POTENTIAL OF BIOGAS PRODUCTION FROM SIZED COTTON YARN WASTE FROM TEXTILE MANUFACTURING PROCESS AT RIVATEX. ELDORET, KENYA

By

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Textile Engineering, Department of Manufacturing, Industrial and Textile Engineering, School of Engineering, Moi University-Eldoret, Kenya

September, 2021

DECLARATION

Declaration by the Student

This thesis entitled "Potential of Biogas Production from Sized Cotton Yarn Waste from Textile Manufacturing Process at Rivatex. Eldoret, Kenya" is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

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DEDICATION

I hereby dedicate this thesis to my loving family. Thank you for never giving up on me and encouraging me towards my goals because your sincere sacrifice towards my education has exposed me to the world of Engineering. If it were not for your love, encouragement, support, and sacrifices, I would have never made it this far. I love and appreciate you.

ABSTRACT

Biogas production is one of the most cost-efficient renewable energy technologies that use biodegradable wastes as feedstock. Sized Cotton Yarn Wastes (CYW) is among the biodegradable wastes that are commonly managed by dumping onto the open land or disposing in sanitary landfills where they undergo anaerobic decomposition. Nevertheless, CYW could be used as the substrate to generate energy in the form of biogas that can be utilized in other activities like powering textiles production. This research aimed to investigate the potential of biogas production from CYW through the solid anaerobic digestion process. The CYW was collected from Rivatex East Africa Limited, Eldoret, Kenya. The specific objectives were to characterize the substrate for biogas production, determine the optimum ratio of total solids (TS) concentration at different ratios of the substrate on biogas volume yield, and analyse the biogas produced to determine its fractional composition. The experiment was carried out in ten reactors of two-litre capacity. The reactors were loaded with varying concentrations from the highest (50%TS) to the lowest (10%TS). The physicochemical characteristics of materials were determined using standard methods. The biogas produced was measured on daily basis (37 days) using the water displacement method. The analysis of CYW before digestion showed that TS, total volatile solids (TVS), and moisture content (MC) were 93.18 ± 0.21%, 82.48 ± 0.51%, and 6.82 ± 0.48% respectively. Analysis of digested sludge showed that TS, TVS, and MC were 16.78 ± 2.66%, 52.84 ± 2.42%, and 83.58 ± 2.72% respectively. The carbon to nitrogen (C/N) ratio of inoculum was 20.5:1, which is in a suitable range to keep the anaerobic digestion (AD) in a stable condition. However, the CYW had high carbon content; resulting in a C/N ratio of 42.5:1. The reactors presented the different biogas yields with 667.57 ± 4.29 mL per g-TVS, 698.88 ± 1.34 mL per g-TVS, 731.87 ± 2.15 mL per g-TVS, 782.87 ± 3.59 mL per g-TVS, 695.93 ± 3.68 mL per g-TVS, 597.14 ± 3.14 mL per g-TVS, 513.40 ± 2.70 mL per g-TVS, 355.41 ± 3.48 mL per g-TVS, 278.72 ± 4.05 mL per g-TVS, and 203.01 ± 3.34 mL per g-TVS respectively. Reactor four (R4) was the one that presented the highest methane (53.98 ± 0.03%) from biogas produced. The average reduced TS and TVS at the end of digestion were 33.35 ± 3.50% and 36.67 ± 3.87%. Based on the degradation characteristics, R4 had the most significant degradation rates of TS (57.78 ± 0.92%) and TVS (62.96 ± 0.40%) respectively while reactor ten had the lowest one (18.41 ± 0.40% TS and 20.83 ± 0.29% TVS). In conclusion, CYW is a suitable substrate for AD due to its high organic matter. Moreover, biogas yield from CYW has a significant positive relationship with the %TS concentration ($p<0.05$). The C/N ratio of CYW reported in this study is higher than the optimal value for AD, therefore, the co-digestion of treated CYW with a nitrogen-rich substrate is recommended to help balance the feed nutrients for higher biogas yield. Further studies are recommended to check for ammonium composition in the residues to determine their suitability for application as fertilizer.

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LIST OF ABBREVIATIONS

AD: Anaerobic Digestion

ANOVA: Analysis of Variance

NMMO: N-Methylmorpholine-N-oxide

OLR: Organic Loading Rate

C: Carbon

N: Nitrogen

CH_4: Methane

g: gram

mL: millilitre

N_2O: Nitrogen Oxide

Kg: kilogram

TVS: Total Volatile Solids

TS: Total Solid

T: Temperature

PVA: Polyvinyl Alcohol

COD: Chemical Oxygen Demand

℃: Degree Celsius

VFAs: Volatile Fatty Acids

pH: potential Hydrogen

HTR: Hydraulic Retention Time

NPK: Nitrogen, Phosphorus, and Potassium

H_2SO_4: Sulphuric Acids

H_2O_2: Hydrogen peroxide

NaOH: Sodium Hydroxide

H_2: Hydrogen

O_2: Oxygen

NH_4OH: Ammonium Hydroxide

eq: Equation

CYW: Sized cotton yarn waste

ppm: parts per millions

L: litre

ACKNOWLEDGEMENTS

I would like to thank the Almighty God for giving me the strength and wisdom to hold on with and complete my thesis. I would love to extend my gratitude to some persons whose efforts have managed to progress and put a landmark in my education. I acknowledge the African Centre of Excellence in Phytochemicals, Textile and Renewable Energy (ACE II-PTRE) for the sincere financial and moral support which helped me uplift my career professional competence, performance, and excellence in the engineering field. Sincere thanks go out to my supervisors Dr. Eric Oyondi Nganyi and Dr. Milton Arimi for their tireless support, patience, guidance, and advice throughout this research. I would also like to thank my lecturers; Prof. Anil Kumar, Dr. David Githinji Njuguna, Dr. Jerry Ocholla, Dr. Charles Nzila, Dr. Obadiah Maube, and Dr. Muchilwa. I'm also thankful to Mr. Xumay Bura Hhaygwawu for providing the scientific ideas during my work. I am thankful to Mr. Alex and Mr. Paul for practical assistance during my experiments. Many thanks go to my colleagues and friends with whom I interacted with during my studies at Moi University. Thank you for making my experience a pleasant one.

CHAPTER ONE
INTRODUCTION

1.1. Background of the study

Since the start of the industrial revolution, the energy requirement for industries has gradually increased worldwide. Population growth and the promotion of living standards have always been key drivers to increase energy demand and fibre consumption (Hasanzadeh et al., 2018; Y. Wang, 2010). Fossil fuel resources such as solid fuels, liquid fuels, and gas fuels represent important world energy resources (Al-Hamamre et al., 2017).

However, increasing world population together with reducing fossil fuel reserves have resulted in global interest to gradually change the energy source from fossils to renewable energy (Rajendran & Balasubramanian, 2011a). One of the environmental problems faced by the planet is that of solid waste management especially biodegradable wastes like textile cotton yarn wastes (CYW). The problem of the final textile industrial wastes has now assumed serious dimensions since it has no saleability and pollutes the atmosphere (Sharma-Shivappa, 2008). Additionally, environmental pollution caused by dumping or landfilling of waste materials in the environment is among the foremost crucial issues the planet is facing today (Deepanraj et al., 2017).

Currently, the management of textile wastes involves recycling them as second-hand textiles, filling materials in the textile industry, composting, landfilling, and burning (Hasanzadeh et al., 2018). Therefore, the annual global production of end life textile wastes is increasing, causing an increased interest in the impact of the disposed of wastes on the environment. However, textile waste is a rich source of energy and substrate for biogas production (Hasanzadeh et al., 2018).

Textile wastes include wastes from streams of fibre, textile and clothing manufacturing processes, commercial service, and consumption (Hu et al., 2018). Textile wastes are mainly composed of cotton and viscose fibres. Reports from previous studies show that cotton has a significant potential to be used as a substrate for biogas production (Achinas et al., 2017; Rasel et al., 2019). The environmental problems caused by organic wastes including cotton wastes should be militated against. One effective way of avoiding these problems is to use cotton wastes as the substrate for biogas production (Papacz, 2011). It is possible to mitigate the negative environmental effects of cotton wastes by using them for the production of biogas.

Moreover, the transformation of complex organic materials into biogas reduces the emission of greenhouse gases and can produce by-products like high-value fertilizer for growing crops (Jeihanipour et al., 2013; Treichel & Fongaro, 2019). Furthermore, concerning emissions, biogas production might be better for the environment than the incineration of wastes. Methane from biogas has different applications (Papacz, 2011; Velmurugan et al., 2014).

Putting all these above advantages into consideration, biogas is one of the principal environmentally friendly energy sources which could substitute fossil fuels (Diane et al., 2009). Biogas represents one of the most important renewable energy sources (Holliger et al., 2016; Triolo et al., 2012). Biogas is often produced from a large range of substrates like industrial, municipal, wastewater, agricultural, and food wastes, moreover plant residues (Phun et al., 2017; Treichel & Fongaro, 2019). Biogas production and its compositions rely on the substrate contents, while their chemical compositions and biodegradability are key factors in the production of biogas and methane (Treichel et al., 2019).

Biogas consists mainly of methane (40-75%), carbon dioxide (25-60%), and other impurities that become inconvenient when not removed (Andriani et al., 2014; Rajendran & Balasubramanian, 2011b). Biogas is mostly produced by anaerobic digestion (AD) via conversion of organic matter through different processes including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Yusuf, 2013). These processes will be described in additional detail in chapter two. Anaerobic digestion is split into three categories depending on the total solid (TS) contents. The low solid reactors contain less than 10% TS with material to water ratio of 1:10 (Kleinheinz & Hernandez, 2016).

The medium solid reactors contain 15-20% TS with material to water ratio of 1:5-7. Finally, the high solid reactors have TS of 22-40% with material to water ratio of 1:2.5-4.5 (MONNET, 2009). Generally, the organic dry matter content that is suitable as substrates for AD ranges from 70% to 95% of TS. It gives a significant advantage over other waste treatment methods. The performance of the AD process is highly dependent on the characteristics of substrates as well as the activity of the microorganisms involved in different degradation steps (Horváth et al., 2016).

The optimization of the AD process has mainly been focused on operational parameters like reactor configuration, mixing, pH, C/N ratio, loading and retention times, temperature, feedstock composition, and pre-treatment methods (Ferguson et al., 2014). Facilities that are available run using mainly industrial wastes as feedstock. Nevertheless, the necessity of expanding AD to a variety of latest substrates has raised attention in key points that should be taken into consideration when new feedstocks are to be used. The use of cotton wastes for the production of high-value compounds including biogas and industrial products provides a method to alleviate disposal issues, reduce consumption of fossil fuels and mitigate adverse impacts on the environment (Sharma-Shivappa, 2008).

From literature, there is very limited work that has used cotton wastes as a substrate for biogas production (Ismail & Talib, 2016). Isci and Demirer, (2007) tested the anaerobic treatability and methane generation potential of various cotton wastes in batch reactors. Results indicated that cotton wastes are often treated anaerobically and are a good source of biogas.

Therefore, due to its large potential for biogas production, cotton certainly merits more research attention for being employed as feedstock in digestion with manures. Ismail and Talib (2016), examined the potential of using recycled medical cotton industrial waste as a source of biogas recovery. However, from the extensive literature survey, no previous study examined the CYW as a possible source for biogas production. The objective of this research work was to investigate the potential of biogas production from CYW through the AD process.

1.2. Problem statement of the study

Solid wastes from municipal and industrial activities are major sources of environmental pollution. Large volumes of those wastes are being generated and are increasing immensely because of a rise in population, urbanization growth, and high consumption rate. The quantity of waste generated is increasing to a level that is difficult to manage. The developing countries are confronted with the difficulties of a tremendous measure of waste management due to the increasing amount of waste disposed and urbanization development. Industrial wastes generated can cause air pollution and global warming at a different rate by releasing gases like CO_2, CH_4, and N_2O. As methane causes global warming 28 times more impact on climate compared to CO_2 (Rutz, 2007; Wellinger et al., 2013; Y. Zheng et al., 2014), therefore, the explanation for the unrestricted release of CH_4 in the process of biogas production and utilization should receive particular attention.

Nitrogen monoxide causes heating 310 times more than CO_2 (Neshat et al., 2017). The various types of wastes are collected in Rivatex East Africa Limited, Eldoret, Kenya at different sections including CYW. Therefore, the problem of those wastes has now assumed serious dimensions since it has no saleability and pollutes the atmosphere.

Normally, the burning, landfilling, and open dumping methods are mostly used as treatment methods for those wastes which increase the worldwide warming problem. Landfilling is a significant practice of disposing of organic solid wastes resulting in emissions of CH_4 and nitrous oxides which contribute to the greenhouse effect. Composting and burning are common methods of treating these wastes. However, composting promotes the emissions of volatile compounds (ketones, aldehydes, ammonia, and CH_4) while incineration can significantly bring out toxic or carcinogenic hydrocarbons to the environment if the exhaust gas is not handled properly (Patinvoh et al., 2017).

Nonetheless, there are guidance rules and standard techniques for the administration of these wastes but by adding on these wastes management options that are both naturally well-disposed and conservative are considered. Subsequently, biogas production through AD is a suitable wastes management option for managing the organic fraction of solid wastes, as biogas production usually leads to reduce pollution and increase energy production. The research work-study was initiated to investigate the feasibility of converting these wastes into biogas.

1.3. Justification of the study

Incineration and open dumping of 48 tons per year of CYW can cause environmental pollution through the discharge of CO_2, CH_4, and N_2O. The need for clean energy and phasing out fossil fuels which have a high amount of greenhouse gas emissions within the atmosphere is continuously increasing.

This necessitates turning to greener alternatives that will have sustainable clean energy production. The use of CYW as feedstock is one of those alternatives. Using this CYW as feedstock would inform plans on waste management and utilization strategies of various materials as feedstock in biogas production.

Anaerobic digestion of CYW is the source of unpolluted energy that may be used for heating purposes. According to Isci & Demirer, (2007), 13 kg of cotton wastes is needed to provide one metre cube (1 m^3) of pure CH_4 in 23 days. Saravanan et al., (2009) also reported that 5 kg of cotton wastes with 5% of cow dung could provide 200 litres of biogas in 45 days. Rajendran and Balasubramanian, (2011), found that one gram (1g) of untreated jean cotton wastes in a very continuously stirred tank reactor of the closed system produced 100 mL of biogas per day.

Therefore, based on the present waste collected in Rivatex, ten metre cubes (10 m^3) of biogas can be produced from CYW in one month and will be used for heating purposes in place of using firewood. Biogas is often enhanced and compressed, very similar to natural gas, and used to power generators and motorized vehicles (Eskicioglu & Ghorbani, 2011). The digestate which is rich in nutrients might be used or sold as a valuable organic fertilizer to substitute chemical fertilizers for soil amendment (Dioha et al., 2013).

Anaerobic digestion improves sterilization, helps in air and water contamination control, and reduces greenhouse gas emissions. Anaerobic digestion diminishes the interest in wood and charcoal for cooking, hence, helps forested regions and natural vegetation. Additionally, it can also help reduce an intense health issue thanks to poor indoor air quality-related to wood and charcoal utilized for cooking (Manyi-loh et al., 2013).

1.4. Objectives of the study

1.4.1. Main objective

- To investigate the potential of biogas production from sized cotton yarn wastes through the solid anaerobic digestion process.

1.4.2. Specific objectives

1. To characterize the substrate for biogas production.
2. To determine the optimum ratio of total solid (%TS) concentration at different ratios of the substrate on biogas volume yield.
3. To analyse the biogas produced to determine its fractional composition.

1.5. Significance and expected output of the study

The characterization of the substrate has shown that the operating parameters influence biogas production. This work will investigate various concentrations of %TS in AD and therefore the corresponding amounts of biogas produced, to work out conditions for optimum biogas production if proper conditions are maintained. The cutting of the CYW into small pieces will improve the microbial activity because the surface area will be increased. Therefore, it will increase the efficiency of biogas production and will improve the entire process economy.

The determination of the biogas composition will give indications of whether it is suitable as a fuel source and reduce greenhouse gas emissions. In keeping with the figure published by Jørgensen & Energi, (2009), atmospheric emissions are reduced by 400 g CO_2 for each one kilo watthour (1 kWh) electricity produced from biomass by biogas production. Biogas-fuelled vehicles can reduce CO_2 emissions by between 75% and 200% compared with fossil fuels (Papacz, 2011).

1.6. Scope of the study

This study will characterize the effect of CYW as a feedstock on methane yield during the solid AD process. The study will be conducted to determine the biogas compositions and find the optimum ratio of the substrate required to give a high biogas yield. The wastes will be collected at Rivatex East Africa Limited, Eldoret, Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.0. Introduction

With the introduction of commercial AD plant designs during the early 1990s, the world has focused on AD of organic wastes. The AD is a method or a process by which the organic matter is biologically decomposed into another form by a range of anaerobic microorganisms with absence or freed from oxygen conditions (Li et al., 2011). The varied microbial populations degrade organic matters which produces biogas and other energy-rich organic compounds as end products. A wide range of materials including municipal, agricultural, industrial wastes, and plant residues are decomposed by AD.

Furthermore, AD has some advantages like low energy requirement for operation and low biomass production, high-efficiency treatment, simple design, and use of non-sophisticated materials. Moreover, it's also considered as a viable technology in the production of renewable energy continuously (Shete & Shinkar, 2017).

Therefore, AD is an environmentally useful technology. Ward et al., (2008) and Naik et al., (2013) reported the advantages of the AD process in reducing the environmental pollution in various ways among which includes reducing CH_4 emission from biomass that stops the exit of CH_4 into the atmosphere while burning off the CH_4 to release carbon-neutral dioxide (no net effect on atmospheric CO_2 and other greenhouses gases). Therefore, biogas will give lower exhaust emissions and then help to boost local air quality.

2.1. Process of biogas formation

Biogas is produced by the decomposition of organic materials under anaerobic conditions with the assistance of anaerobic bacteria. In AD; the organic matter is decomposed by the intensive reaction of a large range of microorganisms in the absence of oxygen to provide the CH_4 and CO_2 as the end-products under ideal conditions (Al-Hamamre et al., 2017). Anaerobic digestion of organic wastes and residues combines both sustainable treatment and renewable energy production.

The process consists of a complex series of reactions that convert an outsized array of polymeric substances such as proteins, carbohydrates, and lipids providing carbon atoms at various oxidation and reduction states; to one carbon molecules in its most oxidized state (CO_2) and its most reduced state (CH_4) (Bruni et al., 2010). Anaerobic digestion is a complex organic process operated by various groups of microorganisms that convert organic matter to biogas through four major steps, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Khalid et al., 2011). The second and third steps are called acid formation steps and the fourth one is termed the methane formation stage.

2.1.1. Hydrolysis process

Biomass is usually made up of large organic polymers. For the bacteria in AD to access the energy potential of the material, these chains must first be broken into smaller constituent parts (Wilson Parawira, 2004). The method of breaking these chains and dissolving the smaller molecules into solution is termed hydrolysis. During the hydrolysis process, the lipids (fats) are converted into fatty acids, carbohydrates (polysaccharides) into simple sugars (monosaccharides), and proteins into amino acids (Rajendran & Balasubramanian, 2011a).

Therefore, the hydrolysis of these polymeric components is the first step in AD. It is carried out by different groups of facultative or obligate fermentative bacteria through the release of extracellular enzymes (Meegoda et al., 2018). Through hydrolysis, complex organic matters are broken into sugars, amino acids, and fatty acids (Gashaw, 2014).

Reactions 2.1 and 2.2 are samples of hydrolysis reactions where cellulose and protein are hydrolysed to soluble sugars and amino acids respectively within the presence of water.

$C_6H_{10}O_5 + H_2O \rightarrow C_6H_{12}O_6$ (2.1)

$-(H_2NCHRCOOH)\ n - + H_2O \rightarrow H_2NCHRCOOH$ (2.2)

Cellulosic hydrolysis shown as reaction 2.1 has been considered the rate-limiting step in AD especially when the substrate contains a high concentration of lignocellulosic materials. The pH, temperature, and solids retention time are some parameters that affect the rate of hydrolysis reaction (Gashaw, 2014). Where the higher rate might be obtained at slightly acidic conditions, high temperature, and long solid retention time (Veeken & Kalyuzhnyi, 2000; H. Wang et al., 2012). Some other reactions take place in the hydrolysis process (Abdelgadir et al., 2014) as shown in equations 2.3-2.6.

Lipids (CH_3 (CH_2) n- COOH) → Fatty Acids ($CH_3(CH_2)$ x- COOH) (2.3)

Polysaccharides (starch) $(C_6H_{10}O_5)$ n → Monosaccharides (CH_2O) x (2.4)

Protein $(C_6H_{10}O_5)$ n → Amino Acids (R-CH (NH_2)-COOH) (2.5)

Nucleic Acids (HO-R-CH_2-COOH) → Purines ($C_5H_4N_4$) & Pyrimidines ($C_4H_4N_2$) (2.6)

2.1.2. Acidogenesis process

The process of acidogenesis results in further breakdown of the remaining components by acidogenic (fermentative) bacteria. Here, volatile fat acids (VFAs) are created together with NH_3, CO_2, and H_2S, furthermore as other by-products (Myovela et al., 2018).

