmental pollution. The application of this process is limited with lack of treatment systems configuration and required a longer time for bio-stabilization of these wastes. Leachate production is less and easy to handle the digestate can be further used as a fertilizers. The dry digestion process is more advantageous than the wet digestion especially, in laboratory and pilot scale studies due to reasonable cost and potential by-products.

SS-AD is the leading treatment for the handling the municipal solid waste (MSW) and typically operates at 15–50% total solids (TS) content. SS-AD provides many benefits over liquid state AD in processing the feedstock such as treating higher organic solids in the single scale digester; producing manure for unindustrialized land as fertilizer and so on (Li et al., 2011). The start-up period of an SS-AD system is the critical phase in the batch digestion process, which requires longer time. The SS-AD of waste digestion is improved by adding one or more substrate, i.e., co-digestion that depends on the characteristics of the waste biomass and process efficiency. The performance of SS-AD is evaluated by its operational characteristics such as carbon to nitrogen ratio, food to inoculum ratio as an initiative. The purpose of co-digestion mainly focuses to maintain the balanced nutrients, C/N ratio and (macro and micronutrients) and dilute inhibitors/toxic compounds to improve methane production (Hartmann et al., 2004). Xu and Li (2012) found out F/E ratio at two achieved higher accumulative methane yields than at higher F/E ratios at 4 and 6 for the same dog food to corn stover ratio. Also, co-digestion yields better-quality methane compared with corn stover or dog food as the sole substrate, due to their enhancements in reactor characteristics. To maximize biogas production, the volumetric loading rate of food waste is also exploited by (Xu and Li 2012) . Increasing the volumetric loading of food waste can be proficient by (a) increasing the F/E ratio with a constant substrate composition (b) increasing the fraction of food waste keeping F/E ratio constant, or (c) both approaches.

2.6 Lignocellulosic biomass as a feedstock for AD

A wide variety of organic materials used as feedstock for anaerobic digestion. The feedstock is selected based on the availability in the regions. In addition, the characteristics of biomass are suitable for the digestion process. Recently, lignocellulosic biomass receives an attention to choose as the feedstock for AD.

Lignocellulosic biomass refers to herbaceous plant, hardwood, softwood and grasses which contains cellulose, hemicellulose and lignin followed by other minor components. Lignocellulosic biomass is the renewable feedstock with the annual production of 10^{10} MT worldwide (Kandasamy et al. 2017). Cellulose is the major structural compound provides strength and stability to plants in the cell wall matrix. Hemicellulose is the constituent of cell wall and polysaccharides of simple structure present in cell wall matrix. Lignin is the aromatic polymer produced through photosynthesis and acts as a protective layer for plants. Apart from cell wall components, water present in the complex form, protein, minerals and other components found as well. The significant variations in hemicellulose and cellulose depend on the nature of the plant. Figure 2.2 summarized the cell wall component (%) in lignocellulosic biomass as the common source of feedstock for digestion (Harmsen et al. 2010). Researchers focuses on cellulosic biofuels is a cost-effective and alternative energy source, which reduce the usage of fossil fuels, balance the energy between cellulosic biofuels and conventional fuels. While choosing the crop for methane production; the factors such as crop availability, maintenance, harvesting and conditioning are the key factors, which influence crop selection (Gupta et al. 2012).

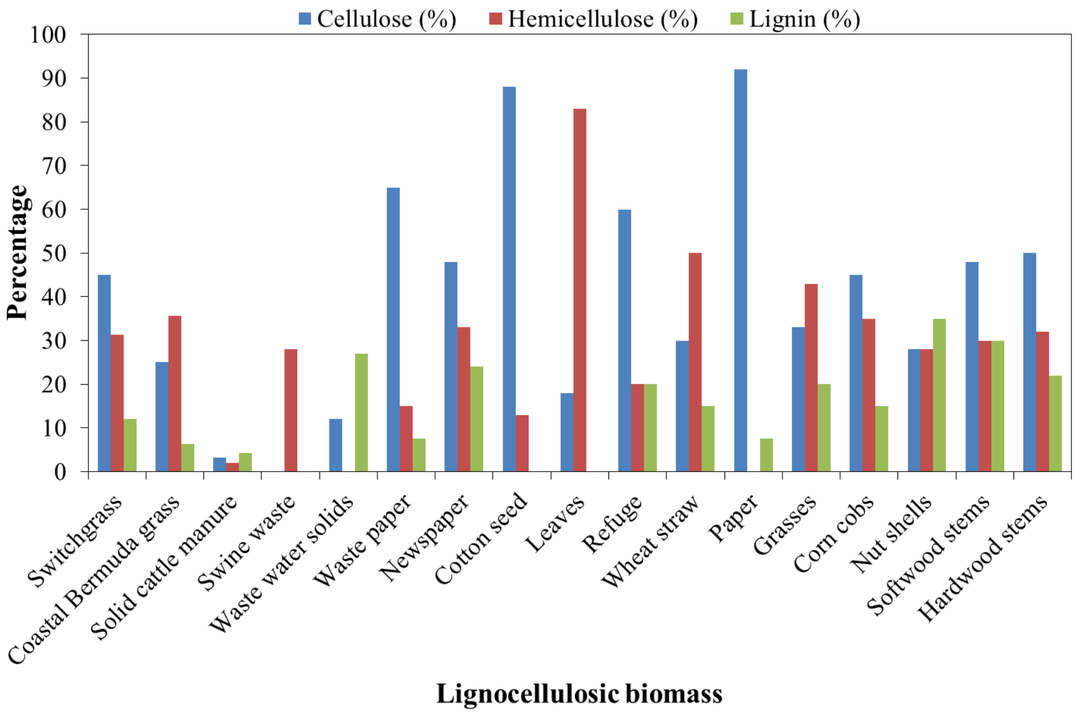


Figure 2.2 Cell wall component of lignocellulosic biomass

Cellulose, also named as β-1-4 glucan is a linear polysaccharide polymer made of cellobiose unit with two glucose molecules (Lo Niee Liew 2011). The chemical formula for cellulose is $(C_6H_{10}O_5)_n$ that depends on the degree of polymerization extends upto 17000 units (Harmsen et al. 2010). Cellulose structure is classified as crystalline and non-crystalline nature. The groups of polymer chains formed the micro-fibrils and unite to form fibres. Cellulose is the hygroscopic material, which absorbs around 10% water in normal condition.

Hemicellulose is made with xylose polysaccharides that is rich in sugars mostly present in hardwood and agricultural residues along with other sugar forms such as glucose, arabinose, galactose and so on. It is the branched chain polymer composed of carbon sugar monomers and extends up to six-carbon chains as glucose. Hemicellulose lacks in the crystalline structure with the branched chain and acetyl group connected to polymer chain is the important aspects in structure and composition. The degree of polymerization exist within 200 units and a minimum around 150 monomeric units (Beyler and Hirschler 2002). Hemicellulose is the water-insoluble component at low temperature rather soluble at elevated temperature and improves solubility with acid concentration.

Lignin is the complex molecules constructed with phenylpropane unit interlinked with cellulose and hemicellulose forms 3D structure. Mainly, four groups of bonds is identified in lignocellulosic biomass namely ether, ester, carbon-to-carbon bond, hydrogen bonds provide inter- and intra-linkage with polymers in the component.

2.7 Pretreatment of lignocellulosic biomass

Lignocellulosic biomass consists of cellulose, hemicellulose and lignin bound together in a matrix. Both cellulose and hemicellulose fractions are commonly known as holocellulose that consist of polymeric sugars. These polymeric sugars are convert to other value added products. Lignin requires vigorous treatment to break down the bondage between the complex groups. Lignin is the recalcitrant nature and slowly degradable component in cell wall. The presence of lignin is providing high resistance to biodegradation. Lignin is less biodegradable at anaerobic conditions, which limits the accessible to cross-linkage with hemicellulose and cellulose. The crystalline structure of cellulose prevents the microorganism disperse into the structure of cell wall. Hemicellulose structure is more random, amorphous and little resistance to hydrolysis when compared to cellulose (Perez et al. 2002). Even though, cellulose and hemicellulose are accessible by microorganisms, the biodegradable performance is lower due to the complex embedded structure. Lignocellulose is surrounds by lignin that shield the cellulose and hemicellulose from the microbial attack (Koch et al. 2010).

Owing to the recalcitrant nature of lignocellulosic biomass, pretreatment employs to simplify the accessibility of enzymatic hydrolysate to cellulose. The effective application of pretreatment results in breaking the barrier and prevents the enzymatic hydrolysis. The pretreatment effectiveness is represented by the increased surface area, lignin reduction and cellulose crystallinity. The fermentable sugar is produce by two approaches (a) hydrolysis of holocellulose (b) Enzymatic hydrolysis for cellulose conversion. Certain obstacle is notified with pretreatment of biomass such as cellulose hydrolysis, inhibitor formation, byproduct conversion and so on. Recent research focuses on lignocellulosic biomass converts them into easily accessible component in an eco-friendly approach. Different kinds of pretreatments are physical, chemical and biological pretreatments or the combinations of these. Selection of pretreatment is based on cost-effectiveness, operating conditions and digestion performances etc. Pretreatment requires sophisticated instruments, energy and operational process. Physical and thermochemical pretreatment requires energy to convert this biomass. The pretreatment is applied intensively to dissociate enzymatic hydrolysis and subsequently produces biogas from lignocellulosic biomass. The limited information are available on the selection of pretreatment and its effects on biomethane potential.

Various pretreatment methods are exist and adopted for biomass, which is physical, chemical and biological pretreatments. Physical and chemical treatment methods are predominant to enhance the degradation of complex organic materials at a faster rate compared with biological methods. Some of the treatment methods are liquid hot water, microwave, and thermal as physical pretreatment. Chemical pretreatment are dilute acids, alkali and other chemicals used for lignocellulosic biomass to improve the accessibility of cell wall component. The effectiveness of pretreatment are categorized based on following criteria: avoiding size reduction, stabilize hemicellulose fractions, control the formation of inhibitors, reducing energy input, and being profitable (Sun and Cheng 2002).

2.7.1 Physical pretreatment

Physical pretreatment is classified as grinding, milling, chipping applied to biomass to reduce the crystallinity in cellulose. The size of the material varies from 0.2 to 2 mm size for milling or grinding and 10-30 mm size for chipping. Milling is done by vibratory ball milling, ordinary ball milling for reducing the crystallinity that improves the digestibility. The power requirement depends on particle size and the characteristics of biomass. The various particle sizes is sorted out by milling and substrate accessibility improves the conversion rate up to 30% compared to coarse milling (Motte et al. 2014).

Pyrolysis is the promising approach for converting biomass to energy. This process is complex and depends on several factors such as biomass composition and heating rate. Pyrolysis decomposes the cellulose rapidly and converts to gaseous products. Lee and Fasina (2009) reported the switchgrass pyrolyzed at 280-370°C using a thermogravimetric analyzer, which quantify the volatilized particles at high temperature to decompose cell wall components converts to gaseous products.

2.7.2 Chemical pretreatment

Chemical pretreatment methods improves the biodegradability of cellulose by removing lignin and hemicellulose or by reducing the degree of polymerization (DP). Chemical pretreatment is classified as ozonolysis, hydrolysis by acid and alkali, oxidative delignification, organosolv processes and so on. Ozone treatment is the method to reduce the lignin content in lignocellulosic biomass, which increases the digestibility of treated materials, unlike the chemical treatments. Ozone used to degrade hemicellulose and lignin in lignocellulosic biomass such as corn stover, hay, pine, cotton straw, sawdust, and bagasse. Ozonation applies to organic waste improves the substrate solubilization and biogas potential from pretreatment (Cesaro and Belgiorno 2013).

Acid pretreatment is developed for the treatment of lignocellulosic material, which improves the enzymatic hydrolysis to release fermentable sugars. Commonly, concentrated acids such as sulphuric acid, hydrochloric acid are used to treat lignocellulosic biomass. The concentrated acid is a most powerful reagent for cellulose hydrolysis, even an acid produces toxic, corrosive, and hazardous to the component and makes the pretreatment process expensive. Sulphuric acid concentration at below 4% applies for most of the treatments, as it effective and inexpensive. Hemicellulose fractions is hydrolyzed with the presence of chemical namely acids and alkaline conditions.

Commercially, sulphuric acids are used for determining furfurals from lignocellulosic biomass. In addition, its application on biomass is to hydrolyze xylose and other sugars and continues to break down to form furfurals. Acid pretreatment is achieved at a high reaction rate and improves the cellulose hydrolysis. Dilute acid is effective to recover and remove the dissolved sugars from hemicellulose and glucose yield from cellulose. Thus, results increase the cell wall (lignin) removal up to 100% during hydrolysis. Cheng et al. (2013) reported acid pretreatment enhances the glucan to glucose conversion during hydrolysis. Recent studies on dilute acid pretreatment aided with temperature application, which favour the cellulose conversion during hydrolysis. The important approach to dilute acid along with temperature such as nitric acid; hydrochloric acid and phosphoric acid are used. Varieties of plant biomass such as corn stover, hardwood maple, birch, aspen, poplar and sweet gum is used and observe the crystallinity index. Consequently, the temperature has fewer impacts on biomass and express on the amorphous cellulose fractions (Kleawkla and Chuenkruth 2016).

Alkaline pretreatment is the approach used to pretreat lignocellulosic materials and depends on the quantity of lignin content. Alkaline pretreatment applied in the optimum temperature and pressure compared to other pretreatment technologies. Compared to acid pretreatment, the alkaline process cause less sugar degradation and many salts can be recovered, namely sodium, potassium, calcium etc. Most of the research studies focuses on pretreatment application to lignocellulosic biomass (Harmsen et al. 2010). However, calcium hydroxide is the effective pretreatment reagent and from the economic point of view. This pretreatment removes most of the amorphous substance such as lignin and hemicellulose, which increase the crystallinity index. Pretreatment is extensively applicable for (i) delignification and high crystallinity (ii) improves enzymatic hydrolysis (iii) crystallinity signifies the improved hydrolysis rate. Sambusiti et al. (2013) studied the effect of sodium hydroxide pretreatment at 4 and 10% at 55°C for 12 hours on different varieties of sweet sorghum observed the changes in cell wall component as cellulose (16-45%), hemicellulose (18-35%) and lignin (50-70%) respectively. The pretreatment of wheat straw and corn stover by oxidative treatment improves the cellulose and lignin content, remove the substantial amount of hemicellulose that improves the access towards the enzymatic hydrolysis (Kaparaju and Felby 2010).

Organosolv method is the promising treatment strategy and provide attention to demonstrate the potential utilization of lignocellulosic material (Toledano et al. 2014). The organic solvents are mixed with inorganic catalyst is used to break down the internal lignin and hemicellulose bonds. The solvents are ethanol, acetone, ethylene glycol used in the process which involve pre-hydrolysis and delignification supports by organic solvents (Kumar et al. 2009a). The cellulose present in biomass partially hydrolyzed into small fractions and remains insoluble in water. Hemicellulose hydrolyzes to soluble components such as oligosaccharides, monosaccharides and acetic acid. The acetic acid lowers the pH level and simulates the acid-catalyzed hydrolysis. The next polymer is the lignin hydrolyzed into low molecular weight fragments, which dissolve in aqueous ethanol (Kumar et al. 2009a). Organosolv pretreatment is applied to lignocellulosic materials namely soft and hardwood, (i.e. elm, pine, agricultural waste, rice straw). Pretreatment at 150°C and 180°C for 30 and 60-minute duration using 75% aqueous ethanol as the organic solvent which result in higher lignin removal in the range (4-40%) and fractions of hemicellulose is also removed as the part of pretreatment (Mirmohamadsadeghi et al. 2014).

2.7.3 Biological pretreatment

Different groups of microorganisms namely, white, brown, soft-rot fungi are commonly used to degrade the cellulose and hemicellulose in the waste materials. The biological treatment uses rot fungi, which are environment-friendly approach, use less energy for the component removal of lignin degradation. Brown rot fungi mainly attacks the cellulose and other fungi group which is used to degrade both cellulose and lignin with the action of lignin degrading enzymes, by regulating carbon and nitrogen sources. A Researcher focus on biological pretreatment applied to corn straw using P. *florida* enzymes over 60 days duration, the degradation is observed for cellulose (28% reduced to 15%), hemicellulose (45% reduced to 36.6%) and lignin (7.5% to 4.8%) with each component (Zhong et al. 2011). This indicates the considerable degradation occurs with hydrolytic microorganisms in a cell wall confirms the maximum product yield from the lignocellulosic biomass.

2.8 Application of pretreated lignocellulosic biomass

Pretreatment methods is investigated and tested to be more effective for methane production especially from lignocellulosic biomass includes wet oxidation, ultrasonication, and incubations as the physical pretreatment (Keshwani et al. 2009). Chemical reagents are acids, alkalis, solvents and some oxidants were used for pretreatment applications to lignocellulosic biomass. Biological methods such as enzymatic and microbiological pretreatments which is combined with heat and pressure is also recommends for lignocellulosic biomass (Raposo et al. 2011). Table 2.2 shows the different application of pretreatment for lignocellulosic materials as a feedstock for biogas production.

Biomass requires uniform size by grinding before biological conversion, which increases the surface area exposed to microbial attack. A Researcher focus on physical methods such as steam explosion, heat treatment show the methane yield at an increased rate from 7 to 20% (Gunaseelan 1997). Chemical pretreatment method that is alkaline treatment distinguishes to break the bond between lignin and hemicellulose, which swells the fibres, increase the size of the pore facilitating hydrolysis. Alkaline pretreatment enhance the methane yield from 4 to 69% (Lo Niee Liew 2011). Alkaline treatment appears to be more effective to break the ester bond between cellulose, hemicellulose and lignin, compared with acid or oxidative pretreatments (Čater et al. 2014). Alkaline pretreatment is pretend to alkali ions which include the reaction with a carbonyl group, neutralize acidic compounds from holocellulose and lignin degradation (Tandukar and Pavlostathis 2015). NaOH is the effective reagent to delignify the lignocellulosic biomass. Monlau et al. (2012) uses different chemical pretreatments for wheat straw to determine its effects on methane yield and the highest yield is obtained as 28% with alkali treated biomass. Even, switchgrass yields the highest by 16% more methane yield with above 55°C pretreatment conditions. The sodium hydroxide pretreatment has an effects on methane with enhancement in yield is studied by (Zheng et al. 2009b). Chemically pretreated corn stover at ambient temperature for 24 hours yield the biogas as 375 L/kg VS obtained with 5% NaOH (Zhu et al. 2015). Finally, the application of pretreatment to feedstock enhances the biogas production and reduces the solid content. In addition to pretreatment, energy requires to balance the pretreatment as well as biogas production.

Table 2.2 Pretreatment of lignocellulosic biomass and its application in anaerobic digestion

Feedstock	Pretreatment			AD system	Biogas production (L/kg VS)	References
	Method	Condition	Duration			
Switchgrass	NaOH (7g/l)	55°C-121°C	30 min to 3 hours	Batch, Mesophilic, 40 days	112 to 140	(Frigon et al. 2012b)
Corn Stover	NaOH (1-5%)	Ambient	Simultaneous alkaline and AD, batch	Mesophilic, 40 days & 22% TS	200 to301	(Zhu et al. 2010)
Corn Stover	NaOH (1-7.5%)	Ambient	24 hours	Batch, 40 days	260 to 375	(Y. Li, J. Zhu, C. Wan 2013)
Corn Stover	NaOH (2-6%)	Ambient	3 days	Batch, 75 days	130 to 215	(Zheng et al. 2009a)
Wheat straw	Steam explosion	160°C-200°C	10 to 20 min	Batch, mesophilic	296 to 331	(Bauer et al. 2009)
Wheat straw	NH_4OH	80°C	24 hours	Batch, thermophilic, 112 days	343 to 365	(Hashimoto 1986)
Hay	(i) $Ca(OH)_2$ - 10% (ii) $(NH_4)_2CO_3$ - 4g/l (iii) Maleic acid- 5.8 g/l	(iv) 85°C (v) 120°C (vi) 150°C	(i) 16 hours (ii) 2 hours (iii) 0.5 hours	Batch, mesophilic and 40 days	230 to 300	(Fernandes et al. 2009)

2.9 Summary of pretreatment on lignocellulosic biomass

Varieties of waste materials could be used as a feedstock for the biogas production by consider the application of pretreatment to lignocellulosic biomass. The choice of pretreatment depends on the biomass type and its composition, by-products recovery. The merits and de-merits of biomass pretreatment is discussed in Table 2.3. Some factors significantly affect the cost of pretreatment methods.

Researchers explore the effectiveness of pretreatment for various lignocellulosic materials with different pretreatments, which shows a substantial reduction in cellulose, hemicellulose and lignin content. Chemical pretreatment shows the significant lignin reduction, which is accessible to sugar. Research studies on sulphuric acid pretreatment show the reduction in xylan about 95% for 2% diluted acid for 3 hours pretreatment meanwhile cellulose to glucose conversion is 24% (Brodeur et al. 2011). In addition, Sodium hydroxide shows the highest percent as 65% delignification and cellulose conversion occurs with 2% diluted concentration at 121°C for 30 minutes. Hydrogen peroxide treatment results in lesser delignification and cellulose conversion are 50% respectively. Wyman et al. (2005) applied various pretreatments to corn stover and highlights the different experimental methods and the biogas yield based on the choice, pretreatment methods for the selected materials on the biomass components are need to be improved.

Table 2.3 Various processes for pretreatment of lignocellulosic biomass

Pretreatment	Merits	De-merits
Comminution	Cellulose crystalinity decreases	Required energy to operate motor
Ozonolysis	Reduce lignin &non-toxic	Ozone requirement is high
Acid hydrolysis	Hydrolyze hemicellulose to xylose & sugars, alters structure or lignin	Cost is high, toxin formation with concentration acid
Alkaline hydrolysis	Remove hemicellulose & lignin, increase the surface area	Long residence time, deposition of salts in biomass surface
Organosolv	Hydrolyze hemicellulose and lignin	Long time to evaporate solvent; high cost
Biological	Degrades hemicellulose & lignin	Slow rate of hydrolysis

2.10 Anaerobic digestion of organic wastes

Anaerobic digestion is the eco-friendly approach either in the way of disposal routes or as a source of alternative energy resources. The most encouraging option for highly biodegradable solid waste is the moisture content which is beyond 50% is considering suitable for biochemical conversion to biomethane potential (Dhar et al. 2016). In recent decades, more attempts are initiated for treating organic fractions of municipal solid waste through biochemical conversion (Table 2.4). This technology is hindered by the significant biodegradable rate of solid waste. Also, it is the rate-limiting step in waste processes with the existence of solids content which gives an obstacle for conversion to soluble compounds (Llore and Lo 2008).

The application of biogas used as a domestic appliance and for the operation of combustion engines make it alternative fuel resources. It can be utilize as a source of energy, liquid and sludge digested as a soil conditioner, lowering the cost of sludge management, ability to stabilize large capacities of diluted organic slurries in a little costs and more economical (Sánchez et al. 2005). This technology has also implemented to treat the agricultural waste, solid waste and other organic sludge used to reduce oxygen demand from the waste, which converts to bioenergy. Therefore, the improvement in biomass utilization for energy production is essential. Co-digestion of mixed substrates offers ecological, technological and economical benefits when it is compared to the single substrate digestion (Shah et al. 2015). However, different types of feedstocks needs to be carefully assorted to use as a feedstock, improves the efficiency of anaerobic digestion (Zhang et al., 2007).

Lignocellulosic biomass is the energy crop used for bioenergy production. Among these energy crops, switchgrass yields more attention towards high profit even with low input conditions, well suited for bioenergy production and provides adequate feedstock supply. It is an efficient method of converting cell wall to bioenergy offers future biogas, which counterbalances as fossil fuels (Uma et al. 2018).

Table 2.4 Summary of a feedstock used in anaerobic digestion

Feedstock	Temp °C	Mode of operation	Methane/ Biogas yield, L/kg	References
Pistachio Hull	Mesophilic	Batch	215 L/kg COD	(Celik and Demirer 2015)
Food waste	Mesophilic	Semi-continuous	216 L/kg VS $_{removed}$	(Ahamed et al. 2015)
Kitchen waste residues	Mesophilic, 41	Batch	479 L/kg TS_{added}	(Gao et al. 2015)
Food waste	Mesophilic & Semi-continuous	Batch	-	(Zhang et al. 2015a)
Kitchen waste slurry	Mesophilic, 39	Pilot-scale	Improves 78% efficiency	(Xiao et al. 2015)
Date palm waste	Mesophilic, 35 &thermophilic, 55	Batch	Biogas recovery 140% with inoculum	(Ismail and Talib 2014)
Food waste	Mesophilic, 35 &thermophilic, 55	Batch	CH_4:70.7%, 440 mL CH_4/g VS	(Ventura et al. 2014)
Food waste	Mesophilic, 35	Continuous	SMY: 468 and 530 mL CH_4/VS /d	(Browne and Murphy 2013)
Food waste	Mesophilic	Semi-continuous	352–450 mL CH_4/g VS/d	(Zhang and Jahng 2012)
Vegetable waste	Mesophilic,35	Batch	387 mL CH_4/g VS	(Velmurugan and Ramanujam 2011)
Food waste	Mesophilic	Batch & continuous	Methane: 62% and 59%	(Chen et al. 2010)
Food waste	Mesophilic	Two-stage	464 mL CH_4/g VS	(Chu et al. 2008)
Food waste	Thermophilic,55	Batch	CH_4:73% (Volume based)	(Zhang et al. 2007)

2.11 Co-digestion of organic wastes

Co-digestion is improved by adding one or more substrate, (i.e., co-digestion). The performance of digestion evaluated based on operational characteristics such as C/N ratio, food to inoculum ratio as an initiative (Hagos et al. 2016). Table 2.5 shows the anaerobic digestion of multiple substrates used to generate biomethane potential by various researchers. The purpose of co-digestion is to balance nutrients C/N ratio, dilute inhibitors/toxic compounds, macro- and micronutrients to improve methane production.

