EVALUATION OF SOLID FOOD WASTE FOR BIOGAS AND MANURE PRODUCTION: A CASE STUDY OF THE UNIVERSITY OF VENDA CANTEEN SOLID FOOD WASTE

By

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ABSTRACT

The ever-increasing food waste generated globally attracts grave concerns due to associated environmental and socio-economic implications. One way to mitigate these adverse consequences is to use the food waste as feedstock for valuable energy and fertilizer generating anaerobic digestion (AD) process. While AD is a widely accepted method for managing and valorising food waste (FW), in South Africa, its use is in the infancy stage. Lack of awareness and adequate knowledge of the potential of FW for biogas and manure production could be the reason for the observed slow uptake of AD in South Africa. This study evaluated the energy and manure production potential of solid FW generated by a typical South African university (the University of Venda) canteen using the AD co-digestion of solid FW and cow dung (CD). In the first part of the study, a 2 x 5 factorial experimental design was used to assess the effect of substrate mixing ratios and temperature on biogas yield, methane content, and biochemical methane potential (BMP) of pure FW and CD and co-digestion of FW and CD for predetermined mixing ratios. FW and CD mixed on a wet mass basis at ratios 0:100, 25:75, 50:50, 75:25, and 100:0 (w/w) were batch incubated at 35±1°C and 55±1°C. Experimental results were subjected to analysis of variance (ANOVA) at a 5% level of significance. Where a significant ANOVA result was obtained, the mean separation was done using Fisher's least significant difference (LSD) test at a 5% level of significance. The obtained results showed that the temperature and substrate mixing ratios significantly ($p < 0.05$) affected biogas yield, biogas methane content, and BMP. The highest biogas yield and BMP were 4903.33 ± 38.84 mL and 301.879 ± 2.07 mLCH_4/g VS at 35 °C, and 7151.67 ± 11.55 mL and 401.88 ± 1.98 mLCH_4/g VS at 55 °C. The lowest biogas and BMP yields were 5301.67 ± 62.51 and 328.278 ± 4.265 mLCH_4/g VS at 55 °C, and 3291.67 ± 81.45 mL and 328.28 ± 4.26 mLCH_4/g VS, respectively, for mono-digestion of FW at 55 ° and 35 °C. Overall, co-digestion of FW and CD produced higher biogas yield and BMP than mono-digestion of the substrates at both test temperatures. These findings can be used to size heated and unheated biogas plants using co-digested FW and CD substrates. In the last part of the study, a 2 x 2 factorial experimental design was used to assess the effect of substrate type and temperature on the quality of bio-slurry. The bio-slurry samples were collected from mono-digestion of FW and CD which were incubated at 35±1°C and 55±1°C. The obtained results indicated that bio-slurry quality was significantly affected ($p < 0.05$) by substrate type, temperature, and the interaction between substrate type and temperature. Bio-slurries had lower total solids (TS %) and (volatile

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solids/total solids) VS/TS% compared to undigested FW and CD. Bio-slurry from the AD of FW and CD at 55 °C had significantly higher ($p < 0.05$) NPK concentrations than bio-slurry obtained from AD at 35 °C. The highest nitrogen (0.25%), phosphorous (1.61%) and potassium (1.25%) concentrations were obtained from bio-slurry obtained the AD of CD at 55 °C. Characterization data for bio-slurry from mono-digested FW and CD can be used to advise biogas plant owners, agricultural extension officers and the public on the application rates of co-digested FW and CD slurry as manure depending on crop nutrient requirements.

Keywords: anaerobic digestion, biochemical methane potential, biogas, bio-slurry, co-digestion, FW, waste management

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DECLARATION ON PLAGIARISM

I, **Prosper Mhlanga (20005859)**, hereby declare that this dissertation for the Master of Science degree in Agriculture in Agricultural Mechanization at the University of Venda hereby submitted by me has not previously been submitted for a degree at this or any other university and that it is my work in design and execution, and that all work sourced from other persons has been referenced.

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DECLARATION ON PUBLICATION

This section presents parts of this dissertation that have been submitted to a Department of Higher Education and Training accredited peer-reviewed journal for consideration for publication. The research reported in the manuscripts are based on the data I collected from the experiments I conducted in this study. I designed the experiments, collected, and analyzed the data and wrote the manuscript. This work was done under the supervision of Prof MO Marenya, Dr D Tinarwo and Dr NT Tavengwa. The asterisk (*) indicates the corresponding author.