Nevertheless, the tip product from hydrolysis (like the soluble sugars and amino acids) is converted to VFAs (formic, acetic acids, propionic, butyric, isobutyric, valeric, isovaleric, caproic, and heptanoic acids) (Gashaw, 2014). Therefore, different chemical pathways are followed in the acidogenesis process. Some examples of acidogenesis pathways are shown in equations 2.7 – 2.9.

$$C_6H_{12}O_6 + 2H_2O \rightarrow \quad 2CH_3COOH + 2CO_2 + 4H_2 \qquad (2.7)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \qquad (2.8)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \qquad (2.9)$$

The AD system has got to be maintained under low H_2 partial pressure to receive a high concentration of ethanoic acid that is favourable for biogas production (Meegoda et al., 2018). This is often favoured by β-oxidation that uses low H_2 partial pressure to transform long-chain fatty acids to acetic or propionic acid (Cazier et al., 2015; Ravindra, 2015).

Muthu et al., (2017) reported on the inhibition of β-oxidation when the AD system is accumulated of H_2 and where, several hydroxylic groups are presented within the cellulose chains, resulting in the formation of H_2 bonds in the same chains or vicinal chains.

Consistent with the previous statement, therefore, reactions 2.8 and 2.9 have taken advantage of the AD process to hold the H_2 concentration within the system and take over reaction 2.7 until the extent of H_2 in the system is back to normal (Ravindra, 2015). Like hydrolysis, acidogenesis is mostly affected by the pH in the AD where the degree of acidification increases with pH. Yu & Fang, (2003) found that a pH of 6-7 in AD gives the advantage of the production of acetate, butyrate, and i-butyrate.

2.1.3. Acetogenesis process

In the acetogenesis stage, the simple molecules created through the acetogenesis phase are further digested by acetogens to produce largely ethanoic acid, as well as CO_2 and H_2 (Vögeli et al., 2014). Hydrogen plays an important intermediate role during this process because the reaction will only occur if the H_2 partial pressure is low enough to thermodynamically allow the conversion of all the acids (Joshua et al., 2014).

The first three steps are known acid formation. The homo-autotrophic acetogenesis is made by acetate from H_2 and CO_2 as shown in reaction 2.13. This step plays an important role as more than 70% of CH_4 from AD is produced from acetic acids (Cazier et al., 2015). The reactions 2.10 and 2.11 are examples of chemical reactions under acetogenesis. As the acetogenic bacteria can convert FVAs to acetic acids (Ravindra, 2015). Moreover, it can also transform ethanol to produce carboxylic acid as shown in reaction 2.12. Homoacetogens also consume CO_2 and H_2 to provide carboxylic as shown in reaction 2.13.

$$H_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (2.10)$$

$$CH_3CH_2CH_2COOH + 2H_2 \rightarrow 2CH_3COOH + 2H_2 \quad (2.11)$$

$$CH_3CH_2OH \rightarrow CH_3COOH + 2H_2 \quad (2.12)$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (2.13)$$

In this process, no organic matter is removed from the liquid phase but converted into a substrate for further process of methanogenesis, and the acetate bacteria convert the acid-phase products into acetate and H_2 that will be utilized by methanogenic bacteria (Meegoda et al., 2018). The methanabacterium suboxydans bacteria account for the decomposition of pentanoic acid to propionic acid while the methanobacterium propionicum bacteria account for the decomposition of propionic acid to acetic acid (Mir et al., 2016a).

Aside from inhibition of acidogenesis reaction, the acetogenesis reaction is also inhibited by high H_2 partial pressure. For this reason, it is important to take care of a vigorous community of homoacetogens which play a function to maintain the H_2 level in the AD process while the remainder of the reactions produce by-products.

2.1.4. Methanogenesis process

The last step is the organic process of methanogenesis where the methanogens use acetic acid and convert them into CH_4, CO_2, and H_2O (Veeken & Kalyuzhnyi, 2000). The pH plays an important role in this process and it should be between 6.5 and 8 values (Manyi-Loh et al., 2013; Muthu et al., 2017). The methanogenesis process can be demonstrated through chemical reactions. Therefore, the reactions 2.14 to 2.16 show the reactions that are responsible to convert acetate, ethanol, and CO_2 into CH_4 (VERMA, 2002).

$$CH_3COOH \text{ (acetic acid)} \rightarrow CH_4 + CO_2 \quad (2.14)$$

$$2C_2H_5OH \text{ (ethanol)} + CO_2 \rightarrow CH_4 + 2CH_3COOH \quad (2.15)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.16)$$

During the CH_4 formation process, the various enzymes play a vital function. They convert carbon oxide (CO) and formate into CH_4. Further, they also help in acetate and carbonyl transformation during the metabolism of CH_4 formation (Meegoda et al., 2018). Methanogens that participate in the production of CH_4 are grouped as strict anaerobic bacteria. The transformation of complex substrates into CH_4 and CO is feasible by the cooperation of four different groups of micro-organisms (Arsova, 2010) and is shown in the following Table 2.1.

Table 2. 1: Conversion of complex organic compounds to CH_4 and CO_2 by four different groups of microorganisms

Microorganisms	Electron donors	Electron acceptors	Product	Reaction type
Methanogenic bacteria	OC	OC	CO_2	Fermentation
Syntrophic bacteria	OC	OC	H_2	Acidogenesis
Acetogenic bacteria	OC/H_2	CO_2	CH_3COOH	Acidogenesis
Methanogenic bacteria	OC/H_2	CO_2	CH_4	Methanogenesis

Source: (Muthu et al., 2017), OC: Organic Carbon

These micro-organisms are classified as primary bacteria, secondary bacteria including syntrophic and acetogenic bacteria, and two remaining forms of methanogens belonging to the domain Archaea which provide different roles during the process of AD degradation of wastes (Shete & Shinkar, 2017). Figure 2.1 summarized the AD process for biogas production through four steps and pre-treatments classification.

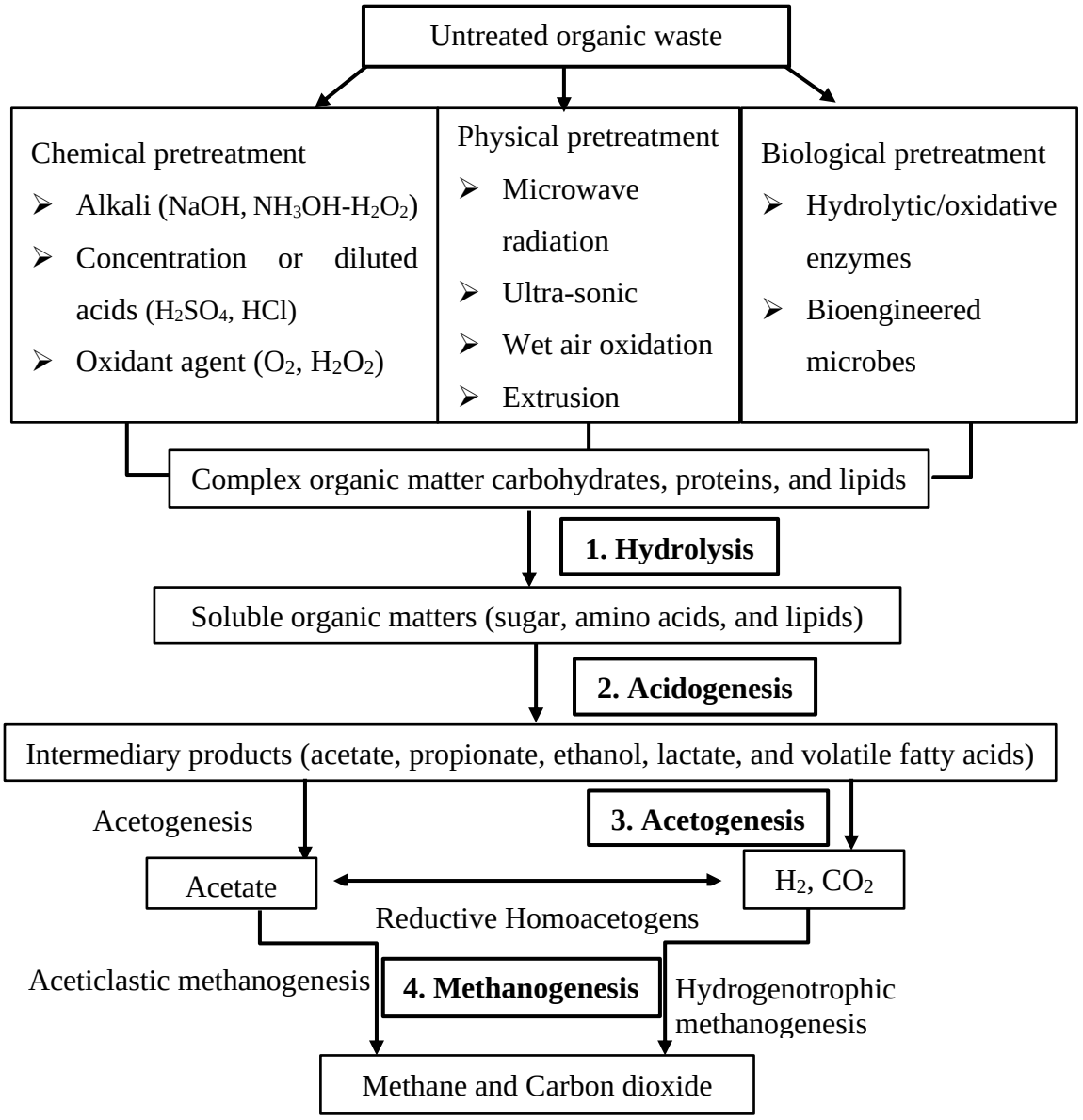


Figure 2. 1: Pre-treatment methods classification and AD process for biogas production modified from (Andriani et al., 2014; Mir et al., 2016a)

2.2. Biogas compositions

The biogas produced during the AD is composed mainly of CH_4 and CO_2 with smaller amounts of H_2S and ammonia (NH_3). Trace amounts of H_2, CO, saturated or halogenated carbohydrates, and O_2 are occasionally present in the biogas as shown in Table 2.2 (Saber & Takach, 2009). The composition of biogas is different depending on the source. The typical composition of biogas, landfill gas, and fossil fuel gas is shown in (Table 2.2). The biogas composition is of course linked to the waste composition and it can vary.

Table 2. 2: Composition of biogas, landfill gas, and Fossil fuel

Constituents	Units	Fossil fuel	Biogas	Landfill gas
Methane	Vol%	91	55-75	45-58
Ethane (C_2H_6)	Vol%	5.1	0	0
Propane (C_3H_8)	Vol%	1.8	0	0
Butane (C_4H_{10})	Vol%	0.9	0	0
Pentane (C_5H_{12})	Vol%	0.3	0	0
Carbon dioxide	Vol%	0.61	25-45	42-55
Nitrogen	Vol%	0.32	0-2	0-3
Volatile organic compound	Vol%	0	0	0.25-0.50
Hydrogen	Vol%	0	0	Trace > 1%
Hydrogen sulphide	ppm	>1	>500	10-200
Ammonia	ppm	0	>100	0
Carbon monoxide	ppm	0	0	Trace

Source (MONNET, 2009; Ray et al., 2013)

The biogas composition mainly depends on the subsequent points:

- The addition of long-chain hydrocarbon compounds: Here, the substrate rich in fat content can help to boost the quality of the biogas that provided the quantities which are reasonable and not large avoiding acidity.
- The structure of the material: the kind of disintegration of the material becomes important if the material is well enclosed to the lignin structure. Thus, the structure should be disrupted instead of cut.
- The number of carbon atoms in the substrate: The CH_4 content increases with a rise within the number of carbon atoms in the substrate as shown in Figure 2.2.

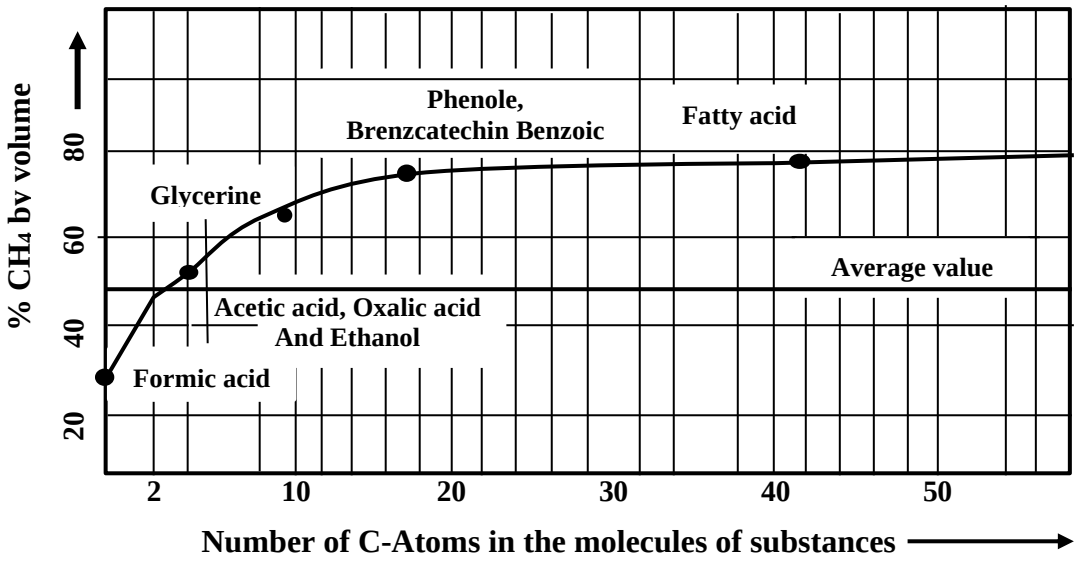


Figure 2. 2: A statistical relation between CH_4 content in biogas and the number of carbon atoms (Rajendran et al., 2011)

- The time of exposure: the anaerobic decomposition of biomass generally improves with the time of reaction. The content of CH_4 increases disproportionately towards the end of duration.
- The activation of the substrate: if the substrate in the reactor is well and homogeneously activated, the fermentation process takes place much faster and as simply as possible. Therefore, the time of reaction may be shorter.
- The preparation of the substrate: the substrate should be prepared and kept well to expedite and intensify the composition (Deublein & Steinhauser, 2011).
- Content of liquid in the reactor: the extent of CO_2 in the gas phase reduces if the content of the liquid in the reactor is high which ends in an exceedingly high concentration of CO_2 dissolved in H_2O.
- The temperature during the process: the upper temperature during the fermentation process corresponds to the lower concentration of CO_2 dissolved in water.

- The pressure: the next concentration of CO_2 dissolved in water is led by high pressure. This will positively influence the standard of biogas if the materials from the underside are removed because CO_2 is discharged. Table 2.3 shows the biogas composition.

Table 2. 3: Approximate biogas composition in AD

Gas	CH_4	CO_2	N_2	H_2	H_2S	O_2	C_xH_y	NH_3
Conc. in %	40-75	25-60	0-10	0-5	0-3	0-3	0-1	0-0.5

Source: (Mir et al., 2016b), Conc.: concentration

2.3. Biogas application

The biogas can be used for all applications designed for fossil fuel subject to some further upgrading, as not all gas appliances require gas with identical quality standards. Biogas can be utilized for heating using boilers. The heat from biogas has many applications like producing steam for industrial processes (Gashaw, 2014). Biogas can be utilized in engines (combined heat and power units). Combined heat and power units are a decent mode of producing efficiently both electricity and heat for the AD plant (Teodorita et al., 2008).

The biogas is used as fuel for vehicles further as lighting and cooking source, the standard requirements are identical to those used for gas (Mir et al., 2016b). The methane contained in the biogas may additionally be used as a fuel for the electric cell (Shete & Shinkar, 2017). Table 2. 4 shows the consumption rates of various biogas applications (Andriani et al., 2015; Vögeli et al., 2014). Generally, biogas has been used as renewable energy and this reduces the dependency on imported fossil fuels and it helps in the reduction of gas emission, mitigation of worldwide warming, and waste reduction (Dioha et al., 2013)

Table 2. 4: Typical utilization rates of biogas in litres per hour (L/h)

Biogas application	Consumption Rate (L/h)
Household cooking stove	200-450
Industrial burners	1000-3000
Refrigerator (100 L) depending on the outside temperature	30-75
Gaslamp, equivalent to 60 W bulb	120-150
Biogas/ diesel engine per brake horsepower (746 watts)	420
Generation of 1 kWh of electricity with biogas/ diesel	700

Finally, biogas is flexible and efficient for the final user. Furthermore, the digestate is employed as a wonderful fertilizer for crops as shown in Figure 2.3 (Ravindra, 2015).

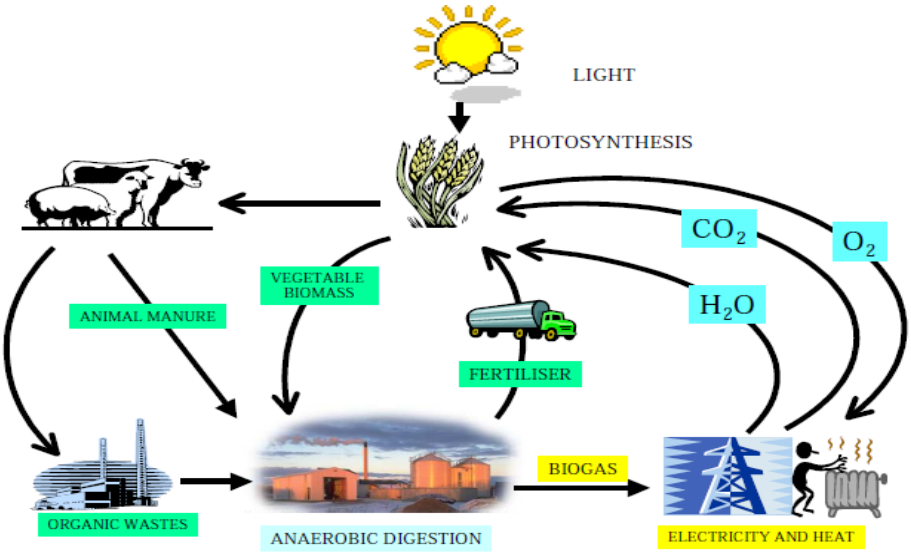


Figure 2. 3:The sustainable cycle of biogas from AD (Seadi et al., 2008)

2.4. Substrate

The substrate that provides carbohydrates, proteins, fats, cellulose, and hemicelluloses is suitable for biogas production (Achinas et al., 2017; Rajendran, 2015). It is therefore important to run fermentation tests before completing the efficient analysis because the biogas production depends highly upon, for instance, the atmospheric phenomenon, the variability, the harvesting time, and the continuance (Abbasi et al., 2012; Deublein & Steinhauser, 2011).

During the choice of the substrate, it is critically important to contemplate the subsequent points:

- The content of the organic substrate should be appropriate for the chosen fermentation process.
- The potential for gas formation and the nutritional value of organic matter should be as high as possible.
- The substrate should be freed from pathogens and other organic matters which should be needed to facilitate the fermentation process.

In similar operating conditions, characteristics of the substrate play a critical role in defining the quantity of biogas produced which might be recovered by AD (Khalid et al., 2013). Substrates that are made better in lipids and easily-degradable carbohydrates exhibit higher biogas production than those of lignocellulosic materials (Cesaro & Belgiorno, 2015). Cotton waste is rich in cellulose and is extremely solid powder content (Rajendran et al., 2011) and so, it contains 40 to 91% of cellulose (Hasanzadeh et al., 2018; Thambiraj & Shankaran, 2017).

Therefore, it might be economically transformed into biogas (Figure 2.4) (AKUNNA, 2018). The CYW contains the starch because the natural starch and its derivatives constitute almost 75% of the sizing agent used to size cotton yarn (Temesgen et al., 2019). Besides, it provides over 70% solid content while other materials like cow dung contain only 15 to 18% (Gholamzad et al., 2014; Raj et al., 2009). Raj et al., (2009) also identified that cotton waste contains a moisture content of around 8.8% (Table 2.5).

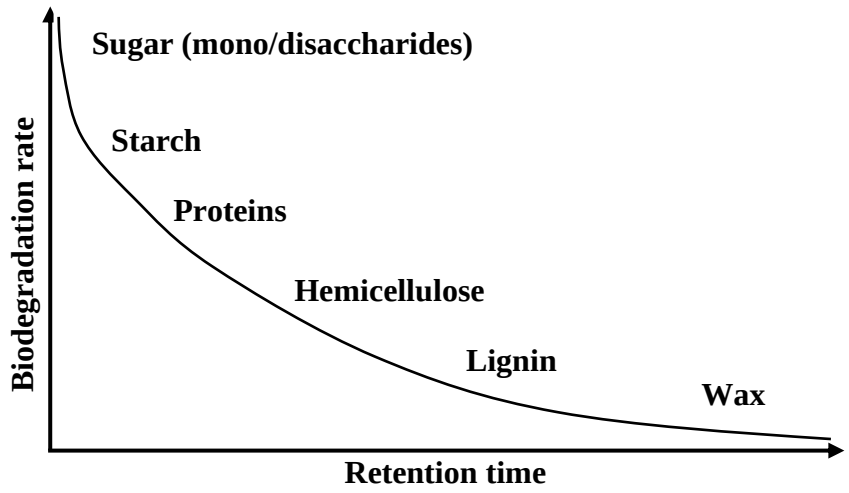


Figure 2. 4: Relationship between the speed of degradation and retention time reckoning on the type of organic compound (AKUNNA, 2018)

Therefore, the cotton wastes are potential material for biogas production if the right conditions are maintained. Biogas production from cotton wastes can be increased by using fresh cow dung as inoculum (Raj et al., 2009).