Table 2.5 Co- digestion performance of multiple substrates

Feedstock	**Temperature, °C**	**Mode of operation**	**Methane/ Biogas yield, L/kg VS**	**References**
Cattle manure: Illama waste	Psychrophilic,16.6	Tubular	Biogas decreased by 50%	(Martí-Herrero et al. 2015)
Food waste: Rice straw	Mesophilic	Batch	GP and methane potential:582 L/kg VS & 392 L/kg VS,	(Yong et al. 2015)
Food waste: leachate	Mesophilic	Batch& Semi-continuous	Mono-digestion:384 Co-d: 456.5 mL/g VS	(Zhang et al. 2015b)
Food waste: Dairy manure	Thermophilic		281-385 $m^3 CH_4$/ton VS	(Zarkadas et al. 2015)
Sludge: Egeria densa	Mesophilic,35	Batch	198.32 ± 2.61 mL/g VS	(Zhen et al. 2015)
Dairy manure: switchgrass	Mesophilic,35	Batch	158.6 mL/g VS; 39% highest than mono-substrate	(Zheng et al. 2015)
Kitchen waste: pig manure	Mesophilic	Batch	409.5 mL/g VS	(Xie et al. 2017)

Food waste: Rice Husk	Mesophilic, 39	Batch	196 mL/g	(Jabeen et al. 2015)
Food waste: Dairy Manure	Mesophilic	Semi-continuous	140±1.53 L CH_4/kg	(Agyeman and Tao 2014)
Café waste: vegetable waste: fruit waste	Mesophilic, 39	Batch	Methane yield: 64%	(Al Mamun et al. 2015)
Sewage sludge: Sugar beet pulp	Mesophilic, 35	Batch	Biodegradability: 48–61.5% on COD removal	(Montañés et al. 2015)
Fruit & vegetable waste: Food waste	Mesophilic	Sequential	4.1% increase in CH_4 at OLR<2.0	(Shen et al. 2013)
Animal manure: Switchgrass	Thermophilic, 55	Batch	337 mL CH_4/g VS/d	(Ahn et al. 2010)
Food waste: Green waste	Mesophilic, 35 Thermophilic, 55	Batch	778, 742, 784 and 396 mL/g VS	(Liu et al. 2009)

2.12 Summary of Literature survey

India is the largest agricultural country, with abundant source of biomass such as animal manure, lignocellulosic biomass and agricultural residues. These residues converted to methane-rich biogas in anaerobic digestion, which provides merits to produce biogas, and simultaneously improved the quality of digestate for their landfills. Compared to conventional anaerobic digestion, solid-state anaerobic digestion shows higher organic loading rate, less quantity, low energy demand, highest volumetric methane production rate. Single substrate digestion has a problem faces with the imbalance of nutrients and inadequate buffering capacity. Various types of raw materials utilizes for co-digestion is the efficient way namely, to digest, balancing the nutrients, reduces the toxic compounds, which improve the biogas production. It provides buffering capacity and acts as a nitrogen source, which enhances digestion and rich in micronutrients necessary for optimum microbial growth. However, the selection of feedstock with different mix ratio for the anaerobic digestion needs optimization for different characteristics to improve the efficiency. Fonoll et al. (2015) reports the co-digestion of sewage sludge and fruit waste yields the highest methane yield about 350 L/kg volatile solids at 60 days hydraulic retention time. It has been reported by Xu and Li (2012) co-digested food waste and corn stover by solid-state anaerobic digestion favour the highest methane yield of 305 L/kg VS obtained with 1:1 ratio of mixed wastes.

Biodegradable organic matter is used as alternative energy source to reduce the use of conventional energy resources. The application of anaerobic digestion for biodegradable wastes improves the bioenergy production. Either process performance improves by adopting the pretreatment methods to lignocellulosic biomass or co-digestion of heterogeneous feedstock recovers the biogas production, overall solids reduction, stabilization and waste reduction, which respond to the problem faced in modern India.

2.13 Research problem identification

Based on the overall literature survey, various problems are identified for the treatment of organic wastes at Indian conditions. With the availability of natural availability of lignocellulosic biomass in Western Ghats region of Karnataka which could be utilized in a sustainable way for the energy conversion in the form of biogas. The efficiency of biogas is produced by utilizing the lignocellulosic biomass as feedstock in anaerobic digestion system. Cell wall matrix of lignocellulosic biomass is limited and encapsulated with lignin, which hinders the accessibility to cellulose and hemicellulose for microbes during decomposition. Furthermore, challenges are faced towards initialization of experiments; microbial accumulation considerably decreases the biodegradability with lignocellulosic biomass.

The accumulated biodegradable waste is toxic and rapid conversion into methane and carbon dioxide in the open environment, which increases GHG emissions. Hence, it is mandatory to search for alternative to handle this waste in the society. High biodegradable waste can be used, as the feedstock for the biogas production via biological routes is the better alternative. Eventhough, this waste is used as a single feedstock for biodegradation, the major constraints are rapid pH fluctuation and volatile acid accumulation at a faster rate which inverses the methanogenic activity (rate limiting step).

Therefore, it is reasonable to examine the effects of mixing heterogeneous wastes used for the anaerobic digestion for more efficient biogas production. Co-digestion is more specific and advantageous with some substrate combination. In addition to, pretreatment technique adopted for lignocellulosic biomass supports the breaking of cell wall and releasing cellulose and hemicellulose for microbes, which increases biodegradability for biomethane potential.

2.14 Research objectives

This study aims to evaluate the performance of digestion in batch and semi-continuous system. The performance is analyzed by applying pretreatment and co-digestion methods to heterogeneous waste biomass in anaerobic digestion system.

The research objectives identified for the present study are:

1. To investigate the effect of pretreatment of substrates in enhancing its anaerobic biodegradability.
2. To evaluate the technical feasibility of solid-state anaerobic digestion of substrates in a single stage reactor operated in batch and semi-continuous mode and to identify the key factors governing the process performance of batch and semi-continuous operation.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 General

This chapter addresses the materials selected, experimental setup and characterization of substrates. In the methodology section, pretreatment of substrates, mode of operation along with experimental design are discussed. Figure 3.1 shows the experimental plan executed for the present study.

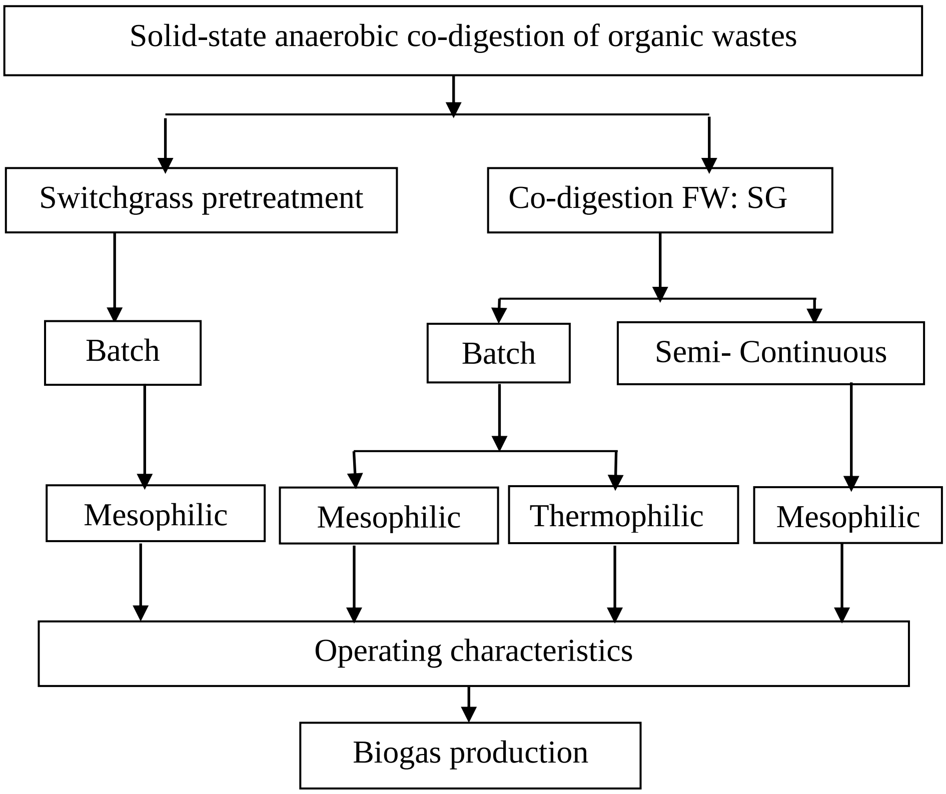


Figure 3.1 Experimental plan

3.1.1 Phase 1: Performance of batch digestion

Batch experiments are categorized in two subsections: (a) Pretreated switchgrass as a substrate for biogas and, (b) co-digestion of food waste and switchgrass at mesophilic and thermophilic conditions. Section (a) focuses on pretreatments applied to switchgrass and determining the effects of pretreated switchgrass on anaerobic biodegradability at mesophilic conditions. Section (b) experiments are conducted using food waste (FW) and switchgrass (SG) as the substrates. These substrates are mixed in different mix ratio and utilizes for biodegradation in mesophilic and thermophilic condition. As a performance evaluation, biomethane potential is observed from both FW and SG combinations as well as from pretreated substrates. Additionally, process parameters (pH, alkalinity, volatile acids, chemical oxygen demand, biogas, and methane content) are evaluated for each study to monitor the digestion performance.

3.1.1.1 Switchgrass pretreatment

The pretreatments are applied to switchgrass in order to access the cell wall matrix of switchgrass. Possibly, various pretreatment improves the utility of SG during anaerobic digestion. The purpose of pretreatment is designed to digest the SG in a faster rate for the better biomethane yield. In this study, two pretreatments are applied to switchgrass i.e., physical and chemical treatments. Physical and chemical pretreatments are liquid hot water (LHW), thermal, 1% NaOH, 1% H_2SO_4 and organosolv treatment are selected for SG. The initial characterizations are analyzed to check the deviations in cell wall matrix and suitability for batch digestion.

3.1.1.2 Co-digestion of food waste and switchgrass

Co-digestion conducted with FW and SG as substrates for biogas production at optimized conditions. Mostly, the substrates are selected based on its availability, high-energy potential and low cost treatment for the maximum biogas yield. Based on those criteria, FW and SG is used as feedstock for batch digestion in mesophilic and thermophilic conditions. Initial characteristics of substrates are analyzed to determine its suitability, with different mix ratio in terms of quality. In addition to that, process parameters namely pH, alkalinity, volatile acid are assessed for both the temperature conditions.

3.1.2 Phase 2: Semi-continuous operation

Experiments were conducted with FW and SG as feedstocks and operated in mesophilic condition to determine the performance on biogas production. At laboratory scale, gradual increase in organic loading and its mix proportion of feedstock in a semi-continuous mode by using single anaerobic digester unit.

3.2 Materials

3.2.1 Feedstock

Feedstocks as food waste and switchgrass were selected for the present work.

Food waste: Food waste consists of cooked vegetables, rice and other proteinaceous content remained as wastes from canteens of NITK campus, Mangalore, Karnataka India. These wastes are collected from the hostels, transferred to the laboratory and store it in a deep freezer. The collected wastes are crushed with a blender to reduce its size for maintaining the uniformity of waste. The initial characteristics of food waste is analyzed and further used as feedstock for digestion.

Switchgrass (SG): Switchgrass is selected based on its availability in the locality. Switchgrass is a lignocellulosic biomass, which is an inedible crop and can be utilized as a feedstock for biogas production. Switchgrass (*Panicum Virgatum*), is a warm seasonal perennial grass which is having hard, deep-rooted bunch that grass grows upto 2.7m height and its leaf size is around 30-90cm. Switchgrass grows in coarse textured soil and wetted areas of all the soil conditions. *SG is a* prominent crop and well adapted for growth in Western Ghats region of Karnataka, India. SG consists of 15%-26% lignin, 31%-37% hemicellulose and 25%-35% cellulose, suggests as a high potential feedstock to convert as a biofuels (Frigon et al. 2012a).

Switchgrass was collected from NITK campus, and used as a feedstock for digestion. Switchgrass is cut into 10 cm size pieces approximately with a chopper. The chopped SG is ground and blended to maintain a uniform size that passes through 0.45 µm sieve. The sieved SG is stored in airtight containers at ambient temperatures until its further use. The initial characteristics of SG determines its suitability towards biodegradation.

3.2.2 Inoculum

Inoculum plays a vital role to digest the organic materials in an anaerobic digester. Inoculum is used as source of microorganisms, influencing the operational process for batch as well as semi-continuous mode during digestion. Anaerobic sludge is collected from UASB unit of United Breweries distilleries, Bikampady Industrial Estate, Mangalore, India. The collected anaerobic sludge is stored at a temperature below 4°C in deep freezer to maintain inactiveness of anaerobic microorganism until its further usage. Further, the quantities of inoculum is kept at 35°C for few weeks, which is used for experiments to improve the process performance and stability during digestion.

3.3 Experimental set-up

3.3.1 Batch mode

Figure 3.2 shows the experimental setup for batch digestion. Bioreactors consisted of one-liter capacity made up of Duran Schott GL45 glass bottles. The bioreactors were capped with a PBT screw-cap, containing a PTFE-coated silicone seal. Each bioreactor is checked to be airtight and resistant to internal pressure before each use. The working volume is taken as 50% of the total volume and the remaining volume is kept at reserved for the collection of biogas. Each digester is filled with inoculum (UASB sludge) which is incubated at 35°C for 2 hours in order to allow the rebalance of CO_2 between liquid and gaseous phase. The known quantity of substrates (different combinations depending on experiments) at 35°C is added for the digestion. Each digester headspace is flushed with a nitrogen gas for 2 minutes with a constant flow in order to ascertain the absence of oxygen in the bioreactor prior to airtight closure. Each experiment is performed under control, consisting of water in the place of the sample in order to determine the biogas produced by the inoculum alone. The pH value is adjusted to neutral range by adding 4% diluted NaOH as buffering reagent which favours biogas production. The biogas volume is measured by using volume displacement setup. The volume of biogas is measured at an interval time of 24-hours from each bioreactor. The net volume of biogas is calculated by the difference in biogas volumes collected between substrates and the reference blank from each reactor. Experiments are stopped when lesser biogas is produced within one week of digestion, i.e. biogas volume, when it reaches 1% of total biogas produced (Pham et al. 2013; Quiñones et al. 2012).

Figure 3.2 Batch digestion set-up

3.3.2 Semi-continuous set-up

Figure 3.3 shows the experimental setup for semi-continuous digester. Two identical laboratory scale semi-continuous reactors are made up of Techno glass with a working volume of 2.5 L used in mesophilic temperature. Among these two, one is designed as control and another for digesting the feedstock at mesophilic condition. The performance of digestion is executed with the retention time of 20, 15 and 10 days subsequently according to loading conditions. A control reactor is fed with fresh inoculum at same retention time. The reactor contents are stirred manually once in a day to avoid phase separation of constituents and to maintain its homogeneity of contents. Feeding and withdrawal are carried out at the every completion of batch, at which the biogas produced is less than 1% of total biogas volume.

Figure 3.3 Semi-continuous mode digester set-up

3.4 Methodology/ Mode of operation

3.4.1 Batch digestion

An experiment is conducted in batch mode, which is categorized based on feedstock (a) Switchgrass pretreatment, and (b) co-digestion.

3.4.1.1 Switchgrass pretreatment and performance evaluation

Two pretreatments i.e. physical and chemical are adopted for SG in this present work. The physical pretreatments are liquid hot water (LHW) and thermal treatments; and chemical pretreatment are 1% NaOH, 1% H_2SO_4 and organosolv treatments. The pretreatment conditions are selected based on literature (Amiri et al. 2014; Antonopoulou and Lyberatos 2013; Cui and Shen 2012; Zhu et al. 2010). Table 3.1 presents the operating conditions for the pretreatment of SG.

Table 3.1 Operating condition of pretreatments

Chemical reagent	Temperature	Concentration (g/100 mL)	Time (Hrs)	pH (Initial)
Raw/ No chemical	35	-	-	7.2
H_2SO_4	35	5	1	2.3
NaOH	35	5	24	12.4
LHW/ No chemical	121	-	0.75	7.2
Thermal/ No chemical	121	5	1	7.1

Figure 3.4 shows the physical and chemical pretreated SG and raw SG. Thermal treatment is carried out by autoclaving the sample at 121°C for 45 minutes for SG. LHW pretreatment is conducted by blending SG and water in the mix ratio of 5% (w/v) solid-liquid ratio, which is boiling at 121°C for 45 minutes. Acid and alkaline pretreatments are performed with 1% H_2SO_4 and 1% NaOH at 5% mix of solid to liquid ratio (w/v) of SG with diluted acid and alkaline reactions respectively. Pretreatment time for acid and alkaline treatments is 60 minutes and 24 hours for acid and alkali reactions with SG in room temperature. Organosolv pretreatment is carried out with 1:8 ratio (w/v) H_2SO_4 reacted at 160°C for 60 minutes and is washed thrice with 75% aqueous ethanol. Finally, all the pretreated switchgrass are neutralized with buffering reagent and utilized as a feedstock for batch digestion. All the biogas volume is measured according to standard temperature and pressure conditions.

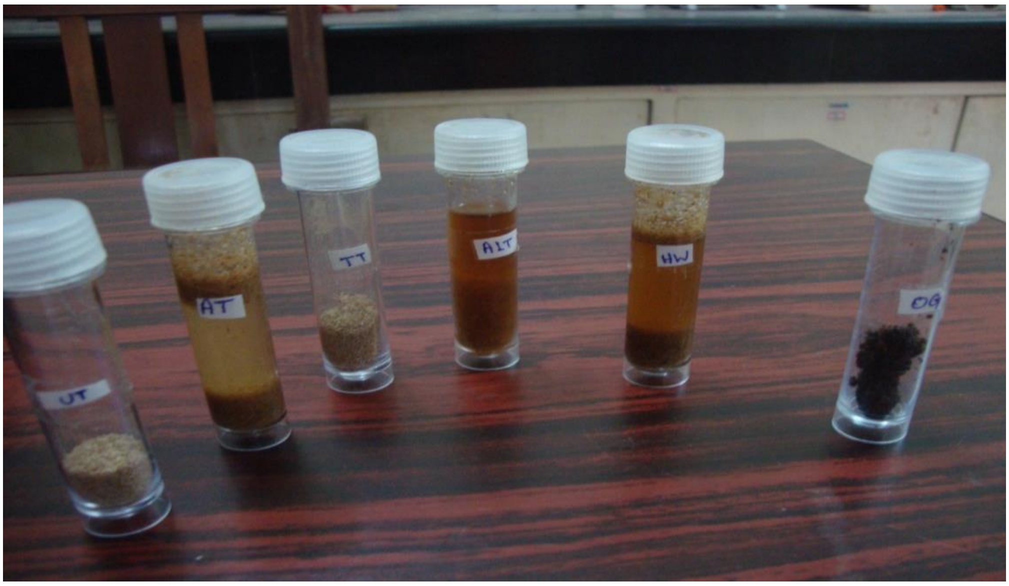


Figure 3.4 Pretreated and raw switchgrass samples

3.4.1.2 Co-digestion test

Three mix ratios (food waste: switchgrass) viz. 1:0, 1:1 and 0:1 each at five different organic loadings (4, 5, 6, 7 and 8 g/L) and two operating conditions (mesophilic and thermophilic) for each mix and organic loading are used in the current study as shown in Table 3.2. The substrate (S) to inoculum (I) ratio is maintained between 1.41 and 1.34 g VS substrate/ g VS inoculum for FW and SG respectively. However, based on the biogas potential and digestion period obtained from Batch A (mesophilic, organic loading: 4 to 8 g/L and 30 day digestion time), and Batch B (thermophilic, organic loading: 4 to 8 g/L) experiments are conducted at 18 day digestion time. In both batches, a thermostat is used to maintain the temperature and had 15 conditions each. Reference or control bio-digesters are operated with only sludge, and the volume of biogas produced in the reference reactor is deducted from the volume of biogas produced by substrates to calculate the net biogas potential of substrate. All the tests are conducted in duplicates. Experiments are stopped at lesser substantial biogas (when the gas production drops below 1% of the total gas yield) within one-week period of digestion (Quiñones et al. 2012).

Table 3.2 Outline of batch experimental methods

Temperature profile	Loading (g/L)	FW: SG (mix ratio) (%)			No. of Conditions
Mesophilic (Batch-A) (35°C)	4	1:0	1:1	0:1	15 conditions & 1 Reference
	5				
	6				
	7				
	8				
Thermophilic (Batch-B) (55°C)	4	1:0	1:1	0:1	15 conditions & 1 Reference
	5				
	6				
	7				
	8				

3.4.2 Semi-continuous digestion

Anaerobic digesters are operated for the retention time of 280 days with simultaneous withdrawal and feeding. Retention time in each trial from T1 to T3, total duration for each batch, loading conditions and the feedstock proportion (FW: SG) for each batch is shown in Table 3.3.

Table 3.3 Experimental plan on semi-continuous mode

Batch	Period (days)	Retention time (Day)	Total duration (Days)	Organic loading, (*g/L*)	Feedstock (FW:SG)
1	T1 (day 1-20)	20	60	4	100% FW
	T2 (day 21-40)	20			
	T3 (day 41-60)	20			
2	T1 (day 61-80)	20	60	5	12:88 (FW:SG)
	T2 (day 81-100)	20			
	T3 (day 101-120)	20			
3	T1 (day 131-150)	20	59	6	25:75 (FW:SG)
	T2 (day 151-170)	20			
	T3 (day 171-189)	19			
4	T1 (day 190-204)	15	47	7	50:50 (FW:SG)
	T2 (day 205-220)	16			
	T3 (day 221-236)	16			
5	T1 (day 241-255)	15	40	8	100% SG
	T2 (day 256-270)	15			
	T3 (day 271-280)	10			

3.5 Characterization of switchgrass

This instrumental characterization is used to identify the morphological deviations and chemical composition of pretreated SG, which is compared with raw SG. The morphological deviation are identified by scanning electron microscopy and the alterations in chemical compositions are characterized from the wavenumber given by Fourier Transform Infrared Spectroscopy (FTIR) analysis.

3.5.1 Scanning electron microscope (SEM)

SEM imaging is used to identify the modifications in morphological characteristics of SG. By comparing the images observed from pretreated SG and raw SG subjected to analyze by SEM imaging (Model: JSM6380). Samples are prepared by mixing with 2.5% glutaraldehyde solution for 10 hours and again treat with one percentage tannic acid for ten minutes and washed with different dilutions as 30%, 70% and 90% ethanol each for ten minutes respectively. The rinsed substrates are dried using hot air oven. Samples are keep in a carbon tape, which is coated with gold, to obtain the images at different magnifications.

3.5.2 Fourier-transform infrared spectroscopy (FTIR)

FTIR is used to analyze the chemical composition and properties of pretreated SG. The spectra for SG are observed in between the wavenumber ranges of 4000 cm^{-1} to 500 cm^{-1} respectively. Samples (approximately 10 mg) are mixed with 10 times of its bulk volume of pure potassium bromide and these mixtures are compressed to make pellets by using a hydraulic press and observe the peaks at different wavenumber. The peak obtained for the different wavenumber represents the functional groups of the cell wall component present in SG.

3.6 Analytical methods

The process parameters are monitored on weekly basis in batch and semi-continuous digestion. The following process parameters such as alkalinity, pH, volatile acids, solids (total& volatile), electrical conductivity are determined for evaluating the performance of digestion. All the parameters are analyzed as per APHA standard methods (APHA 1999) Table 3.4 shows the analytical methods for process parameters using digestion.

Table 3.4 Analysis of process parameters

Parameters	Method/ Instrument
pH	pH meter electrode
EC	Conductivity meter electrode
Temperature	Thermometer
Solids (TS& VS)	Gravimetric methods (Method:2540)
SCFA (compositional)	GC method
t- volatile fatty acids	Titration method
Alkalinity (mg/L)	Titrimetric method
Chemical Oxygen Demand (COD)	Closed reflux method
NH_4-N (mg/L)	Standard method 4500B: colorimetric method
TKN (mg/L)	Standard method 4500B: Kjeldahl method
Proteins	Lowry method
Biogas volume	Volume displacement setup
Biogas composition	Gas Chromatography

3.6.1 Solids content

Solid content are classified as total and volatile solids (TS & VS) respectively. Approximately, 10 g of samples is kept in a dish and weighed as (W_1). Samples are evaporated in a weighed dish until dried to the constant weight in a hot air oven at 103°C-105°C which is further cooled down to room temperature and weighed (W_2). Again, the samples are allowed to elevate the temperature at 550 °C for two hours in a muffle furnace and further, brought down to room temperature, weighed (W_3) to calculate total and volatile solids. The VS is calculated from the combusted organic matter by weighing the ash content deducted from TS content.

3.6.2 pH and Electrical conductivity

Different aspects of complex microbial metabolism are influenced by pH, measured with potentiometric methods. The samples is centrifuged, filtered, which follows the potentiometric titration for determing the pH. pH is measured by using a glass electrode which has been calibrated and corrected to the standard temperature (25°C). Glass electrode is directly dipped into the sample, until readings has to remain constant for a minimum period of 30 seconds. The similar procedure is followed for measuring the electrical conductivity of the samples.

3.6.3 Chemical Oxygen Demand (COD)

The COD is determined by the standard protocol (5220C) using closed reflux method. About 2.5 mL of the samples are mixed with 3.5 mL sulphuric acid reagent and 1.5 mL of potassium dichromate digestion solution in the vials. These COD vials are closed tightly and inverted for several times for the complete mixing of the content. These vials are refluxed in a closed digester maintained at 150°C for 2 hours and allowed to cool down to optimum condition. The content is transferred for titrating against freshly prepared 0.1N Ferrous Ammonium Sulphate (FAS) using ferroin indicator and noticed for the colour change that is observed from blue-green to reddish brown rapidly within few seconds. Similarly, the above method is adopted for distilled water to determine the blank. COD expressed in mg/L is calculated from Eq. 3.1

$$\textbf{COD, mg } \mathbf{O_2/L} = \frac{(\text{A-B})*\text{M}*8000}{\text{mL of sample}} \qquad \textbf{Eq. 3.1}$$

Where, A= mL tirant (FAS) consumed for blank

B= mL FAS consumed for sample

M= Molarity of FAS

Constant value 8000= m eq. The weight of oxygen×1000 mL/L

3.6.4 Alkalinity and t-volatile fatty acids

Alkalinity is determined by the titrimetric method (APHA 1999). The known quantity of samples is titrated against 0.2N sulphuric acid using phenolphthalein or methyl orange indicator. Alkalinity is calculated from Eq. 3.2

$$\textbf{Alkalinity as } \mathbf{CaCO_3}, \frac{\text{mg}}{\text{L}} = \frac{\text{mL of acid used}*\text{N}*50000}{\text{mL of sample}} \qquad \textbf{Eq. 3.2}$$

t-volatile fatty acid*:* About 200 mL sample is centrifuged for 10 minutes. The supernatant liquor about 100 mL diluted with equal quantity (100 mL) of distilled water. Later, 5 mL sulphuric acid is added and mixed well to maintain acidic solution. Titrate against 0.1N NaOH using phenolphthalein indicator and note the colour change from colourless to pink colour. The t-volatile fatty acids is determined from Eq. 3.3

$$\textbf{Volatile acid as } \mathbf{CH_3COOH}, \frac{mg}{L} = \frac{\text{mL of NaOH*N*60000}}{\text{mL of sample*f}} \qquad \textbf{Eq. 3.3}$$

Where ‘f’ is the dilution factor,

‘N’ is the normality of NaOH

3.6.5 Volatile fatty acid composition

Volatile fatty acids (VFA) are quantified using gas chromatography (GC) (Model: Thermo-1110). Samples are collected and centrifuge it at 3000 rpm for 15 minutes. About 5 mL of supernatant is transferred into vials, add diethyl ether and acidified using 65% nitric acid. The content is allowed to react for a few minutes and a little quantity of magnesium sulphate is added to absorb the moisture content. Separate the ether phase that is used for GC injection. VFA consist of group of compounds such as acetic, propionic, i-butyric, butyric and valeric acids, iso-valeric acids are determined by GC method. The conditions adopted to determine VFA by GC method is described in Table 3.5

Table 3.5 Specifications for VFA composition

Description	Specifications
Column	Capillary BP21, 30 mm×0.53×0.5 mm. 0.25 µm
Detector	Flame Ionization Detector
Carrier gas	Nitrogen
Split	1:30
Oven Temperature	80°C
Injector/ Detector temperature	80-220°C, 80°C/min increased @ 4°C/min to 220°C and maintained at 2 min. 210°C and 220°C
Calibration	VFA standard mix (acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, caproic& heptanoic acids with conc range of 10mM in deionized water)
Sample volume	5 µL

3.6.6 Nitrogen and protein content

Total Kjeldahl nitrogen is analyzed by the digestion-titration method. About 250 mL of diluted sample is placed in the digestion flask. Later 10 mL of concentrated sulphuric acid reagent and catalyst are added to bring it to heat for 2 hours. Further it is distillated and brought down to optimum conditions. Then 50 mL of distilled water and sodium hydroxide is added to recover ammonia from these samples. Around 20 mL of the sample extract is titrated against sulphuric acid with methylene blue indicator. Similar procedures are followed for the reference blank. Nitrogen is calculated from the Eq. 3.4.

$$\textbf{Total N}, \frac{\textbf{mg}}{\textbf{L}} = \frac{\textbf{(A-B)*280}}{\textbf{mL of sample}} \qquad \textbf{Eq. 3.4}$$

Where, A= Volume of H_2SO_4 required for sample, mL

B=Volume of H_2SO_4 required for blank, mL

Ammonia nitrogen is determined by spectrophotometric method. Ammonia is reacted with the Nessler reagent, stabilized by potassium sodium tartrate, and detected at the wavelength 430 nm, which is proportional to ammonia.