Manuscript

Prosper Mhlanga*, Moses Okoth Marenya, Nikita Tawanda Tavengwa, David Tinarwo. **Anaerobic co-digestion of canteen food waste and cow dung at 35 °C and 55 °C.** Submitted to Energy Sources, Part A: Recovery, Utilisation and Environmental Effects Submission ID: 224777588

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Characterization of canteen food waste bio-slurry for bio-fertilizer production

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
AMPTS II	Automatic Methane Potential Test System
ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists
APHA	American Public Health Association
°C	Degrees Celsius
$C_3H_6O_3$	Lactic acid
Ca	Calcium
CBY	Cumulative biogas yield
CD	Cow dung
$CH_3CH_2CH_2COOH$	Butyric acid
CH_3CH_2COOH	Propionic acid
CH_3CH_2OH	Ethanol
CH_3COOH	Acetic acid
CH_3OH	Methanol
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CSIR	Council for Scientific and Industrial Research
CSTR	Continuous stirred-tank reactor
Cu	Copper
CV	Coefficient of variation
DMY	Daily methane yield

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EC	Electrical conductivity
Fe	Iron
FW	Food waste
GC	Gas chromatography
GLM	General linear model
h	Hour
H_2S	Hydrogen sulphide
$HClO_4$	Perchloric acid
HCOOH	Formic acid
HNO_3	Nitric acid
HRT	Hydraulic retention time
IC	Ion chromatography
IR	Infrared
K	Potassium
LSD	Least significance difference
M1	Weight of the dried residue + dish
M2	Weight of the dish
M3	Weight of the wet sample + dish
M4	Weight of the residue + dish after ignition
MC	Moisture content
Mg	Magnesium
M_{in}	Mass of the inoculum
Min	Minutes
mLCH4/gVS	Millilitres of methane per gram of volatile solids

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Mn	Manganese
M_{sub}	Mass of the substrate
MSW	Municipal solid waste
n	Corresponding day during anaerobic digestion
N	Nitrogen
Na	Sodium
NaOH	Sodium hydroxide
NH_3	Ammonia
NO_3	Nitrate
P	Phosphorous
RT	Retention time
S	Sulphur
SD	Standard deviation
SMY	Specific methane yield
SR	Substrate mixing ratio
ST	Substrate type
T°C	Temperature
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids
VS_{in}	Volatile solids content in the inoculum
VS_{sub}	Volatile solids content in the substrate
w/the w	weight for weight (proportion of a particular substance within a mixture measured by weight)

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XRF	X-ray fluorescence
Zn	Zinc

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CHAPTER 1

INTRODUCTION

1.1 Background

Residential, institutional, and industrial sectors generate large quantities of FW annually. Yearly, about 1.3 billion tons of FW are generated worldwide. This amount is equivalent to one–third of food produced for human consumption globally (FAO, 2013). If used in AD, the amount of FW generated globally can generate 370 m^3 of biogas per dry tonne at about 65% methane content (Palaniswamy et al., 2013). According to Dlamini et al. (2019) 59 million tons of municipal solid waste was generated in South Africa in 2011, of which 13% was classified as organic waste. Several studies (Mosisa & Tibba, 2018; Starovoytova, 2018; Ugwu et al., 2020) showed that universities contribute significantly to FW generation. At Rhodes University (South Africa), about 450 tons of FW were generated in 2015 (Paintera et al., 2016). Approximately 3.5 tons of FW were generated every week in 2016 at the Stellenbosch University campus (South Africa) based on the study by Paritosh et al. (2017). The large quantities of FW generated present major waste management concerns and need addressing using environmental methods and techniques.

Conventional methods for FW management are incineration, compositing, and landfilling (Raimi et al., 2020; Karak et al., 2012; Silva et al., 2020). Incineration is energy-intensive due to the high moisture content in FW, which makes it an expensive technology to implement, especially in developing countries (Ahamed et al., 2016). Apart from having a high energy demand, incineration of FW releases dioxins which may cause severe health and environmental issues (Paritosh et al., 2017). Composting is a simple and affordable method for FW management, but it also produces gaseous emissions, which are detrimental to the environment (Cerda et al., 2018). In several developed countries, like Germany, Italy, the United Kingdom, Denmark, Finland, and Sweden (Dawkins & Allan, 2012), landfilling has been banned due to adverse environmental and economic impacts.

Landfilled FW decomposes to form methane, ammonia, and volatile organic compounds. Methane has 25 times more global warming potential than carbon dioxide (Solomon et al., 2007). Decomposed FW also forms leachate, which can contaminate water bodies. With over 90% of

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solid waste being disposed of at landfills, local municipalities run out of landfill space (IWMSA, 2019; Niklasson & Skogfors, 2018). The Council for Scientific and Industrial Research (CSIR) estimated that FW in South Africa accounted for about 2.1% of the country's gross domestic product in 2015, which accounts for energy and resources used in the food supply chain from production to disposal. FW as a substrate for AD can ease environmental and economic problems associated with conventional waste management.