Table 2. 5: Cotton waste contents

Contents	Percentage
Moisture	8.80
Ash % by weight	7.20
Ether extractive	12.00
Non-cellulose	16.00
Cellulose	54.00
Nitrogen	0.80
Metals and other	3.20

2.5. Process and Bioreactors

2.5.1. Batch process

The batch process is also called a closed system process because fresh inoculum, substrate, and enough amounts of nutrients are added at the start of the process and do not get refilled once the process is started.

Therefore, they are then allowed to undergo all the degradation steps sequentially resulting in the formation of biogas (MONNET, 2009). It is better for the substrates which contain a high solids content (30-40%) and it is digested in a gas tight-container (Naik & Wung, 2013). After the completion of the batch process, all the slurry and therefore the digested materials are removed, and thereafter a reactor is discharged and loaded with a replacement batch (VERMA, 2002). During this process, gas production increases initially and then gets diminished. In the starting stage microorganisms get adapted to the environment and start growing; later biomass growth remains constant.

At the final stage, the biomass starts dying because of a scarcity of nutrients and other factors like attenuation in substrate and pH, therefore, the biogas yield starts decreasing. Anaerobic batch digestion is helpful because it is often performed with a straightforward and simple to handle the process, inexpensive equipment, with the waste of %TS as high as 90% with small attention during loading and unloading of the reactor. It is used in laboratory-scale studies for assessing the speed at which a material can be digested and determining the yields of biogas obtained (Khalid et al., 2013; Wilson Parawira, 2004; Rajendran, 2015; Rossano & Cividino, 2013).

There are three types of batch processes which include a single-stage system, sequential system or multi-stage system (Figure 2.5), and upflow anaerobic sludge blanket (UASB) reactor. The single-stage system involves re-circulating the leachate at the highest of the same reactor which is equivalent to a partial mixing, therefore, all the stage of the AD takes place in a single sealed reactor (Velmurugan et al., 2014).

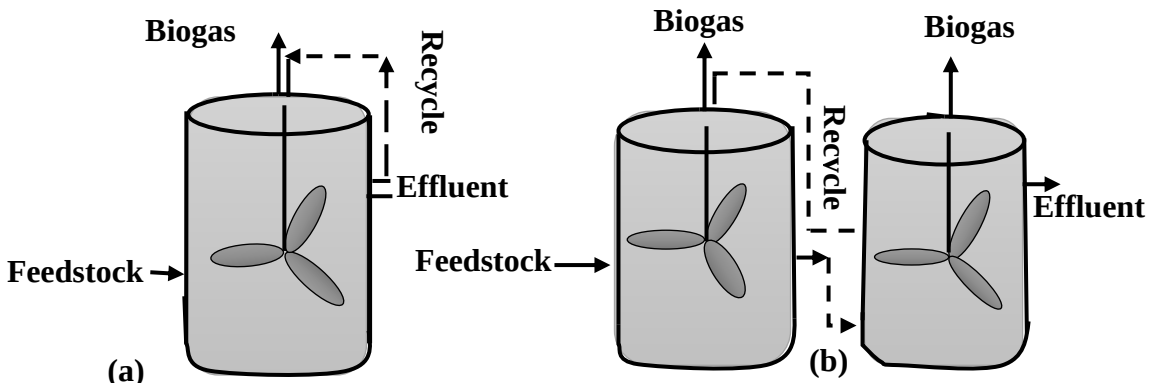


Figure 2. 5: Design variations in (a) one-stage and (b) two-stage reactors (Nizami & Murphy, 2010).

The sequential or multi-stage system comprises two or more reactors. The leachate from the first reactor containing a high level of organic acids is re-circulated to the second reactor where methanogenesis occurs (Anahita et al., 2019). The leachate from the methanogenesis reactor having small or no acids is combined with a pH-buffering agent and re-circulated to the first reactor. This ensures the inoculation between the two reactors that eliminates fresh waste with seed materials.

Hence, separate reactors are employed for both acid and CH_4 formation to bring maximum control over microorganisms during acid and CH_4 formation (Khalid et al., 2013). The third type is that of the hybrid batch-UASB process which is extremely the same as the multi-stage process with two reactors with biomass retention (Ravindra, 2015).

2.5.2. Continuous process

In the continuous process, the reactors are continuously fed and withdrawn simultaneously to stabilize the gas production (Rossano & Cividino, 2013). The continuous process reactor requires regular intervals during substrate feeding and a constant removal rate of comparable output.

The materials which contain a minimum of 20% of solids content in the container are preferable for this sort of reactor (Naik & Wung, 2013). The substrate and nutrient are added continuously to maintain the constant growth of the bacteria and this continuous addition turns to extend the duration of the exponential phase due to constant growth and exhaustion of biomass (Rajendran & Balasubramanian, 2011a). Mixing is completed with the assistance of an agitate and regular flow is maintained. A high flow would result in washing away of the biomass. Temperature and pH are maintained to stabilize gas production. The gas which is continually produced from the reactor is collected in a gas holder (Karray et al., 2017).

2.5.3. Dry and wet digesters

Baere, (2016) and Rutz, (2007) reported that the anaerobic reactor type in which the feedstock used consists of 20-40% of dry matter is called dry AD whereas those with below 20% of dry matter are called wet AD. One-stage dry batch reactors are typically used whereby the high TS feedstock is entered into a vessel without any diluted solution (Figure 2.6). The leachate or water is employed by recirculation (Baere, 2016).

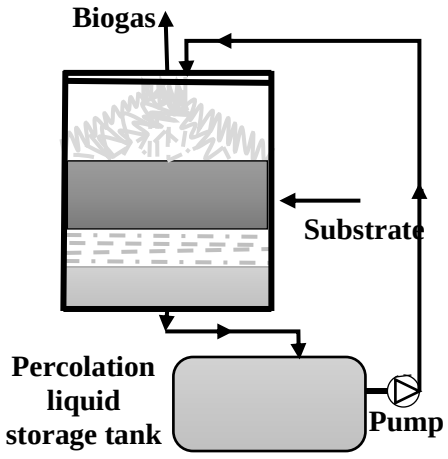


Figure 2. 6: One-stage dry batch reactor with a sprinkling of liquor in a very controlled system (Nizami & Murphy, 2010)

The vertical continuous stirred tank reactor configuration is that the most commonly used configuration that accounts for 90% of the newly erected wet reactors (Wilson Parawira, 2004; Rutz, 2007). Parasitic energy demands for the wet reactor are higher compared to the dry reactor (Wellinger et al., 2013) because of the necessity to dilute the substrate and mixed reactors for the entire retention time. The dry batch reactor has more advantages compared to others. These include simplicity, robustness, flexibility, inexpensive equipment, and ease in assessing the rate of digestion (Chatterjee & Mazumder, 2016; Khalid et al., 2011).

2.6. Factors affecting the biogas production

Anaerobic digestion is a highly complicated process to provide biogas from organic wastes. This process proceeds through the interactions of the many biotic (microbial community) and abiotic (reactor parameters) factors yet to be made clear and comprehensible (Khalid et al., 2013). To take advantage of AD to its full potential and achieve the most potential of this technology, much more about the basic process parameters needs to be explored (Jain et al., 2015; Neshat et al., 2017). Precise control of a number of these parameters is important as any deviation from their optimum levels can even cease the entire process (Neshat et al., 2017).

2.6.1. Temperature

Temperature plays a critical role in the biogas production process. It is a strong influence not only on the quality but also on the quantity of biogas production. It affects the rate of the reaction, the solubility of the heavy metals, CO_2, and also the composition of the gas (Dioha et al., 2013; Kaur & Chauhan, 2017). The speed of the reaction is improved by raising the ambient temperature. This would result in a rise in biogas production (Rajendran, 2015).

The effect of temperature can occur in the metabolic activity of organisms and other factors like settling characteristics of solids and gas transfer rate. Temperature variations can affect hydrolytic bacteria which are liable for the degradation of complex materials (Abdelgadir et al., 2014). The temperature tolerance for AD is ± 4℃ for mesophilic and ± 1℃ for thermophilic conditions (MONNET, 2009; Sarker et al., 2019; Zhang et al., 2007). The three temperature ranges where the bacteria have high activity are shown in Table 2.6 (Climent et al., 2007; Mir et al., 2016b; Rutz, 2007).

Table 2. 6: Thermal stages and typical retention times

Thermal stage	Process temperatures	Minimum retention time
Psychrophilic	< 20℃	70 to 80 days
Mesophilic	25 to 45℃	30 to 40 days
Thermophilic	45 to 65℃	15 to 20 days

Mesophilic operations have the advantage of suitable operating performance, stability, and less sensitivity to inhibitors (25–40℃). Neshat et al., (2017) found that both mesophilic and thermophilic temperature regimes showed a high potential for methane production. However, thermophilic reactors are harder to maintain and less attractive for commercial applications because they require large amounts of energy for heating.

Nevertheless, the thermal destruction of pathogenic bacteria at elevated temperatures is taken into a giant advantage because it allows a high loading rate and high yield of biogas production (Rumana, 2013).The thermophilic AD reactor shortens the desired retention time because it quickens the reactors of degradation of the organic materials (Arsova, 2010). For instance, the mesophilic temperature range is perfect for more methane-forming microorganisms and it operates with robust microbial consortia which tolerate greater changes in the environment and are slightly stable (Abdelgadir et al., 2014; Arsova, 2010).

Therefore, to reduce the process failure, smaller reactors, poorly insulated reactors or reactors in cold climates are not used for warmth fluctuation. These could be beneficial if the reactor is run within the mesophilic range (Nayono, 2009). The temperature must remain constant (Gregor & Grilc, 2012). Figure 2.7 shows the rate of AD process versus temperature. Anaerobic digestion plants aim to supply biogas for energy applications, thus the consumption of energy to heat the reactors for thermophilic digestion is not economical (Rossano & Cividino, 2013). Working in at mesophilic condition needs less energy and may enhance process stability and reduce pathogen inactivation

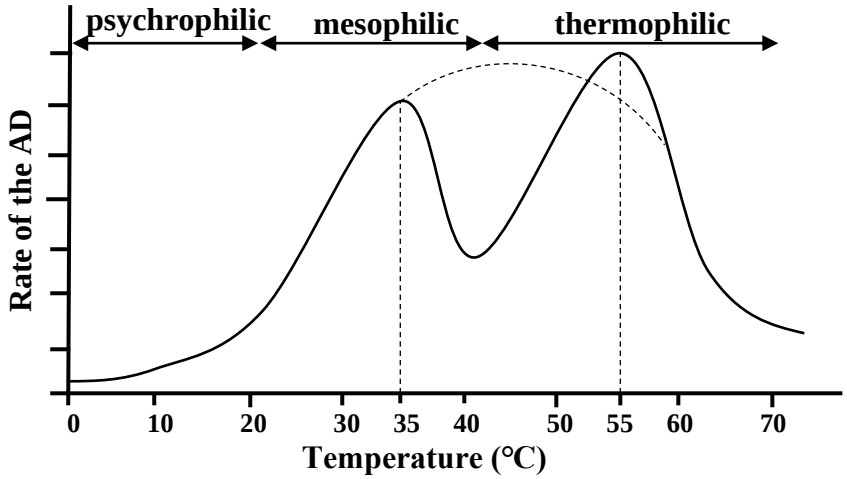


Figure 2. 7: Rate of AD process vs temperature (Velmurugan et al., 2014)

Furthermore, it requires lower investment costs. Although it requires an extended retention time. The stability of mesophilic temperature makes it more attractive in current AD for commercial-scale plants (Kaur & Chauhan, 2017; Mir et al., 2016b).

2.6.2. Potential of hydrogen (pH)

pH is one of the numerous factors that play a key role in the biogas production process. Different stages of the AD system have different optimal pH values. The little changes in pH would lead to adverse effects in the optimum results (Geogor & Grilc, 2012).

The pH value changes in response to the biological transformations during different stages of the AD process. The microorganisms involved in biogas production can be classified into two main classes based on pH. One is an acidogenic species and the other is methanogens. Methanogens are more sensitive to pH compared to other microbes in the microbial community (Rajendran, 2015). Their optimal pH ranges between (7.8 -8.2).

Acidogenic bacteria are less sensitive to pH and their optimal value lies in the range of (5.5 -6.5) (Vögeli et al., 2014). In the combined culture of both methanogens and acidogenic species, the optimum pH ranges are between (6.8 -7.4) (Ferguson et al., 2014). Since methanogens are considered as the rate-limiting step, it's necessary to maintain the pH close to neutral because high or lower pH can affect bacterial activities (Guarino et al., 2016).

Neshat et al., (2017) investigated the effect of pH in the range of 6.9–8.9 on the anaerobic co-digestion of palm pressed fibre and cattle manure to get the simplest condition for the activity of methanogenic microorganisms. They found that the optimum pH range was 6.8–7 at which, the best CH_4 production yield was achieved. In another study undertaken by Cheng and Zhong, (2014) who investigated the optimum pH for AD of the cotton stalk and cattle manure, either as a sole substrate or co-substrate, observed an optimal value of 6.5.

Therefore, constant pH is important in the start-up phase as fresh wastes should go first through the stage of hydrolysis and acidogenesis before any CH_4 can be formed that may lower the pH. Moreover, it has been identified that the utmost range of pH for getting maximal biogas production in AD is between 6.5 and 7.5 value (Manyi-Loh et al., 2013; Muthu et al., 2017; Yu & Fang, 2003).

2.6.3. Carbon to nitrogen (C/N) ratio

The C/N ratio plays a critical role in the biogas production process. It is a vital parameter in estimating nutrient deficiency and ammonia inhibition (Kaur & Chauhan, 2017). There is a particular need for carbon and nitrogen for microorganisms in any growth environment as well as AD systems. Reports suggest different values for the optimum range of C/N but it lies in the range of 20–30 (Risberg et al., 2013). When the C/N ratio is too low it would lead to a decrease in biogas production.

On the opposite hand, if the C/N ratio is too high, it would cause a lack of nitrogen and can harm the protein formation (Dioha et al., 2013). This would result in the structural metabolism of the microorganisms. A high C/N ratio will have nitrogen starvation by biomass and will affect bacterial activities (Rajendran, 2015). The optimum range of C/N ratio for AD is 30:1 and the minimum is 20:1, thus, outside optimum ratio, the concentration of VFAs increases that increases the pH which inhibits bacterial activities, and below minimum ratio also inhabits the bacterial activities (Guarino et al., 2016). Since the aim is to attain low biomass production and high biogas production, a low amount of nutrients is sufficient for this process (Tanimu et al., 2014).

2.6.4. Hydraulic Retention Time (HRT) and Organic Loading Rate (OLR)

Retention time is described as the period spent by the substrate in the reactor for complete digestion. It is also called duration (Meegoda et al., 2018). Hydraulic retention time is the average period that the substrate resides in the AD and OLR describes the quantity of organic matter expressed in g COD/L or g TS/L or g VS/L added to the reactor per reactor volume and unit time (Kaur & Chauhan, 2017). Hydraulic retention time is inversely proportional to OLR and both are very useful parameters that determine the design and performance of the reactor (Manyi-Loh et al., 2013).

Hydraulic retention time is dependent on the reactor volume and also the volume of substrate fed per time calculated in line with the subsequent equation (eq 2.1) (Seadi et al., 2008).

$$HRT = \frac{V_R}{V} \qquad \text{eq 2.1}$$

Where HRT: Hydraulic Retention Time (days), V_R: digester volume (m^3), and V: volume of substrate fed per time unit (m^3/d).

Based on equation 2.1 above, increasing the organic loading reduces the HRT. Therefore, the retention time must be sufficiently long to ensure that the number of microorganisms removed with the digestate is not more than the quantity of produced microorganisms.

2.6.5. Mixing

Mixing is the most significant factor to keep the substrate in contact with microorganisms. It helps in accelerating the process by exposing substrate material to the bacteria. Besides, mixing ensures homogeneous temperature distribution and improves the bacterial population's ability to get nutrients. It is therefore important to inoculate the flesh material with microbes (Kaur & Chauhan, 2017). Mixing may be done either mechanically or by recycling produced sludge (Velmurugan et al., 2014). The key advantages of mixing are:

- The mixture is homogeneous and preserves the uniformity of substrate concentration, temperature, and other environmental factors.
- To reduce the scum formation at the surface and also the development of temperature gradients within the reactor (Velmurugan et al., 2014).

Then the various feedstocks should be mixed before feeding the reactor to ensure sufficient homogeneity (Rajendran, 2015). The filamentous microorganisms in the reactor bring out the scum and foam formation (Vögeli et al., 2014).

Mixing prevents sludge formation at the underside of the reactor (Velmurugan et al., 2014). The type of mixing and the way it is performed vary with the type of reactor and TS content in a reactor.

2.6.6. Substrate to water ratio

The total ratio of substrate to water is a vital design parameter for the prepared feedstock, which allows the classifying of AD as high solids or dry digestion and low solids or wet digestion (Naik & Wung, 2013). Dry matter content requirement in AD should be about 40% for hydrolytic and acidogenic bacteria and less than 30% for methanogenic bacteria (Deublein & Steinhauser, 2011). A substrate to water ratio affects biogas production.

Therefore, the AD process can perform better at the pure material to water ratios between 1:4 and 1:10 (Tasneem et al., 2012). Anaerobic reactors are divided into three categories based on the solid contents; the low solid reactors contain less than 10% TS with material to water ratio of 1:10 (Kleinheinz & Hernandez, 2016), medium solid reactors solids range from 15-20% TS with material to water ratio of 1:5-1:7, and high solid reactors range from 22-40% TS with material to water ratio of 1:2.5-1:4.5. It is also known by name of dry digestion depending on the sort of substrate used. Therefore, the volume of the reactor decreases because the TS content increases, owing to lower water requirements (MONNET, 2009; Nayono, 2009; Velmurugan et al., 2014; Ward et al., 2008).

2.6.7. Inoculation and start-up

Inoculation is considered the most important factor for improving methane production and stabilizing the reactor of AD. It also affects the physical and chemical properties of fermentation during an AD (Ma, 2019).

When starting the digestion for the first time, the reactor has to be inoculated with favourable bacteria for the AD process. Based on different reports, diluted cow dung is required to inoculate substrates (Dennis, 2015; Njogu et al., 2015). In general, the cow dung is used as inoculation, the better biogas production (Dennis, 2015; Ma, 2019). During the start-up phase, the bacteria population must be gradually acclimatized to the feedstock (Liu et al., 2018). The successful operation of an AD requires the activity of an abundant and diverse population of methane-forming bacteria (Z. Z. Ismail & Talib, 2016). Thus, seeding the reactor which is heated at normal temperature with flesh cow dung is also helpful (Vögeli et al., 2014). This could be achieved by regularly feeding the load and allowing sufficient time to attain a balanced microorganism population (Xu et al., 2013). The loading to the reactor should proceed slowly during the start-up. The control of pH and alkalinity are important factors for the proper AD process (Gerardi, 2003).

Overloading occurs from either feeding an excessive amount of biodegradable organic matter compared to the active population capable of digesting it or rapidly changing reactor conditions. Such disturbances specifically affect methanogenic bacteria, whereas the acidogenic bacteria which are more tolerant continue to work and produce the acids (Wu et al., 2016). This eventually results in an acidification of the reactor that inhabits the activity of methanogens. Such an imbalance between the acidogenic and methanogenic bacteria can cause the reactor to fail (Ma, 2019). The use of cow manure can avoid this because it increases the buffer capacity thereby reducing the risk of acidification (Vögeli et al., 2014). The AD start-up should be done smoothly. Furthermore, the time between the initial feeding substrate within the reactor and stable operation should be short as much as possible (Brown & Li, 2013; Cheng & Zhong, 2014).

2.6.8. Total Solids

The total solids can be used to measure the dry matter in a sludge, irrespective of its organic or inorganic nature. It is only the organic matter that contributes to biogas production. It is often presented in literature either as a percentage or concentration (Meegoda et al., 2018). The TS content of solid waste influences AD performance, especially biogas and CH_4 production efficiency (Yi et al., 2014). TS is a crucial attribute of reactor operation.

Furthermore, improved biogas yields were reported in continuous high-TS reactors compared to low-TS reactors operating on the identical retention time (Meegoda et al., 2018). Generally, the organic solid content which are suitable substrates for AD are in ranges from 70% to 95% of TS. Substrates with less than 60% of organic dry matter content are not considered as suitable substrates for AD (Vögeli et al., 2014).

2.6.9. Total Volatile Solids

The total volatile solids (TVS) is one way for the measurement of the organic fraction of TS. It is also called the organic dry matter and it contributes to biogas production, although a more accurate description would be the quantity of matter during a sludge that is lost on ignition (Vögeli et al., 2014). Therefore, TVS is the parameter that is commonly used to characterize the organic waste for AD. The TVS content is set by combusting the remaining solids produced from TS measurement at 550 ℃, though some volatilization may have already occurred during the measurement of the TS (Meegoda et al., 2018). Figure 2.8 shows the classification of feedstock material.