Organic nitrogen can be estimated by subtracting total Kjeldahl nitrogen and ammonia nitrogen. Protein is calculated by multiplying the numerical value 6.25 with organic nitrogen.

3.6.7 Biogas and methane content

Biogas composition mainly consists of methane and carbon dioxide, which is analyzed by gas chromatography (GC). Table 3.6 shows the specifications of GC method to determine biogas composition. Biogas sample is collected by injecting the needle through septa and drawing the plunger out until the pressure in the headspace is dropped to ambient pressure. The volume of gas collected in the syringe is injected into the gas sampling port of GC. Biogas composition is determined by GC (TRACE-1110) equipped with spherocarb is used at 120°C to separate 'O', N_2, CH_4 and CO_2. Nitrogen is used for carrier gas and the flow rate of nitrogen is 2.0 mL/min. The split-less ratio of gas sample in inlet chamber is used to control the amount of biogas flow into column and prevent unconventional peak. The gaseous compound is detected using a thermal conductivity detector. The temperatures are 150°C for injector and 150°C for detector. The calibration is carried out with the standard gas composed of 14.37% N_2, 32.88% CO_2, and 52.75% CH_4.

Table 3.6 GC method for biogas composition

Description	Specifications
Column	Spherocarb, Mesh size 80/100, length-8'1/8". Maximum temperature: 225°C
Detector	Thermal conductivity detector
Carrier gas	Nitrogen
N_2 flow volume	36 mL/min
Split	Splitless
Oven Temperature	120°C
Injector/ Detector temperature	150°C/ 150°C
Calibration standard	Gas mixture:14.37% N_2, 32.88% CO_2, and 52.75% CH_4
Sample volume	2.5mL

3.6.8 Cellulose, Hemicellulose and lignin determination

Cell wall component is classified, as cellulose, hemicellulose and lignin determined by the following procedure:

Cellulose: Approximately, 0.5± 0.2g of the sample is weighed, mixed with 100 mL acid detergent reagent and reflux for 60 minutes. Later, samples are rinsed thrice with hot deionized water and again by using acetone to remove the acids present in detergent solutions. The residues are weighed after drying at 105°C in hot air oven for 5 hours and cooled to room temperature. Dried residues are ignited at 550°C for one hour and cellulose content is calculated by the gravimetric method (Moller 2009).

Hemicellulose: About 5g of the sample is refluxed with 350 mL of diluted ethanol (4:1, based on volume) for 60 minutes, filtered and the dried residues are weighed (R_1). Phosphate buffer is added to the sample for the protein removal and pH is maintained at 4.5 by adding hydrochloric acid. Solid residues are separated by centrifuging, and are rinsed with water and ethanol. The extractions of hemicellulose with final residue are allowed to react with 2.5 M NaOH solution for two hours in a shaker. The sample is centrifuged and solid residues are dried and weighed (R_2) to calculate hemicellulose content from Eq. 3.5 (Aravantinos-zafiris et al. 1994).

$$\textbf{Hemicellulose (\%)}=\frac{(R_1-R_2)*100}{W_1} \qquad \textbf{Eq. 3.5}$$

Lignin is classified into soluble and insoluble form which is determined by two-step hydrolysis process (Sluiter et al. 2011). The insoluble lignin is determined by the gravimetric method and soluble lignin reacted with 72% sulphuric acid and diluted to 3% respectively. The diluted acid solution is boiled for 4 hours and allowed to cool down to room temperature. Then filtrate obtained after vacuum filtration is measured for absorbance at 205 nm to determine soluble lignin.

3.6.9 Digestion performance index (DPI)

Digestion Performance Index is used to determine the relationship between biomethane potential achieved from the experimental and theoretical data. DPI applied to the substrates as well as its combinations for determining the effect on BMP. Performance Index ratings are named as synergistic, antagonistic and independent interactions correlated with S/I ratio (Nielfa et al. 2015). The stoichiometric equation is based on the atomic composition of waste materials (BMPth) as per Eq. 3.6. It is used to calculate the theoretical methane composition by considering the elements C, O, H and N. Theoretical methane yield is calculated by Boyles equation (Raposo et al. 2011). DPI calculated with the ratio between experimental biomethane yields to theoretical biomethane potential yield expressed in terms of volatile solids percentage calculated from Eq. 3.7.

$$\mathbf{BMP_{th}}=\frac{22.4\,(^{n}/_{2}+^{a}/_{8}-^{b}/_{4}-^{3c}/_{8})}{12n+a+16b+14c} \qquad \textbf{Eq. 3.6}$$

$$\mathbf{DPI}=\frac{\text{Experimental methane yield},\frac{mL}{g}VS}{\text{Theoretical methane yield},\frac{mL}{g}VS} \qquad \textbf{Eq. 3.7}$$

3.6.10 Anaerobic Toxicity Assay (ATA)

Anaerobic toxicity assay are determined from biogas volume expressed in terms of percentage inhibition on biogas. Inhibition study is used to determine the inhibited biogas produced during a five day test period as per the Eq. 3.8 given by Haak et al.(2016).

$$\textbf{Inhibition (\%)=1-}\frac{V_{gas,test}}{V_{gas,\ control}}\textbf{*100} \qquad \textbf{Eq. 3.8}$$

Where, $V_{gas,\ test}$ = Volume of biogas produced from sample

$V_{gas,\ control}$ = Volume of biogas produced from blank

If the biogas volume is observed as negative, value refers as “no inhibitions” equivalent to zero values and positive value representing the presence of inhibitions.

CHAPTER 4

RESULT AND DISCUSSION

4.1 General

This chapter explains the results obtained from the laboratory-scale experiment i.e. the pretreatment of switchgrass and its performance on batch digestion. Additionally, results are discussed for the co-digestion of food waste (FW) and switchgrass (SG) by batch and semi-continuous digestion. The main objective (1) provides more insight into the switchgrass pretreatment by physical and chemical methods and subsequent biodegradation in batch anerobic reactors. Objective (2) focuses on biodegradation of food waste and switchgrass with different mix ratios by batch and semi-continuous digestion. The analytical procedures of operating parameters (pH, alkalinity, volatile fatty acids) are executed as per the details given in chapter 3.

To achieve these objectives, comprehensive experiments were conducted at various stages. The first part, describes about the detailed characteristics of feedstock and inoculum. The second part provides physical and chemical pretreatment of switchgrass, its characterization is depicted through graphs, and detailed explanation is provided in detail. This is to accomplish the help of studying the performance of pretreated switchgrass on biodegradation by batch digestion. The third part consists of laboratory scale experiments on performance evaluation of co-digestion of (FW) and (SG). Experiments were carried out with FW and SG at different mix ratio at mesophilic and thermophilic condition for determining the performance of biogas production by batch digestion. The fourth part consist of similar experiments as like "third part" on co-digestion of FW and SG with semi-continuous digestion process. The fifth part deals with effects of operational parameters on the performance of biogas production. The results of these experiments are depicted graphically.

4.2 Initial characteristics of substrates

Initial characteristics of inoculum and feedstock are presented in Table 4.1. The characteristics of biomass reflect on the performance of biogas production. The

VS/TS ratio represents the volatile nature of biomass converting into biomethane. The operational parameters such as volatile acids, alkalinity, pH and nitrogen are the indicators to monitor the process of digestion.

Table 4.1 Initial Characteristics of Inoculum and substrate

Parameter	Units	Anaerobic sludge	Food waste	Switchgrass
pH	NU	6.92	5.33	7.12
Conductivity	mS/cm	6.86	5.24	4.64
TS	% (DM)	9.53	78.76	96.49
VS	% (DM)	6.56	76.40	88.12
VS/TS	(%)	68.84	97.02	91.33
COD	mg/L	3168	16250	ND
TKN	% (TS)	1.62	2.176	47.92
TAN	mg/L	498.00	413.20	ND
TA	mg/L	4860.20	42.00	ND
Total VFA	mg/L	1215.05	3860.00	ND
Ash	(% TS)	19.92	NA	0.30
VFA/TA ratio	%	25.00	9.16	ND
Cellulose	%	-	-	32.00
Hemicellulose	%	-	-	20.50
Lignin	%	-	-	26.96

4.3 Characterization of pretreated switchgrass

The pretreated switchgrass shows the changes in structural morphology, identified by scanning electron microscopy. And the functional groups of the pretreated switchgrass is analysed by FTIR respectively.

4.3.1 SEM of Switchgrass

Figure 4.1 shows the surface morphology of raw and pre-treated switchgrass by using SEM. The surface of raw switchgrass shows squeezed, impermeable and fibrillar structure consisting of cell wall component (Figure 4.1(a)). Porous structure in Figure 4.1(b) shows the relocated cell wall matrix by acid pretreatment. A ruptured cuticle surface observed in Figure 4.1(c) is formed due to the removal of silica layer from cell wall, which increases the digestibility of switchgrass. Similarly, the surface of organosolv treated SG (Figure 4.1(d)) exhibits a disrupted surface with tears, representing the removal of hemicellulose (Obama et al. 2012). Figure 4.1(e) shows the rugged surface owing to the mass solubility of biomass of LHW pretreated SG (Sant'Anna and Souza 2012). The exposed structural breakage attained with TT-switchgrass indicates the cell wall defoliation as a function of temperature in Figure 4.1(f). Pretreatment confirms the modification that occurs in the cell wall (cellulose, hemicellulose and lignin) binding. Modification occurred in the cell wall improves the accessibility of cellulose & hemicellulose by microbes, thus enhancing the hydrolysis during digestion.

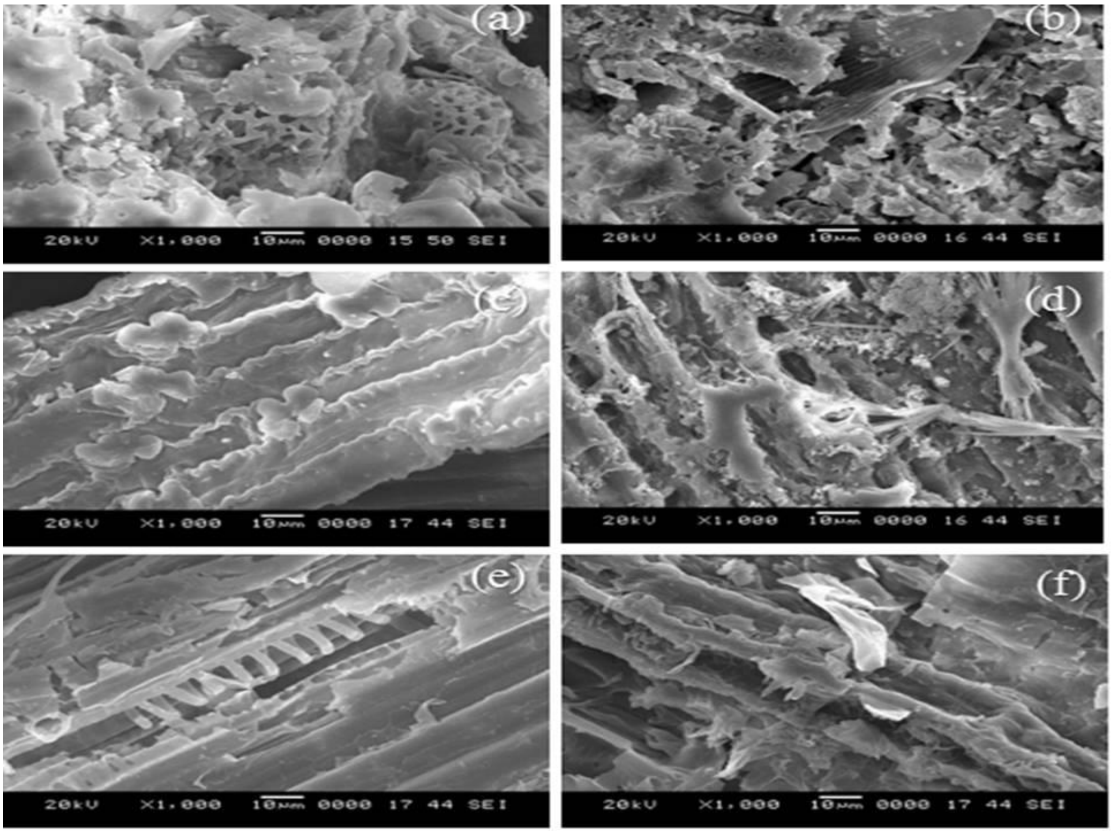


Figure 4.1 SEM images: (a) raw (b) AT-Acid treated (c) ALT-NaOH treated; (d) OT-organosolv treated; (e) LHW-Liquid hot water; (f) TT-Thermally treated switchgrass

4.3.2 FTIR Analysis

FTIR spectroscopy is performed to analyze and compare the functional group changes in raw and pretreated switchgrass. Figure 4.2 shows the FTIR Spectra for the raw and pretreated SG samples. Table 4.2 represents the absorption band through FTIR spectrum of switchgrass. The assignment of FTIR peaks corresponding to the functional group of lignocellulosic component are according to the literatures (Boeriu et al. 2004; Kumar et al. 2009b; Monlau et al. 2015; Narendar and Priya Dasan 2014; Zheng et al. 2009a) is listed in Table 4.2. It is obvious that the FTIR spectra between raw and pretreated SG shows the clear differences in terms of intensity and shape (Figure 4.2). The spectral range 3200-3600 cm^{-1} and 898 cm^{-1} represents the O-H, C-H stretching, which confirms the presence of glucose in the form of cellulose (Phitsuwan et al. 2017). At 1000-1150 cm^{-1} represents C-O-C group stretching that contains holocellulose vibrational characteristics observed from all pretreated switchgrass. The peak at 1630 cm^{-1} and 1510 cm^{-1} indicates C=C stretching in the aromatic ring which solubilizes the lignin (Monlau et al. 2012). The intensity at 1510 cm^{-1} is higher for thermal and acid treated SG that represents the presence of lignin. At same wavenumber 1630 cm^{-1}, peak intensity is indicated, as H lignin/carbohydrate is lesser for alkali, LHW, OG-treated SG. The signal around 1720 cm^{-1} corresponding to the C=O functional group is a characteristic peak of ester linked acetyl, feruloyl and ρ-coumaroyl groups between hemicellulose and lignin (Zheng et al. 2009a). The absence of peak near to 1720 cm^{-1} indicates the removal of lignin through ester bond cleavage by the pretreatment. The bending peaks at 1430 cm^{-1} indicates the presence of cellulose, 1370 cm^{-1}, and 898 cm^{-1} specifies the presence of carbohydrates. Spectral range at 1040-1080 cm^{-1} and 3400-3500 cm^{-1} represents the functional group of C-O-C and O-H stretching, which confirms the cellulose and lignin present in all the samples. Peaks at 1057 cm^{-1} show the cellulose accessible for glycosidic bridge attained with all pretreated switchgrass. Spectrum at 1042 cm^{-1} represents the polysaccharides accessed with the crystalline and amorphous bounded region. The signal at 900 cm^{-1} is attributed to β-1,4-glycosidic linkages revealing the structure of cellulose (Sathitsuksanoh et al. 2011). The results are consistent with chemical composition and confirm the reduction in lignin, thus increases in cellulose content after the pretreatment. The application of pretreatment effectively breaks the cell wall matrix confirmed by the changes in functional group, which is attributed to delignification. In addition to the intensity of cellulose peak that increases as compared to untreated SG, and due to the alteration in hemicellulose and lignin, it might be beneficial to improve the anaerobic digestibility of SG.

Table 4.2 Absorption band through FTIR spectrum of switchgrass

Wave number (cm^{-1})	Functional group	Components
3200-3600	O-H stretching	Cellulose
2900	C-H stretching	Cellulose
1720	C=O stretching acetyl/carboxylic acid	Hemicellulose & lignin
1630	C=C stretching of the aromatic ring	Lignin
1598	C=C stretching of the aromatic ring	Lignin
1510	C=C stretching of the aromatic ring	Lignin
1430	$-CH_2$ bending	Cellulose
1375	C-H deformation	Cellulose
1315	$-CH_2$ wagging vibration	Cellulose and Hemicellulose
1230	C-O-H deformation, C-O stretching of phenolics and C-C-O stretching of ester	Hemicellulose & Lignin
1000-1158	C-O-C stretching	Cellulose & Lignin
898	Glucose ring stretching, C-H	Cellulose

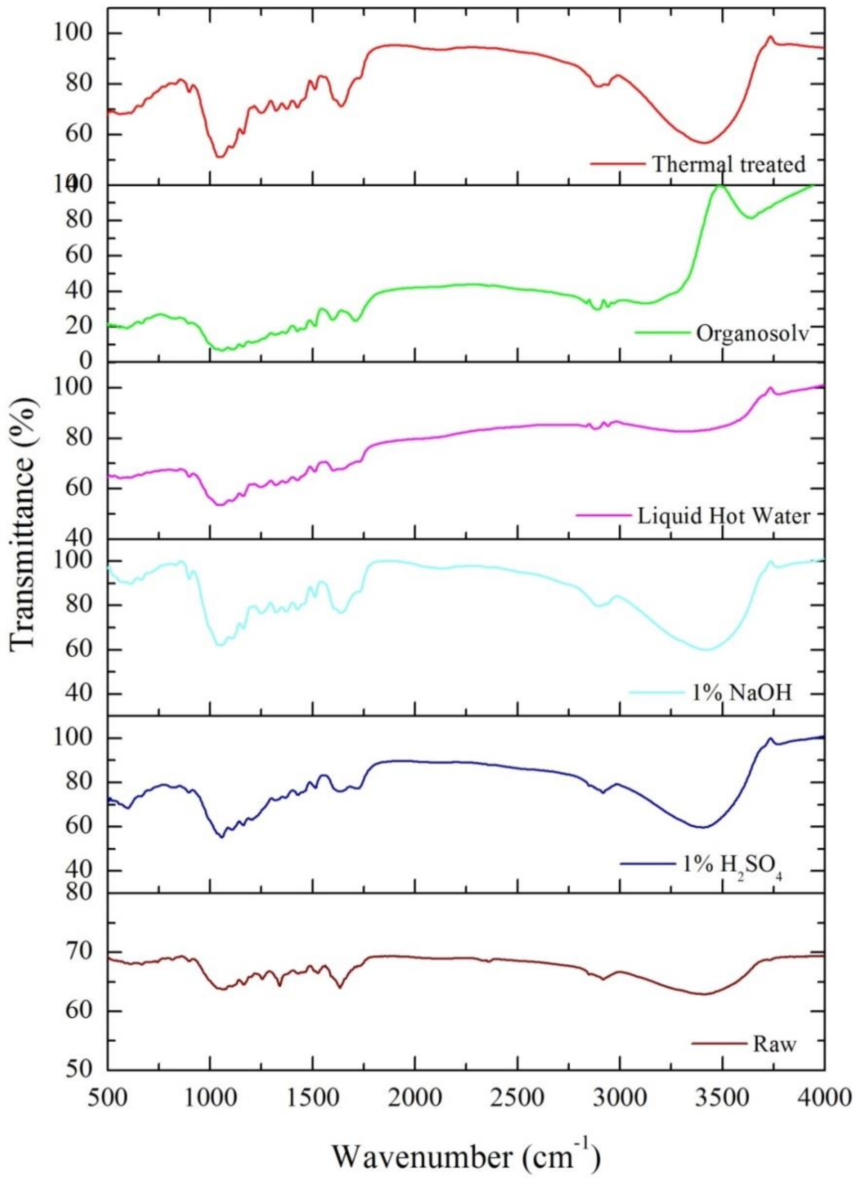


Figure 4.2 FTIR spectra of pretreated switchgrass and raw SG

4.4 Batch digestion

Batch digestion is performed in two phases to determine the biogas potential with substrates that is (1) pretreated SG (2) co-digestion FW: SG. The process parameters are monitored frequently to maintain the stable performance in mesophilic condition for pretreated SG. Similarly, process parameters were evaluated for FW and SG at different mix ratios at mesophilic and thermophilic conditions.

4.4.1 Performance evaluation of pretreated SG

4.4.1.1 Biogas production in batch studies

Biogas produced from raw and pretreated switchgrass is presented in Figure 4.3. The maximum biogas volume from all the digesters is attained within ten days, from the beginning of the experiment. The biogas production is maximum on day 5 for all substrates that is in the range between 70 to 200 mL. The lowest biogas volume is observed for acid treated SG and the highest biogas volume is attained with organosolv pretreated switchgrass. Maximum cumulative biogas yield is obtained as 583 mL/g VS for alkali-treated SG. The highest biogas yield shows the efficacy of alkali treatment in breaking the cell wall of switchgrass and making cellulose and hemicellulose accessible to microbes during digestion. Acid and LHW-treated SG yields less biogas, when compared to raw SG and its methane content is relatively low. The biogas production depends on the pretreatment condition, substrate quality and operating condition (Antonopoulou and Lyberatos 2013; Labatut et al. 2011).

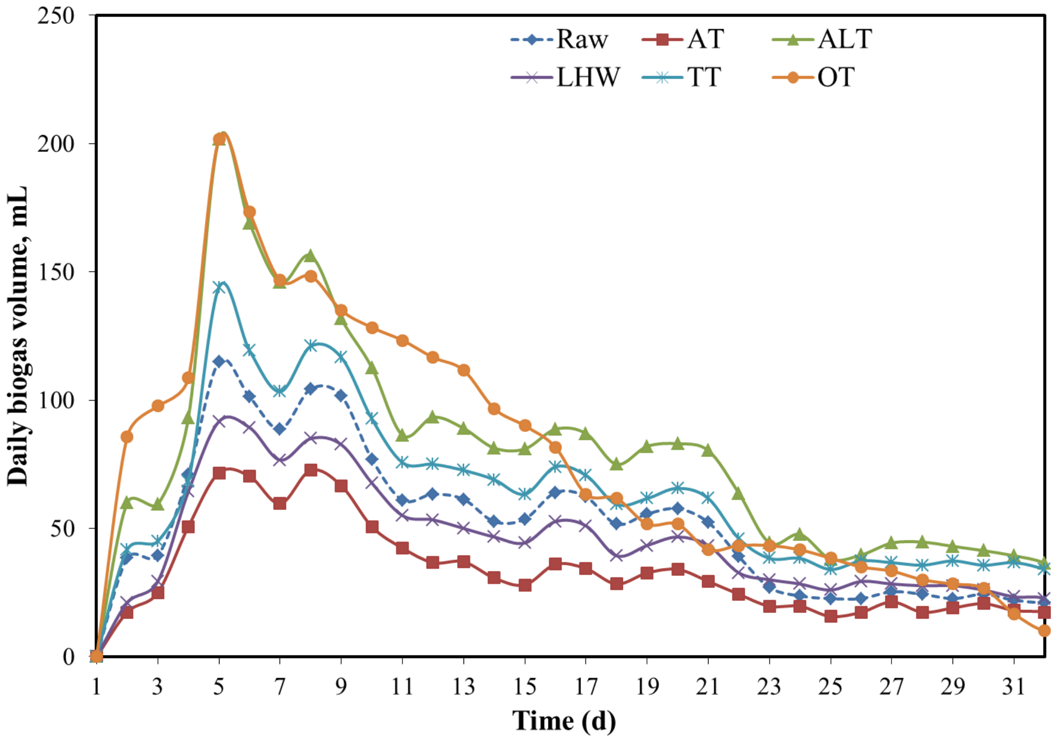


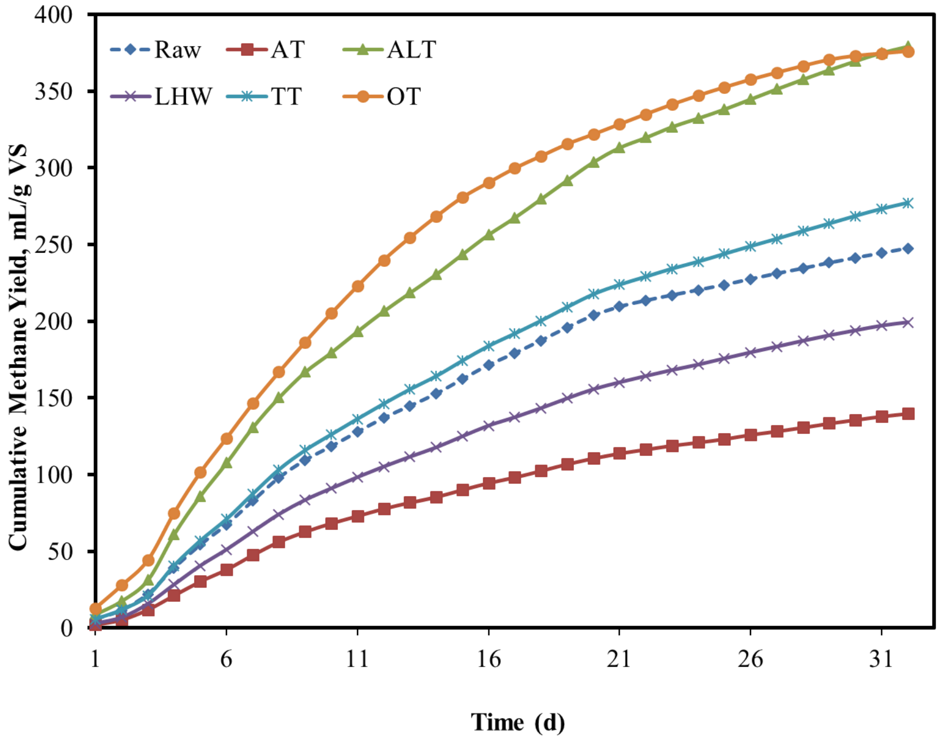
Figure 4.3 Daily biogas volume

4.4.1.2 Biomethane potential

Results show the methane yield (mL/g VS) obtained for all the pretreated and raw SG in Figure 4.4 (i). Experiments were conducted for 32 days, beyond which the volume of biogas production was terminated in all the reactors. The highest methane yield attained is 379 mL/g VS, for alkaline treated SG and organosolv treated SG. This high methane yield can be attributed to lignin degradation, which in turn could have released easily accessible cellulose and hemicellulose to microbes for utilization (Zhu et al. 2010). The lowest methane yield is 140 mL/g VS and 199 mL/g VS obtained for acid and LHW treated SG, which inferred the presence of acid concentration and hydrothermal pre-treatment that was unfavourable for microorganism during digestion.