Disposing FW in landfills is a waste of resources it can be used to produce energy and manure using AD. AD is a proven and viable technology for FW management (Xu et al., 2015). AD converts FW into biogas, a green energy source and bio-slurry which is nutrient-rich manure for plants. The AD process has a lower cost and lowers residual waste production than thermochemical methods like incineration (Ahamed et al., 2016). In South Africa, AD is predominantly used to treat wastewater and animal manure, specifically CD (Achinas & Euverink, 2019; Mukumba et al., 2016). Despite its well-known benefits, there is limited application of AD using FW singly or in combination with other biodegradable materials as a substrate to produce biogas and manure, thus providing an environmentally friendly way of handling FW.

Several studies in South Africa have focused on quantifying FW and the challenges associated with its management, such as waste collection and recycling (de Lange & Nahman, 2015; Luke & Jason, 2015; Painter et al., 2016). Limited attention has been paid to FW treatment using AD. While AD is a widely used technology for FW management in developed countries such as Japan, China, USA and Germany, South Africa is yet to adopt AD and realize its benefits (Dlamini et al., 2019). Based on the foregoing background, this study aimed to characterize and evaluate the energy and manure production potential of FW generated from a typical South African university through AD using mono-digestion of FW and CD, and the co-digestion of FW and CD at predetermined ratios.

1.2 Hypotheses

The study tested the following null hypotheses:

1. The quantity and quality of biogas generated from solid FW are the same as that generated by the CD at predetermined and fixed mesophilic and thermophilic temperatures.

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2. The quality of bio-slurry produced from the AD of FW is the same as that generated from CD at predetermined and fixed set mesophilic and thermophilic temperatures.

1.3 Objectives

The specific objectives of the study were to:

1. Characterize FW generated at the University of Venda canteen.
2. Determine the quantity and quality of biogas generated from the AD of pure FW and CD and co-digestion of FW and CD at predetermined mixing ratios.
3. Determine the quality of bio-slurry from mono-digestion of FW and CD.

1.4 Outline of dissertation structure

This dissertation was organised into five chapters.

Chapter 1	Provided a general overview of the justification of the study, research hypothesis, and objectives.
Chapter 2	This chapter reviewed the sources of FW, characterization, and conventional methods for energy recovery. The chapter also discusses the AD process, outlining its advantages and factors that affect the process. Methods employed to determine the BMP are also discussed in this chapter.
Chapter 3	This chapter investigated the effect of temperature and co-digestion of FW and CD at predetermined ratios on biogas yield and quality.
Chapter 4	This chapter investigated the effect of substrate type and temperature on the physicochemical properties of bio-slurry.
Chapter 5	The chapter highlighted the findings of this study and gives recommendations arising from the study.

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CHAPTER 2

LITERATURE REVIEW

2.1 Food waste overview

FW is all food, edible and inedible parts, removed from the food supply chain to be recycled or disposed of, including composting, under-ploughed/unharvested crops, AD, bioenergy production, cogeneration, incineration, disposal in sewers, landfills, or the sea (Bos-Brouwers et al., 2014). Historically, FW has been defined as food losses that occur at the retail and consumption stage. However, FW can occur at any stage of the food supply chain, i.e., production, processing, distribution, storage, sale, preparation, cooking, and serving of food, as shown in Figure 2.1.