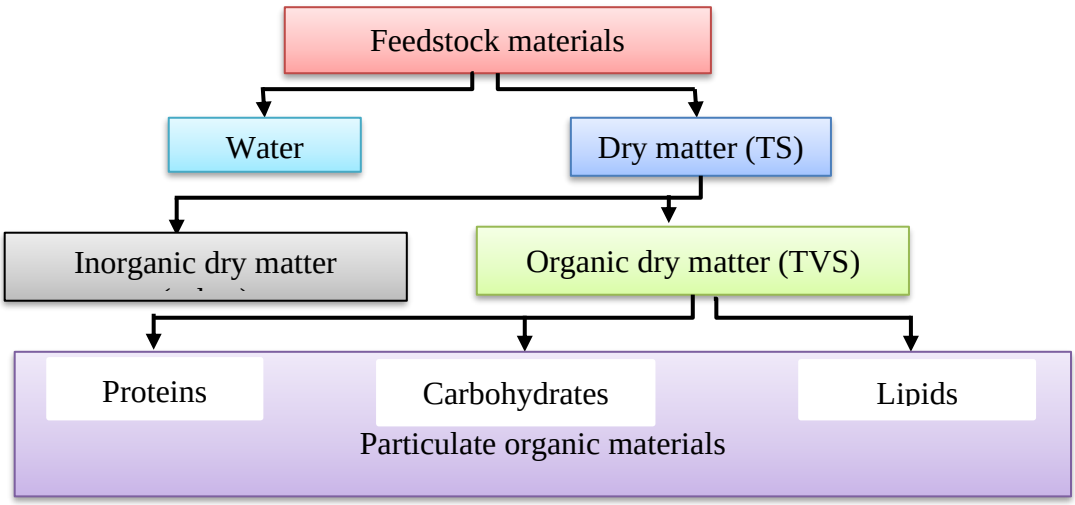


Figure 2. 8: Classification of feedstock materials supported dry matter (Vögeli et al., 2014)

2.6.10. Water content

Water is a necessary material for the survival of microorganisms. It is necessary for the movement of organisms and extracellular activity. Besides, it facilitates the breakdown of the substrate (Jeihanipour et al., 2013). However, a high-moisture content (MC) can result in a decrease in the slurry temperature and eventually result in a decrease in biogas production. On the opposite hand, the lower water content would lead to the excessive acid formation which would affect the fermentation process (Rajendran, 2015).

An AD process was applied at different moisture levels mostly between 70 and 80 % MC. It has been reported that the high yield of biogas rates occurs at 60-80% humidity (Hernández-Berriel et al., 2008). Therefore, the reactor which operated at 70% of MC produces more CH_4 than the reactor operated at 80% of MC (Khalid et al., 2013; Le et al., 2011). Therefore, MC plays a necessary function during pre-treatment reactions, helping in chemical and enzymatic reactions, reducing the slurry by raising the lubricity of the particles, having a medium for solubilization of sugars, and mass transfer by diffusion (Modenbach & Nokes, 2012).

2.7. Pre-treatment

Most substrates are not ideal for biogas production for several reasons: (a) They can't be digested by microorganisms; (b) Digestion is feasible but very slow; and (c) Inhibitors are present in the material, or inhibitory compounds are produced during microbial degradation (Treichel & Fongaro, 2019). Pre-treatment aims to facilitate the digestion process by removing these barriers and making the organic contents of the substrate easily accessible and usable by the microbial community. The pre-treatment techniques are used to improve hydrolysis and AD performance. They also decrease the sludge volume, enhance the degradation of VS, and thus increase biogas production (Deepanraj et al., 2017).

The process aims to get rid of undesirable materials like large items and inert materials to permit a better digestate quality more efficient digestion and this prevents failure in the process (Vögeli et al., 2014). The pre-treatment of cotton wastes is carried out to facilitate the hydrolysis of the cellulose component existing in the substrate. Cellulose encompasses a highly crystalline structure because of the presence of extensive chemical bonds and inter-chain in the cellulose structure (Ismail & Talib, 2016).

The circumstances like accessible surface area, cellulose crystallinity, and lignin content of lignocellulosic matter limit the digestibility of materials (Treichel & Fongaro, 2019). Figure 2.9 shows the general pre-treatment process. Therefore, pre-treatment before AD is crucial to overcome the restrictions imposed by the hydrolysis rate (Treichel & Fongaro, 2019). Pre-treatments are often classified as thermal, physical or mechanical, chemical, biological, ultrasonic, and enzymatic pre-treatment and various combinations (Ariunbaatar et al., 2014, Yunqin et al., 2010).

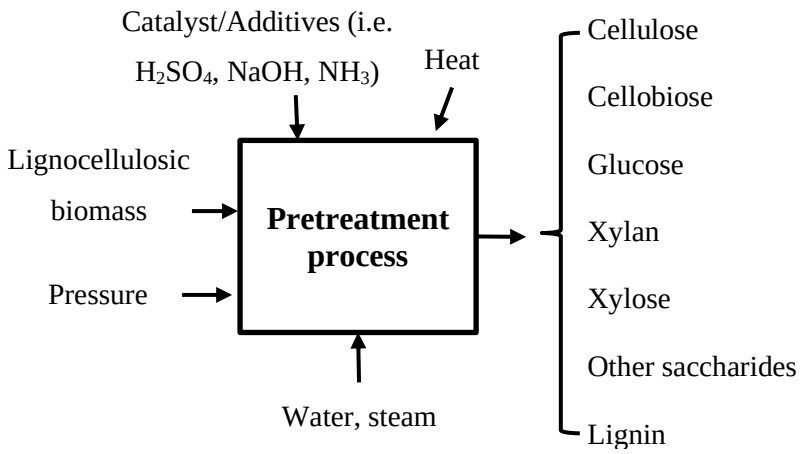


Figure 2. 9: General pre-treatment process (Modenbach & Nokes, 2012)

Mechanical pre-treatments can reduce the particle size of the substrates, produce no microbial inhibitors, and typically cause increased CH_4 production (Teghammar et al., 2010). The functions of physical pre-treatment of raw material are mainly to increase the surface area and size of the pores, rupture the structure of the biomass, and therefore decrease the crystallinity (Climent et al., 2007).

CHAPTER THREE
RESEARCH METHODOLOGY

3.1. Introduction

This chapter gives details about research design, procedures, and materials used in biogas production from CYW. It also explains the methods of chemical analysis used, physical parameters of substrates, the experimental conditions, and daily analysis of biogas.

The type of reactor designed and operation criteria selection depended on the feedstock characteristics and financial aspects among others. Figure 3.1 represents the methodology of this study.

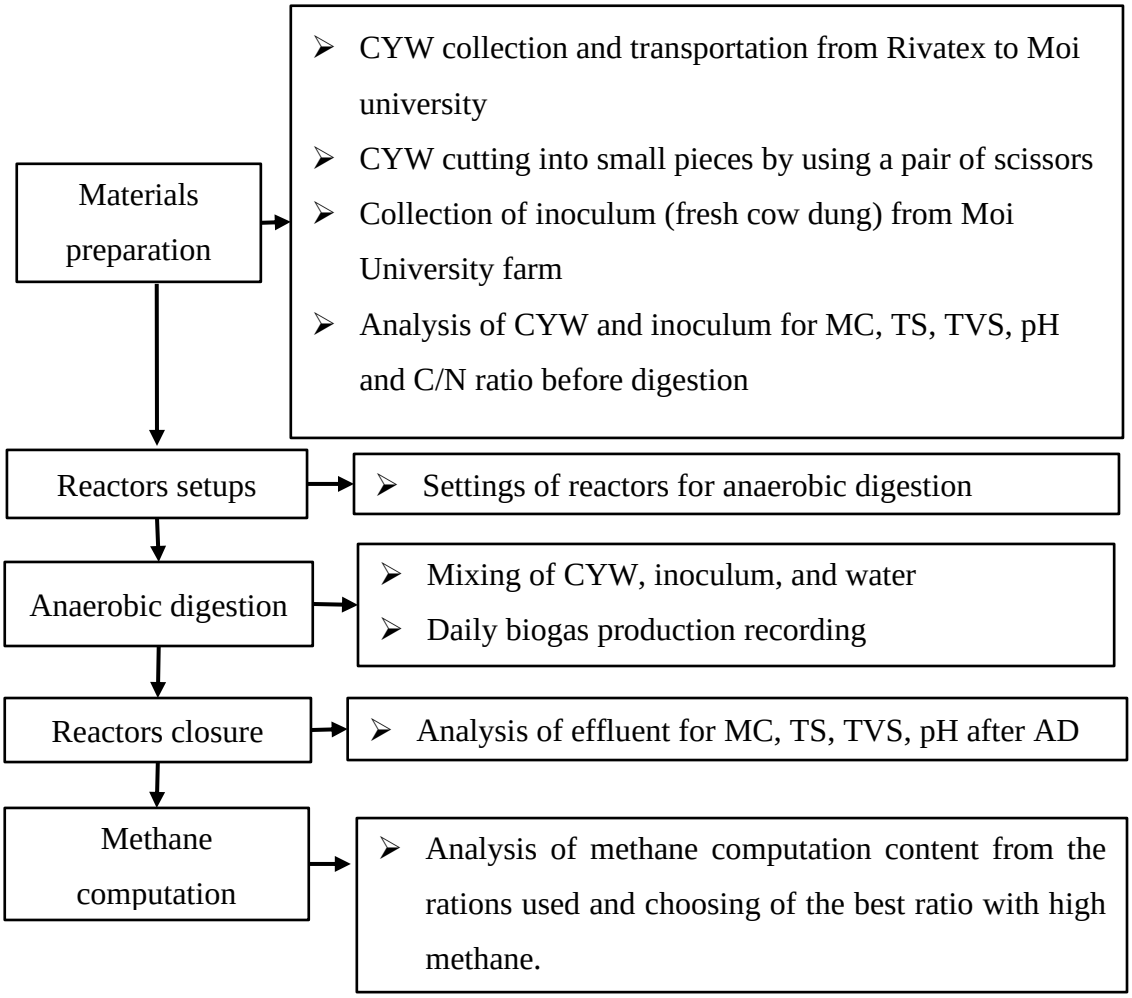


Figure 3. 1: Process flow diagram

Each mode of operation has its advantage and downside, and during this case, this research study focuses on the mode of the reactor. The results obtained from the experiments concerning solid concentration were used for comparison between biogas production and %TS concentration within the reactor.

3.2. The materials

The tools and materials used in this research included; oven, furnace, scissors, conical flasks (250 mL, 500 mL, and 1000 mL), silicone sealant (clear and black), digestion tube, aluminium foil, aspiration plastic bottles used as reactors (2 L), flexible rubber piper (5 mL of diameter) and non-return air valves, buckets (20 L and 15 L), a syringe (5 mL, 10 mL, and 60 mL), sawdust, pH meter, thermometer, gas sampling bag, gas chromatography, and multi-gas detector.

3.2.1. Sawdust

The sawdust was collected at carpentry, dried under the sun, and kept in the laboratory ready to be used. The sawdust stored within the laboratory aimed to prevent heat loss by occupying the space adjacent to the reactors. Sawdust can maintain the temperature within and ensure that there are only minimal changes due to its physical properties. It was also necessary that the outer wall temperature of the reactor at a given depth be almost the same as that of sawdust (Terradas et al., 2014). Therefore, the sawdust was used to control temperature, that is, to keep the temperature within the desired range.

3.2.2. Non-return air valves

Non-return air valves were used to ensure one direction flow of gases from the headspace of anaerobic fermentation broths.

3.2.3. Reactors

The aspiration bottles of two litres were used as the reactors (Figure 3.2). Igoni et al., (2008) reported that the batch reactors of the lab-scale experiment are suitable as the reactors have only to be loaded once and may not even need to be stirred. Here, the reactors were painted with black paint as shown in Figure 3.2 (B) to extend the reactor insulation because the insulation can increase the reactor temperature by 1.5-2.1℃ (Pham et al., 2014).

(A) **(B)**

Figure 3. 2: (A) and (B): Reactor preparation before painting and after painting.

3.2.4. Gas sampling Bag

The gas was collected and stored in five litres (5 L) gas sampling bag. The gas sampling bag used in this experiment is illustrated in Figure 3. 3.

Figure 3. 3: Gas Sample Bag

3.2.5. Gas Chromatograph (GC)

A GC-Shimadzu 2010 was used in the quantitative analysis of biogas yields from all the reactors. The specifications and settings of this GC are as shown in Table 3.1.

Table 3. 1: GC Analysis Parameters

GC	**Shimadzu GC 2010**
Injector	Temperature: 150℃; Split ratio: 5.1; Total Flow: 81.2 ml/min; Purge flow: 3 ml/min
Column	ZB-Wax; Length: 30 m; Inner Diameter: 0.32 mm; Film thickness: 0.25 µm
Colum	2 min initial hold time, 40℃ to 220℃ at 20℃/min, 4 min final hold time (Temperature program); Column flow: 12.82 ml/min; Linear velocity: 120.6 cm/sec
Nitrogen (Carrier gas)	Temperature: 200℃; Pressure: 227.9 kPa; Total Flow: 81.2 ml/min; Purge flow: 3 ml/min
H_2 (Detector)	Temperature: 250℃; Flow: 80 ml/min; Make up flow: 20 ml/min
Injection volume	2 µL
Detector	Flame Ionization Detector (FID)
Software	GC Solution

3.2.6. Multi-gas detector (gas analyser)

A multi-gas detector SKY2000-M4-WH model was used in the quantitative analysis of biogas yields from all the reactors (Table 3.2). Figure 3.4 shows the multi-gas detector used in this experiment.

Figure 3. 4: A multi-gas detector used in the analysis of biogas yield

Table 3. 2: The specifications and settings of a multi-gas detector

Gas type: CO_2/CH_4/O_2/H_2S	
Gas Detected	CO_2, O_2, H_2S, CH_4
Detection principle	Electrochemistry, Catalytic combustion
Sampling Method	Pumping suction, the flow rate can up to 1L/min, ten grades of pumping suction for selection.
Measure Range	CO_2: 0-1000 ppm, O_2: 0-30%VOL, H_2S: 0-100 ppm, CH_4: 0-100% LEL (LEL: Lower Explosive Limit)
Resolution	CO_2: 1 ppm, O_2: 0.01% VOL, H_2S: 0.1 ppm, CH_4: 1% LEL
Model	SKY2000-M4-WH
Number	200812B1
Voltage	3.7 V
Range	Standard
Precision	3%~5% F.S. (Full Scare)
Linearity error	≤±1%

3.3. Substrate and inoculum collection

The solid wastes (CYW) that were used as the substrate for this study were collected from Rivatex East Africa Limited, Eldoret, Kenya (Figure 3.5) while the inoculum was obtained from Moi University main campus farm at normal temperature (Figure 3.6). Measured 20% of the full volume working reactor was used as inoculum. The inoculum was kept in refrigeration at 4℃ for two days before use to reduce degradation and preserve the integrity of microorganisms within the material.

Figure 3. 5: Cotton yarn wastes bulk sample at Rivatex East Africa Limited before collection.

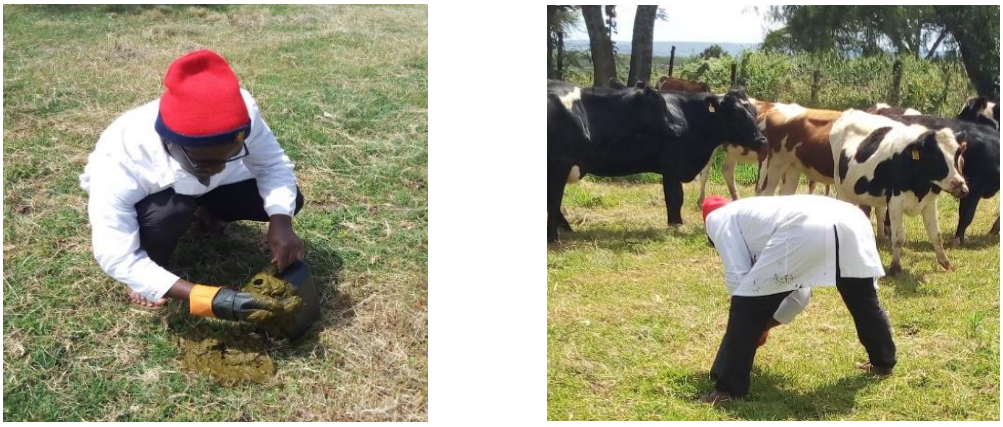


Figure 3. 6: The fresh cow dung sample collection from Moi University main campus farm

3.3.1. Substrate preparation

The CYW was sliced into small pieces as shown in Figure 3.7 to produce the substrate with appropriate size using the scissor to facilitate biological degradability (Y. Wang, 2010). They were kept in the laboratory for one week.

The CYW and inoculum were characterized for TS, TVS, C/N, and pH in line with standard methods. This has been detailed in the next section.


Figure 3. 7: Cotton yarn wastes bulk sample after cutting into small pieces.

3.3.2. The characterization of the substrate

The biogas production from any substrate is extremely dependent on the C/N ratio of the material, pH, temperature, TS, and TVS (Dioha et al., 2013).

The pH of the substrate and digestate was determined using a portable pen-type pH meter probe (PH-009(I)A) as described in Table 3.3.

Table 3. 3: The pH meter specifications

Specifications	Range	Resolution	Accuracy	Temperature compensation	Operating temperature
Values	0.0-14.0 pH	0.1 pH	± 0.1 pH	0℃-50℃	0℃-50℃

The materials analysis procedure was as given in Figure 3.8.

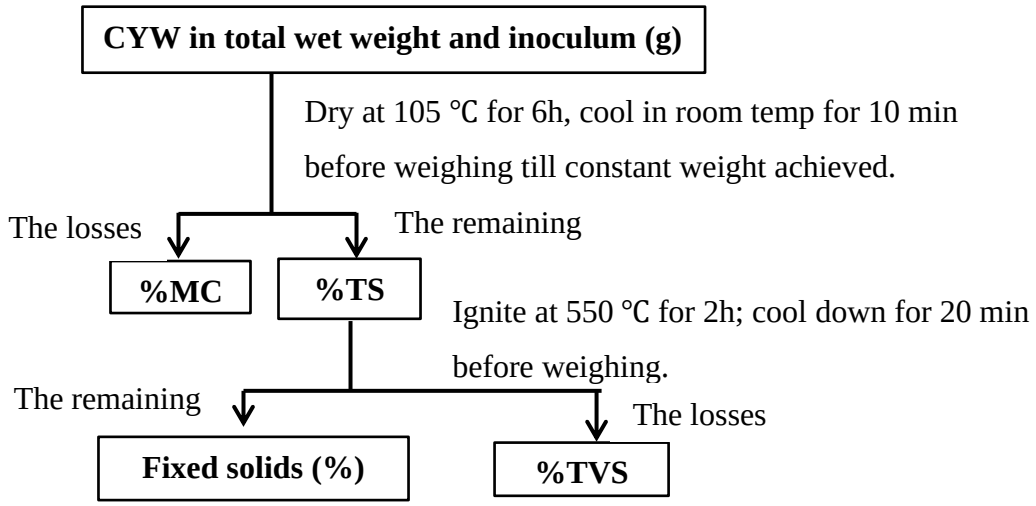


Figure 3. 8: Substrate analysis procedures

3.3.2.1. Substrate moisture content analysis

Moisture content is determined by the amount of water lost from dry matter upon drying to constant weight; it is expressed as the weight per unit of the dry substrate or as the volume of water per unit bulk volume of the substrate. Samples were taken from either raw or digestate (effluents) waste. The crucible was filled with 10 g of sample and placed in the oven at 105 ℃ for 6 hours, the losses in mass were recorded. The identical procedure was repeated until the constant weight was achieved. Then the percentage of moisture content (MC) on a wet basis was calculated using equation 3.1.

$$MC = \frac{W-D}{W} \times 100 \qquad \text{eq 3.1}$$

Where W: wet weight (weight of CYW used in hydrolysis, D: dry weight (after oven drying at 105 ℃ at a constant mass)

3.3.2.2. Total solids and total volatile solids analysis.

The water content or dry weight of the material was measured using TS measurement. The TVS was measured to calculate the quantity of carbon or the volatile organic compounds present within the material which was converted into ash.

The equipment needed for the measurement of TS and TVS were crucibles, analytical balance, oven, and muffle furnace. The crucibles were marked for ease of identification. The marked crucibles were then placed in an oven, daily before the analysis experiments were done. Figure 3.9 shows the TS determination processes. The crucibles were then transferred to room temperature and cooled down for 10 minutes (Rajendran & Balasubramanian, 2011a).

(A) **(B)** **(C)**

Figure 3. 9: (A), (B), and (C) show the determination of the weight of the crucible with material by using analytical balance before putting the samples into the oven and after setting the oven at 105 ℃.

The weight of empty crucibles was noted using an analytical balance. The material that the TS and TVS were to be quantified was weighed with the crucible. The crucibles were then covered with aluminium foil and transferred to an oven at 105 ℃ for six hours.

The crucibles were then covered with foil containing the dry material and allowed to cool down at room temperature for about 10 minutes. The crucibles with dry material were weighed using an analytical balance and then placed in a muffle furnace maintained at a temperature of 550 ℃ for two hours as shown in Figure 3.10 (Karimi et al., 2018). The crucible with ashes was allowed to cool down to room temperature for about 20 minutes.

The crucible was always covered with foil before measurements to avoid change in weight due to moisture from the atmosphere. The measurements were repeated thrice and average values were recorded. The subsequent formulas (eq 3.2 and 3.3) were then used to determine the TS and TVS.

$$TS = \frac{W3-W1}{W2-W1} \times 100 \qquad \text{eq 3.2}$$

$$TVS = \frac{W3-W4}{W3-W1} \times 100 \qquad \text{eq 3.3}$$

Where; W1: Weight of crucible, W2: Weight of wet material and crucible, W3: Weight of dry material and crucible at 105 ℃ ovens, W4: Weight of material and crucible after ignition at 550 ℃ (Hasanzadeh et al., 2018).