Figure 4.4 (ii) shows the average percentage of methane, carbon dioxide, and methane-to-carbon dioxide ratio for all the substrate combinations. The CH_4/CO_2 ratio is 1.5 for physical pretreated SG while 1.8 to 2.0 for chemical pretreated SG, except for acid-treated SG (1.3). Pretreatment with alkali and organosolv improved the methane yield of switchgrass, which is in line with the pretreatment results obtained for biomass such as corn stover, agricultural residue, wheat straw and municipal solid waste (Zheng et al. 2014). This technique is compared with other pretreatment methods in terms of chemical requirements, applicability. However, no drastic fluctuations, or inhibitors are notified during the digestion process.

Methane composition was obtained for raw (65%), acid (57%), alkali (65%), LHW (61%), thermal (60%) and organosolv (67%)-treated switchgrass respectively. However, their cumulative volume of gas production varies and this is shown in Figure 4.3. Possibly, the formation of maillard (chemical reaction between amino acids and reduced sugars) reactions results in the biodegradability reduction, which showed apparent effect between lignocellulosic biomass and the pretreatment conditions. The biomethane potential depends on various factors like inoculum sources and inhibitors which affected the digestion performance in the batch system.



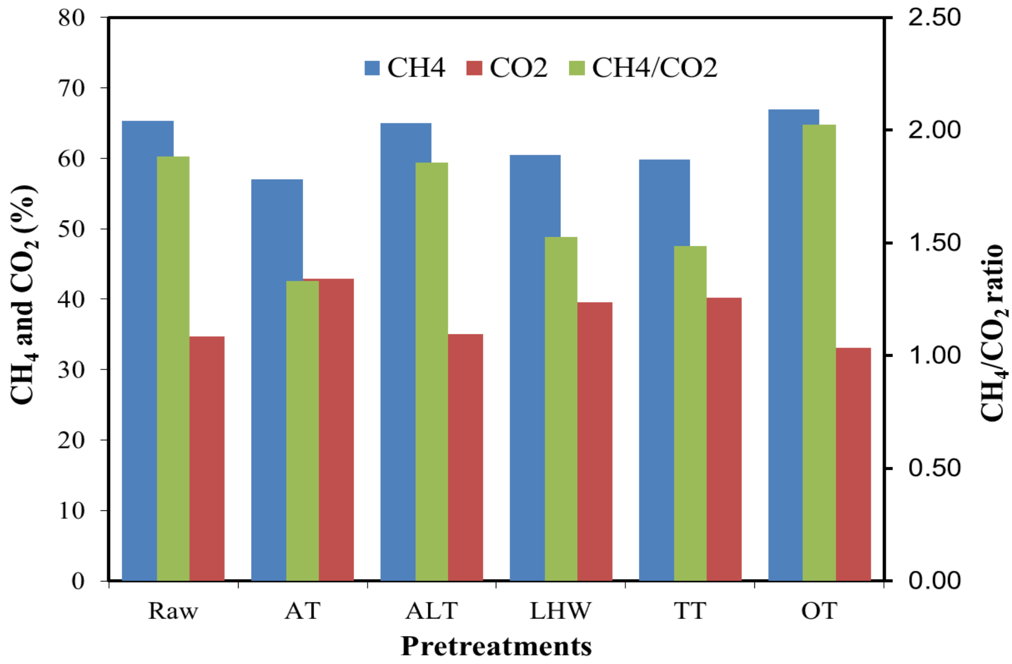


Figure 4.4 (i) Cumulative Methane yield (ii) Impacts of pretreatment on average methane & Carbon dioxide.

4.4.1.3 Effects of pretreatment on pH, alkalinity, and volatile acid

A continuous monitoring of the pH, alkalinity, and volatile acids is required for better performance of the digestion system in order to improve methane potential. The pH observed for pretreated switchgrass is 6.0-8.0 and for raw SG is in the range 6.5 to 7.0 with small fluctuation over the digestion period. Alkalinity and volatile acids concentration are the indicators to assess the performance of the digestion system and helps in determining the buffering reagent required for the process in the digester. Alkalinity is observed in the range between 5.42-8.25 g $CaCO_3$/L and the volatile acid concentration ranges from 4.35 to 6.37 g/L during the digestion for the raw and pretreated SG. In particular, volatile acid to alkalinity ratio has a key role in monitoring the process of digestion. The volatile acid production occurs during the pH between 5.5 to 6.5 and stages of microbial growth occurs between acidogenesis to methanogenesis which leads to the reduction in volatile acid (Zhai et al. 2015). Methane-forming micro-organisms and the presence of excess alkalinity acts as buffer to maintain the pH and stabilize the digestion process (Meng et al. 2014). As the digestion continues, hydraulic retention time that is more than ten days, yields higher consumption of acids and a favourable environment to methanogens for further conversion of biomethane potential (Ahn et al. 2010; Zhu et al. 2010).

Results show, the volatile acid to alkalinity ratio varies from 0.6 to 0.9 for the raw and pretreated SG. However, for a favourable microbial activity, volatile acid to alkalinity ratio is around 0.34 at constant temperature and pressure respectively. If a volatile acid is produced continuously instead of methane then that fluctuates the process by insufficient buffering capacity that will disturb the performance of digestion. The range of pH at 7.0-7.5 favours the methanogens for methane production (Zheng et al. 2015). At pH equivalent to 6.0, methanogenic bacteria builds acid within a shorter duration owing to hydrolysis. Alkali ion consumption refers to the balance between alkalinity and volatile acid that improves the biogas yield. At pH values above 6.5 and with the presence of excess buffer restoring bicarbonate/carbonate ions existing with sodium alkali becomes toxic to bioreactor during the process of digestion (Gerardi 2003).

4.4.1.4 *Inhibition studies*

Inhibition studies were performed to assess the possibility of toxicity in the digester due to the addition of pre-treated substrate by determining inhibition percentage as per the Eqn. 3.8 (Haak et al. 2016; Owen et al. 1979). The control produced cumulative biogas volume of 44mL and 99 mL for the retention time of 2 days and 4 days respectively. If the inhibition value shows, the negative sign it indicates that no inhibition occurs with pretreated sample. The result of the inhibition occurred with acid and LHW-treated SG on the second day is further neutralized by buffering reagent. The raw and pretreated samples produce positive biogas volume without any inhibition, which proves better digestibility, occurred with switchgrass.

Further, no sign of inhibition is observed for all the samples throughout the batch digestion. Research is limited on the inhibition studies from biogas potential during the start-up of the digestion. Alkaline pretreatments adopted for the SG that yield maximum biogas is confirmed as the highest negative inhibition value obtained for 4 days running time. Similarly, a lower rate of biogas produced from acid pretreatment confirmed the inhibition from this study.

In the present study, alkaline pretreatment method is adopted for the SG, providing maximum biogas yield, confirming the highest negative inhibition value obtained for 4 days retention time.

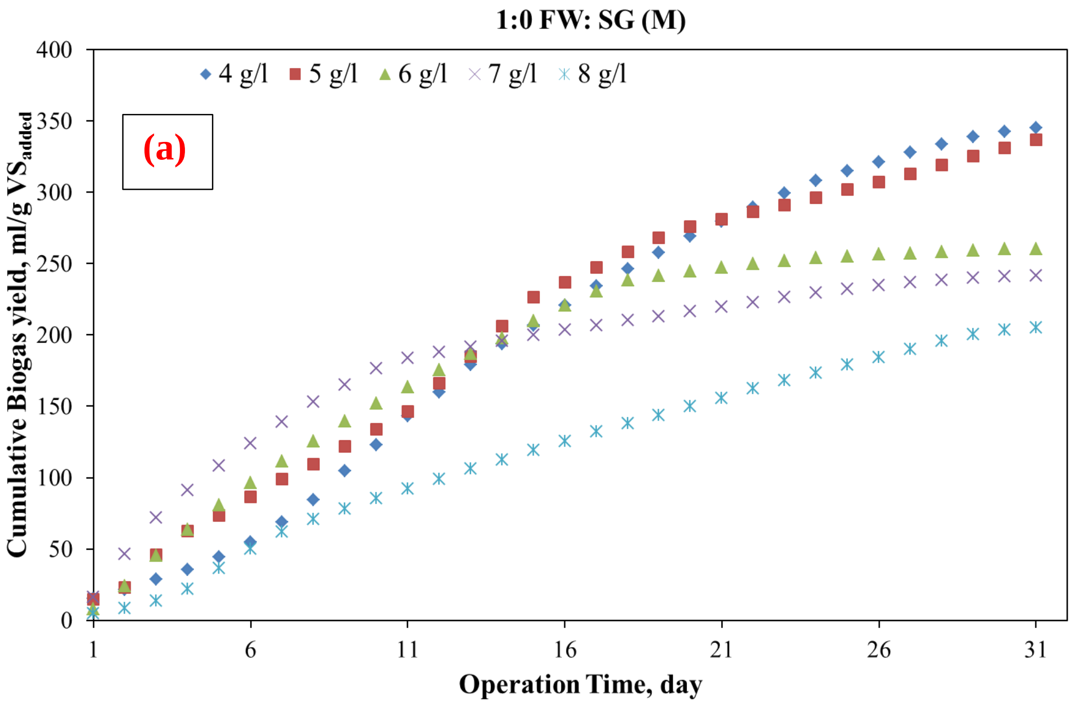
4.4.2 Co-digestion of switchgrass with food waste

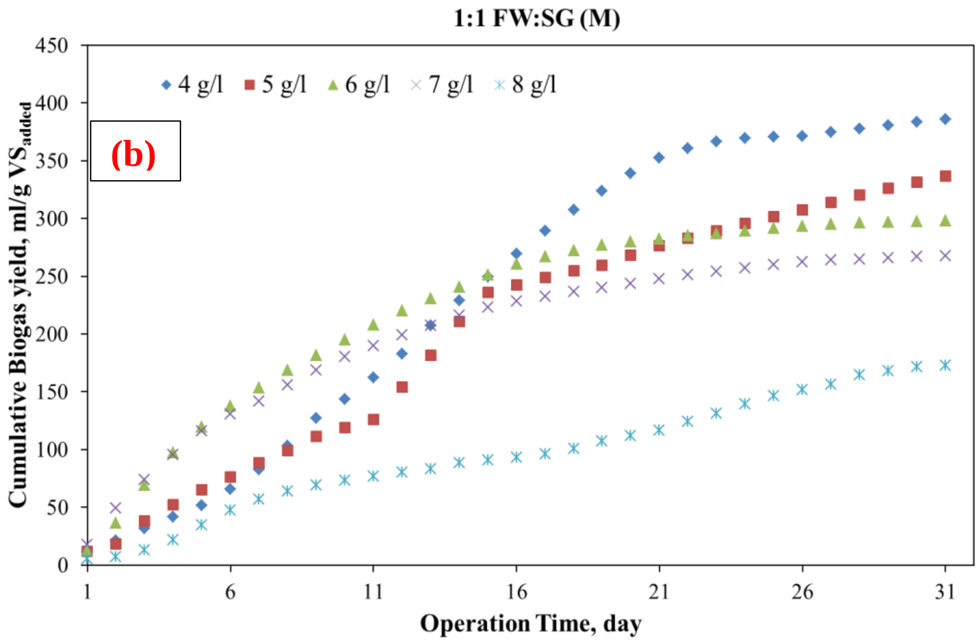
4.4.2.1 *Performance on biogas yield*

The cumulative biogas yield of FW and SG at different mix ratios as a function of digestion time for 30 and 18 days are shown in Figure 4.5 and 4.6.

Highest biogas yield is obtained within 15 days for mesophilic and 10 days for thermophilic conditions. Food waste produces the average biogas yield in the range 205 to 345 mL/g VS for the loading from 4-8 g/L at the mesophilic condition (Figure 4.5 a). The biogas yield (386 mL/g VS) obtained with OLR of 4 g/L at (1:1 FW: SG) is the highest among all the loading conditions (Figure 4.5 b). The yield of biogas is 128 to 331 mL/g VS for switchgrass in single substrate digestion at 35°C (Figure 4.5

c). Around 68-72% of total biogas yield is achieved within 15 days of digestion. The performance of digester stability and biogas yield are better with co-digest mix proportion of food waste and switchgrass which are in line with the results obtained (El-Mashad and Zhang 2010).





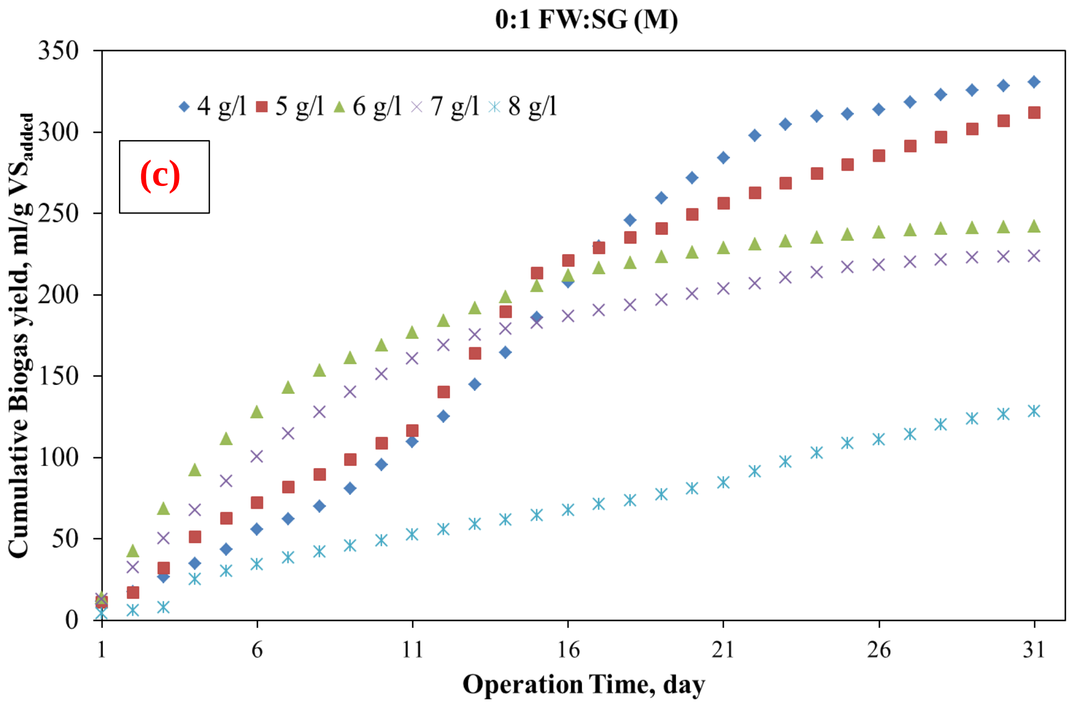


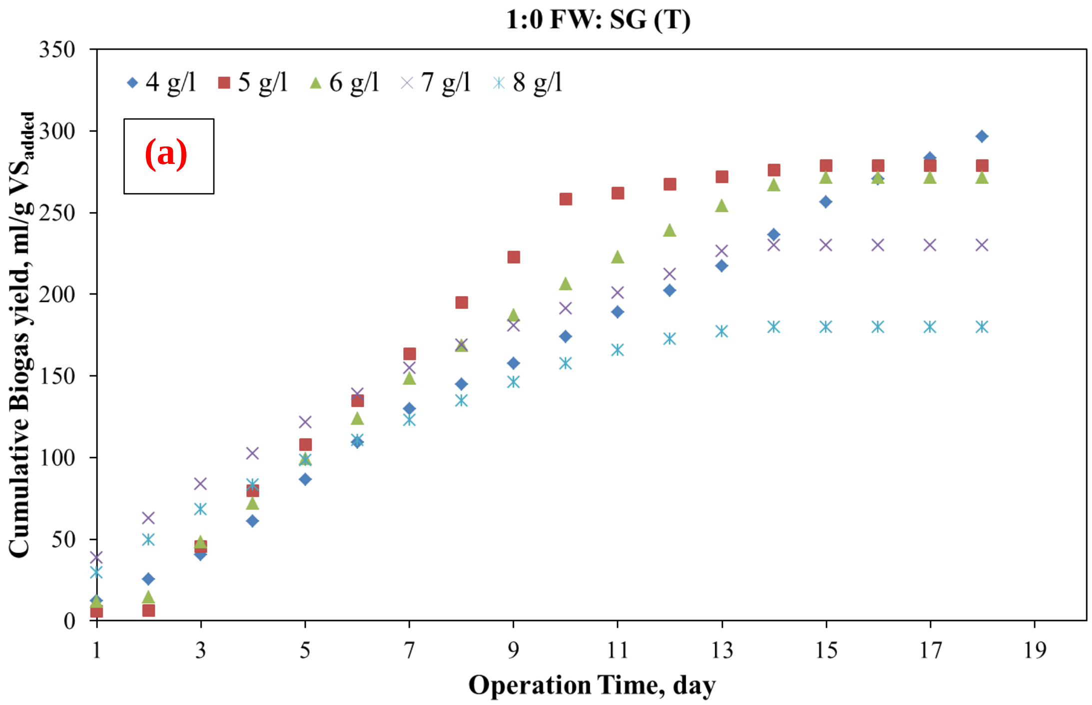
Figure 4.5 Cumulative biogas yield at mesophilic conditions (a) 1:0 FW: SG (b) 1:1 FW: SG (c) 0:1 FW: SG for different loading condition over digestion time

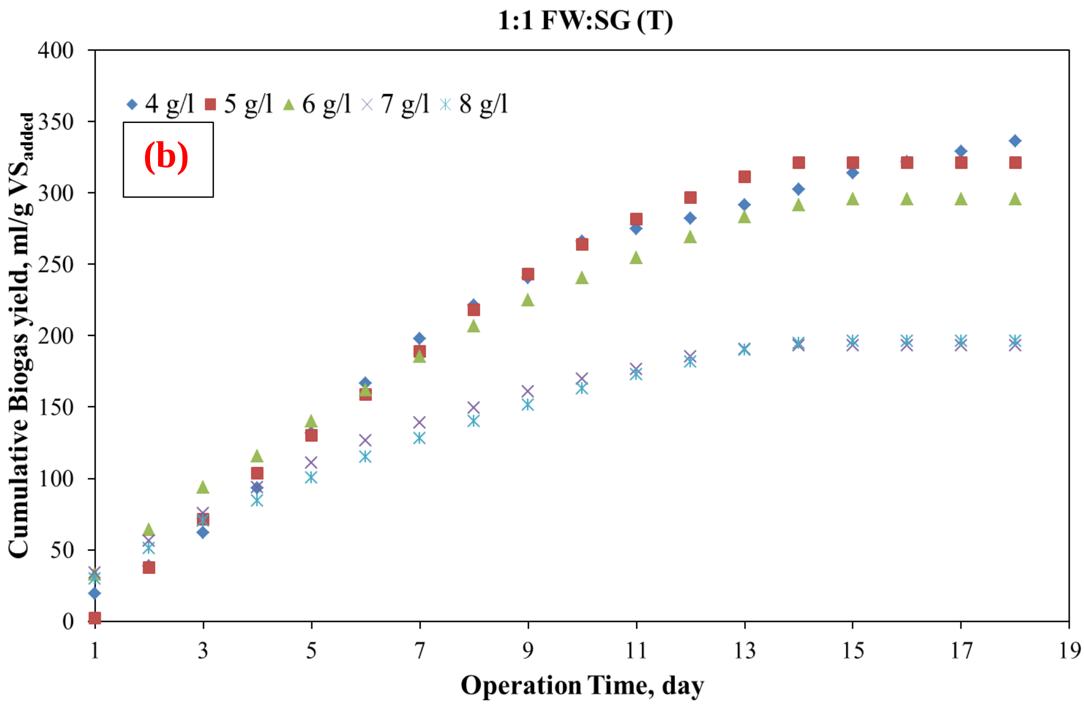
Likewise, biogas yield is between 180 to 296 mL/g VS for food waste at 55°C for 18 days digestion time. Thermophilic digestion yields the maximum biogas of 336 mL/g VS on 4 g/L at an equal mix proportion of FW and SG, however the maximum biogas yield at 4g/L loading in mesophilic condition is 386 mL/g VS. A similar trend of biogas is obtained for switchgrass at thermophilic operation, and the biogas yield is in the range of 109 to 251 mL/g VS respectively. In comparison with single substrate digestion i.e. with FW or SG, co-digestion with FW and SG yields the highest biogas from both the temperature conditions.

Results show that biogas yield under thermophilic condition is almost 90% in comparison to mesophilic condition and is achieved in 18 days (thermophilic) against 32 days (mesophilic). Lesser biogas yield occurred with a gradual increase in the feeding rate in both conditions.

On the other hand, the distress with methanogenic population occurs at shorter digestion time and unfavourable temperature, which affects the stability of the system. The substrate degradation is more rapid at thermophilic digester than mesophilic, and biogas yield is achieved in 18 days under thermophilic, whereas 32 days in mesophilic conditions. However, hydrolysis occurs at a faster rate for the quickly digestible substrate, especially food waste which initiates volatile acid production and sometimes inhibits the methanogenesis process (Suksong et al. 2016). Biomass utilized by acid-producing microorganisms (acidogenesis) reacts immediately with the microorganism to produce the intermediate compounds, which are volatile and leads to inadequate buffering, and the function of methanogens gets inhibited. Therefore, biogas production delays due to the imbalance between acidification and methanation phase. Co-digestion improves the balancing between acid and methane phase yielding the better performance. Multiple factors include the characteristics of biomass, the design of digester and operational parameters that can impact the biomethane potential (Zheng et al. 2015). It has been noted that the anaerobic digestion of switchgrass for biomethane production of 250 L/kg VS depending on seasonal variations (Frigon et al. 2012b) which are almost similar to our present study. Similarly, ensiled switchgrass yields the methane potential as 266 to 300 L/kg VS from switchgrass (Masse et al. 2010). Switchgrass produces greater methane yield

(102-145 L/kg VS) for thermophiles than the methane yield (88-113 L/kg VS) from mesophilic condition (Sheets et al. 2015).





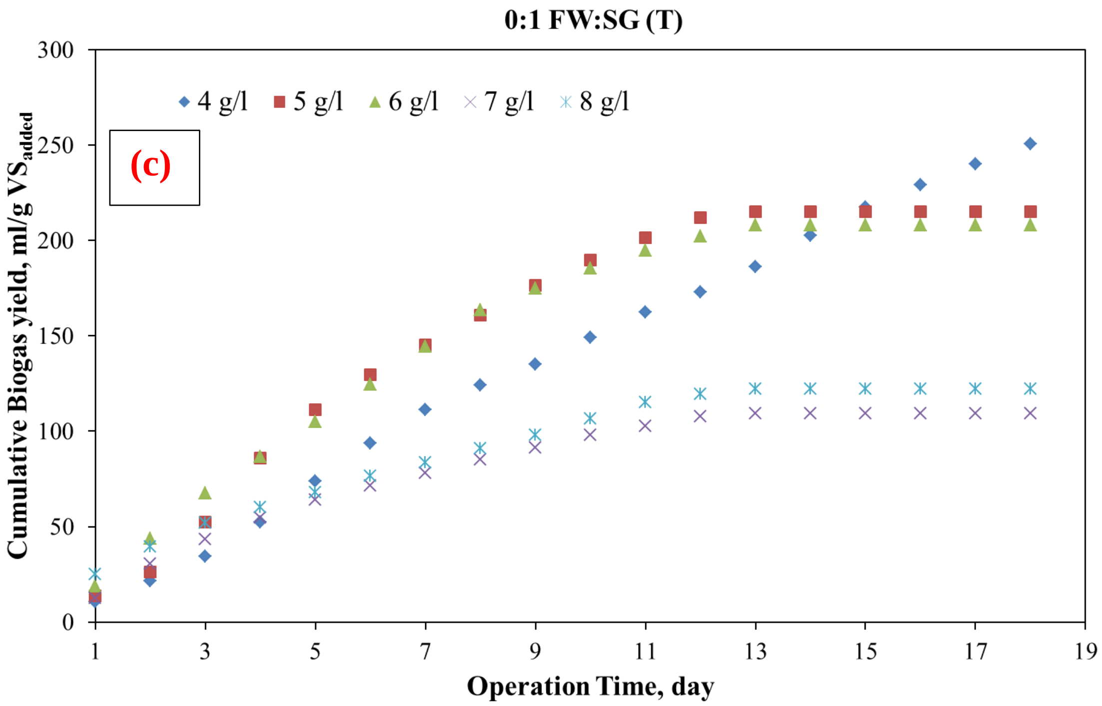
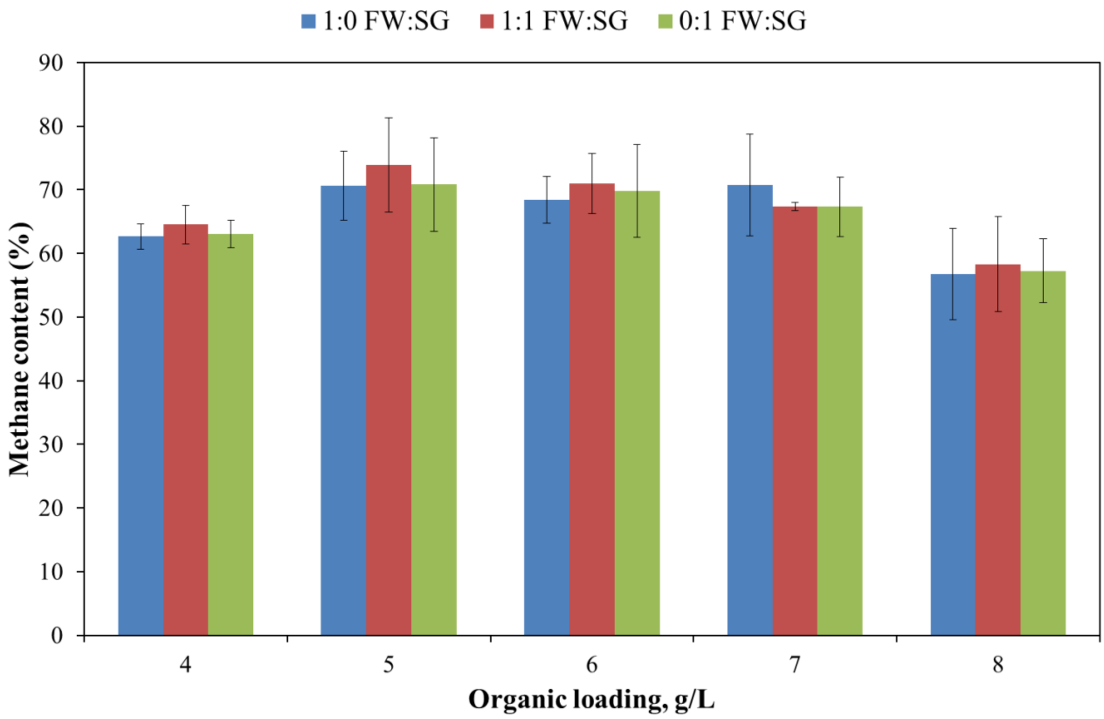


Figure 4.6 Cumulative Biogas yield at thermophilic conditions (a) 1:0 FW: SG (b) 1:1 FW: SG (c) 0:1 FW: SG for different loading condition over digestion time

4.4.2.2 Performance on biomethane content

Figure 4.7 showed the methane content obtained for the loading from 4-8 g/L. The methane content is found stable in the range of 50-75% for the entire mix ratio. Highest percentage i.e. 75 and 72 percentage (n=2) of methane content is obtained at 5g/L (1:1 FW: SG) for mesophilic and thermophilic condition respectively. The lowest methane potential observed is 52% (mesophilic) and 48% (thermophilic) for 1:0 (FW: SG) and 0:1 (FW: SG), respectively, at a loading of 8 g/L. The reason for the lowest methane content attained with food waste could be due to faster biodegradability and accumulation of volatile acids in the digester. An anaerobic digestion inhibition is observed with a 100% SG due to lack of survival of microorganism at the thermophilic condition and attributed to the building of volatile organic acid of pH 6.5 with switchgrass till the end of experiment.



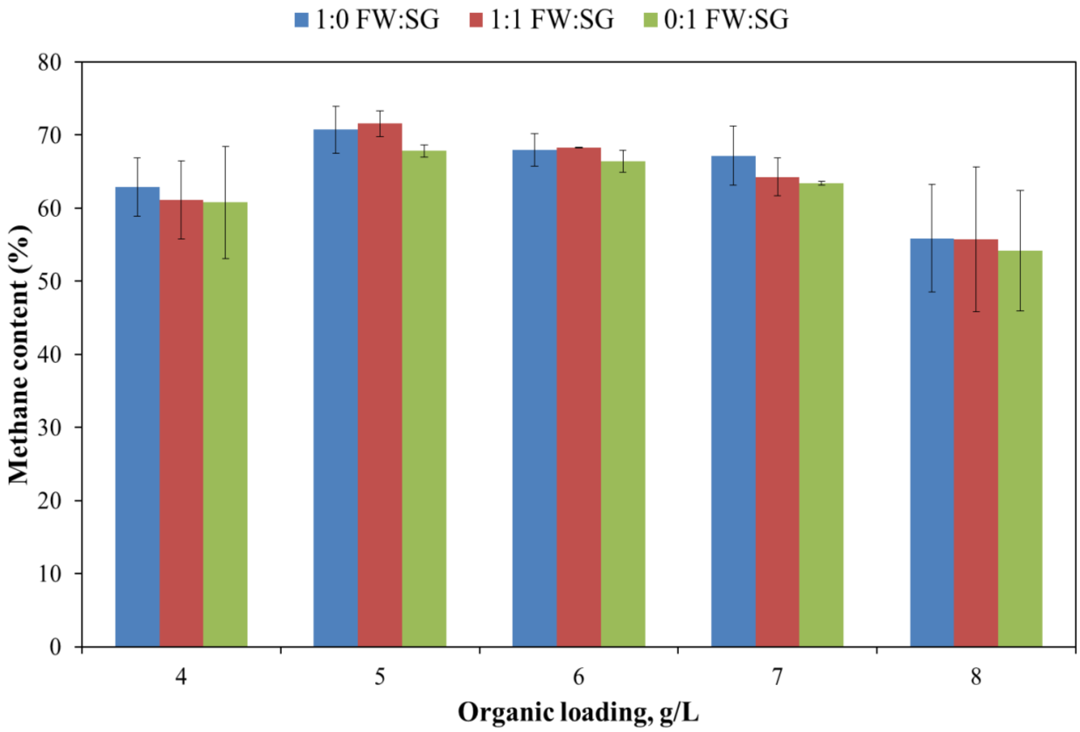


Figure 4.7 Methane content (a) Mesophilic (b) Thermophilic. Error bar represents the standard deviation on mix proportion at (n=2)

The consistency of the digestion is determined by studying the correlation between pH and VFAs. Buffering capacity is maintained in a bioreactor, with available buffer supplements to prevent souring and afford stability to the process. Results are also applicable for methane potential assessment in large-scale digesters especially with food waste, switchgrass or both mixtures.

Moreover, FW produces high methane due to the presence of highly biodegradable and nutrient content present in the nature of waste. The co-digestion result shows an increase of 3 to 5% compared to individual FW or SG digestion. The results show the mix ratio of 1:1 for methane yield, best with an equal proportion of highly biodegradable waste with the slow biodegradable waste. The reason for the highest methane yield depends upon the biodegradability of feedstock. The lowest methane potential is observed as 52% with the feed of 100% food waste with 8 g/L VS at mesophilic conditions. Lowest methane is attained with a single substrate at 100% feed, due to the faster biodegradability and production of volatile acid in an anaerobic digester. The volatile acid and methane were inversely proportional to each other depending on the products. The minimum methane potential is 48%, which is detected with a loading rate of 8 g/L with a single substrate digestion of switchgrass under thermophilic condition. As digestion continues, inhibition occurs with 100% SG due to lack of survival of micro-organism at the thermophilic condition and attributed to the building of volatile organic acid at the range of pH 6.5 with switchgrass until the end of the experiments (Wickham et al. 2016). The methane potential of biogas produced from lignocellulosic biomass is evaluated with batch digester that is comparatively around 77% correlated with the present study (Molinuevo-Salces et al. 2014). A statistical test performed for the methane content with mesophilic and thermophilic conditions which shows p-value greater than 0.05. There is no significant difference observed between the methane content for FW: SG at 1:0; 1:1 and 0:1 at 5% significance level respectively.

The process parameters such as operating temperature, loading rate and substrate to inoculum ratio significantly affects the methane potential (Yong et al. 2015). Food waste plays a major role for the substrate utilization for the production of methane potential instead of VFA produced by degrading FW related to system reliability and balance. Buffering capacity is present in bioreactor with buffer supplements to prevent souring and afford stability to the process. Specific quantity of inoculum is used as a source based on volatile solid content for loading condition. Probably, most promising environmental condition shows the presence of intermediate products and dissolved solids in the batch system (Siciliano et al. 2016).

4.4.2.3 Digestion performance index

Digestion performance index is analyzed to measure the methane potential with respect to theoretical methane yield based on the performance index. Table 4.3 shows the DPI for different loading at the mesophilic and thermophilic conditions. The digestion at a loading 5 g/L shows the synergetic effects with an equal proportion of FW and SG at both temperature profiles. In addition, 100 % SG (0:1) yields a synergistic effect at 5 g/L, which means biomethane potential obtained from the experimental condition is favorable with sufficient buffering and well-balanced pH in the anaerobic system. Except for co-digestion, individual substrates obtained the value less than one, this means, conditions are least suitable for effective digestion, thus emphasizing the importance of co-digestion of heterogeneous waste. Except for co-digestion, individual substrates obtained the value less than one that showed the antagonistic effects of having competitive effects on system imbalance between volatile acid and pH variation nearer to neutral range resulting in possible inhibitions. To reduce the inhibition levels, the digestion process is more consistent with mixing, feed and retention time, this help the digester function more significantly. It permits to balance the function throughout the reactor and prevents toxin accumulation as the blemish layer on the surface. Overmixing leads to a reduction in surface tension of solids accumulating over the liquid.

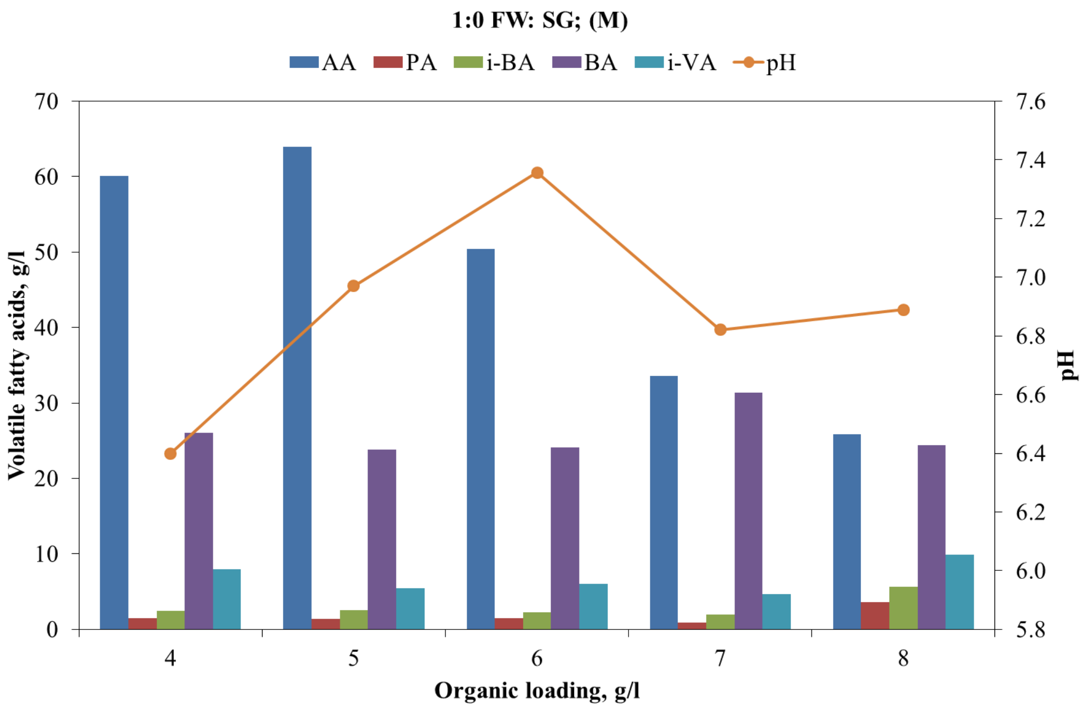
Retention time depends on the feed of solids, the operating temperature with the different mode of operation. Usually, mesophilic requires 20 to 25 days and 10-15 days for thermophilic operation in which solid requires the highest retention than liquid. Higher retention time required for solids are due to the presence of nutrients and have high potent on methane-producing bacteria. Ultimately, slight process hindrance occurs as the function of digester significantly affects the role of short chain fatty acid and pH in an anaerobic digester.

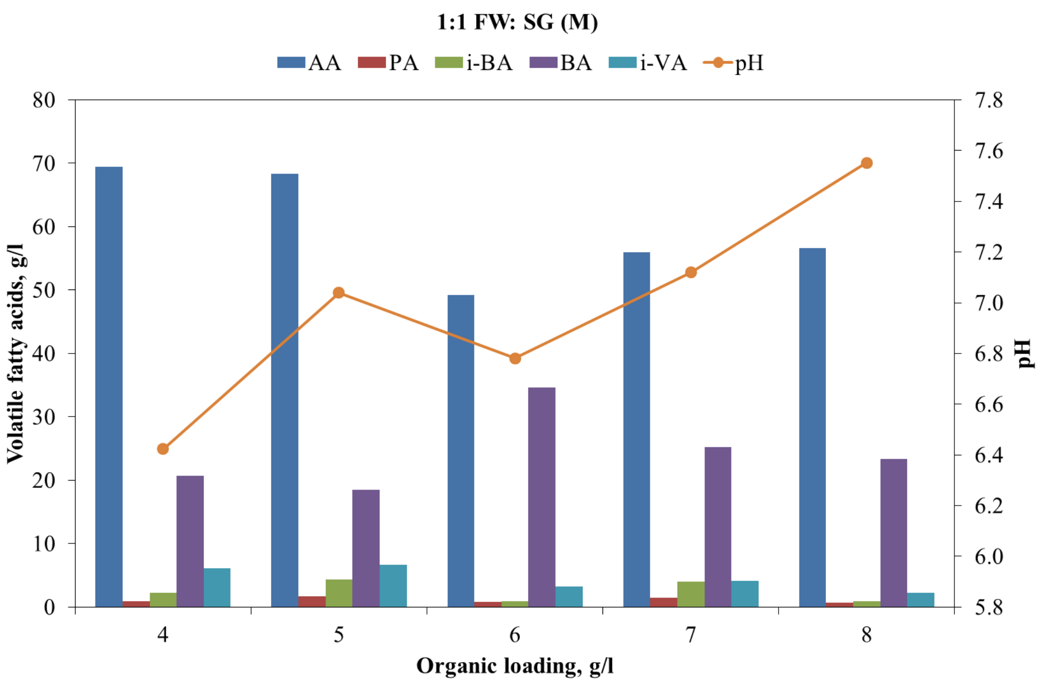
Table 4.3 Digestion Performance Index on FW and SG

DPI	Mesophilic			Thermophilic		
FW: SG Loading	1:0	1:1	0:1	1:0	1:1	0:1
4	0.83	0.88	0.91	0.69	0.88	0.62
5	0.98	**1.05**	**1.06**	0.79	**1.05**	0.66
6	0.72	0.77	0.81	0.74	0.77	0.63
7	0.72	0.77	0.71	0.63	0.77	0.31
8	0.42	0.44	0.31	0.36	0.44	0.27

4.4.2.4 Role of pH and volatile acids on digestion performance

Figure 4.8 and 4.9 shows a similar trend of pH and VFA with loading from 4-8 g/L at the mesophilic and thermophilic conditions. The pH shows the variations in trend line with a gradual increase in loadings from 4 to 8 g/L with mesophilic and thermophilic temperatures. For single substrate, FW and SG show the average pH of 6.89 and 7.10 whereas for 1:1 FW: SG is obtained as 6.98 at mesophiles. In the case of thermophilic, pH is 6.78, 7.00 and 6.76 for FW, SG and 1:1 FW: SG digestion. Maintaining the optimum pH for both thermophilic and mesophilic temperatures throughout the digestion satisfies the biomethane potential. The methane yield is approximately 67% for mesophiles and 63% for thermophiles from all loading (4 g/L to 8 g/L) conditions. The balance between acidogenic and methanogenic phase is well maintained which is strongly depends on the buffering capacity during digestion. In addition, the role of pH is considered as the bridge indicator between VFA and the fraction of CO_2 in bio-digester (Liu et al. 2008). As soon as unease occurs with stable digestion, owing to retention time or organic solids it tends to increase. Acetate converts promptly to methane and carbon dioxide. The other VFA concentrations such as propionate, butyrate and valerate and its isomeric forms considerably remain constant for longer digestion time.


1:0 FW: SG; (M)
AA
PA
i-BA
BA
i-VA
pH
Volatile fatty acids, g/l
pH
Organic loading, g/l
0
10
20
30
40
50
60
70
5.8
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
7.6
4
5
6
7
8


1:1 FW: SG (M)
AA
PA
i-BA
BA
i-VA
pH
Volatile fatty acids, g/l
pH
Organic loading, g/l
0
10
20
30
40
50
60
70
80
5.8
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
7.6
7.8
4
5
6
7
8

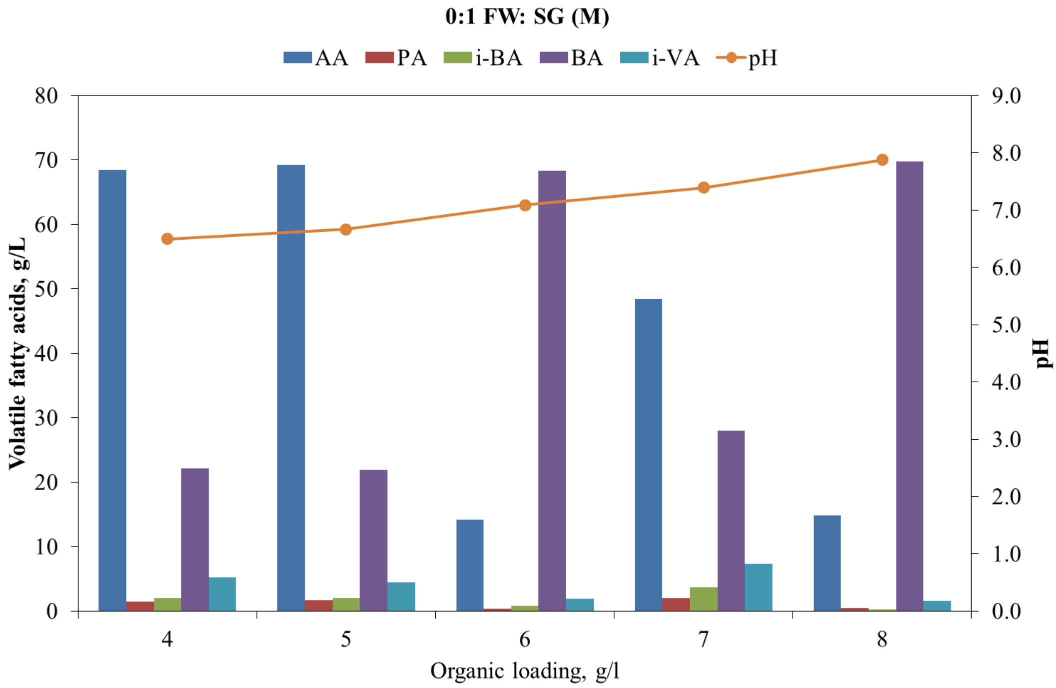
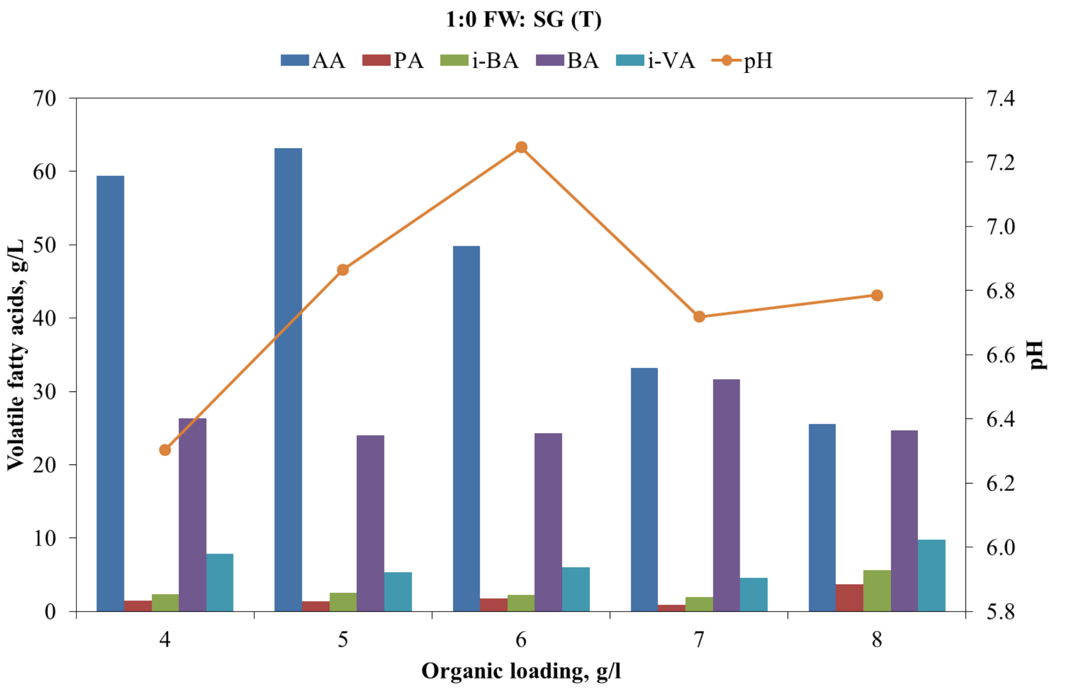
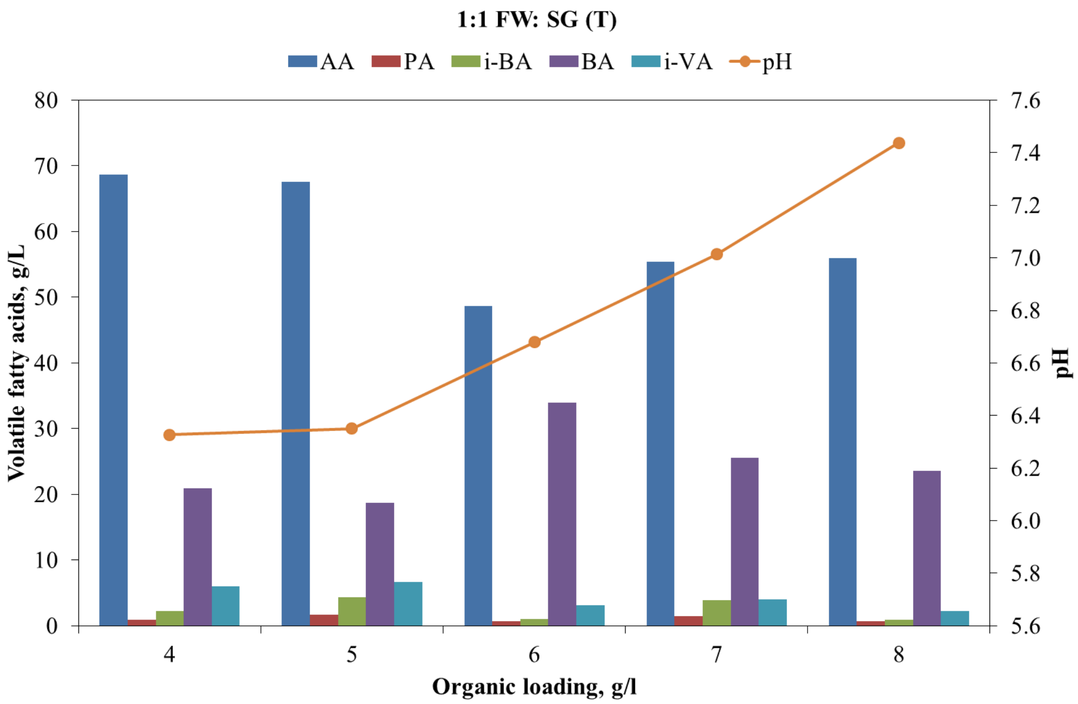


Figure 4.8 pH and VFA concentration on organic loading condition at mesophilic (1:0; 1:1; 0:1 FW: SG)

The solid content during the initial stages of digestion supplies a strong organic acid during digestion that occurs in a system, which is utilized by methanogens, and accelerates the methane yield. In connection with the above, the imbalance on stability is maintained by monitoring the volatile acid concentration in the digestion system. Major components of VFA were acetic, propionic, butyric and valeric acid that play a dynamic role on methane content. The results show the acetic and butyric acid concentrations are high during digestion, and the remaining acids (propionic, valeric and i-forms) are present at a lower concentration. The possibilities of VFA accumulation occur from insoluble macromolecular organic polymers such as carbohydrates, protein and fat by hydrolyzing micro-organisms and hence VFA is consumed by methanogens (Zhai et al. 2015). In addition, three parameters pH, alkalinity and volatile acids are interrelated with each other, and are responsible for conversion as hydrogen, methane and carbon dioxide respectively. In reality, the acetic acid and hydrogen are formed by acidogenic and acetogenic microbial activity utilized by methanogens and methane, which are produced during stable operating conditions. Subsequently, volatile acids maintains the anaerobic digester with the presence of a lower concentration of carbonates which is less consumed by microbes through steady maintenance of pH in the system (Gunaseelan 1995).





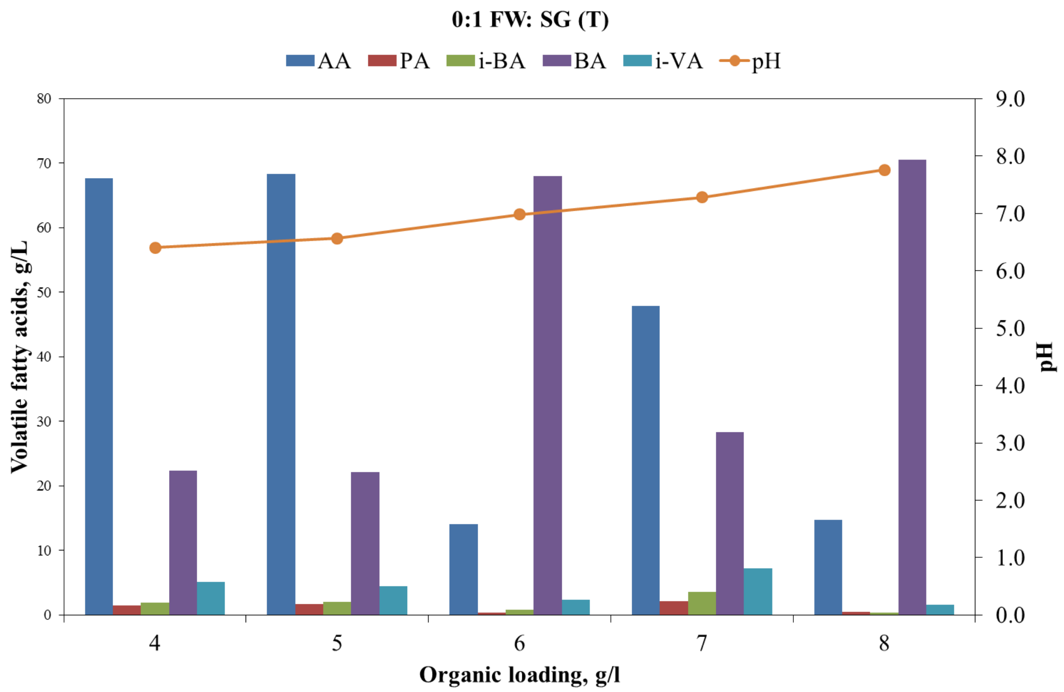


Figure 4.9 pH and VFA concentration on organic loading condition at Thermophilic (1:0; 1:1; 0:1 FW: SG).

4.4.2.5 Relationship between pH, volatile acids and methane content

Methane content shows the identical trend for all the mix ratios and loadings in mesophilic and thermophilic condition. Methane produced is in the range of 52% to 79% for 30 days retention time in mesophiles and 48 to 73% with 18 days retention time for thermophilic conditions respectively. The VFA accumulation occurs during acidification with both temperatures indicates a mild variation in methane profile. VFA showed a significant relationship between pH and methane irrespective of acid production (Figure 4.9). The value of methane confirmed the satisfactory relationship with pH. The pH observed above 6.0, showed good methane profile at both temperatures. The methane production is optimum with the pH at 6.5 to 8.5 for suitable methanogenic activity. It is highly recommended to maintain the pH in an appropriate range to produce methane content. In addition, temperature profile plays an active role and is related to the volatile acid production during digestion.

The temperatures at 35°C and 55°C correlate with tVFA produced with 4-8 g/L loading condition. At thermophilic temperature (55°C), butyric acid concentration shows the pH 6.8 as similar as mesophilic conditions. About i-butyric acid, pH above 6.2 shows a less concentration at either 35°C or 55°C that exists in isomeric form and even follow butyric acid concentration. The iso-Valeric acid is the indicator for determining its process instability in comparison with all acids, even though the volatile acids do not affect the methane production. The presence of i-valeric acid as in the isomeric form of valerate is in less concentration and this is observed in our study. Therefore, the amount of valerate confirmed the stability of acid accumulation in the system. The propionic acids produced from all the digestibility condition are similar. In connection with the above results, fatty acid producing microorganisms do not show direct consequence with the temperature profile and minor changes occur in the diversity of microbial consortia. Lesser VFA level is described by enhancing re-utilization and lower production due to the decay phase of biodiversity of mixed culture at thermophilic condition (Stein et al. 2017). The combined VFA and other physical conditions stress the behaviour of a microbial consortium.

The other portion of volatile acid composition is acetic acid, which plays a significant role and has obtained the maximum amongst all the components with both mesophilic and thermophilic conditions. Therefore, possibly the presence of acetic acid shows higher concentration that inhibits the system even with a pH at 7 respectively. Methanogens are extremely sensitive to pH but acetogens shows less susceptibility and function at extensive pH range. Consequently, this acid acclimation indicates the imbalance between acid producer and the consumers connected with the buffering capacity of the digester. Considering the inhibitory effect of acetic acid, the addition of sufficient buffer maintains the optimum concentration of volatile acids in the fermentation system (Franke-Whittle et al. 2014).