(A) **(B)** **(C)**

Figure 3. 10: (A), (B), and (C) show data recording, the dry matter within the furnace and furnace settled at 550 ℃.

3.3.2.3. Substrate ash analysis

Ash contains various minerals in several concentrations required during bacteria metabolism. Ten grams (10 g) of CYW was weighed into a crucible and dried at 105 ℃ by an oven heater for six hours and then the dry matter was burnt at 550 ℃ by a muffle furnace for two hours. The residue was then weighed and the percentage ash content (%A) was calculated as follows (eq 3.4) (B. P. Ismail, 2017).

$$\%A = \frac{M(Cb+A)-MCb}{M(Cb+sample)-MCb} \times 100 \quad \text{eq 3.4}$$

Where $M(Cb + A)$: Mass of crucible and ash, $M(Cb + sample)$: Mass of the crucible and original sample, MCb: Mass of the crucible, Cb: Crucible, and A: Ash

3.3.2.4. Total solids and total volatile solids reduction analysis

The substrate balance analysis is shown in Figure 3.11. The loading substrate has a total weight of W_0 and a dry weight of D_0. After entering the reactor and digested, the reduction of TS and TVS occurred. Therefore, the effluent encompasses a total weight of W_1 and a dry weight of M_1 which were less than that of W_0 and D_0, respectively.

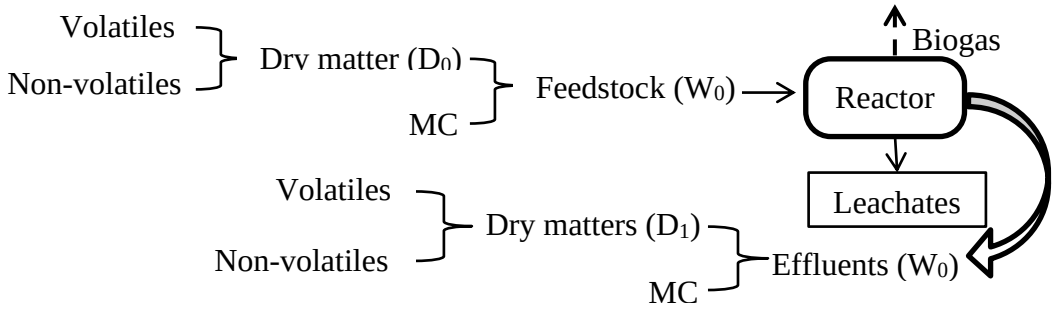


Figure 3. 11: Materials balance analysis in the anaerobic digestion process

Equations 3.5 and 3.6 were used to estimate the %TS and %TVS reduction (Switzenbaum et al., 2003, Spellman, 2016).

a) $TS\ reduction\ = \frac{TS_0-TS_1}{TS_0} \times 100\%$ eq 3.5

Where TS_0= TS load charged into the reactor (%) and TS_1= TS load discharged out of the reactor (%)

b) $TVS\ reduction = \frac{TVS_0-TVS_1}{TVS_0} \times 100\%$ eq 3.6

where TVS_0 = TVS load charged into the reactor (%) and TVS_1 = TVS load discharged out of the reactor (%).

3.3.2.5. Total carbon analysis

Total carbon analysis was determined using the Walkey-Black potassium dichromate method as described by (Bakr and El-ashry, 2018, MYOVELA, 2018). Whereby 1 g of dry samples was put in 250 mL conical flasks and 10 mL of 1N $K_2Cr_2O_7$ was added and swirled three times. Then 15 mL of H_2SO_4 was added during a fume hood and swirled again three times. The flasks were allowed to stand for 30 min, so 150 mL distilled water was added, followed by the addition of 5 mL of Ortho-phosphoric acid. The contents were titrated with 0.5 N ferrous ammonium sulphate solutions until the colour changed from blue to green. Simultaneously, an empty digestion tube (blank) was run without a sample and organic carbon was calculated using equation 3.7.

$$\%C = ((B - S) \times (V \times 0.3 \times 1.3)) \div W \qquad \text{eq 3.7}$$

Where %C = percentage of organic carbon, B = Blank reading (mL), S = Sample reading (mL), W = Weight of sample weighed (1g), V = Volume of 1N $K_2Cr_2O_2$ (mL) and 1.3 = a correction factor which is normally applied to the result to adjust the organic carbon recovery (77% recovery).

3.3.2.6. Total nitrogen analysis

The Kheldahl method was used to determine the total nitrogen content which involved the sample digestion and volumetric determination whereby about 1 g of sample was weighed into a digestion flask along with a catalyst composed of 5 g of K_2SO_4 and 0.5 g of $CuSO_4$, and 10 mL of concentrated of H_2SO_4 (98%). The mixture was heated in an exceeding fume hood at 420 ℃ till the digest colour turned blue signifying the end of the digestion process. Then the digest was cooled to room temperature, transferred to a 100 mL volumetric flask, and topped up to the mark with distilled water. An empty digestion tube (blank) with the catalysts and acid was also made.

Ten millilitres (10 mL) of the diluted digest was transferred into a distilling flask and washed with about 3 mL distilled water. Fifteen millilitres (15 mL) of 40% of NaOH was added and this was also washed with about 3 mL distilled water. Distillation was done to a volume of about 60 mL distillate. The distillate was titrated using 0.02 N-HCl to the orange colour of the mixed indicator (Methyl orange) which signified the end point. The total nitrogen was calculated by using equation 3.8.

$$\%NT = (V_1 - V_2) \times N \times F \times 0.014 \times (100/V) \times (100/W) \quad \text{eq 3.8}$$

Where NT = total nitrogen, V_1 = Titer for the sample (mL), V_2 = Titer for blank (mL), N = Normality of standard HCl solution, F = Factor of standard HCl solution, V = Volume used for distillation, and W = Weight of sample taken (g)

In summary, Table 3.4 shows the standard method used in this thesis to determine the substrate characteristics which is the American Public and Health Association (APHA).

Table 3. 4: Methods used for CYW and inoculum characterization

Characteristic items	Methods used
pH	Pen type pH meter (PH-009(I)A)
Moisture content	Methods 2540 B (APHA 2012)
Total solids	Methods 2540 B (APHA, 2012)
Ash content	Methods 2540 E (APHA, 2012)
Total volatile solids	Methods 2540 E (APHA, 2012)
C/N ratio	Methods 4500 B (APHA, 2012)

3.4. Methodology design

3.4.1. Introduction

The following steps were applied during this method; treated CYW with water at different ratios and inoculum. The slurry was mixed and put in two litres (2 L) reactors. The mixing was done thoroughly by handshaking under ambient temperature (room temperature).

3.4.2. Model of biogas plant

The experiment was carried out in batch-type laboratory-scale reactors at Moi University main campus. Thirty reactors of 2 L total volume, 12 cm diameter, and 25 cm height each made of aspiration plastic bottles were used for this experiment. Ten reactors were run in triplicate for each test run as reported by previous researchers (Hasanzadeh et al., 2018). The reactors were closed with suitable rubber plugs and a few holes were drilled in the centre of the plug for water displacement and collecting biogas. The flexible rubber piper, digestion tubes, and syringes were used to make water in and out of the conical flask (1000 mL) for measurements of displaced water (Figures 3.13 and 3.15).

The quantities were calculated to get the final volume of 1000 mg of mixtures. All the reactors with 50% working volume (1000 g) were run concurrently. One-half (50%) of the working volume was chosen because of the high-volume occupation of CYW. Moreover, a headspace for the gas should take enough space during the fermentation process. The pH was in the range of 7.2 ± 0.3 in all mentioned reactors. The batch reactors were buried in a bucket filled with sawdust at depth of 30 cm to attenuate the temperature fluctuation during the day and night (Figure 3.12).

The operating temperature was in the mesophilic range of 27 ± 3 ℃ which is suitable for biogas production (Teodorita Al et al., 2008). The CYW was mixed with water to produce ratios (substrate/ water) 1 (R_1), 2 (R_2), 3 (R_3), 4 (R_4), 5 (R_5), 6 (R_6), 7 (R_7), 8 (R_8), 9 (R_9) and 10 (R_{10}). The control reactor with only a sample of inoculum was used and digested to verify the amount of biogas produced using such substrate and making it possible to determine the difference when using the flesh manure (inoculum) mixed within the substrate. Each of these reactors was connected to a conical flask of one little (1 L) by a versatile rubber pipe, that was used to collect the produced biogas (Figure 3.15).

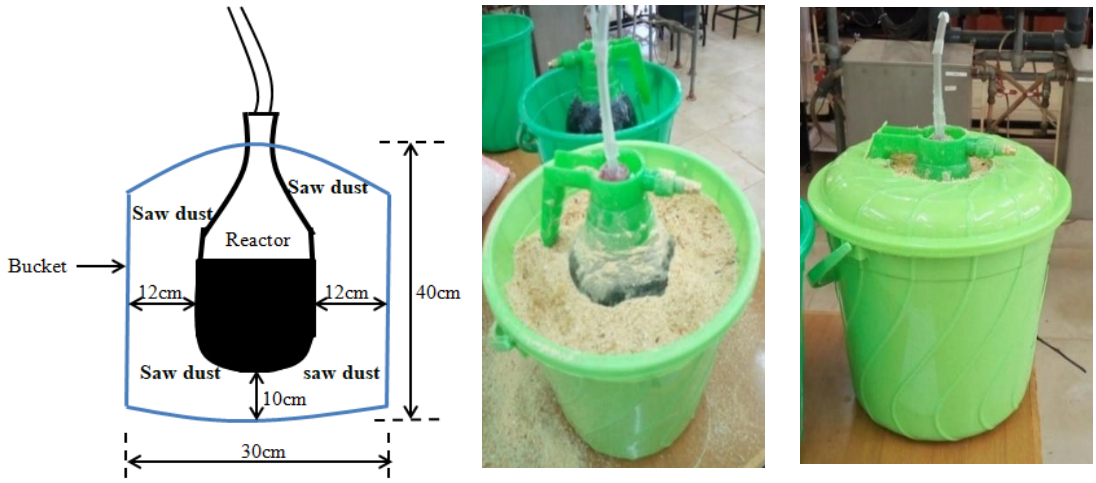


Figure 3. 12: Reactor buried in a bucket filled with sawdust

The volume of biogas collected in the conical flask was measured by the water displacement method every day. The schematic configuration of the anaerobic biogas reactor is given in Figure 3.13.

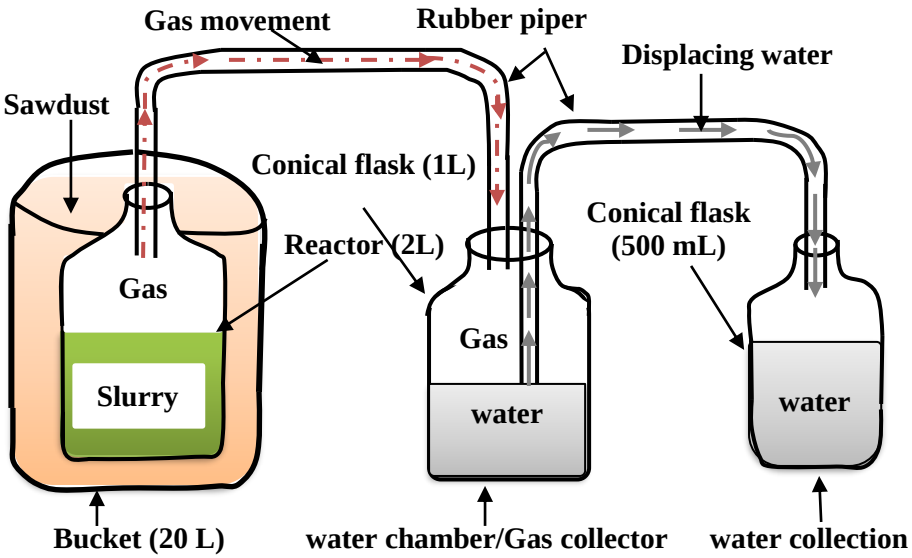


Figure 3. 13: Overview for model biogas plant

3.4.3. The experiment conditions

In the experiment, ten mixtures were prepared by mixing the CYW in varying proportions with water. The reactors loading was differentiated using a mixture with TS concentration of 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:6, and 1:10 on TS content basis (high, medium, and low solids content) as shown in Table 3.5.

This choice of loading was according to information presented in the literature which provided data associated with solid content in three ranges of loading which can be applied on this kind of substrate (Abbasi et al., 2012; MONNET, 2009). Moreover, 1:1.5-4 values were selected from the 1:2.5–4.5 range, 1:6 was selected from the 1:5-7 range and 1:10 was selected from the 1:10 range.

Furthermore, the ratio of 1:1 was chosen as an extreme value for more investigation. The CYW had 93.18 ± 0.21% initial TS in the samples. Later, the fresh cow dung with 7.14 ± 0.22% TS was added. Finally, water was added to fit the initial TS contents per the reactors: 50%, 40%, 33%, 28%, 25%, 22%, 20%, 18%, 14%, and 10% TS and mixture composition (Table 3.5). The reactors were loaded with respective amounts of mixtures to get feed total working volume.

Table 3. 5: CYW to water ratios for anaerobic digestion

Reactor	CYW ratio (% TS)	Water ratio (%)	CYW to water ratio	CYW in gram	Water in gram
R_1	50	50	1:1	400	400
R_2	40	60	1:1.5	320	480
R_3	33	67	1:2	264	536
R_4	28	72	1:2.5	224	576
R_5	25	75	1:3	200	600
R_6	22	78	1:3.5	176	624
R_7	20	80	1:4	160	640
R_8	16	84	1:5	128	672
R_9	14	86	1:6	112	688
R_{10}	10	90	1:10	80	720

Figure 3.14 shows the prepared reactors filled with slurry and water displacement.

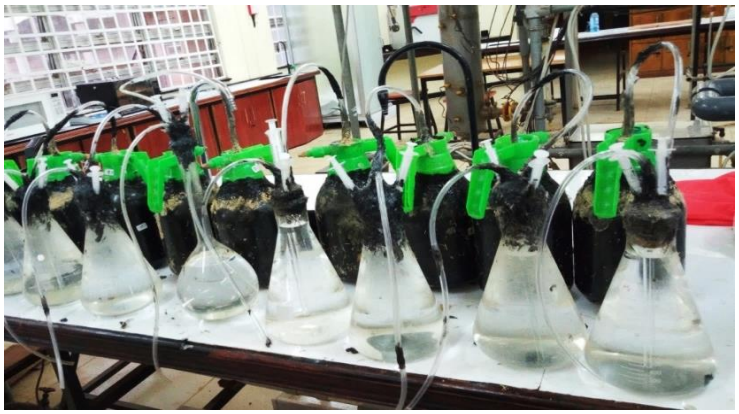


Figure 3. 14: The prepared reactors filled with slurry and water displacement

Finally, each reactor was then filled with 200 g of fresh cow dung as inoculum. This was done only at the initial stage. The reactors were sealed and arranged for the complete setup (Figure 3.15). The CYW and inoculum were analysed by TS, TVS, C/N, MC, and pH before digestion, and also the mixtures were prepared consistent with those for characterizations. The anaerobic batch reactors were digested at different type ratios (dry digestion). Thus, it was heated under sawdust to achieve the required temperature in normal conditions (Chaula et al., 2014; Kubler, 1982). The reactors were closed till the degradation time was complete. When there was no more biogas production, the reactors were open for reactor effluents analysis.

Figure 3. 15: Full biogas set up

3.5. The biogas production analysis

The volume of biogas produced was measured with the water displacement method at ambient temperatures daily. The conical flask filled with water (1 L) and connected to a reactor so that when gas was produced, water moved to a different conical flask (500 mL) then the displaced water was collected and measured using a measuring cylinder (Figure 3.15). The biogas yield (Y) was calculated using equation 3.9 while the total volatile solids removed was calculated using equation 3.10 (Jaroenpoj, 2015).

$$y = \frac{biogas\ production\ (mL)}{TVS_{removed}\ (g)} \quad \text{eq 3.9}$$

$$TVS_{removed} = TVS_{initial} - TVS_{final} \quad \text{eq 3.10}$$

The sample was taken by injecting the syringe into gas sampling bags; the Gas Chromatograph (GC) with flame ionization equipped with the column was used to measure the methane content, and a multi-gas detector was also used to measure the biogas compositions. The injector, detector, oven with 150℃, 250℃, and 200℃ were set respectively, where a syringe that carries gas sample was injected into the GC using nitrogen gas as a gas carrier accountable for obtaining any security clearances within the column.

3.6. Data analysis

In all kinds of analyses, it is necessary to determine the parameters that are responsible for a large variation in the output responses and in quantifying the variation. All numerical results were subjected to preliminary statistical analysis where they were averaged, and the results presented as means ± standard deviations of triplicates. Graphical presentations were drawn from the averaged results. All statistical work was done in Microsoft Excel 2016 (Microsoft Corporation, USA) and Past statistical software (version 4.03).

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1. The substrate characterization results

Characteristics of feedstocks and important process parameters are crucial in designing and operating anaerobic reactors. The initial characteristics of feedstock strongly affect biogas production and anaerobic stability (Phun et al., 2017). The biodegradability of the organic substrate is determined by the physicochemical characteristics (Aslanzadeh, 2014). The CYW and inoculum were individually characterized for their initial pH, C/N ratio, MC, TS, TVS, organic carbon, total nitrogen, and ash content before they were prepared for digestion. The CYW and inoculum characterization results are tabulated in Table 4.1.

Table 4. 1: Physicochemical characteristics of YCW and inoculum before digestion

Characteristic items	CYW	Inoculum
pH	7.1 ± 0.20	6.4 ± 0.21
%MC	6.82 ± 0.28	92.67 ± 0.27
%TS	93.18 ± 0.21	7.14 ± 0.22
%TVS (% of TS)	82.48 ± 0.21	88.64 ± 0. 23
%A (% of TS)	17.52 ± 0.22	11.36 ± 0.22
Total Carbon (% TC)	35.7 ± 0.18	32.25 ± 0.21
Total Nitrogen (% TN)	0.84 ± 0.09	1.57 ± 0.12
C/N ratio	42.50 ± 0.15	20.50 ± 0.10

Values are presented as means ± standard deviations of triplicates.

For ease of analysis of Table 4.1, the literature suggested that biogas production depends extensively on the compositions of substrates (Bambokela et al., 2016). The biogas production should be mainly determined by dry matter content from the substrate for biochemical reactions (Filer et al., 2019).

Raposo et al., (2011) reported that various factors affect anaerobic biodegradability which include the inoculum, the substrate characteristics, and experimental factors. The results showed that the MC for CYW was low (6.82 ± 0.28%) due to the high solid content which has been presented in CYW. Le et al., (2011) and Khalid et al., (2013) reported that the substrate which contains 70-80% of MC is suitable for AD. Therefore, the MC of raw CYW was out of this range. Nevertheless, the MC of mixed substrates was within the range of 50-90% which was within the preferable range of AD.

Initially, the TS content of CYW and inoculum was 93.18 ± 0.21% and 7.14 ± 0.22%, respectively (Table 4.1.) which are in the range of results reported by (Cheng & Zhong, 2014; Twizerimana et al., 2020) for CYW. Then, the TS content of mixed materials was between 10 and 50% (Table 4.2). It is often categorized as low, medium, and high solid content (Anahita et al., 2019, MONNET, 2009).

Table 4. 2: The results of mixed CYW and inoculum at initial states

	Parameters			
Reactors	**pH**	**MC (%)**	**TS (%)**	**TVS (%)**
R_1	7.1 ± 0.20	50	50	85.62 ± 0.28
R_2	6.9 ± 0.20	60	40	82.87 ± 0.24
R_3	7.2 ± 0.21	67	33	83.75 ± 0.25
R_4	7.2 ± 0.15	72	28	85.10 ± 0.24
R_5	6.9 ± 0.22	75	25	80.25 ± 0.24
R_6	6.8 ± 0.11	78	22	81.85 ± 0.22
R_7	7.3 ± 0.22	80	20	79.75 ± 0.21
R_8	7.4 ± 0.21	84	16	78.10 ± 0.22
R_9	6.8 ± 0.22	86	14	71.69 ± 0.28
R_{10}	6.9 ± 0.21	90	10	70.98 ± 0.25

Values are presented as means ± standard deviations of triplicates.

The potential of gas production from the substrate can usually be captivated with the TVS loading in the reactor and therefore the percentage of TVS reduction through digestion (Adebayo & Odedele, 2020). Therefore, the substrate characterized by a high concentration of TVS is the best for AD. As a result, the TVS of CYW and inoculum were 82.48 ± 0.21 and 88.64 ± 0.23% respectively (Table 4.1). However, the TVS of the mixed substrate was 71-85 ± 0.29% (Table 4.2). This is in agreement with the recommended value for biogas production (70-95%) by Getahun and Gebrehiwot, (2014). This shows that an oversized fraction of CYW is biodegradable and thus it can be a good feedstock for biogas production. The process could be operated at high TS concentration with no operational problem.