4.4.2.6 Effect of t-VFA to total alkalinity ratio

Table 4.4 shows the data for initial and final VFA/Alkalinity ratio for the batch digestion. The presence of alkalinity prevents pH fluctuation in a bio-digester. Stability depends on pH, which is significant to monitor the relationship between alkalinity and volatile acids of the system. A volatile acid to alkalinity is a valid indicator to monitor consistency of the digestion. Methanogens survival at 0.2-0.3 was satisfactory with VFA/Alkalinity (Meng et al. 2014). The digesters required VA/Alkalinity at 0.34 or lesser at a constant temperature. The VFA to alkalinity ratio expressed the balance between acidification and methanation phase. Mesophilic conditions attained is 0.2 to 0.9 whereas 0.3-1.5 for the thermophilic condition, which, means higher production acid to alkalinity ratio showed the unbalanced state between acidogenesis and methanogenesis which complicates during digestion. The ratio above 0.5 indicates the unstableness, the process performance with pH upsets with fluctuations. Also, the presence of higher volatile acids leads to rapid accumulation of acidogens rather than methanogens which inhibits the production of methane content (Zheng et al. 2015).

A drastic drop in pH occurs with insufficient buffering capacity and inhibits the function of methanogens by interrupting the performance of digestion systems. The optimum pH between 7.0 - 7.5, which favors methanogenic bacteria, can function within this range. At, pH 6.2, all methane-forming bacteria mechanize well and lead to a build-up of volatile acids. These acids build-up in an anaerobic digester occur at lower pH to optimum pH levels. While temperature is related to the VFA/alkalinity ratio, the release of acids occurs in the shorter retention time with thermophilic owing to faster hydrolysis rate than at mesophiles during digestion. Steady-state is maintained with the volatile solid consumption by methanogens and it signifies the existence of alkali residues sustaining the buffering capacity of the system (Ahn et al. 2010). The consumption of alkali by microbes shows the indication between volatile acid and pH for maximum biogas yield that indicates stable performance. In addition, the presence of excess buffer causes the development of alkaline ions to boost the nitrogen content and wedge the pH in the system. When pH is increased above 6.2, it can be restored by bicarbonate/carbonate salts, which exist as co-forms with sodium and potassium respectively. An assorted group of carbonate/bicarbonates are toxic above 100-200 mg/L resulting in encapsulated white clusters formed on the surface of the bioreactor (Gerardi 2003).

Table 4.4 t-VFA and Alkalinity in batch operation

Process parameters	Mesophilic			Thermophilic		
	1:0	1:1	0:1	1:0	1:1	0:1
Loading (g/L)		4 to 8			4 to 8	
i-Alkalinity (g/L)	7.0 ~9.3	5.4~10.0	5.1~10.4	4.1~6.7	4.1~7.5	3.8~7.8
f-Alkalinity (g/L)	4.4~8.8	3.4~9.5	2.97~9.9	2.2~6.6	2.3~7.2	2.2~7.4
i-VFA (g/L)	6.4~6.7	2.4~7.0	1.8~7.1	4.7~8.3	3.0~8.7	2.3~8.9
f-VFA (g/L)	0.9~4.9	1.4~3.8	1.3~3.5	1.2~6.1	1.7~4.7	1.5~4.4
i- tVFA/ alk.	0.7~0.9	0.5~0.7	0.4~0.7	1.2~1.6	0.8~1.2	0.6~1.2
f-tVFA / alk	0.2~0.9	0.3~0.8	0.26~0.9	0.4~1.5	0.5~1.3	0.4~1.5

4.5 Semi-continuous digestion

The aim of semi-continuous digestion is focused on co-digesting food waste and switchgrass at different loading and mix ratios for biogas production. The experiments were conducted in mesophilic (35°C) temperature at specific time intervals. Process parameters such as pH, alkalinity, ammonia nitrogen, VA-to-TA ratio were evaluated for the performance of digestion. Additionally, environmental significance on co-digestion of substrates is a viable alternative for bioenergy production.

4.5.1 Performance on biogas yield and biomethane potential

The maximum biogas yield is 628 mL/g VS for 4 g/L loading. The average biogas yield for the loadings were 5 g/L (544 mL/g VS), 6 g/L (457 mL/g VS), 7g/L (422 mL/g VS) and 8 g/L (335 mL/g VS). Figure 4.10 depicts the cumulative biogas yield obtained from T1 to T3 loading with FW and SG at semi-continuous digestion. Biogas yield is stable for every T1 to T3 and a gradual decrease in yield is observed with the increase in loading conditions. The average biogas volume ranges between 1900 to 2500 mL over the digestion time of 15-20 days with each loading condition and maximum volume obtained for the loading 4 g/L for the trials. It had also been reported that co-digestion of multiple substrates improve the system stability and increase the total biogas yield. Various researchers focused on anaerobic co-digestion of food waste and yard waste at a specific ratio which could improve the operating characteristics (Brown and Li 2013; Li et al. 2014).

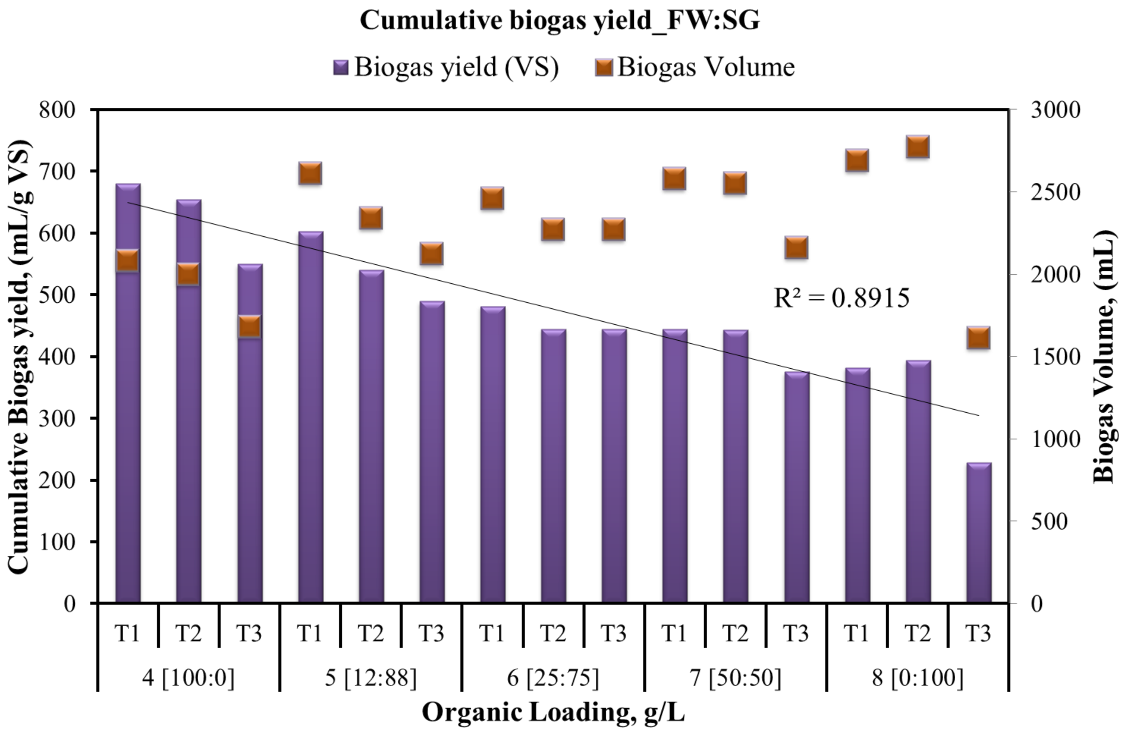


Figure 4.10 Cumulative biogas yield for semi-continuous digestion

Figure 4.11 shows the biomethane profile for the semi-continuous digestion. Methane and carbon dioxide for all the trials were in the range of 57% to 74% and 30% to 45% respectively. The methane percentage obtained is 4g/L (65.18%), 5g/L (67.15%), 6g/L (70.42%), 7g/L (70.08%), and 8g/L (56.89%) for the loading from 4-8 g/L. The hydrolytic carbon dioxide producing micro-organisms were active below the loading 6 g/L. The process efficiency improves the biogas productivity at 4 and 5 g/L loading and is satisfactory with heterogeneous co-substrates.

Methane content shows the highest potential for food waste, which is due to fast rate of hydrolysis, and stable performance that occurs with co-substrate digestion. Methane content is lower for switchgrass (less biodegradable) alone, which is below 60%, occurs in semi-continuous operation. It has been noticed that biomethane yield obtained from a mixture of organic waste found at 50% to 60% and declined in the methane potential with increased loading rates. Likewise, Li et al. (2014) has noticed that methane yield is lower for the continuous operation compared to a batch digestion with chicken manure (fast biodegradable) and corn stover (slow biodegradable) as a feedstock which may be unfavorable because of high energy density, easily degradable substrates and the green waste etc.

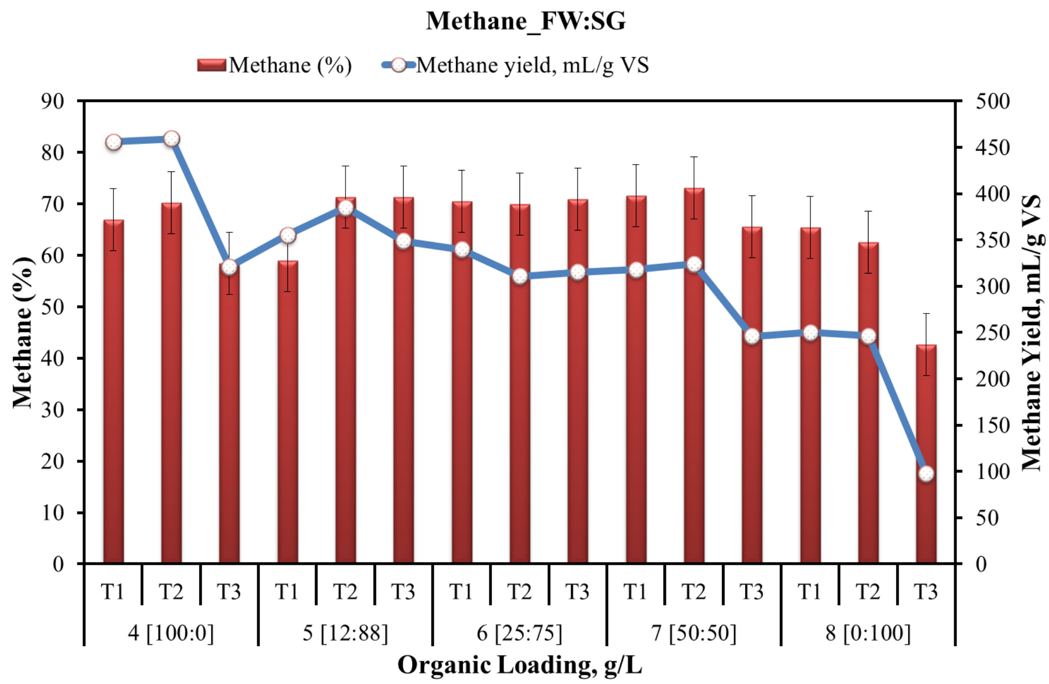
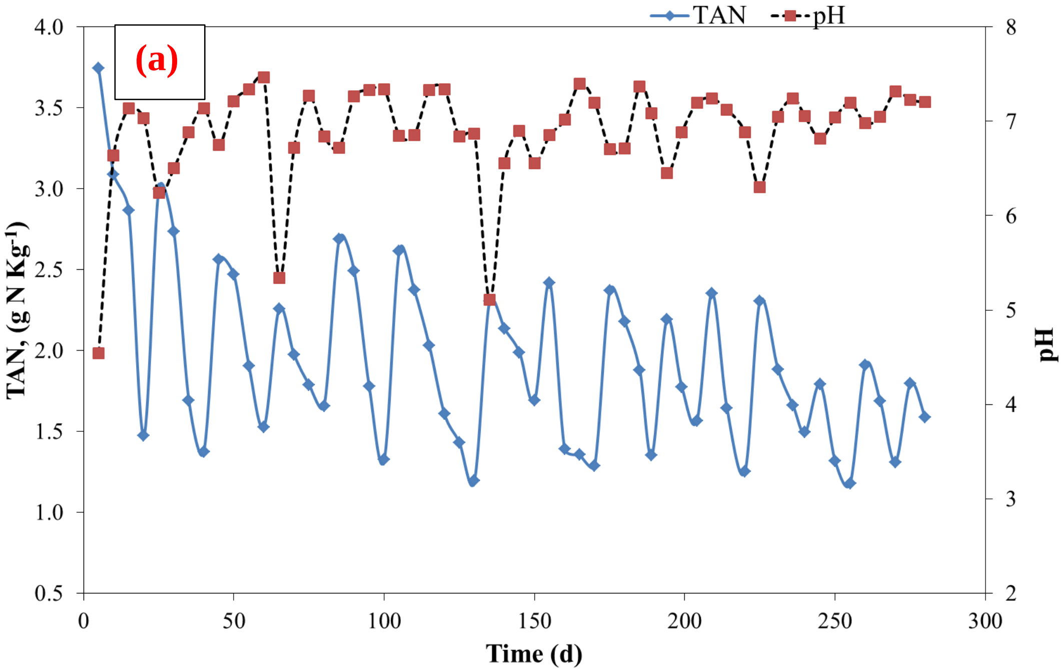


Figure 4.11 Methane content

4.5.2 Effects of process parameter on semi-continuous digestion

The effect of process parameter is responsible for monitoring the performance of substrate degradation throughout digestion. The pH below 6.5 represents the acidogenic phase. The pH is maintained in the range of 6.3 to 7.3 for the co-digestion mix in anaerobic digester. At the initial stages, drop occurs to some extent due to hydrolytic acidification of organic compounds. Also, pH drop caused by the presence of complex fatty acid compounds in food waste becomes toxic to methanogens and limits the conversion of acidogenic to methanogenic phase (Tian et al. 2015).

Figure 4.12 (a) shows the TAN and pH over time at organic loading rate from 4-8 g/L. At loading 4 g/L, TAN Varies from 1.4-3.7 g N kg^{-1} and ammonia concentrations ranges from 1.17 to 2.68 g/kg for the remaining loadings of 5-8 g/L. Alkalinity concentration is obtained in the range of 4.37 to 7.89 g $CaCO_3$ $kg^{-1}$and it is higher for the loading 4 g/L compared to other loading conditions. The pH maintained between 4.0 and 7.0, shows the range for acidity-alkalinity condition of the digestion process. The gradual degradation of ammonia is observed with each loading rate, which is then converted to organic compounds. Alkalinity is closely interrelated with ammonia nitrogen. The relationship between alkalinity and volatile acid-to-alkalinity ratio in respect to time were shown in Figure 4.12 (b). The volatile acid/alkalinity ratio is 0.34-0.78 for the loading 4 g/L and other loading are in the ratios 5g/L (0.19 to 0.71), 6 g/L (0.39 to 0.69), 7 g/L (0.42 to 0.83) and 8 g/L (0.39 to 0.59) respectively. This ratio indicates that the system is stable even at increasing loading rates. According to the overall results, the increasing loading rate influences the process stability confirmed by alkalinity, pH, and volatile acids during digestion.



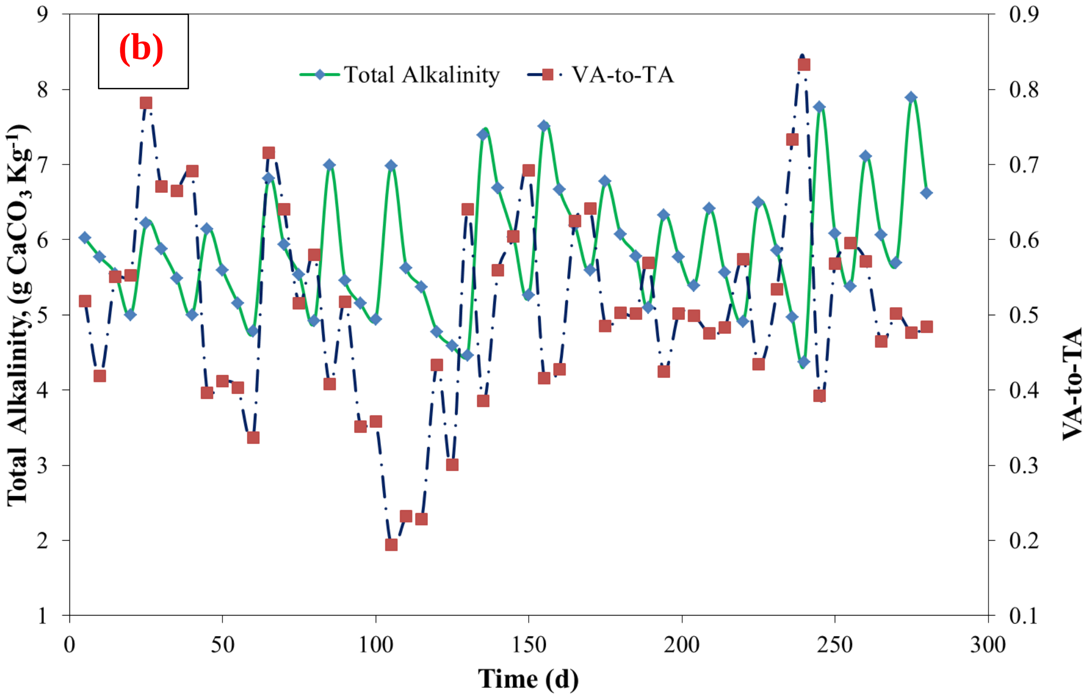


Figure 4.12 (a) Trend of TAN, pH Vs time (b) Trend of Alkalinity, VA-to-TA Vs Time

4.5.3 Environmental significance on co-digestion of food waste and switchgrass

The synergistic effect in co-digestion of food waste and switchgrass improves the methane yield. Assuming, from the food waste produced from hostels as1 ton and 1 ton of switchgrass that is taken into consideration. 35% of electrical efficiency and 50% of thermal efficiency is assumed to evaluate the net energy output (Dahunsi et al. 2017; Jugal Sukhesh and Venkateswara Rao 2018). The bioenergy calculated for FW: SG loading rate of 4-8 g/L were 1900 to 2500 mL of biogas in the semi-continuous mode. Methane produced from all the loadings were in the range 57-71 %.

The average value of methane production is 71% (320 mL/g CH_4). Then the co-digestion mixture is estimated to generate 32,000 m^3 CH_4 yield. The Combined Heat and Power (CHP) system facility can produce electrical energy and thermal energy from organic wastes for the methane production. The electrical energy is used for street lighting and other domestic purposes for the economic development. The net thermal energy is used for boiling and other industrial utility. The methane production by anaerobic co-digestion reduces the payback period of investment. Co-digestion adopted by single digester makes an economically viable alternative for bioenergy production. The results agree for the utilization of CHP system implementation to evaluate the electrical and thermal efficiencies by 35% and 50%. CHP systems are widespread across many countries for energy conversion in a well-balanced system. The concept of co-digestion simplifies the different substrate combination with integrated organic waste management for the hygienic environment.

CHAPTER 5

SUMMARY AND CONCLUSION

Organic waste conversion continues to be of major importance in the field of solid waste management. This study is performed with the objective to investigate the performance of anaerobic digestion potential in food waste (FW) and switchgrass (SG) by pretreatment and co-digestion on laboratory scale. The efficiency of pretreatment and co-digestion is assessed to compare the operational performance of anaerobic digesters by treating switchgrass and food waste in batch and semi-continuous digestion respectively.

Based on the present study, the following conclusions are drawn:

5.1 Batch digestion

5.1.1 Switchgrass pretreatment and performance evaluation

- Physical and chemical pretreatments such as LHW, thermal, 1% acid, 1% alkaline, liquid hot water, thermal and organosolv were adopted for switchgrass. The pretreated switchgrass shows the modification in morphological and chemical composition that was analyzed through SEM, and FTIR. The change that occurred in cell wall matrix improves the accessibility, which enhances hydrolysis during digestion.
- Maximum biogas yield is 583 mL/g VS obtained from alkali treated SG, which shows the efficacy of alkali treated SG towards accessibility to cellulose and hemicellulose during digestion.
- Biomethane yield from raw switchgrass is 248 mL/kg VS in batch digestion. A rise in biomethane yield by 53%, 52%, and 12% for alkaline, organosolv and thermal pretreated switchgrass is observed. However, a decrease by 44% and 20% is noticed for LHW and acid pretreated switchgrass as compared with raw switchgrass. A higher biomethane yield confirms the enhanced biodegradability of switchgrass with alkaline and organosolv pretreatments.

- As per inhibition study, acid treated SG and LHW treated SG showed least biomethane yield due to a faster rate of hydrolysis and the acid accumulation in acidogenesis stage, which causes inhibition in methanogen and retards the methane yield. Therefore, chemical pretreatments such as alkaline and organosolv are the best treatment methods for SG, compared to the other pretreatments (acid treated and LHW). The biomethane potential depends on various factors such as inoculum sources and the effect of inhibitors that has an impact on digestion performance during batch digestion.
- There was no substantial dissimilarity observed in methane content produced from raw and pretreated switchgrass, except the slight variation observed from acid-pretreated switchgrass.
- The operational parameters such as pH, alkalinity, and volatile acids are the mandatory functions to be maintained to balance between parameters that yield better performance on methane potential. The digester performance showed the pH between 6.0 to 8.0 and alkalinity in the range 5.42 to 8.25 g $CaCO_3$/L, there is a balanced bridge indicator between volatile fatty acids and alkalinity reactions throughout the digestion.
- For inhibition study on biogas volume, least volume of biogas is observed with acid and LHW pretreated SG.

5.1.2 Co-digestion test

- Batch digestion is the realistic approach for digesting FW and SG in mesophilic (35°C) and thermophilic (55°C) conditions. The biogas yield obtained in the range 205 to 345 mL/g VS for FW and 128 to 331 mL/g VS for SG in the single substrate digestion in mesophilic condition. The equal proportion of FW: SG yields the maximum biogas of 337 mL/g volatile solids. Approximately, 68 % to 72% of biogas yield is achieved within 15 days of the total digestion period.
- Similarly, FW yield of the cumulative biogas is in the range between 180 to 296 mL/g VS and 109 to 251 mL/g VS for SG at thermophilic condition. Compared to single substrate digestion either FW or SG, co-digestion yields the highest biogas yield at thermophilic condition. The degradation of

feedstock occurs more rapid in thermophiles. The possible distress of microbial population occurred over the shorter detention time that affects the process stability of digestion.

- The maximum biomethane yields are 267 and 234 mL/g VS produced from 1:1 mix ratio FW and SG at both mesophilic and thermophilic profiles. Co-digestion is an environment-friendly approach to recover methane at various temperature profiles which reduces GHG emissions.
- The highest percentage of methane content is 75% and 72% produced from (1:1 FW: SG) both temperature profiles. Gradual increase in loading yields a least methane potential observed from both temperatures.
- The digestion performance depends on various operational parameters (pH, alkalinity and volatile acid) which stabilizes the biomethane yield. Results confirm that, the equal proportion of FW: SG yields better methane by maintaining balance between the operational parameters in the single digester.
- Digestion performance index shows the synergetic effects with 5 g/L (1:1 FW: SG) at both 35°C and 55°C means biomethane potential that is favourable with sufficient buffering and well-balanced anaerobic system emphasizing the importance of co-digestion of heterogeneous wastes.
- Process parameter such as pH and volatile acid plays a vital role, which maintains the satisfactory relationship with methane content. The balance between acidogenic and methanogenic phase reflects on the function of bio-digester.
- The volatile fatty acids such as acetic, propionic, butyric and valeric acids show a dynamic role on the performance of digestion. The acetic and butyric acid concentration yields highest concentration and other acids are present in the trace level.
- A t-Volatile Fatty Acid to Alkalinity (t-VFA/alk) ratio is the indicator to monitor the consistency between acidification and methanation balance in the digestion. The t-VFA/alk ratio is obtained as 0.2 to 0.9 for mesophilic, whereas 0.3-1.5 for the thermophilic condition. Temperature is related to VFA/alkalinity ratio that means, the release of volatile acids occur with thermophilic than mesophilic in shorter retention time.

- Result concludes that thermophilic digestion offers efficient biomethane potential over mesophilic in shorter operation time. The organic waste utilization rate is contributing in better nutrient consumption from microbes, which favors the nutrient level, environmental condition and these are key factors to monitor the performance of digestion.

5.2 Semi-continuous digestion

- The performance of FW and SG co-digestion were analyzed for the organic loading of 4 to 8 g/L with different mix ratio at mesophilic condition in laboratory scale. The results yielded 628 mL/g VS of biogas with 70% methane content obtained for the loading of 4g/L and it was observed that biogas yield reduces with high loading rates.
- Co-digestion of food waste and switchgrass also gives an average of about 500 mL/g VS of biogas yield. The results suggest that co-digestion could be a reliable option to deal with a mixture of waste with different characteristics.
- pH is maintained in the range between 6.3 to 7.3 throughout the digestion. At initial stages of loading, the drop in pH is observed in the digester that is the representation of acidification, produced from organic acids. The organic acids are neutralized by adding buffering reagents, which converts the acidogenic phase to methanation phase during the process of digestion.
- The volatile acids-to-alkalinity ratio maintain in the range between 0.2 to 0.8 for the loading from 4 to 8 g/L respectively.
- The overall results conclude that the process stability has positive response on increased organic loading which is confirmed by interlinking of the operational parameters (alkalinity, pH, and volatile acids) during digestion.
- Overall, the average methane content is 71% produced from FW and SG along with favourable operational parameters. Approximately, methane yield is estimated as 32000m^3 by assuming FW and SG production about 1 ton when each is taken for consideration. Combined Heat and Power (CHP) system facility implements 35% electrical energy and 50% thermal energy widespread across countries for the energy conversion in the well-balanced system.

- This application of electrical energy is used for domestic purposes, and thermal energy minimizes the need of natural biofuels. This concept of co-digestion simplifies the organic waste management for the hygienic environment.

5.3 Scope for future work

The anaerobic bioreactor could be scaled-up for small-scale industrial units and municipal treatment plants to treat the waste at higher organic loading rates for the biogas production and biomethane potential.

Studies on microbial consortium and their behaviour on bioreactors could be correlated with the modelling studies for recovering the methane content from heterogeneous organic wastes

Modelling is an excellent tool for pilot plant design, operation, and their modification in biochemical reactions in anaerobic digester. To optimize the biological treatments (i.e. model) or identifying the best valorization pathway for the organic waste digestion. Certainly, this developed tool can be used as an indicator for predicting aerobic/anaerobic biodegradability of organic residue that are the potent contributions to organic carbon stock.

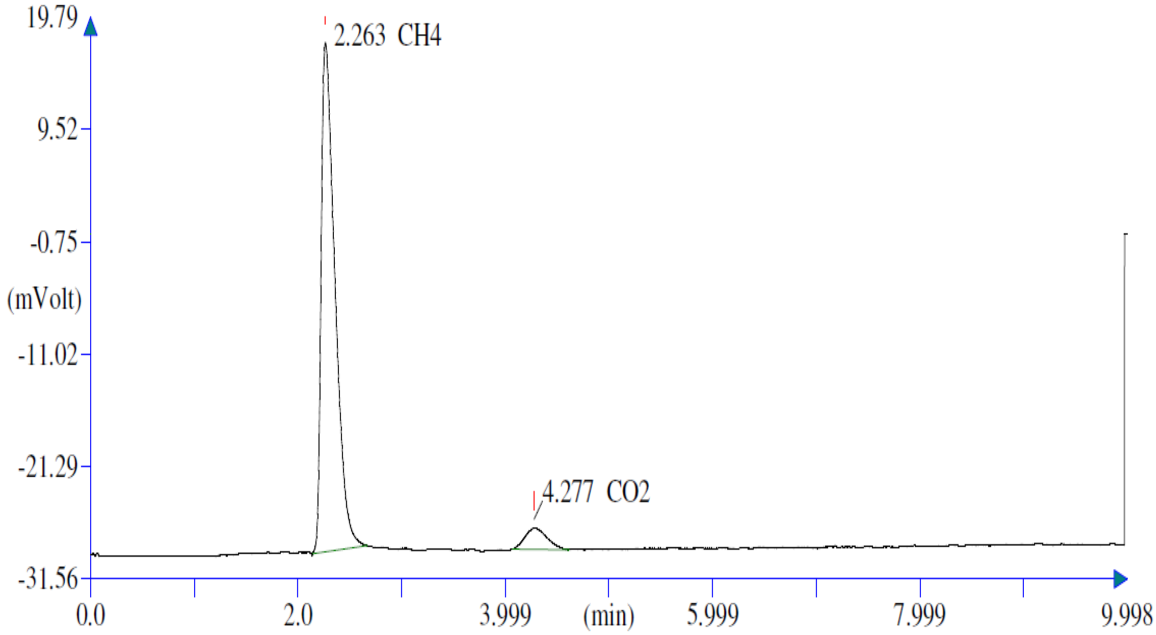
Further, it can be used to encourage clean and pollution free environment. Methane content is streamlined and reformed to other form of bioenergy sources, which could be utilized for transportation applications to develop self-sustainable system.

APPENDICES

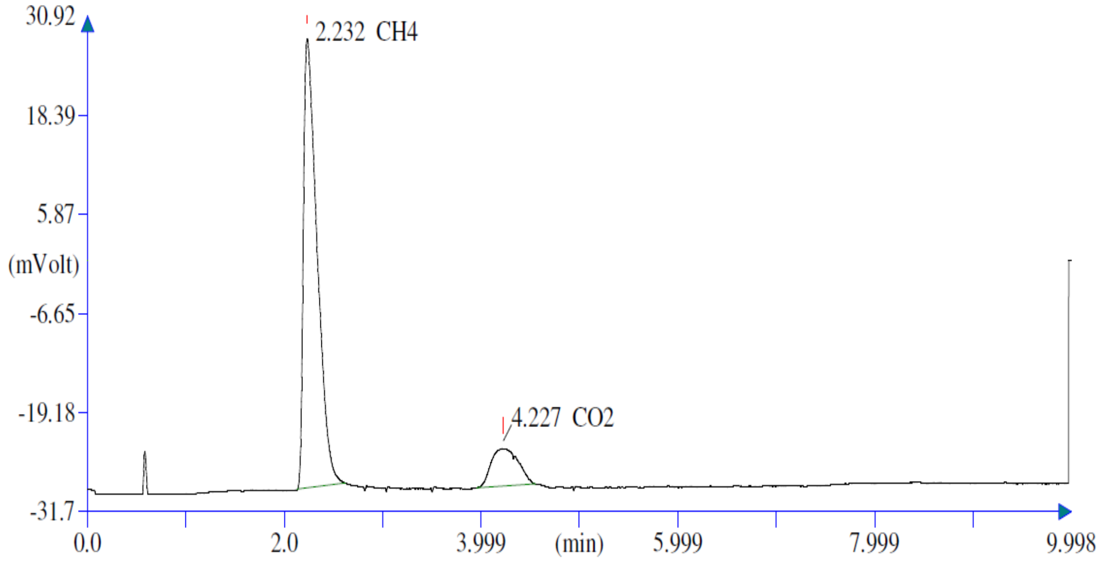
APPENDIX A

Biogas composition data from Gas chromatography

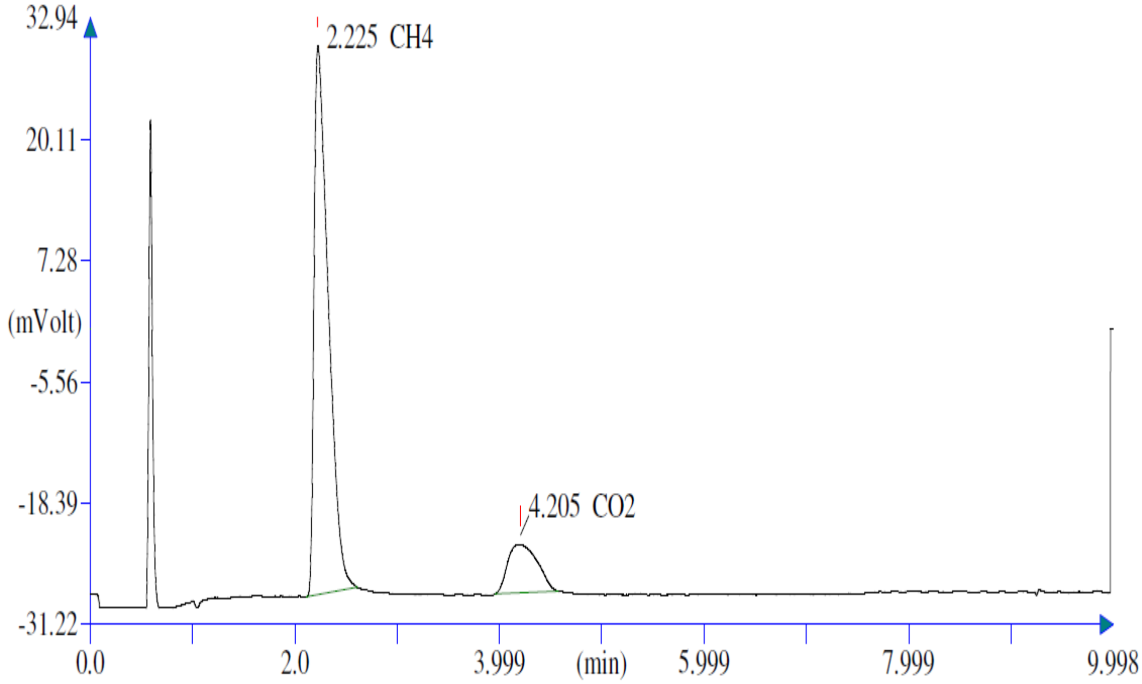
1. **FW : SG (1:0) by batch mode**



2. **Co-digestion of food waste and switchgrass FW:SG (1:0)**

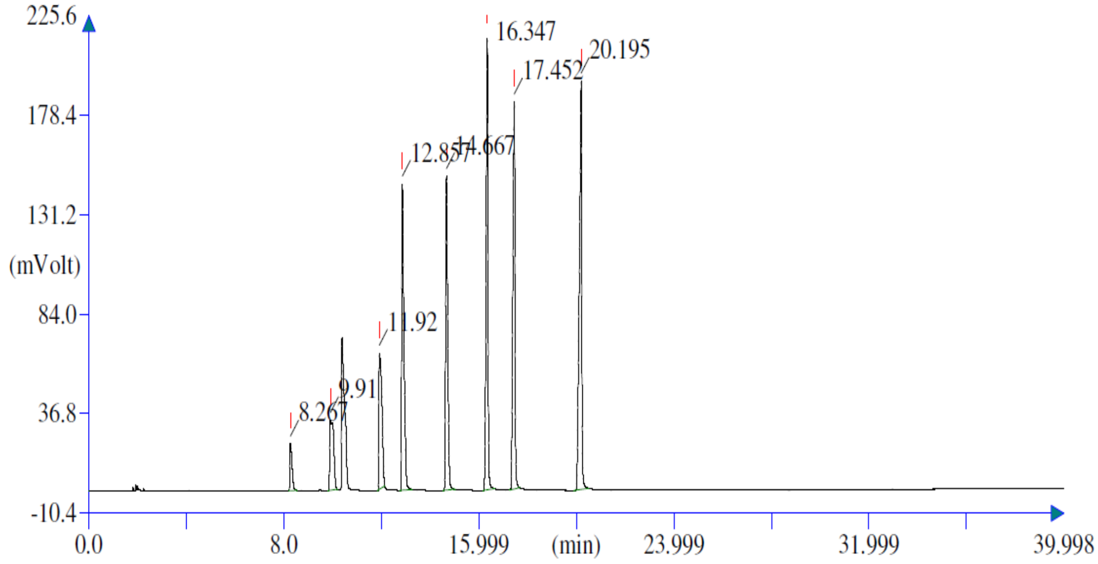


3. FW:SG (0:1) by batch mode

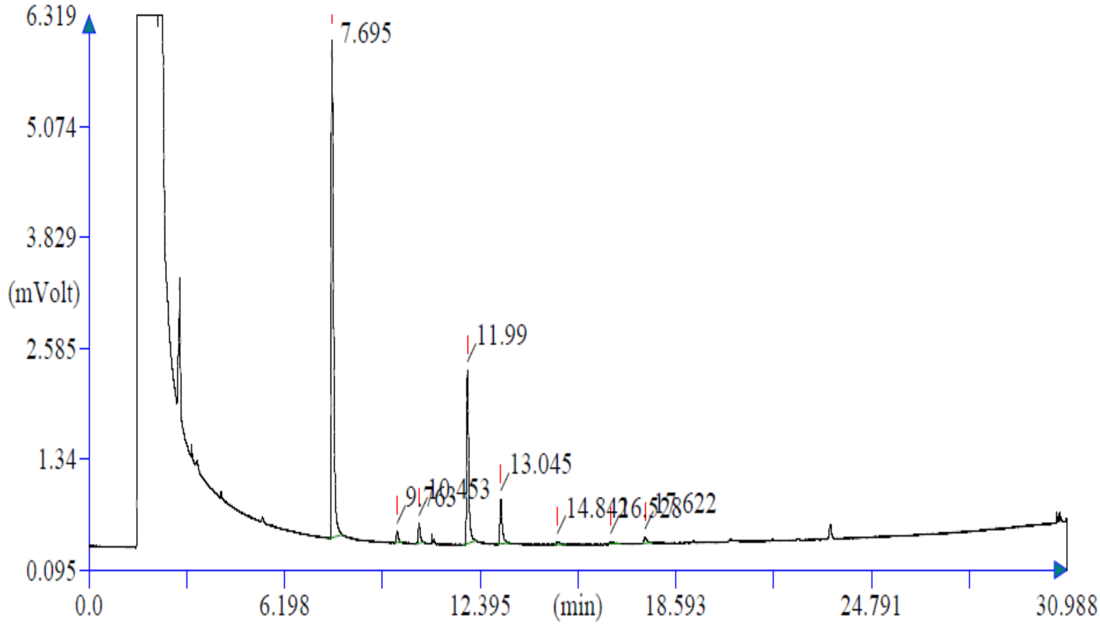


APPENDIX B

1. Standard volatile fatty acid composition



2. Sample data



REFERENCES

Abbasi, T., Tauseef, S. M., and Abbasi, S. A. (2012). "Biogas energy." *Biogas Energy*, 1–169.

Agyeman, F. O., and Tao, W. (2014). "Anaerobic co-digestion of food waste and dairy manure: Effects of food waste particle size and organic loading rate." *J. Environ. Manage.*, 133, 268–274.

Ahamed, A., Chen, C. L., Rajagopal, R., Wu, D., Mao, Y., Ho, I. J. R., Lim, J. W., and Wang, J. Y. (2015). "Multi-phased anaerobic baffled reactor treating food waste." *Bioresour. Technol.*, 182, 239–244.

Ahn, H. K., Smith, M. C., Kondrad, S. L., and White, J. W. (2010). "Evaluation of Biogas Production Potential by Dry Anaerobic Digestion of Switchgrass – Animal Manure Mixtures." *Appl. Biochem. Biotechnol.*, (160), 965–975.

Alibardi, L., and Cossu, R. (2016). "Effects of carbohydrate, protein and lipid content of organic waste on hydrogen production and fermentation products." *Waste Manag.*, 47, 69–77.

Amiri, H., Karimi, K., and Zilouei, H. (2014). "Organosolv pretreatment of rice straw for efficient acetone, butanol, and ethanol production." *Bioresour. Technol.*, 152, 450–456.

Antonopoulou, G., and Lyberatos, G. (2013). "Effect of pretreatment of sweet sorghum biomass on methane generation." *Waste and Biomass Valorization*, 4(3), 583–591.

APHA. (1999). "Standard Methods for the Examination of Water and Wastewater." *Am. Water Work. Assoc.*

Aravantinos-zafiris, G., Orepoulou, V., Tzia, C., and D., C. T. (1994). "Fibre Fraction from Orange Peel residues after pectin Extraction." *Leb. Wiss u Technol.*, 27, 468–471.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., and Lens, P. N. L. (2014).

"Pretreatment methods to enhance anaerobic digestion of organic solid waste." *Appl. Energy*, 123, 143–156.

Ariunbaatar, J., Scotto, E., Perta, D., Panico, A., Frunzo, L., Esposito, G., Lens, P. N. L., and Pirozzi, F. (2015). "Effect of ammoniacal nitrogen on one-stage and two-stage anaerobic digestion of food waste." *Waste Manag.*, 38, 388–398.

Bajpai, P. (2017). "Basics of Anaerobic Digestion Process." *Anaerob. Technol. pulp abd Pap. Ind.*, 7–13.

Banks, C. J., Chesshire, M., Heaven, S., and Arnold, R. (2011). "Anaerobic digestion of source-segregated domestic food waste : Performance assessment by mass and energy balance." *Bioresour. Technol.*, 102(2), 612–620.

Bauer, A., Bösch, P., Friedl, A., and Amon, T. (2009). "Analysis of methane potentials of steam-exploded wheat straw and estimation of energy yields of combined ethanol and methane production." *J. Biotechnol.*, 142(1), 50–55.

Beyler, C. L., and Hirschler, M. M. (2002). "Thermal Decomposition of Polymers." *SFPE Handb. Fire Prot. Eng. 2*, 110–131.

Boe, K., Batstone, D. J., Steyer, J. P., and Angelidaki, I. (2010). "State indicators for monitoring the anaerobic digestion process." *Water Res*, 44(20), 5973–5980.

Boeriu, C. G., Bravo, D., Gosselink, R. J. A., and Dam, J. E. G. Van. (2004). "Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy." 20, 205–218.

Bolzonella, D., Pavan, P., Battistoni, P., and Cecchi, F. (2005). "Mesophilic anaerobic digestion of waste activated sludge: Influence of the solid retention time in the wastewater treatment process." *Process Biochem.*, 40(3-4), 1453–1460.

Böske, J., Wirth, B., Garlipp, F., Mumme, J., and Weghe, H. Van Den. (2015). "Upflow anaerobic solid-state (UASS) digestion of horse manure : Thermophilic vs . mesophilic performance." *Bioresour. Technol.*, 175, 8–16.

Bouallagui, H., Lahdheb, H., Romdan, E. Ben, Rachdi, B., and Hamdi, M. (2009). "Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition." *J. Environ. Manage.*, 90(5), 1844–1849.

Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K. B., and Ramakrishnan, S. (2011). "Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass : A Review." 2011.

Broughton, J. M., Cannon, M. D., and Bartelink, E. J. (2010). "Evolutionary Ecology, Resource Depression, and Niche Construction Theory: Applications to Central California Hunter-Gatherers and Mimbres-Mogollon Agriculturalists." *J. Archaeol. Method Theory*, 17(4), 371–421.

Brown, D., and Li, Y. (2013). "Solid state anaerobic co-digestion of yard waste and food waste for biogas production." *Bioresour. Technol.*, 127, 275–280.

Browne, J. D., and Murphy, J. D. (2013). "Assessment of the resource associated with biomethane from food waste." *Appl. Energy*, 104, 170–177.

Buyukkamaci, N., and Filibeli, A. (2004). "Volatile fatty acid formation in an anaerobic hybrid reactor." *Process Biochem.*, 39(11), 1491–1494.

Capson-Tojo, G., Torres, A., Muñoz, R., Bartacek, J., and Jeison, D. (2017). "Mesophilic and thermophilic anaerobic digestion of lipid-extracted microalgae N. gaditana for methane production." *Renew. Energy*, 105, 539–546.

Castillo-Gonzalez, A., Burrola-Barraza, M. E., Dominguez-Viveros, J., and Chavez-Martinez, A. (2014). "Rumen microorganisms and fermentation." *Arch Med Vet*, 46, 349–361.

Čater, M., Zorec, M., and Marinšek Logar, R. (2014). "Methods for Improving Anaerobic Lignocellulosic Substrates Degradation for Enhanced Biogas Production." *Springer Sci. Rev.*, 2(1-2), 51–61.

Celik, İ., and Demirer, G. N. (2015). "Biogas production from pistachio (Pistacia vera L.) processing waste." *Biocatal. Agric. Biotechnol.*, 4(4), 767–772.

Cesaro, A., and Belgiorno, V. (2013). "Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste." *Ultrason. Sonochem.*, 20(3), 931–936.

Chanakya, H N, Ramachandra, T. V, and Vijayachamundeeswari, M. (2006).

Anaerobic digestion and reuse of digested products of selected components of urban solid waste.

Chen, X., Romano, R. T., and Zhang, R. (2010). "Anaerobic digestion of food wastes for biogas production." *Int. J. Agric. Biol. Eng.*, 3(4), 61–72.

Cheng, J.-Q., Chen, Y.-C., and Cheng, J. J. (2013). "Dilute acid pretreatment of paulownia for bioethanol production." *Chem. Ind. For. Prod.*, 33(3), 110–114.

Chu, C. F., Li, Y. Y., Xu, K. Q., Ebie, Y., Inamori, Y., and Kong, H. N. (2008). "A pH- and temperature-phased two-stage process for hydrogen and methane production from food waste." *Int. J. Hydrogen Energy*, 33(18), 4739–4746.

Cioabla, A. E., Ionel, I., Dumitrel, G. A., and Popescu, F. (2012). "Comparative study on factors affecting anaerobic digestion of agricultural vegetal residues." *Biotechnol. Biofuels*, 5(ii), 1–9.

Cui, M., and Shen, J. (2012). "Effects of acid and alkaline pretreatments on the biohydrogen production from grass by anaerobic dark fermentation." *Int. J. Hydrogen Energy*, 37(1), 1120–1124.

Dahunsi, S. O., Oranusi, S., and Efeovbokhan, V. E. (2017). "Optimization of pretreatment, process performance, mass and energy balance in the anaerobic digestion of Arachis hypogaea (Peanut) hull." *Energy Convers. Manag.*, 139, 260–275.

Dhar, H., Kumar, P., Kumar, S., Mukherjee, S., and Vaidya, A. N. (2016). "Effect of organic loading rate during anaerobic digestion of municipal solid waste." *Bioresour. Technol.*, 217, 56–61.

El-Mashad, H. M., and Zhang, R. (2010). "Biogas production from co-digestion of dairy manure and food waste." *Bioresour. Technol.*, 101(11), 4021–4028.

Fernandes, T. V., Klaasse Bos, G. J., Zeeman, G., Sanders, J. P. M., and Lier, J. B. van. (2009). "Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass." *Bioresour. Technol.*, 100(9), 2575–2579.

Ferrer, I., Garfí, M., Uggetti, E., Ferrer-Martí, L., Calderon, A., and Velo, E. (2011).

"Biogas production in low-cost household digesters at the Peruvian Andes." *Biomass and Bioenergy*, 35(5), 1668–1674.

Fonoll, X., Astals, S., Dosta, J., and Mata-alvarez, J. (2015). "Anaerobic co-digestion of sewage sludge and fruit wastes : Evaluation of the transitory states when the co-substrate is changed." *Chem. Eng. J.*, 262, 1268–1274.

Forster-Carneiro, T., Pérez, M., Romero, L. I., and Sales, D. (2007). "Dry-thermophilic anaerobic digestion of organic fraction of the municipal solid waste: Focusing on the inoculum sources." *Bioresour. Technol.*, 98(17), 3195–3203.

Franke-Whittle, I. H., Walter, A., Ebner, C., and Insam, H. (2014). "Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities." *Waste Manag.*, 34(11), 2080–2089.

Frigon, J. C., Roy, C., and Guiot, S. R. (2012a). "Anaerobic co-digestion of dairy manure with mulched switchgrass for improvement of the methane yield." *Bioprocess Biosyst. Eng.*, 35(3), 341–349.

Frigon, J.-C., Mehta, P., and Guiot, S. R. (2012b). "Impact of mechanical, chemical and enzymatic pre-treatments on the methane yield from the anaerobic digestion of switchgrass." *Biomass and Bioenergy*, 36, 1–11.

Gao, S., Huang, Y., Yang, L., Wang, H., Zhao, M., and Xu, Z. (2015). "Evaluation the anaerobic digestion performance of solid residual kitchen waste by NaHCO3 buffering." *Energy Convers. Manag.*, 93, 166–174.

Gerardi, M. H. (2003). *The Microbiology of Anaerobic Digesters*. *Wiley-Interscience*.

Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R., Lens, P. N. L., and Esposito, G. (2015). "A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products." *Appl. Energy*, 144, 73–95.

Goswami, A. (2018). "Food wastage crisis in India." *Clean India J.*, 3–5.

Gu, Y., Zhang, Y., and Zhou, X. (2015). "Effect of Ca(OH)2 pretreatment on extruded rice straw anaerobic digestion." *Bioresour. Technol.*, 196, 116–122.

Gunaseelan, N. (1997). “Anaerobic digestion of biomass for methane production: A review.” *Biomass and Bioenergy*, 13(97), 83–114.

Gunaseelan, V. N. (1995). “Effects of inoculum/substrate ratio and pretreatments on methane yield from parthenium.” *Biomass and Bioenergy*, 8(1), 39–44.

Gupta, P., Shekhar, R., Sachan, A., Vidyarthi, A. S., and Gupta, A. (2012). “A re-appraisal on intensification of biogas production.” *Renew. Sustain. Energy Rev.*, 16, 4908–4916.

Haak, L., Roy, R., and Pagilla, K. (2016). “Toxicity and biogas production potential of refinery waste sludge for anaerobic digestion.” *Chemosphere*, 144, 1170–1176.

Hagos, K., Zong, J., Li, D., Liu, C., and Lu, X. (2016). “Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives.” *Renew. Sustain. Energy Rev.*, (March).

Harmsen, P., Huijgen, W., Bermudez, L., and Bakker, R. (2010). “Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass.” *Energy*, 1–49.

Harmsen, P., Lips, S., and Bakker, R. (2013). *Pretreatment of lignocellulose for biotechnological production of lactic acid: Research Review*.

Hashimoto, A. G. (1986). “Pretreatment of wheat straw for fermentation to methane.” *Biotechnol. Bioeng.*, 28(12), 1857–1866.

Hashimoto, A. G., and Hruska, R. L. (1982). “Methane production from beef cattle manure: effects of temperature, hydraulic retention time and influent substrate concentration on Kinetic parameter (K)*.” *Biotechnol. Bioeng.*, XXIV, 2039–2052.

Hendriks, A. T. W. M., and Zeeman, G. (2009). “Pretreatments to enhance the digestibility of lignocellulosic biomass.” *Bioresour. Technol.*, 100(1), 10–18.

Hult, K., and Holmquist, M. (1997). “Kinetics, Molecular Modeling and synthetic Applications with Microbial Lipases.” *Acad. Press*, 286, 386–405.

Ismail, Z. Z., and Talib, A. R. (2014). “Assessment of anaerobic co-digestion of agro wastes for biogas recovery: A bench scale application todate palm wastes.” *Int. J.*

energy Environ., 5(5), 591–600.

Jabeen, M., Yousaf, S., Haider, M. R., and Malik, R. N. (2015). "High-solids anaerobic co-digestion of food waste and rice husk at different organic loading rates." *Int. Biodeterior. Biodegradation*, 1–5.

Jard, G., Jackowiak, D., Carrere, H., Delgenes, J. P., Torrijos, M., Steyer, J. P., and Dumas, C. (2012). "Batch and semi-continuous anaerobic digestion of Palmaria palmata: Comparison with Saccharina latissima and inhibition studies." *Chem. Eng. J.*, 209, 513–519.

Jayasinghe, P. A., Hettiaratchi, J. P. A., Mehrotra, A. K., and Kumar, S. (2011). "Effect of enzyme additions on methane production and lignin degradation of landfilled sample of municipal solid waste." *Bioresour. Technol.*, 102(7), 4633–4637.

Jenkins, B. M., Williams, R. B., Adams, L. S., Peace, C., Petersen, G., and Leary, M. (2008). "Current Anaerobic Digestion Technologies Used for Treatment of Municipal Organic Solid Waste."

Jugal Sukhesh, M., and Venkateswara Rao, P. (2018). "Synergistic effect in anaerobic co-digestion of rice straw and dairy manure - a batch kinetic study." *Energy Sources, Part A Recover. Util. Environ. Eff.*, 7036, 1–12.

Kandasamy, M., Hamawand, I., Bowtell, L., Seneweera, S., Chakrabarty, S., Yusaf, T., Shakoor, Z., Algayyim, S., and Eberhard, F. (2017). "Investigation of ethanol production potential from lignocellulosic material without enzymatic hydrolysis using the ultrasound technique." *Energies*, 10(1).

Kaparaju, P., and Felby, C. (2010). "Characterization of lignin during oxidative and hydrothermal pre-treatment processes of wheat straw and corn stover." *Bioresour. Technol.*, 101(9), 3175–3181.

Keshwani, D. R., Cheng, J. J., Keshwani, D. R., and Cheng, J. J. (2009). "Switchgrass for Bioethanol and Other Value-Added Applications : A Review Switchgrass for Bioethanol and Other."

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., and Dawson, L. (2011). "The anaerobic digestion of solid organic waste." *Waste Manag.*, 31(8), 1737–1744.

Kim, J. K., Oh, B. R., Chun, Y. N., and Kim, S. W. (2006). "Effects of Temperature and Hydraulic Retention Time on Anaerobic Digestion of Food Waste." 102(4), 328–332.

Kim, S. B., and Lee, Y. Y. (2002). "Diffusion of sulfuric acid within lignocellulosic biomass particles and its impact on dilute-acid pretreatment." 83, 165–171.

Kleawkla, A., and Chuenkruth, P. (2016). "Reducing Sugar Production from Agricultural Wastes by Acid Hydrolysis." *Trans Tech Publ. Switz.*, 675-676, 31–34.

Klinke, H. B., Ahring, B. K., Schmidt, A. S., and Thomsen, A. B. (2002). "Characterization of degradation products from alkaline wet oxidation of wheat straw." *Bioresour. Technol.*, 82, 15–26.

Koch, K., Lübken, M., Gehring, T., Wichern, M., and Horn, H. (2010). "Biogas from grass silage - Measurements and modeling with ADM1." *Bioresour. Technol.*, 101(21), 8158–8165.

Kothari, R., Pandey, A. K., Kumar, S., Tyagi, V. V., and Tyagi, S. K. (2014). "Different aspects of dry anaerobic digestion for bio-energy: An overview." *Renew. Sustain. Energy Rev.*, 39, 174–195.

Kumar P, Barrett D M, D. elwiche M. J. and S. P. (2009). "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production." *Ind Eng Chem Res*, 48, 3713 – 3729.