The C/N ratio of inoculum was 20.50 ± 0.10 which was in a very suitable range to keep the AD in a very stable condition (Bambokela et al., 2016). This result was within the range of values of the study conducted by (Ren et al., 2019; Safari et al., 2018) where they found that the C/N ratio of cow dung was within the range of 20 to 22 ± 0.66. It was also in agreement with the value 20.47 ± 1.25 reported by Yitayal et al., (2017). The optimum C/N ratios are between 20:1 and 35:1 as reported by previous studies (Matheri et al., 2017, Patinvoh et al., 2016).

Others have reported even more wide-spread C/N ratios between 10:1 and 30:1 are reported (Habiba et al., 2009; Patinvoh et al., 2016). Therefore, the optimum C/N ratio will vary depending on the type of substrate. The substrate with a high C/N ratio has poor buffering capacity while low C/N leads to the accumulation of ammonia and increasing the pH which becomes toxic to methanogens (Anahita et al., 2019). Despite that, the inoculum (fresh cow dung) was suitable to provide the buffer capacity of the digestion process (Gu et al., 2014; Manyi-Loh et al., 2013).

However, there is a possible variation in a buffering capacity that will lead to a variety of substrate compositions (Anahita et al., 2019). Additionally, Gu et al., (2014) suggested that an acceptable inoculum can provide nitrogen, micronutrients, and macronutrients for AD. There seems to be a collective conclusion that when selecting inoculum, priority should be the source already adapted to the substrate. However, the CYW contains high carbon content, leading to a C/N ratio of 42.50 ± 0.15 which is much higher than the expected value (20 to 30) for AD. Even though the nitrogen content agreed with the result reported by (Sendilvelan et al., 2017).

The biogas production rate was highly influenced by the quantity of carbon and nitrogen balance in feeding material (Muhayodin et al., 2020; W Parawira et al., 2004). The C/N ratio of CYW was not in the accepted optimum C/N ratio range (20-30:1) of the substrate in AD (Goswami et al., 2016). The higher C/N ratio of CYW as compared to the optimum range can result in low biogas production (Wei et al., 2019), while the lipid content should be high because of the shortage of nitrogen in the biochemical conversion procedure of organic matter into biogas (Lee et al., 2020).

A high C/N ratio will have nitrogen starvation by the substrate and can affect the bacterial activities (Anahita et al., 2019). Even so, for solid wastes with a high C/N ratio, the ammonia inhibition effect can be compensated by dilution with water which lowers the concentration of potential inhibitors (Chen et al., 2008; Drosg, 2013). Consistent with Einarsson & Persson, (2017), it is smart to think about both the C/N ratio and TS content as both contribute a crucial potential for biogas production. Relaxing maximum TS content always encompasses a strong positive effect on biogas production (Hao et al., 2016; Phun et al., 2017). Against this, the relative effect is especially large under a stricter constraint on the minimum C/N ratio.

Compare the results presented in Table 2.5 from literature to results presented from Table 4.1 from this research shows that MC from Table 2.5 is high, however, ash content and nitrogen from Table 2.5 are lower than those from Table 4.1. Nevertheless, the investigation on the potential of biogas production from CYW was investigated. Consequently, the use of cow dung as inoculum can improve the biogas production and reduce the anaerobic fermentation process (Zongyan et al., 2018). Hence, the characteristics exhibited by CYW indicate that it was readily biodegradable because of the high TS and TVS content of the solid fractions. The high solids content means CYW provides more efficient use of reactor volume so that the required reactor volume is less. It also implies that more of the CYW is consumed by reactor bacteria and yielding more biogas. This indicated that the CYW employed in this study is a suitable substrate for biogas production through AD.

4.2. Biogas production and biogas composition

The cumulative biogas and the daily biogas yield were calculated to gauge the effect of %TS concentration on the AD of CYW. One of the specific objectives of this research was to ascertain the performance of the AD process when operated at different loading ratios of TS concentration. For this reason, it is important to assess the process performance in terms of biogas composition and its production. The research was executed in triplicate. The values from the study were then averaged and the cumulative volume of biogas production was ascertained.

The experimental results showed the daily biogas yields of reactors R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, and R_{10} as illustrated in Figure 4.1 (A and B). Furthermore, Appendix one shows the average daily biogas yields for R_1 to R_{10}. The retention time for the reactors was between 15 days to 37 days (Appendix one).

This was within the range of 23 days reported by Isci and Demirer, (2007) and below 8 days reported by Saravanan et al., (2009) where they produced biogas from cotton waste in 23 and 45 days respectively. It was found that R_4 produced the very best (782.68 ± 3.59 mL per g-TVS) within the whole period of digestion. Reactor ten (R_{10}) had the least biogas yield of 203.01 ± 3.34 mL/g-TVS during the whole experiment period. These results are in agreement with those reported by other authors where they tested the potential of biogas production from different sorts of cotton wastes (Isci & Demirer, 2007; Raj et al., 2009; Rajendran & Balasubramanian, 2011b).

The low biogas yield from R_{10} was due to the low substrate loaded within the reactor because the biogas production increases by increasing the substrate loaded into a reactor. Filer et al., (2019) showed that if the substrate loaded is too low, there is a possibility of low quantities of biogas being produced because of the low metabolic activity of the microorganisms leading to low biogas yield. However, this has been contradicted by Ojolo et al., (2008) who showed that the total biogas produced was not proportional to the amount of substrate loaded but the measure of TS digested. Furthermore, the control reactor which contained the sole inoculum produced 89.48 mL per g-TVS within the digestion period. The reactors (R_8, R_9, and R_{10}) gave the assembly of biogas in 15 days, this could be due to the distribution of VFA led to a disturbance of acetogenesis and methanogenesis pathways (Isaksson, 2018; Wainaina et al., 2019).

This shortage of AD process can be attributed to an accumulation of both ethanoic acid and butyric acids resulting from fermentation pathway and propionic acids which are known to be difficult to degrade (Motte et al., 2013). Thus, it seems that the microorganisms within the reactors are not sufficient to sustain the anaerobic degradation progress.

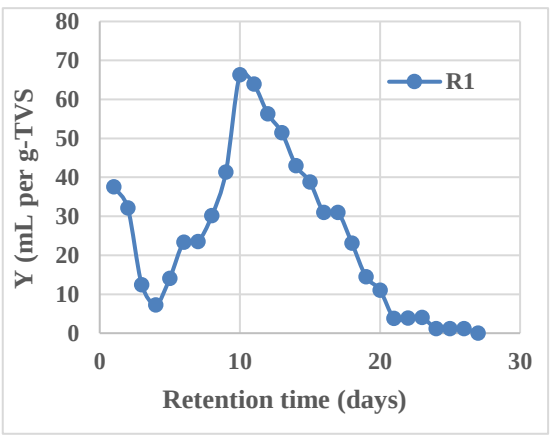
On the opposite hand, the lower gas production measured in R_8, R_9, and R_{10} could also be explained based on methanogenic bacteria which can be more at risk to unfavourable conditions (higher toxic effects exerted by VFA and ammonia) because of high water content and have lower growth rates (Pantini et al., 2015). For that reason, the inhibition was likely because of an imbalance within the growth rate of acidogenic bacteria, which led to an accumulation of degradation by-products within the reactors (Jnr, 2011).

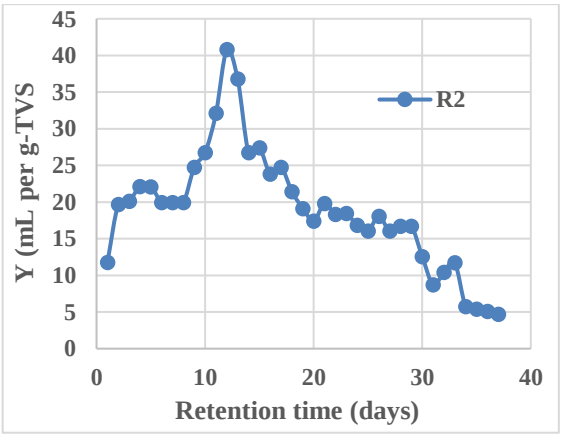
Aside, the inhibition of methanogenic bacteria could also be due to the high C/N ratio and ammonia content (Musa et al., 2014). Therefore, it is likely that the interaction between ammonia, VFA, and pH could have led to an inhibited steady-state condition in which the process was running stably but with a really low biogas yield (Chen et al., 2008). Each of the reactors has two peaks, while the worth peaks and positions are different as shown in Figure 4. 1 (A and B). The first high peak of biogas production could come from air mass inside the reactors because of the presence of CO_2, H_2S, and other gases that are formed.

Lately, a study pointed that the formation of two peaks could be explained by the high content of chemical oxygen demand within the substrate which can be biodegraded rapidly and contributed to the first peak (Li et al., 2015). The peaks of biogas production in the first few days could also be associated with an easily biodegradable substrate that presents into CYW (high solid content, carbohydrates, proteins, and starch) (W Parawira et al., 2004; Wei et al., 2019). It was also noted that, after the conversion of the easily biodegradable fraction, the system needed to start over the degradation of more complex compounds with a greater level of difficulty for biodegradation (W Parawira et al., 2004; Xia et al., 2018); a fact that will be evidenced by lowering the production of biogas.

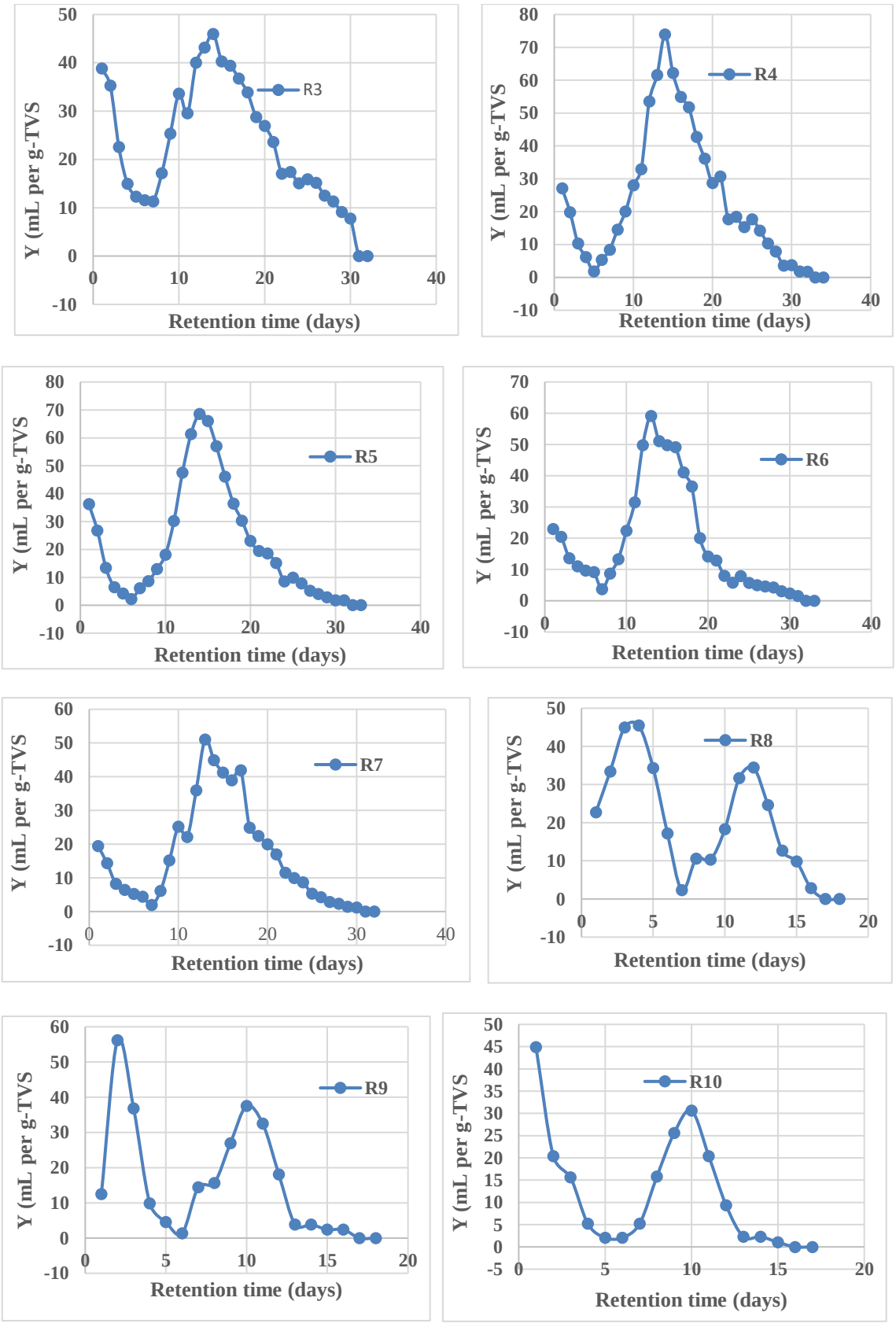
Another reasonable explanation of these peaks of biogas is that the lack of oxygen at the start of the experiment is caused by nitrogen flow in the reactor headspace (W Parawira et al., 2004). The earliest production peaks in the reactors are also related to the capacity for adaptation to the AD process of the microorganisms already present within the inoculum. The study of Gu et al., 2014 reported that the rapid production of biogas within the period was due to the high amount of organic matter available in the reactor. According to the above literature explanation, these could also be the explanations behind two peaks of biogas production. Furthermore, all the reactors displayed very similar trends in biogas production. Biogas production increased rapidly on the first few days then sharply inclined to a low level in five and seven days (Figure 4.1).

Afterward, the biogas production started again increasing slowly and reached a high peak value on day 10 and day 14. Within the range of five to fifteen days, biogas production significantly increased because of the exponential growth of microorganisms and their higher adaptation to the change of the concentration of substrate. Thenceforward, biogas production began to decrease until the end of the experiment and this was due to the stationary phase of microbial growth (Budiyono et al., 2014). The rates of biogas production differed appreciably with the increase in the TS concentration as is shown in Figure 4. 1 (A and B)





(A)



(B)

Figure 4. 1: (A and B) Daily biogas yield (Y) (mL per g-TVS) of R_1 to R_{10}

Therefore, CYW was digested in three phases including a start-up phase (1-7 days), stable biogas production phase (8-15 days), and decline phase (after 15 days). The start-up phase is critical for ensuring a rapid transfer to the stable gas production stage (M. Zheng et al., 2009). Moreover, a close range of biogas production was observed on day 10 and day 14 for all the reactors. Within the AD process, both degradation and production of organic solid material occurred at the same time in the reactor through the decomposition process of organic compounds and also the reproductive process of anaerobic microorganisms (Komemoto et al., 2009).

The production of the anaerobic microorganisms may additionally have occurred during this experiment. Hence, the best daily biogas production was observed on day 10 and day 14. Additionally, the R_4 showed the continuous production of biogas with the best biogas of 73.88 mL per g-TVS recorded on day 14 followed by R_1, and R_5 with the production of 66.34 mL per g-TVS and 68.57 mL per g-TVS recorded on day 10 and 14 respectively. Figure 4.2 shows the daily cumulative biogas yield for all reactors with error bars. Reactor (R_2) has also less but continuous production with the trend of adjusting from time to time with a peak biogas production of 40.80 mL per g-TVS recorded on day 12.

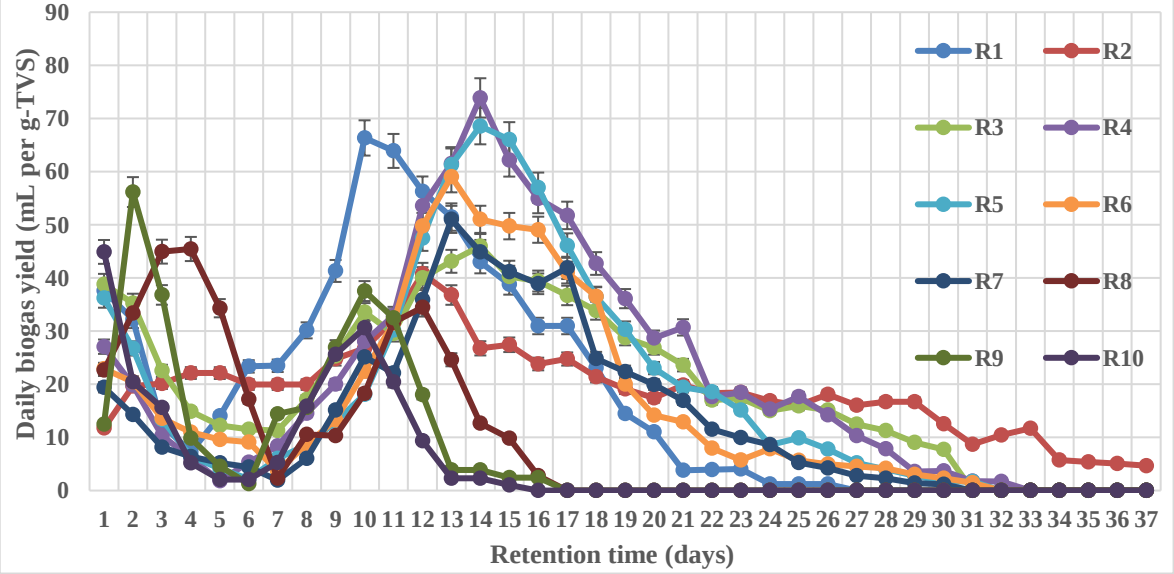


Figure 4. 2: The cumulative daily biogas yield

From this, results of R_2 are good because the production is continuous over the whole period of operation but can't be applied since the production is low. Figure 4.2 is often seen that biogas production was not ended at the same time. This is often showed that the carbons contained by all of the reactors' constituents are not equally degraded or converted to biogas production.

4.2.1. The ratios result

The maximum biogas productivity, biodegradability, and therefore the high rate of biogas production was reached at a ratio of 1:2.5 (28:72%). At this ratio, the ultimate average biogas yield was 782.68 ± 3.59 mL per g-TVS in 32 days of digestion. The typical biogas yield was 667.57 ± 4.29 mL per g-TVS, 698.88 ± 1.34 mL per g-TVS, 731.87 ± 2.15 mL per g-TVS and 695.93 ± 3.68 mL per g-TVS for ratio 1:1 (50:50%), 1:1.5 (40:60%), 1:2 (33:67%) and 1:3 (25:75%) respectively in 29-37 days. The biogas production started within 24 hours for all runs.

This was because while the wastes were at the dumpsites, microbial actions had begun on them (Ojolo. et al., 2008). All ratios started with high production for the first days and showed a trend in production from day 5 to day 7. Then, the production began to extend sharply to the peak value on day 10 and day 14 and so started decreasing till the end of the experiment setup period. Ratio one (1:1) showed a trend in biogas production on day 4 while the high production of biogas was recorded on day 10 (66.34 mL per g-TVS).

The ratio three (1:2), six (1:3.5), seven (1:4), and eight (1:5) showed a trend in biogas production on day 7 besides because the peak production of biogas recorded on day 14 for ratio three with a biogas yield of 45.97 mL per g-TVS whereas the peak production of biogas recorded on day 13 for ratio six and seven with biogas yield of 59.06 mL per g-TVS and 37.53 mL per g-TVS respectively.

The peak production for ratio eight was recorded on day 12 with the amount of 34.46 mL per g-TVS. The ratios nine (1:6) and ten (1:10) showed the trend in production on day 6 although the peak production was recorded on day 10 with biogas of 37.53 mL per g-TVS and 30.62 mL per g-TVS respectively.

From the results, the ratios eight, nine, and ten have shown that there was a shortage in biogas production and biodegradability which should suggest the potential inhibition of the methanogenic activity (Patinvoh et al., 2016). Furthermore, the results confirmed that the reactors should run at a ratio of 1:2.5 (28:72%), like the one that gave high biogas production. This result was in agreement with the work of Khalid et al., (2013) who reported that AD contained MC of 70-80% produced maximum biogas yield.

4.2.2. The effect of solid concentration on biogas produced

This research work examined the varied concentrations of %TS of CYW which were digested and therefore the corresponding biogas yield, to determine the suitable value of %TS for optimum biogas production. The effect of necessities on the solid contents on biogas production was studied by varying the TS from 10 to 50%. The results showed that the quantity of biogas produced was associated with %TS concentration (Figure 4.3).

There was a gradual increase in biogas as well as CH_4 content with a corresponding increase in %TS. This showed that there was gradual acclimatization of microbial communities to the conditions in the reactors and possibly a replacement predominant microbial community for high solid-state digestion was formed (Patinvoh et al., 2017). But, as the process continues, a time comes when any minimal increase in finished concentration would not contribute to the increased volume of biogas produced (Figure 4.3). This result might suggest that the CYW decomposed physico-chemically under low water content.

Additionally, this is predicted due to the function of water content in reactors since the TS content is going to be inversely related to water content. As was observed by Budiyono et al., (2014), water content is one of the vital factors affecting AD of solid wastes. Here, there are two main reasons mentioned; first, water makes possible the movement and growth of bacteria facilitating the dissolution and transport of nutrients; and secondary, water reduces the limitation of mass transfer of non-homogenous or particulate substrate. Furthermore, changing %TS concentrations would go together with the change of pH of the substrates which could also affect the quantity of biogas produced (Rico et al., 2011; Sathish et al., 2019).

The same trend was observed by Deepanraj et al., (2016), who reported that multi-response optimization of process parameters in biogas production from waste using Taguchi. In their study, they found that there was a decrease in biogas yield by volatile solid removal efficiency by 1.12% and chemical oxygen demand removal efficiency by 12.85% when the solid concentration was changed from 7.5% to 10% due to poor microbial substrate contact with an increased amount of %TS present in the reactor. Parawira et al., (2004) have also reported that the biogas yield from potato solids waste was increased because the TS increased from 10% to 40% and so decreased by increasing the TS from 50% to 80%.

This can be possible because when %TS increases, the amount of water decreases, thus reducing the level of microbial activity which then affects the amount of biogas production, particularly at a higher value of TS concentration. Igoni et al., (2008) showed that slurry with high TS concentration was more acidic than that of lower TS concentration, which is an additional reason why the higher value of TS wouldn't significantly affect the volume of biogas produced.

Furthermore, the results showed that the CYW composition diluted to 28% TS content produced optimum biogas yield (782.68 ± 3.59 mL per g-TVS) compared to 50%, 40%, 33%, 25%, 22%, 20%, 16% 14% and 10% as shown in Table 4.3. The results obtained confirm that reactors should digestate at this 28% TS.

Table 4. 3: The biogas yields (Y) with standard deviation for R1 to R10

R	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Y	667.57 ± 4.29	698.88 ± 1.34	731.87 ± 2.15	782.68 ± 3.59	695.93 ± 3.68	597.14 ± 3.14	513.40 ± 2.70	355.41 ± 3.45	278.72 ± 4.05	203.01 ± 3.34

Y: yields (mL per g-TVS) and R: Reactor

In conclusion, there is a significant correlation between the %TS concentration and biogas yield ($p<0.05$) as shown in Table 4.4.

Table 4. 4: Anova results showing the relationship between %TS and biogas yield (Y measured in mL per g-TVS)

ANOVA: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
%TS	10	258	25.8	153.066		
Y	10	5524.94	552.494	42181		
ANOVA						
SV	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
BG	138703 2	1	1387032	65.527631	0.01146	4.413
WG	381008	18	21167.14			
Total	176804 0	19				

Where SV = Source of Variance, BG = Between Group and WG = Within Group

This might be because the CYW is rich in nutrients and contains an adequate amount of carbon, oxygen, hydrogen, phosphorous, potassium, and a few trace elements which are very essential for the expansion of anaerobic bacterium represented in this reactor

(Maamri & Amrani, 2014).

This might have optimized the interaction between acetogens and methanogens which is the most critical step in the biomethanation process (Angelidaki et al., 2011; Krishania et al., 2013). However, the various optimum concentrations of TS caused biogas production at a unique rate. Generally, with increasing the %TS loading rate, the total biogas yields increased (Figure 4.3). Thus, the rise of %TS resulted in higher biogas production. These results are consistent with those reported by other authors that indicate a rise in biogas production because the TS content in the mixture increases until it reaches an optimal production point (Budiyono et al., 2014; Camarena-Martínez et al., 2020; Safari et al., 2018; Teleszewski & Żukowski, 2018).

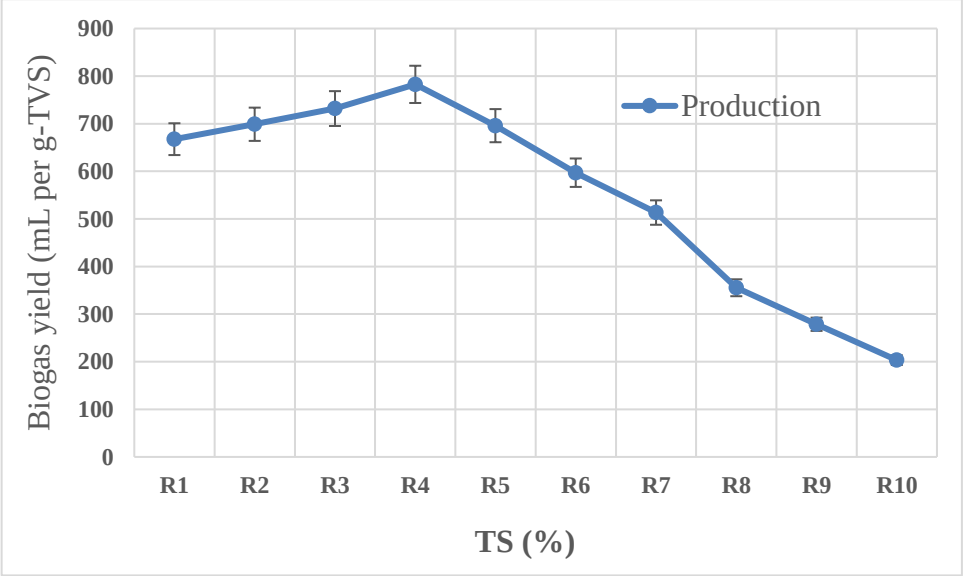


Figure 4. 3: Effect of TS on biogas yields. R1 to R10 represent 50, 40, 33, 28, 25, 22, 20, 16, 14 and 10% respectively

4.2.3. The biogas compositions

The measurement of quantity and composition of biogas produced in terms of CH_4 and CO_2 content is crucial and fundamental to evaluate the stability of the process. The methane content was almost zero for all experiments for the primary days. It was lower in the first days due to the dilution of biogas with air on digestion liquor inside the reactor.

This was similar to the results from a study conducted by (L. Zhang et al., 2011) in which rapid, increased daily biogas production was achieved on the first day with low CH_4 concentration and high CO_2 concentration. The main reason was acidogenesis during this era (Nakamura et al., 2020; Ren et al., 2019). During the first days of fermentation, the bacteria have not reached the CH_4 or methanogenesis formation stage but one can confirm the stage of hydrolysis and acidogenesis because they have produced gases like CO_2, H_2S, and air or other gases. All gases are normally produced simultaneously at a relentless rate, when hydrolysis, acidogenesis, and methanogenesis processes work properly, CO_2 and air then cause pressure within the reactor to increase (Yitayal et al., 2017). That air was pushed out of the reactor in time by the biogas produced and CH_4 also reached 50% (Table 4.5).

Table 4. 5:The Biogas Compositions with standard deviation (Multi-gas detector, Gas type: $CO_2/CH_4/O_2/H_2S$)

Compositions	R_1	R_2	R_3	R_4	R_5
CH_4 (%)	42.12 ± 0.03	45.34 ± 0.02	47.15 ± 0.02	53.98 ± 0.03	45.62 ± 0.02
CO_2 (%)	47.72 ± 0.02	42.73 ± 0.03	39.30 ± 0.02	35.67 ± 0.04	43.52 ± 0.03
O_2 (%)	0.34 ± 0.01	1.10 ± 0.03	2.05 ± 0.02	2.21 ± 0.01	2.67 ± 0.02
H_2S (ppm)	235 ± 0.20	120 ± 0.30	105 ± 0.40	79 ± 0.30	115 ± 0.40
Others (%)	9.82 ± 0.04	10.83 ± 0.03	11.50 ± 0.04	8.14 ± 0.03	8.19 ± 0.03

All treatment tests produced biogas with CH_4 concentrations above 40%, showing their quality in terms of calorific value. The total solids concentration influenced the biogas production as more CH_4 yield was observed within the substrate of 28% TS than 25%TS, 33% TS, 40%TS, and 50% TS. The biogas in the R_4 contained 22% CH_4 content on the second week, with an increase to 48% on the third week.

Similarly, the biogas in the R_1, R_2, R_3, and R_5 contained 18%, 19%, 21%, and 15% CH_4 content on the second week, with the content rising to 40, 42, 45, and 43% CH_4 on the third week respectively. These results are in the range of those of Jeihanipour et al., (2010) who found that the CH_4 content of treated cotton/polyester with fresh N-Methylmorpholine-N-oxide was 31.28% to 53.02%. The trend patterns of the CH_4 composition for all reactors were almost identical.

Conversely, H_2S concentration was low for all the tests, which is consistent with findings from Jaroenpoj, (2015). The removal of the gas is extremely important because it corrodes pipes and engines; being also toxic to humans. However, the fact that the CO_2 in biogas was found increasing implies that the acidifying microorganisms are prevailing on the methanogens which will result in VFA accumulation (Goswami et al., 2016; Isaksson, 2018). From the findings during this research, CO_2 wasn't only produced from the acidification of the system, but it had been also the assembly of aerobic reactions occurring inside the reactors.

This issue has occurred during the feeding process by which there was an opportunity of air that may go inside the reactor due to the reactor configuration. This statement was proved by comparing the CH_4 concentration during the first days of operation. Besides CH_4, CO_2, H_2S, and O_2, other chemical compounds were found in biogas samples that were not quantified separately by gas chromatography and multi-gas detectors for instance water vapour, air, hydrogen, and carbon. Noor et al., (2014) described the presence of those constituents in biogas at a degree not up to CH_4 and CO_2, however, in line with Wesley et al., (2017), the raw biogas has got to be purified to extend its quality and applicability standards.

The gas mixture is saturated with vapour and will contain dust particles and a trace amount of H_2, N_2, CO, and halogenated compounds looking at the feedstock and process conditions. The largest peak within the sample showed by GC are the solvent (CH_4) peaks (100%) and the other peaks, relative to the solvent peak are extremely small and can't be observed during this spectrum. This huge peak contains a great area, and every other peak is a trace and not of interest (Appendices 2-5). Those traces of peaks are not identified by GC because the GC column used was for the sole CH_4 peak. In short, these compounds made up at most 9.50 ± 0.03% of the biogas composition (Table 4.6).

Table 4. 6: Composition of biogas from anaerobic digestion process (Multi-gas detector, Gas type: CO_2/CH_4/O_2/H_2S)

Components	Volume percentages	Unit
CH_4	42-53 ± 0.02	Vol.%
CO_2	47-35 ± 0.03	Vol.%
O_2	1-2 ± 0.02	Vol.%
H_2S	130-150 ± 80	ppm
Others	8-11 ± 0.03	Vol.%

It may be seen from Table 4. 6 that when CH_4 content in the biogas is high, CO_2 content is low. The proportion of the CH_4 increased together with the operating time, whereas the CO_2 decreased at an analogous rate. This can be because the biogas production is related to the %TVS reduction (Jaroenpoj, 2015).

Also, the two principal pathways of CH_4 formation in AD are the conversion of H_2 and CO_2 to CH_4 and H_2O, and also the conversion of acetate to CH_4 and CO_2 (Abobaker et al., 2019). In an early stage of the digestion process, more CO_2 content is made and in the last stage, the methanogens use CO_2, H_2, formate, acetate, methanol, and CO to produce CH_4 (Enzmann et al., 2018; Stevens et al., 2012).

For that reason, it is expected that in the first days of the digestion period, high CO_2 content is observed and in the later digestion period, higher CH_4 content is obtained with the corresponding decrease in CO_2 content. The methane content, however, continues to be lower due to two reasons. First, the period of 30 days' fermentation might not be sufficient enough for CYW to permit the methanogenesis process to be completed and a longer fermentation time is required. Secondly, the AD during this study was conducted at an average temperature below 30℃.

Previous studies showed that the optimum temperature of 35-38℃ is required to realize high gas CH_4 production (Abobaker et al., 2019; Jankowska et al., 2017; S. Wang et al., 2019). The succeeding study is required to check the biogas production of CYW at this optimum temperature using different biological substrates co-digestion.

4.3. The characterization effluent results

The results of effluent characterization are shown in Table 4.7. The effluent showed that pH was in the acidity range (4.7 ± 0.18) as shown in Table 4.7. The solids content of effluent was reduced from R_1 up to R_{10}. This was since the sample was wet which implies that less water but higher TS content; here the bacteria used water as a medium of movement. Against this, higher water less TS content due to the high accessibility of bacteria to CYW. The water was increased from R_1 up to R_{10}. This was because of water addition and water came from the hydrolysis process during the digestion.

Table 4. 7: Effluent characteristic results

	Parameters					
R	**pH**	**MC (%)**	**TS (%)**	**TVS (%TS)**	**TS loss (%)**	**TVS loss (%)**
R_1	4.7 ± 0.16	64.15±0.23	35.85±0.62	59.81±0.71	28.30±0.29	30.14 ± 0.31
R_2	4.5 ± 0.20	75.15±0.43	24.82± 0.52	49.62±0.68	37.95±0.42	40.12 ± 0.35
R_3	5.1 ± 0.17	77.87±0.62	22.13± 0.43	48.39±0.91	32.93±0.62	42.22 ± 0.25
R_4	4.8 ± 0.21	86.18±0.52	11.82± 0.21	31.52±0.61	57.78±0.92	62.96 ± 0.40
R_5	5.3 ± 0.23	87.87±0.68	14.82± 0.32	44.75±0.31	40.72±0.41	44.23 ± 0.47
R_6	4.9 ± 0.18	86.00±0.72	13.97± 0.54	49.99±0.45	36.50±0.35	38.92 ± 0.51
R_7	5.1 ± 0.30	88.49±0.81	13.51± 0.45	51.74±0.65	32.45±0.42	35.12 ± 0.42
R_8	4.8 ± 0.11	88.18±0.93	11.82± 0.71	56.13±0.93	26.12±0.52	28.13 ± 0.31
R_9	3.9 ± 0.09	90.13±1.08	10.87± 0.36	54.44±0.82	22.35±0.38	24.06 ± 0.32
R_{10}	3.7±0.10	91.84±0.97	8.16 ± 0.25	56.19±0.95	18.41±0.40	20.83 ± 0.29

Values are presented as means ± standard deviations of triplicates

4.3.1. The total solids and total volatile solids reduction

The destruction of organic matter is the primary objective of AD (Phun et al., 2017). Determining the physicochemical properties of effluents is vital to access the reusability of the CYW and determine the most suitable variety of process for its treatment, and it can reflect the AD efficiency of fermentation experiments (Lv et al., 2018).

The results of physicochemical properties of effluents over 15 and 37 days of the experiment have shown that there was decreasing in TS and TVS (Figures 4. 6 and 4.7), which can result in utilization of the CYW by the microorganisms. This can be in agreement with the result of Olanrewaju & Olubanjo, (2019), who explained that there was a reduction in TS and TVS as biogas yield increases.

The reduction of TS and TVS is evidence of the efficiency of AD, especially in the

reduction of TVS concentration related to high biogas production. Therefore, TS and TVS must be measured to see the general process efficiency.

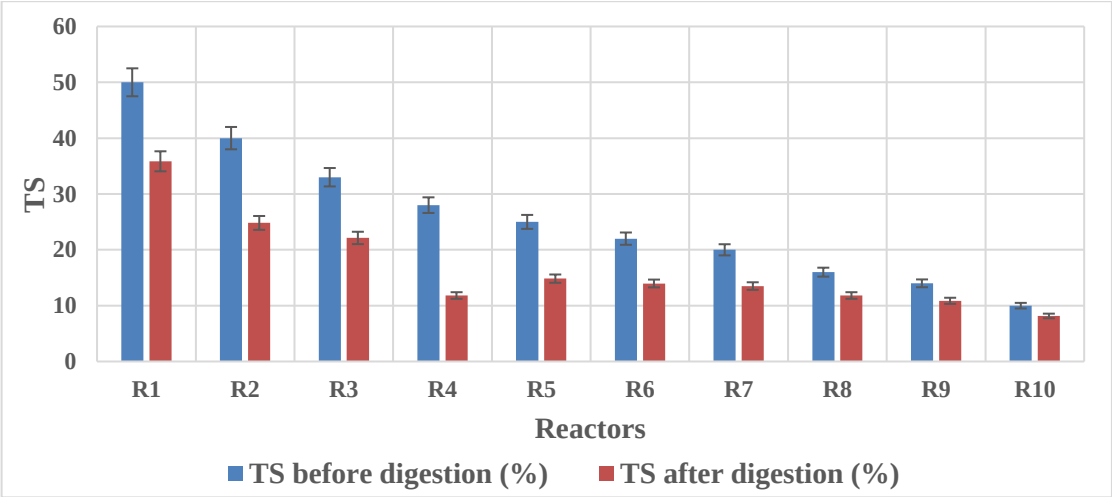


Figure 4. 4: Content variation of TS before and after anaerobic digestion

Figures 4.4 and 4.5 show the TS and TVS content variation before and after AD: the above values (TVS values) are nearly identical altogether test ratios before AD but exhibit large differences after digestion reflects their different digestion abilities.

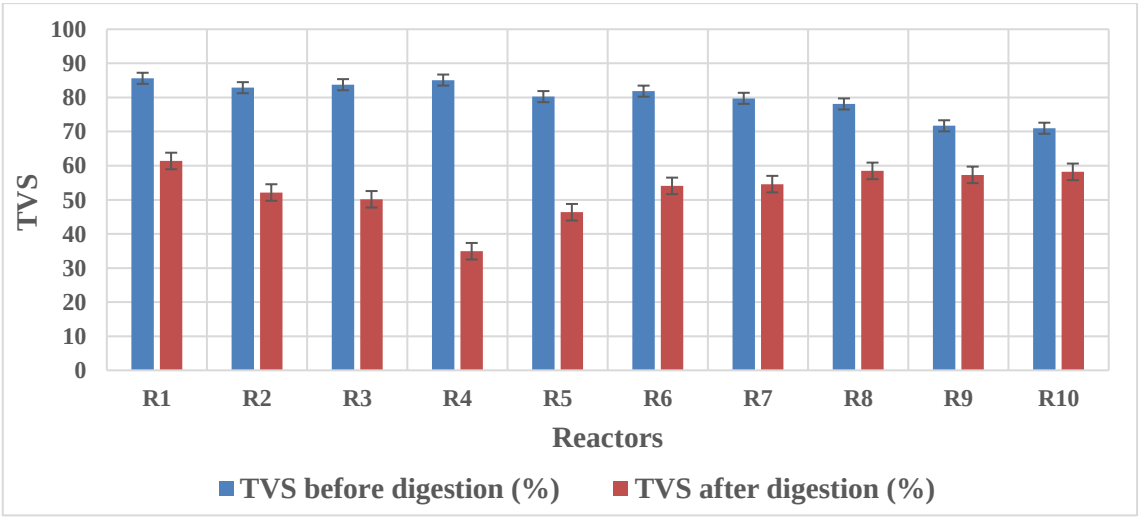


Figure 4. 5: Content variation of TVS before and after the anaerobic digestion

Figure 4.4 describes the change of TS before and after digestion.

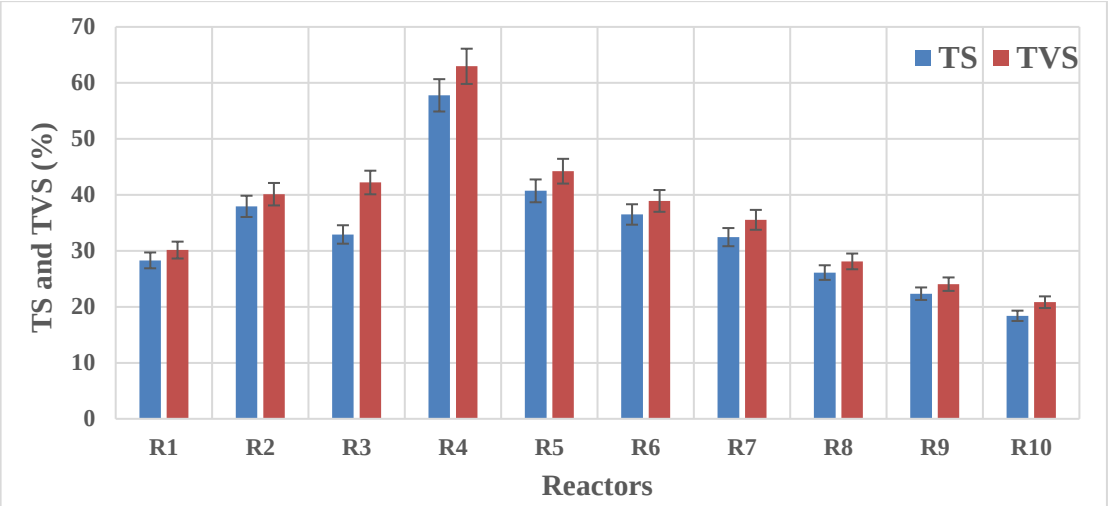


Figure 4. 6: The TS and TVS reduction after digestion

According to the TS content variation during digestion, the degradation of TS in the ten test ratios were 28.30 ± 0.29, 37.95 ± 0.42, 32.93 ± 0.62, 57.78 ± 0.92, 40.72 ± 0.41, 36.50 ± 0.35, 32.45 ± 0.42, 26.12 ± 0.52, 22.35 ± 0.38 and 18.41 ± 0.40% for R_1 to R_{10} respectively which means that the degradation increased in line with biogas production. The TS reduced in which the biogas production increased (Figure 4.6). The R_4 showed the best degradation rate of TS while R_{10} showed the least with 57.78 ± 0.92% and 18.41 ± 0.40% respectively after 32 days of AD.

Figure 4.5 depicts the TVS variation in ten test ratios before and after digestion. Per the TVS contents before and after digestion, the TVS removal rates of ratios were 30.14 ± 0.31, 40.12 ±0.35, 42.22 ± 0.25, 62.96 ± 0.40, 44.23 ± 0.47, 38.92 ± 0.51, 35.12 ± 0.42, 28.13 ± 0.31, 24.06 ± 0.32 and 20.83 ± 0.29% for R_1 to R_{10} respectively. Similarly, R_4 had the very best TVS removal rate and R_{10} had the least removal. The higher loss of TVS more than the TS was a good indication of the high uptake rate of the organic fraction of TS by methanogenic bacteria. The TVS parameter indicates that the organic fraction of the substrate was degraded during the digestion process, showing the action of methanogenic bacteria (Muhayodin et al., 2020).