Kumar, A., Kumar, N., Baredar, P., and Shukla, A. (2015). "A review on biomass energy resources, potential, conversion and policy in India." *Renew. Sustain. Energy Rev.*, 45, 530–539.

Kumar, P., Barrett, D. M., Delwiche, M. J., Stroeve, P., Kumar, P., Barrett, D. M., Delwiche, M. J., and Stroeve, P. (2009a). "Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production." *Ind. Eng. Chem. Res.*

Kumar, R., Mago, G., Balan, V., and Wyman, C. E. (2009b). "Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies.” *Bioresour. Technol.*, 100(17), 3948–3962.

Labatut, R. A., Angenent, L. T., and Scott, N. R. (2011). “Biochemical methane potential and biodegradability of complex organic substrates.” *Bioresour. Technol.*, 102(3), 2255–64.

Lalman, J. A., and Bagley, D. M. (2004). “Extracting Long-Chain Fatty Acids from a Fermentation Medium.” *AOCS Press*, 81(2), 105–111.

Lee, S., and Fasina, O. (2009). “TG-FTIR analysis of switchgrass pyrolysis.” *J. Anal. Appl. Pyrolysis*, 86, 39–43.

Lei, Z., Chen, J., Zhang, Z., and Sugiura, N. (2010). “Methane production from rice straw with acclimated anaerobic sludge: Effect of phosphate supplementation.” *Bioresour. Technol.*

Lerdrattranataywee, W., and Kaosol, T. (2015). *Effect of Mixing Time on Anaerobic Co-digestion of Palm Oil Mill Waste and Block Rubber Wastewater. Energy Procedia*, Elsevier B.V.

Li, Y., Jin, Y., Borrion, A., Li, H., and Li, J. (2017). “Effects of organic composition on the anaerobic biodegradability of food waste.” *Bioresour. Technol.*, 243, 836–845.

Li, Y., Park, S. Y., and Zhu, J. (2011). “Solid-state anaerobic digestion for methane production from organic waste.” *Renew. Sustain. Energy Rev.*, 15(1), 821–826.

Li, Y., Zhang, R., He, Y., Zhang, C., Liu, X., Chen, C., and Liu, G. (2014). “Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR).” *Bioresour. Technol.*, 156, 342–347.

Liew, L. N., Shi, J., and Li, Y. (2011). “Enhancing the solid-state anaerobic digestion of fallen leaves through simultaneous alkaline treatment.” *Bioresour. Technol.*, 102(19), 8828–8834.

Liu, C., Li, H., Zhang, Y., and Liu, C. (2016). “Improve biogas production from low-organic-content sludge through high-solids anaerobic co-digestion with food waste.” *Bioresour. Technol.*, 219.

Liu, C., Wang, W., Anwar, N., Ma, Z., Liu, G., and Zhang, R. (2017). “Effect of Organic Loading Rate on Anaerobic Digestion of Food Waste under Mesophilic and Thermophilic Conditions.” *Energy and Fuels*, 31(3), 2976–2984.

Liu, C., Yuan, X., Guang-ming Zeng, Wen-wei Li, and Jing Li. (2008). “Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste.” *Bioresour. Technol.*, 99, 882–888.

Liu, G., Zhang, R., El-mashad, H. M., and Dong, R. (2009). “Effect of feed to inoculum ratios on biogas yields of food and green wastes.” *Bioresour. Technol.*, 100(21), 5103–5108.

Liu, H. W., Walter, H. K., Vogt, G. M., Vogt, H. S., and Holbein, B. E. (2002). “Steam pressure disruption of municipal solid waste enhances anaerobic digestion kinetics and biogas yield.” *Biotechnol. Bioeng.*, 77(2), 121–130.

Liu, X., Wang, W., Gao, X., Zhou, Y., and Shen, R. (2012). “Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste.” *Waste Manag.*, 32(2), 249–255.

Llore, C. E., and Lo, M. (2008). “Effect of alkaline pretreatment on anaerobic digestion of solid wastes.” 28, 2229–2234.

Lu, X., Xi, B., Zhang, Y., and Angelidaki, I. (2011). “Microwave pretreatment of rape straw for bioethanol production : Focus on energy efficiency.” *Bioresour Technol.*, 102, 7937–7940.

Luo, G., Xie, L., Zhou, Q., and Angelidaki, I. (2011). “Enhancement of bioenergy production from organic wastes by two-stage anaerobic hydrogen and methane production process.” *Bioresour. Technol.*, 102(18), 8700–8706.

Madsen, T., and Rasmussen, H. B. (1996). “A method for screening the potential toxicity of organic chemical to methanogenic gas production.” *water Sci. Technol.*, 33(6), 213–220.

Mamun, M. R. Al, Torii, S., Rashed, M., Mamun, A., and Torii, S. (2015). “Anaerobic co-digestion technology in solid wastes treatment for biomethane generation.” *Int. J. Sustain. Energy*, 6451(May 2015), 1–11.

Mane, A. B., Rao, B., and Rao, A. B. (2015). “Characterisation of Fruit and Vegetable Waste for Maximizing the Biogas Yield.” *Int. J. Adv. Technol. Eng. Sci.*, 3(01), 1892–1903.

Manyi-loh, C. E., Mamphweli, S. N., Meyer, E. L., and Okoh, A. I. (2013). “Microbial Anaerobic Digestion (Bio-Digesters) as an Approach to the Decontamination of Animal Wastes in Pollution Control and the Generation of Renewable Energy.” 4390–4417.

Martí-Herrero, J., Alvarez, R., Cespedes, R., Rojas, M. R., Conde, V., Aliaga, L., Balboa, M., and Danov, S. (2015). “Cow, sheep and llama manure at psychrophilic anaerobic co-digestion with low cost tubular digesters in cold climate and high altitude.” *Bioresour. Technol.*, 181, 238–246.

Masse, D., Gilbert, Y., Savoie, P., Belanger, G., Parent, G. G. G., and Babineau, D. (2010). “Methane yield from switchgrass harvested at different stages of development in Eastern Canada.” *Bioresour. Technol.*, 101(24), 9536–9541.

Meng, Y., Shen, F., and Yuan, H. (2014). “Start-up and operation strategies on the liquefied food waste anaerobic digestion and a full-scale case application.” 2333–2341.

Mirmohamadsadeghi, S., Karimi, K., Zamani, A., Amiri, H., and Horváth, I. S. (2014). “Enhanced solid-state biogas production from lignocellulosic biomass by organosolv pretreatment.” *Biomed Res. Int.*, 2014.

Molinuevo-Salces, B., Larsen, S. U., Ahring, B. K., and Uellendahl, H. (2014). “Biogas production from catch crops: Increased yield by combined harvest of catch crops and straw and preservation by ensiling.” *Biomass and Bioenergy*, 79, 3–11.

Moller, J. (2009). “Gravimetric Determination of Acid Detergent Fiber and Lignin in Feed: Interlaboratory Study.” *J. AOAC Int.*, 92(1), 74–90.

Monlau, F., Barakat, A., Steyer, J. P., and Carrere, H. (2012). “Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks.” *Bioresour. Technol.*, 120, 241–247.

Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J., and Carrère, H. (2013). “Lignocellulosic Materials Into Biohydrogen and Biomethane: Impact of Structural Features and Pretreatment.” *Crit. Rev. Environ. Sci. Technol.*, 43(3), 260–322.

Monlau, F., Sambusiti, C., Antoniou, N., Zabaniotou, A., Solhy, A., and Barakat, A. (2015). “Pyrochars from bioenergy residue as novel bio-adsorbents for lignocellulosic hydrolysate detoxification.” *Bioresour. Technol.*, 187, 379–386.

Montañés, R., Solera, R., and Pérez, M. (2015). “Anaerobic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: effect of temperature.” *Bioresour Technol*, 180, 177–184.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., and Ladisch, M. (2005). “Features of promising technologies for pretreatment of lignocellulosic biomass.” *Bioresour. Technol.*, 96(6), 673–686.

Motte, J., Escudié, R., Hamelin, J., Steyer, J., Bernet, N., Delgenes, J., and Dumas, C. (2014). “Substrate milling pretreatment as a key parameter for Solid-State Anaerobic Digestion optimization.” *Bioresour. Technol.*, 173, 185–192.

Motte, J.-C., Trably, E., Escudié, R., Hamelin, J., Steyer, J.-P., Bernet, N., Delgenes, J.-P., and Dumas, C. (2013). “Total solids content: a key parameter of metabolic pathways in dry anaerobic digestion.” *Biotechnol. Biofuels*, 6(1), 164.

Mumme, J., Linke, B., and Tölle, R. (2010). “Novel upflow anaerobic solid-state (UASS) reactor.” *Bioresour. Technol.*, 101(2), 592–599.

Narendar, R., and Priya Dasan, K. (2014). “Chemical treatments of coir pith: Morphology, chemical composition, thermal and water retention behavior.” *Compos. Part B Eng.*, 56, 770–779.

Nartker, S., Ammerman, M., Aurandt, J., Stogsdil, M., Hayden, O., and Antle, C. (2014). “Increasing biogas production from sewage sludge anaerobic co-digestion process by adding crude glycerol from biodiesel industry.” *Waste Manag.*, 34(12), 2567–2571.

Niee Liew, Lo. (2011). “Solid- State Anaerobic Digestion of Lignocellulosic Biomass for Biogas Production.” *Master’s Thesis*.

Nielfa, A., Cano, R., and Fdz-Polanco, M. (2015). “Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge.” *Biotechnol. Reports*, 5(1).

Obama, P., Ricochon, G., Muniglia, L., and Brosse, N. (2012). “Combination of enzymatic hydrolysis and ethanol organosolv pretreatments: Effect on lignin structures, delignification yields and cellulose-to-glucose conversion.” *Bioresour. Technol.*, 112, 156–163.

Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y., McCarty, P. L., W. F. Owen, D. C. Stuckey, J. B. Healy, JR., L. Y. Y. and P. L. M., Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y., and McCarty, P. L. (1979). “Bioassay for monitoring biochemical methane potential and anaerobic toxicity.” *Water Res.*, 13(6), 485–492.

Paritosh, K., Kushwaha, S. K., Yadav, M., Pareek, N., Chawade, A., and Vivekanand, V. (2017). “Food Waste to Energy : An Overview of Sustainable Approaches for Food Waste Management and Nutrient Recycling.” *BioMed reserach Int.*, 1–19.

Pena, M. J., Tuomivaara, S. T., Urbanowicz, B. R., Malcolm A O’Neill, and York, W. S. (2012). “Methods for Structural Characterization of the Products of Cellulose- and Xyloglucan-Hydrolyzing Enzymes.” *Methods Enzymol.*, 121–139.

Perez, J., Munoz-Dorado, J., la Rubia, T. de, and Martinez, J. (2002). “Biodegradation and biological treatments of cellulose , hemicellulose and lignin : an overview.” *Int. J. Microbiol.*, (5), 53–63.

Pham, C. H., Triolo, J. M., Cu, T. T. T., Pedersen, L., and Sommer, S. G. (2013). “Validation and recommendation of methods to measure biogas production potential of animal manure.” *Asian-Australasian J. Anim. Sci.*, 26(6), 864–873.

Phitsuwan, P., Permsriburasuk, C., Baramee, S., Teeravivattanakit, T., and Ratanakhanokchai, K. (2017). “Structural Analysis of Alkaline Pretreated Rice Straw for Ethanol Production.” *Int. J. Polym. Sci.*, 2017, 1–9.

Quiñones, T. S., Plöchl, M., Budde, J., and Heiermann, M. (2012). "Results of batch anaerobic digestion test - effect of enzyme addition." *Agric. Eng. Int. CIGR J.*, 14(1), 38–50.

Rahman, M. A., Møller, H. B., Saha, C. K., Alam, M. M., Wahid, R., and Feng, L. (2017). "Optimal ratio for anaerobic co-digestion of poultry droppings and lignocellulosic-rich substrates for enhanced biogas production." *Energy Sustain. Dev.*, 39, 59–66.

Rajagopal, R., Masse, D. I., and Singh, G. (2013). "A critical review on inhibition of anaerobic digestion process by excess ammonia." *Bioresour. Technol.*, 143, 632–641.

Raposo, F., Fernandez-Cegri, V., Rubia, M. A. D. la, Borja, R., Beline, F., Cavinato, C., Demirer, G., Fernandez, B., Fernandez-Polanco, M., Frigon, J. C., Ganesh, R., Kaparaju, P., Koubova, J., Mendez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., Wierinck, I., and Wilde, V. De. (2011). "Biochemical methane potential (BMP) of solid organic substrates : evaluation of anaerobic biodegradability using data from an international interlaboratory study." *J chem Technol Biotechnol*, 86, 1088–1098.

Sambusiti, C., Ficara, E., Malpei, F., Steyer, J. P., and Carrère, H. (2013). "Effect of sodium hydroxide pretreatment on physical, chemical characteristics and methane production of five varieties of sorghum." *Energy*, 55, 449–456.

Sambusiti, C., Monlau, F., Ficara, E., Musatti, A., Rollini, M., Barakat, A., and Malpei, F. (2015). "Comparison of various post-treatments for recovering methane from agricultural digestate." *Fuel Process. Technol.*, 137, 359–365.

Sánchez, E., Borja, R., Travieso, L., Martín, A., and Colmenarejo, M. F. (2005). "Effect of organic loading rate on the stability, operational parameters and performance of a secondary upflow anaerobic sludge bed reactor treating piggery waste." *Bioresour. Technol.*, 96(3), 335–344.

Sang, H., Hee, C., Moon, S., Yong, J., and Kim, J. Y. (2013). "Effect of long chain fatty acids removal as a pretreatment on the anaerobic digestion of food waste." 82–89.

Sant'Anna, C., and Souza, W. De. (2012). "Microscopy as a Tool to Follow Deconstruction of Lignocellulosic Biomass." *Curr. Microsc. Contrib. to Adv. Sci. Technol.*, 639–645.

Sathitsuksanoh, N., Zhu, Z., Wi, S., and Percival Zhang, Y. H. (2011). "Cellulose solvent-based biomass pretreatment breaks highly ordered hydrogen bonds in cellulose fibers of switchgrass." *Biotechnol. Bioeng.*, 108(3), 521–529.

Shah, F. A., Mahmood, Q., Rashid, N., Pervez, A., and Raja, I. A. (2015). "Co-digestion , pretreatment and digester design for enhanced methanogenesis." *Renew. Sustain. Energy Rev.*, 42, 627–642.

Sheets, J. P., Ge, X., and Li, Y. (2015). "Effect of limited air exposure and comparative performance between thermophilic and mesophilic solid-state anaerobic digestion of switchgrass." *Bioresour. Technol.*, 180, 296–303.

Shen, F., Yuan, H., Pang, Y., Chen, S., Zhu, B., Zou, D., Liu, Y., Ma, J., Yu, L., and Li, X. (2013). "Performances of anaerobic co-digestion of fruit & vegetable waste (FVW) and food waste (FW): Single-phase vs. two-phase." *Bioresour. Technol.*, 144.

Shi, J., Wang, Z., Stiverson, J. A., Yu, Z., and Li, Y. (2013). "Reactor performance and microbial community dynamics during solid-state anaerobic digestion of corn stover at mesophilic and thermophilic conditions." *Bioresour. Technol.*, 136, 574–581.

Shi, J., Xu, F., Wang, Z., Stiverson, J. A., Yu, Z., and Li, Y. (2014). "Effects of microbial and non-microbial factors of liquid anaerobic digestion effluent as inoculum on solid-state anaerobic digestion of corn stover." *Bioresour. Technol.*, 157, 188–196.

Shu, C., Jaiswal, R., and Shih, J. (2015). "Improving Biodegradation of Rice Straw Using Alkaline and Aspergillus niger Pretreatment for Methane Production by Anaerobic Co-Digestion." *Bioprocess. Biotech.*, 5(10).

Siciliano, A., Stillitano, M. A., and Rosa, S. De. (2016). "Biogas production from wet olive mill wastes pretreated with hydrogen peroxide in alkaline conditions." *Renew. Energy*, 85, 903–916.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Nrel, D. C. (2011). *Determination of Structural Carbohydrates and Lignin in Biomass*.

Smith, D. B., and Almquist, C. B. (2014). "The anaerobic co-digestion of fruit and vegetable waste and horse manure mixtures in a bench- scale , two-phase anaerobic digestion system." *Environ. Technol.*, 35(7), 859–867.

Song, Z., Yang, G., Liu, X., Yan, Z., Yuan, Y., and Liao, Y. (2014). "Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion." *PLoS One*, 9(4), 1–8.

Stamatelatou, K., and Antonopoulou, G. (2011). "Production of biogas via anaerobic digestion." *Handb. biofuels Prod.*, Woodhead Publishing Limited, 266–304.

Stein, U. H., Wimmer, B., Ortner, M., Fuchs, W., and Bochmann, G. (2017). "Maximizing the production of butyric acid from food waste as a precursor for ABE-fermentation." *Sci. Total Environ.*, 598, 993–1000.

Stroot Peter G., KD, M., RI, M., and L., R. (2001). "Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions--I. Digester performance." *Water Res.*, 35(7), 1804–1816.

Suksong, W., Jehlee, A., Singkhala, A., Kongjan, P., Prasertsan, P., Imai, T., and O-Thong, S. (2016). "Thermophilic solid-state anaerobic digestion of solid waste residues from palm oil mill industry for biogas production." *Ind. Crop. Prod.*, 1–10.

Sun, Y., and Cheng, J. (2002). "Hydrolysis of lignocellulosic materials for ethanol production : a review." *Bioresour. Technol.*, 83, 1–11.

Tandukar, M., and Pavlostathis, S. G. (2015). "Co-digestion of municipal sludge and external organic wastes for enhanced biogas production under realistic plant constraints." *Water Res.*, 1–14.

Teater, C., Yue, Z., Maclellan, J., Liu, Y., and Liao, W. (2011). "Assessing solid digestate from anaerobic digestion as feedstock for ethanol production." *Bioresour. Technol.*, 102(2), 1856–1862.

Tian, H., Duan, N., Lin, C., Li, X., and Zhong, M. (2015). "Anaerobic co-digestion of kitchen waste and pig manure with different mixing ratios.” *J. Biosci. Bioeng.*, 120(1), 51–57.

Toledano, A., Serrano, L., Pineda, A., Romero, A. A., Luque, R., and Labidi, J. (2014). “Microwave-assisted depolymerisation of organosolv lignin via mild hydrogen-free hydrogenolysis : Catalyst screening.” *"Applied Catal. B, Environ.*, 145, 43–55.

Torrijos, M., Thalla, A. K., Sousbie, P., Bosque, F., and Delgene, J. P. (2008). “Anaerobic digestion of residues from production and refining of vegetable oils as an alternative to conventional solutions.” *Water Sci. Technol.*, 58, 1871–1878.

Uma, S., Thalla, A. K., and Devatha, C. P. (2018). “Co-digestion of Food Waste and Switchgrass for Biogas Potential: Effects of Process Parameters.” *Waste and Biomass Valorization*, 1–13.

Velmurugan, B., and Ramanujam, R. A. (2011). “Anaerobic Digestion of Vegetable Wastes for Biogas Production in a Fed-Batch Reactor.” *Int. J. Emerg. Sci*, 1(3), 478–486.

Ventura, J.-R. S., Lee, J., and Jahng, D. (2014). “A comparative study on the alternating mesophilic and thermophilic two-stage anaerobic digestion of food waste.” *J. Environ. Sci.*, 26(6), 1274–1283.

Vindis, P. (2009). “The impact of mesophilic and thermophilic anaerobic digestion on biogas production.” 36(2), 192–198.

Wang, Y., Zhang, Y., Wang, J., and Meng, L. (2009). “Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria.” *Biomass and Bioenergy*, 33(5), 848–853.

Ward, A. J., Hobbs, P. J., Holliman, P. J., and Jones, D. L. (2008). “Optimisation of the anaerobic digestion of agricultural resources.” *Bioresour. Technol.*, 99(17), 7928–7940.

Weiland, P. (2001). “Anaerobic waste digestion in Germany – Status and recent developments.” 415–421.

Wickham, R., Galway, B., Bustamante, H., and Nghiem, L. D. (2016). “Biomethane potential evaluation of co-digestion of sewage sludge and organic wastes.” *Int. Biodeterior. Biodegradation*, 1–6.

Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., and Lee, Y. Y. (2005). “Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover.” *Bioresour. Technol.*, 96(18 SPEC. ISS.), 2026–2032.

Xiao, X., Huang, Z., Ruan, W., Yan, L., and Miao, H. (2015). “Evaluation and characterization during the anaerobic digestion of high-strength kitchen waste slurry via a pilot-scale anaerobic membrane bioreactor.” *Bioresour. Technol.*, 193, 234–242.

Xie, T., Xie, S., Sivakumar, M., and Nghiem, L. D. (2017). “Relationship between the synergistic/antagonistic effect of anaerobic co-digestion and organic loading.” *Int. Biodeterior. Biodegrad.*, 124, 1–7.

Xu, F., and Li, Y. (2012). “Solid-state co-digestion of expired dog food and corn stover for methane production.” *Bioresour. Technol.*

Y. Li, J. Zhu, C. Wan, S. Y. P. (2013). “Solid-state anaerobic digestion of corn stover for biogas production.” *Am. Soc. Agric. Biol. Eng.*, 54(4), 1415–1421.

Yong, Z., Dong, Y., Zhang, X., and Tan, T. (2015). “Anaerobic co-digestion of food waste and straw for biogas production.” *Renew. Energy*, 78, 527–530.

Zarkadas, I. S., Sofikiti, A. S., Voudrias, E. A., and Pilidis, G. A. (2015). “Thermophilic anaerobic digestion of pasteurised food wastes and dairy cattle manure in batch and large volume laboratory digesters: Focussing on mixing ratios.” *Renew. Energy*, 80.

Zeshan, Karthikeyan, O. P., and Visvanathan, C. (2012). “Effect of C/N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester.” *Bioresour. Technol.*, 113, 294–302.

Zhai, N., Zhang, T., Yin, D., Yang, G., and Wang, X. (2015). “Effect of initial pH on anaerobic co-digestion of kitchen waste and cow manure.” *Waste Manag.*, 38, 126–

131.

Zhang, L., and Jahng, D. (2012). "Long-term anaerobic digestion of food waste stabilized by trace elements." *Waste Manag.*, 32(8), 1509–1515.

Zhang, L., Lee, Y., and Jahng, D. (2011). "Anaerobic co-digestion of food waste and piggery wastewater : Focusing on the role of trace elements." *Bioresour. Technol.*, 102(8), 5048–5059.

Zhang, R., El-Mashad, H. M., Hartman, K., Wang, F., Liu, G., Choate, C., and Gamble, P. (2007). "Characterization of food waste as feedstock for anaerobic digestion." *Bioresour. Technol.*, 98(4), 929–935.

Zhang, W., Zhang, L., and Li, A. (2015a). "Enhanced anaerobic digestion of food waste by trace metal elements supplementation and reduced metals dosage by green chelating agent [S , S] -EDDS via improving metals bioavailability." *Water Res.*, 84, 266–277.

Zhang, W., Zhang, L., and Li, A. (2015b). "Anaerobic co-digestion of food waste with MSW incineration plant fresh leachate : process performance and synergistic effects." *Chem. Eng. J.*, 259, 795–805.

Zhen, G., Lu, X., Kobayashi, T., Li, Y., Xu, K., and Zhao, Y. (2015). "Mesophilic anaerobic co-digestion of waste activated sludge and Egeria densa : Performance assessment and kinetic analysis." *Appl. Energy*, 148, 78–86.

Zheng, M., Li, X., Li, L., Yang, X., and He, Y. (2009a). "Enhancing anaerobic biogasification of corn stover through wet state NaOH pretreatment." *Bioresour. Technol.*, 100(21), 5140–5145.

Zheng, Y., Pan, Z., and Zhang, R. (2009b). "Overview of biomass pretreatment for cellulosic ethanol production." 2(3), 51–68.

Zheng, Y., Zhao, J., Xu, F., and Li, Y. (2014). "Pretreatment of lignocellulosic biomass for enhanced biogas production." *Prog. Energy Combust. Sci.*, 42(1), 35–53.

Zheng, Z., Liu, J., Yuan, X., Wang, X., Zhu, W., Yang, F., and Cui, Z. (2015). "Effect of dairy manure to switchgrass co-digestion ratio on methane production and the bacterial community in batch anaerobic digestion." *Appl. Energy*, 151, 249–257.

Zhong, W., Zhang, Z., Luo, Y., Sun, S., Qiao, W., and Xiao, M. (2011). "Effect of biological pretreatments in enhancing corn straw biogas production." *Bioresour. Technol.*, 102(24), 11177–11182.

Zhu, J., Wan, C., and Li, Y. (2010). "Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment." *Bioresour. Technol.*, 101(19), 7523–7528.

Zhu, J., Yang, L., and Li, Y. (2015). "Comparison of premixing methods for solid-state anaerobic digestion of corn stover." *Bioresour. Technol.*, 175.

LIST OF PUBLICATIONS

JOURNALS

Uma, S., Thalla, A. K., and Devatha, C. P. (2018). “Co-digestion of Food Waste and Switchgrass for Biogas Potential: Effects of Process Parameters.” Waste and Biomass Valorization (Springer), 1–13. *DOI: 10.1007/s12649-018-0508-2*

Arun Kumar Thalla, **Uma Sakthivel** and C P Devatha “Characterization of physical and chemical pretreated switchgrass to assess the performance on biomethane potential” communicated to International Journal of Environment and Waste Management, Inderscience Publication – ***In Review***

CONFERENCES

S. Uma and Arun Kumar Thalla. “Digestibility of food waste for biogas production” has been accepted and published in proceedings at Global conference on Renewable energy (GCRE-2016), 04-06, March, 2016, NIT Patna, India

Arun Kumar Thalla, **Uma S** and C P Devatha. “Anaerobic treatability of switchgrass for biogas production” 5th International Engineering Symposium-IES 2016

BIO-DATA

Name of the Scholar: Uma S.

Registration No.: 145027CV14F11

Date of Birth: 24.01.1985

Email: uma.nitk@gmail.com

Contact No.:9791265267

Corresponding Address: 112A, Velan Kudil, Padasalai Street

Eachanari (PO) Coimbatore-641021

Uma Sakthivel is a full time PhD student worked under the supervision of Dr. Arun Kumar Thalla and Dr. C P Devatha in Department of Civil Engineering, National Institute of Technology Karnataka through the financial support of Ministry of Human Resources Development, Government of India Institute fellowship during 2014-2019. Her doctoral research aims to investigate the biogas production from the organic substrates co-digestion and its application orientation for the replacement of alternative natural bioenergy systems. She is the life member of Indian society for Technical Associations New Delhi, India. She worked as lecturer and Assistant professor in various engineering colleges at Coimbatore, TamilNadu during 2009-2014.

She holds master's degree in Environmental Engineering from Government college of Technology Coimbatore Tamil Nadu, investigated biogas production from biodegradable waste -as a part of master thesis during 2007-2009.The master's thesis work entitled "Performance Evaluation on anaerobic digestion of banana waste along with domestic wastewater" is published in proceeding "Technological Innovations for Sustainable infrastructure (TISI-2015)", NIT Calicut, Kerala India. She completed her Bachelor's degree in Civil Engineering from Thanthai Periyar Government Institute of Technology Vellore, Tamil Nadu during 2003-2007.

(S. Uma)