Therefore, the TS removal was dependent upon the solids content, which implies that higher solids content produced low TS removal ($p<0.05$). This might be caused by the low accessibility of the substrates by the microorganism movement due to less water in the reactor. Sample biodegradation efficiency is slightly increased by controlling the substrate property.

There was no significant ($p>0.05$) difference in either TS or TVS removal ratios between all ratios. The difference in the digestion efficiency between samples during this study might depend upon an interaction (water/solid content ratio, nutrients equilibrium, moisture balance, and/or inhibitory material dilution adjustment) during the substrate digestion (Lee et al., 2020). In this study, the anaerobic treatment was favoured when it had been conducted with a 28% TS content compared to a similar process with 50% TS, 40% TS, 33% TS, 25% TS, 22% TS, 20% TS, 16% TS, 14% TS, and 10% TS respectively. As a summary, at the starting of the digestion process, the TS and TVS content values in each reactor were high compared to the end of the digestion process. From the represented data, it is clear that both contents TS and TVS are significantly reduced ($p<0.05$).

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The physicochemical characteristics of CYW showed that it has potential to be used as a substrate for biogas production. CYW had average TS content of 93.18 ± 0.21% and TVS of 82.48 ± 21% respectively which is suitable for biogas production. The removal efficiencies of TS and TVS by the AD for R_4 were highest in the runs tested at 57.78 ± 0.92% in TS and 62.96 ± 0.40% in TVS respectively. The percentage TS removal for R_1, R_2, R_3, R_5, R_6, R_7, R_8, R_9, and R_{10} were 28.30 ± 0.29%, 37.95 ± 0.42%, 32.93 ± 0.62%, 40.72 ± 0.41%, 36.50 ± 0.35%, 32.45 ± 0.42%, 26.12 ± 0.52%, 22.35 ± 0.38% and 18.41 ± 0.40% respectively while the percentage TVS removal for the same were 30.14 ± 0.31%, 40.12 ± 0.35%, 42.22 ± 0.25%, 44.23 ± 0.47%, 38.92 ± 0.51%, 35.12 ± 0.42%, 28.13 ± 0.31%, 24.06 ± 70.32% and 20.83 ± 0.29% respectively.

However, the C/N ratio of the CYW was 42.50:1 which was far beyond that required for AD. Each of the reactors had two peaks of biogas production which were associated with the easily biodegradable substrate that presents into CYW, while the value peaks and positions are different. All the reactors showed very similar trends in biogas production. The results obtained showed that the quantity of biogas produced was associated with the %TS in the reactors.

There was a gradual increase in biogas production as well as CH_4 content with a corresponding increase in %TS up to optimal value. The reactor (28% TS) showed the best biogas volume yield (782.68 ± 3.59 mL per g-TVS) with 53.98 ± 0.03% CH_4 content and also gave the best daily biogas production (73.88 mL per g-TVS). The results obtained confirm that digesters should run at 28% TS for maximum biogas generation.

Generally, the biogas contained CH_4 and CO_2 content of 42-53 ± 0.02% and 47-35 ± 0.03% respectively. However, obtaining high yields remains a serious major challenge. One solution is to optimize the method, adjusting the number of the physical and chemical parameters and make co-digestion with an organic substrate with a low C/N ratio.

5.2. Recommendations

The C/N ratio of CYW reported during this study was 42.50:1 which was way too high compared to the optimum recommended C/N ratio for AD, therefore, the utilization of treated CYW as a co-substrate with nitrogen-rich substrate could help balance the nutrients. Improvement in biogas yield has been observed at 28%TS of CYW, therefore, other different TS ratios on this waste should be tried.

Since biogas is a mixture of gases, some of them are flammable and others are non-flammable. It has been observed that the gas contained high CO_2 content and a tiny amount of H_2S gas which is a poisonous gas generated through sulphate available in CYW during a touch together with biogas. Hence, biogas requires treatment to enhance its quality so that it is often utilized as a safe energy source. In future investigations, cost-effective removal of H_2S and other harmful gas in biogas is the key task behind the fixation of cost level which should be analysed well with refinement methods.

The sludge materials rich in minerals are often used for various purposes as bio-fertilizer. However, the residues from this study have not been tested for ammonium composition. The high concentrations of NH_3 can work like phytotoxic materials, which inhibit the expansion of plants. Further studies are recommended to check for ammonium composition in the residues to determine their suitability for application as fertilizer.

The other important parameters essential for the optimum functioning of an AD-like temperature, pH, loading rate should be considered for further studies to attain the optimum value for the AD. Thus, the increasing volumes of solids organic waste should become a valuable commodity, as an energy resource that should be managed properly through AD.

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APPENDICES

APPENDIX ONE: THE AVERAGE DAILY BIOGAS YIELD

	The average daily biogas yields (mL per g-TVS)									
D	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**R_7**	**R_8**	**R_9**	**R_{10}**
1	37.59	11.77	38.83	27.07	36.21	22.89	19.43	22.69	12.51	44.9
2	32.17	19.66	35.27	19.81	26.74	20.44	14.32	33.4	56.16	20.41
3	12.4	20.11	22.54	10.32	13.37	13.55	8.17	44.95	36.81	15.62
4	7.24	22.11	14.92	6.14	6.46	10.99	6.42	45.45	9.86	5.21
5	14.08	22.1	12.27	1.84	4.14	9.6	5.2	34.31	4.51	2.08
6	23.36	19.94	11.55	5.32	2.21	9.13	4.39	17.16	1.31	2.08
7	23.48	19.93	11.31	8.37	6.05	3.7	1.93	2.28	14.43	5.24
8	30.14	19.93	17.12	14.51	8.6	8.69	6.07	10.55	15.63	15.83
9	41.32	24.75	25.3	20.02	12.92	13.25	15.11	10.3	26.95	25.62
10	66.34	26.76	33.59	28.01	18.11	22.4	25.12	18.28	37.53	30.62
11	63.9	32.11	29.51	32.92	30.19	31.5	22.12	31.65	32.48	20.41
12	56.28	40.8	40.03	53.55	47.47	49.75	35.92	34.46	18.04	9.37
13	51.46	36.79	43.13	61.54	61.3	59.06	51.02	24.61	3.85	2.29
14	43.02	26.76	45.97	73.88	68.57	51.03	44.91	12.66	3.85	2.29
15	38.8	27.43	40.28	62.18	66.01	49.75	41.18	9.85	2.4	1.04
16	30.96	23.81	39.41	54.92	56.98	49.07	38.9	2.81	2.4	0
17	30.96	24.75	36.71	51.77	46.09	41.02	41.89	0	0	0
18	23.08	21.41	33.84	42.72	36.44	36.53	24.85	0	0	0
19	14.5	19.13	28.76	36.11	30.31	20.01	22.39	0	0	0
20	11.07	17.41	26.91	28.68	23.04	14.16	19.94	0	0	0
21	3.84	19.81	23.6	30.7	19.42	12.89	16.95	0	0	0
22	3.91	18.33	17	17.69	18.55	7.94	11.51	0	0	0
23	4.04	18.46	17.37	18.42	15.11	5.77	9.93	0	0	0
24	1.21	16.86	15.02	15.31	8.55	7.85	8.61	0	0	0
25	1.21	16.06	15.89	17.69	9.91	5.66	5.27	0	0	0
26	1.21	18.06	15.14	14.25	7.77	4.93	4.22	0	0	0
27	0	16.06	12.47	10.32	5.18	4.56	2.81	0	0	0
28	0	16.73	11.31	7.87	3.97	4.2	2.28	0	0	0
29	0	16.73	9.08	3.57	2.81	3.04	1.4	0	0	0
30	0	12.57	7.74	3.68	1.73	2.32	1.14	0	0	0

31	0	8.72	0	1.78	1.72	1.46	0	0	0	0
32	0	10.43	0	1.72	0	0	0	0	0	0
33	0	11.71	0	0	0	0	0	0	0	0
34	0	5.75	0	0	0	0	0	0	0	0
35	0	5.38	0	0	0	0	0	0	0	0
36	0	5.08	0	0	0	0	0	0	0	0
37	0	4.68	0	0	0	0	0	0	0	0
S	667.57	698.88	731.87	782.68	695.93	597.14	513.4	355.4	278.7	203.0
M	25.675 76923	18.888 64865	24.395 66667	24.458 75	22.449 35484	19.262 58065	17.113 33333	22.21 3125	17.42	13.53 4
SD	19.913 75187	8.1582 41628	11.815 25597	20.383 8837	20.509 16249	17.494 50256	14.838 12966	13.92 9072	16.20 3451	12.96 36088
SE	3.9827 50373	1.3412 06662	2.1571 60739	3.6033 95597	3.6835 54324	3.1421 05416	2.7090 59443	3.482 2680	4.050 8627	3.347 18941

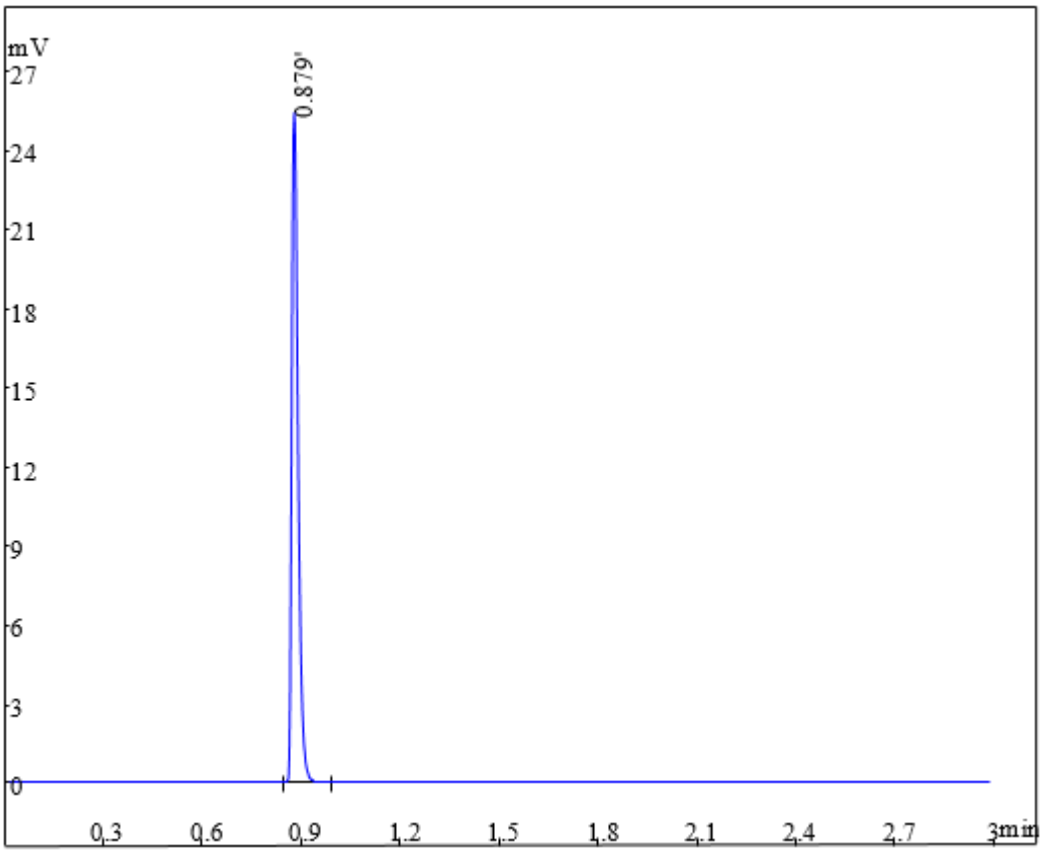
Where D = Days, R_1 to R_{10} = Reactor one to ten, S = Sum, M = Mean, SD = Standard Deviation, and SE = Standard Error

APPENDIX TWO: METHANE PEAK REPORT OF R_1

Printing time: Fri Nov 06 09:51:57 2020

Injection time: Wed Nov 04 13:05:29 2020

File opened: C:\Users\PC\Desktop\Peak-ABC\program\Maurice R1 (20201104 13;05;29).



Rank	Time	Name	Area%	Area
1	0.879		100	29459
Total			100	29459

APPENDIX THREE: METHANE PEAK REPORT OF R_2

Printing time: Fri Nov 06 09:50:45 2020

Injection time: Wed Nov 04 13:11:39 2020

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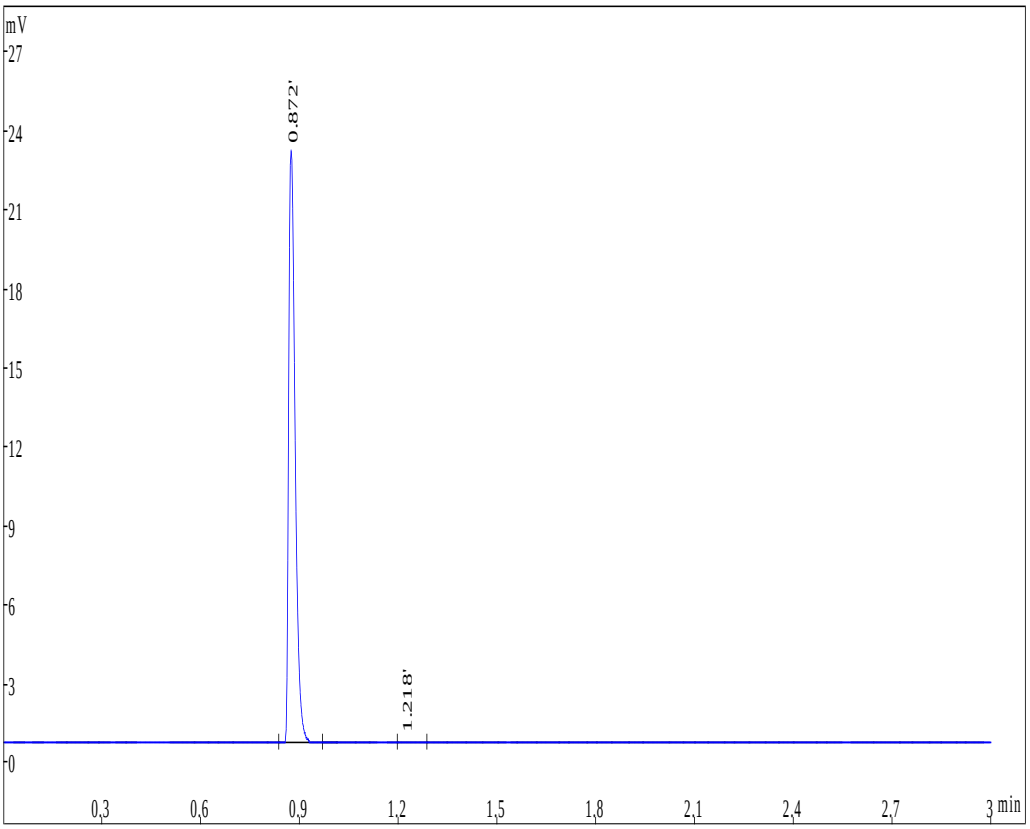

Rank	Time	Name	Area%	Area
1	0.872		99.65	28885
2	1.218		0.3491	101
Total			100	28986

APPENDIX FOUR: METHANE PEAK REPORT OF R_3

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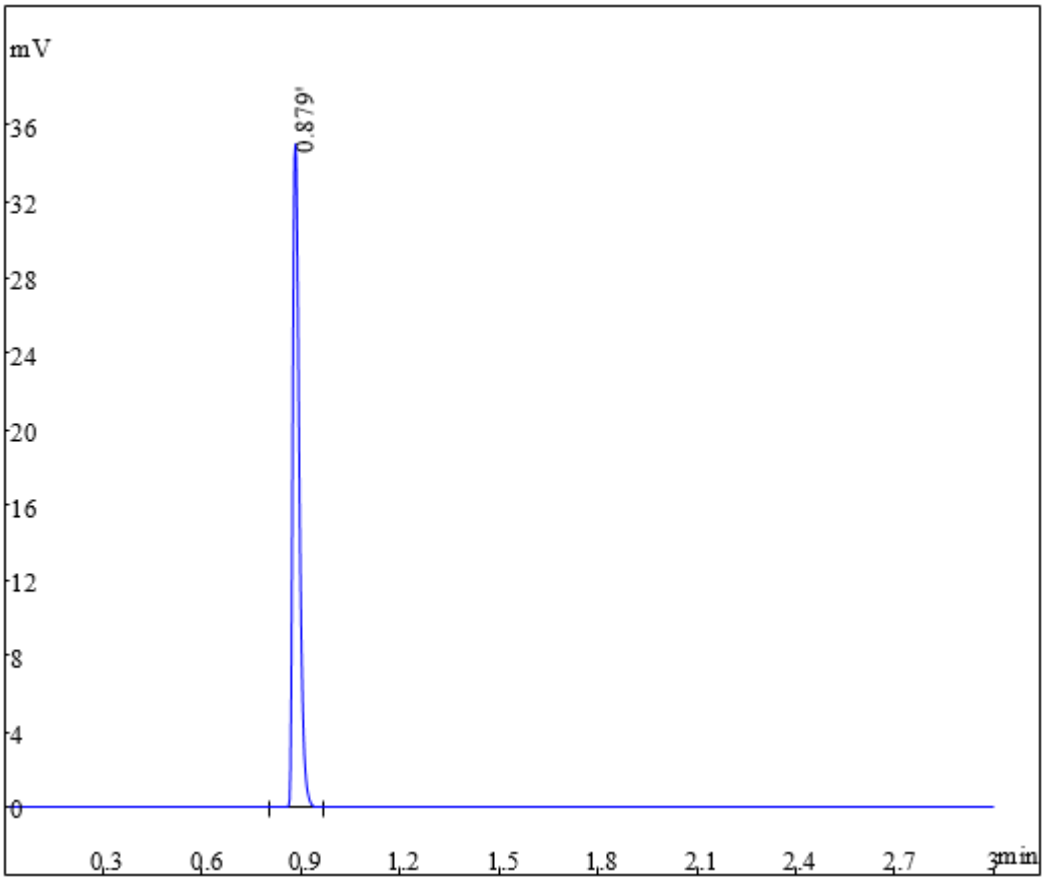

Rank	Time	Name	Area%	Area
3	0.879		100	41629
Total			100	41629

APPENDIX FIVE: METHANE PEAK REPORT OF R_4

Printing time: Fri Nov 06 09:42:06 2020

Injection time: Wed Nov 04 13:22:28 2020

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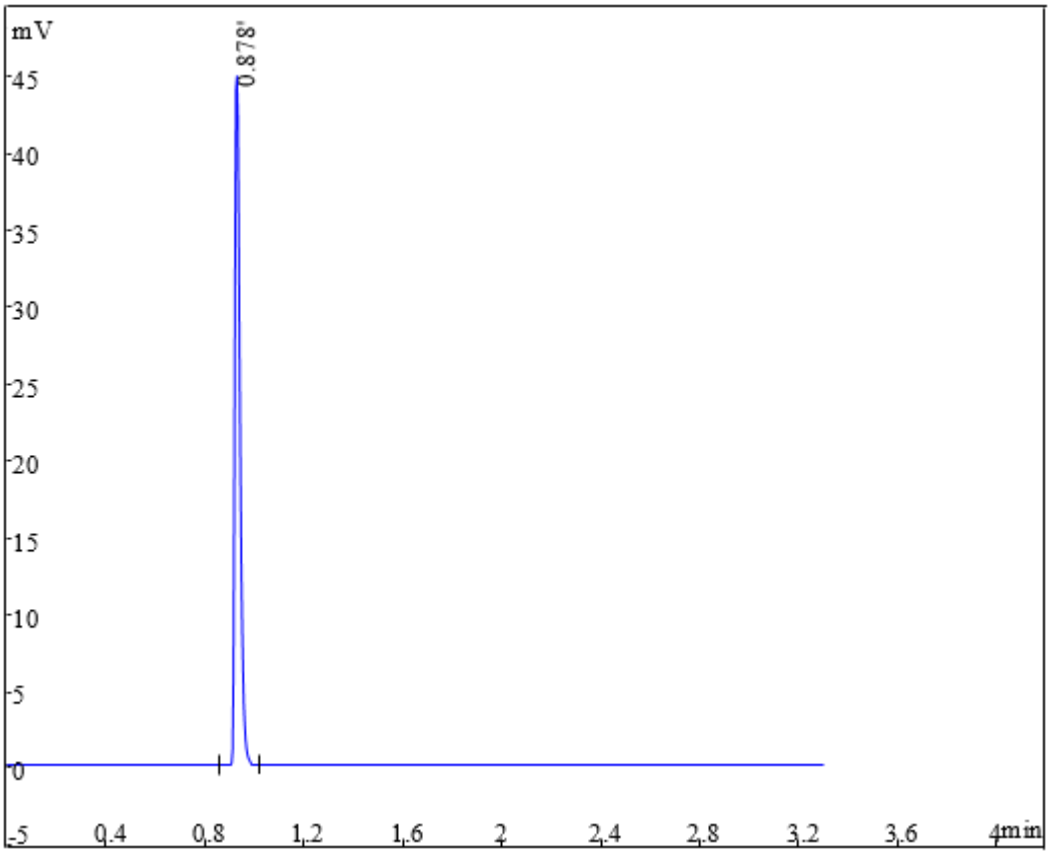

Rank	Time	Name	Area%	Area
1	0.878		100	49139
Total			100	49139