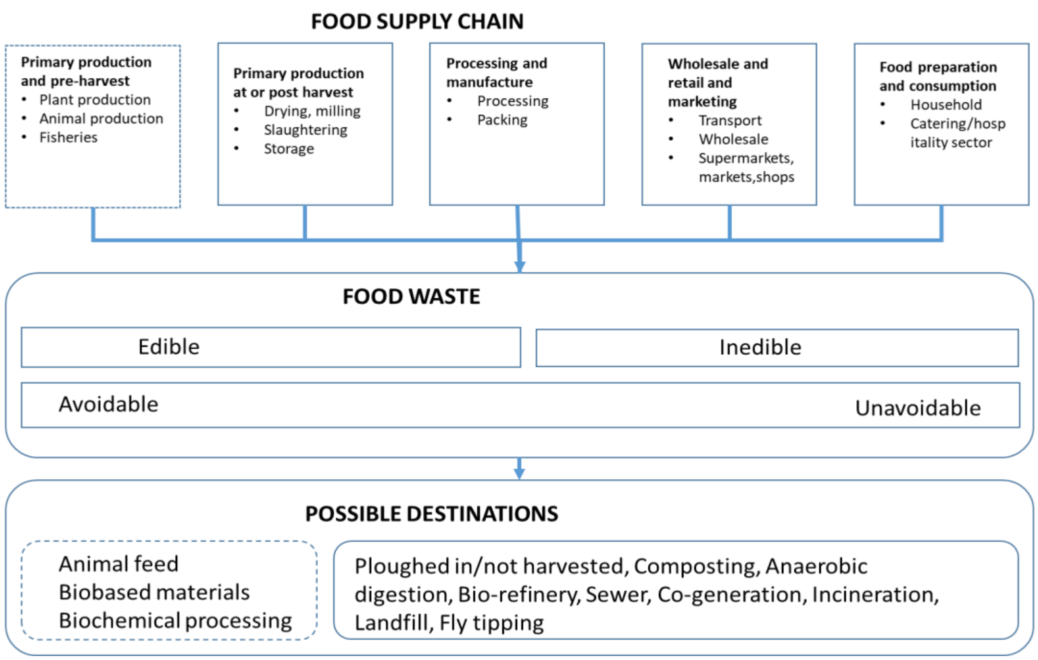

Figure 2.1 Food supply chain and food waste destinations (Banks et al., 2018)

Most FW is generated at the consumption stage in households, cafeterias, food outlets, canteens, and restaurants (Dölekoğlu & Var, 2019). Most of this waste consists of inedible parts, such as bones and peels. The amount and composition of food wasted are influenced by various factors, including eating habits, the standard of living, and changes in seasons (Ayeleru et al., 2016). Despite the difficulty of quantifying food waste streams, available research suggests that 25 − 65% of municipal solid waste is composed of food waste and varies based on origin (Banks et al., 2018).

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Several techniques are used for food waste management. Anaerobic digestion can be used to produce bio-hydrogen or bio-methane for energy production, or to produce specific chemical compounds for plastic, chemical or pharmaceutical applications (Girotto et al., 2015). Food wastes can be recycled for use as animal feed or for composting. Composting is used to recover nutrients and sequester carbon by forming humic substances. Composting generates compost, which can substitute for synthetic fertilizer that requires energy-intensive production and generates emissions (Al-Rumaihi et al., 2020). Landfilling and incineration are the least desirable techniques as they have adverse environmental impacts and risks, such as odours, fires, VOCs, groundwater contamination, and global climate change (Pham et al., 2015). Thermal treatment methods, although providing for energy recovery, is limited by the low heating values of food waste (Scherhaufer et al., 2018). Food waste management vary from country to country, the specific stage of the supply chain and the characteristics of organic waste.

Numerous studies (Bong et al., 2018; Ho & Chu, 2019; Shin et al., 2015; Yadav et al., 2016; Zhang et al., 2007) conducted on food waste characterization were motivated by variability in its characteristics. FW characteristics depend on the source of food waste, handling of FW, climatic conditions, and difference in waste treatment methods used. A review conducted by Fisgativa et al. (2016) on food waste characterization showed high variability between food waste samples. The study estimated that 24% of the difference in chemical characteristics was attributed to the geographical origin of the food waste. Wang et al. (2017) reported that food waste composition depends on the collection source. Characterization of food waste should be source-specific to design energy recovery systems properly. Depending on the waste composition, energy contained in the organic waste can be recovered through either biochemical or thermochemical conversion (Abdalqader & Hamad, 2012). Bio-chemical conversion is the most suitable method for treating organic waste with high moisture content and thermochemical conversion for low moisture content waste (Lhanafi et al., 2018).

2.2 Energy recovery from FW

Food waste is an under-utilized resource with great potential for energy generation and organic fertilizer production. Recovering energy from food waste can be a critical part of a sustainable human society's economic development (Ingrao et al., 2018). The energy in food waste can be

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recovered from food waste via two pathways, viz., thermochemical/thermal and bio-chemical processes, as indicated in Fig 2.2 (Pham et al., 2014).

2.2.1 Thermal and thermo-chemical energy recovery

Incineration, pyrolysis, gasification, hydrothermal carbonization and incineration are among the thermal and thermochemical methods of recovering energy from food waste (Pham et al., 2015). During incineration, food waste is subjected to temperatures of 760°C − 870°C and the energy recovered is used to generate power or in combined heat and power systems (Otitoju & Seng, 2014). Pyrolysis and gasification processes convert food waste into energy through thermal decomposition. The primary products of the processes are bio-oil and syngas (carbon dioxide and hydrogen). In pyrolysis, temperatures of 649°C − 1204°C are employed in the absence of oxygen (Elkhalifa et al., 2019), and gasification requires higher temperatures of 760°C − 1537°C in the presence of air, oxygen, or steam under a pressurized system (1 − 30 bars) (Grycová et al., 2016). Hydrothermal carbonization involves the conversion of food waste into hydrochar, which is rich in energy under autogenous pressures and low temperatures of 180°C − 350°C (Tradler et al., 2018). Hydrochar has similar fuel characteristics to lignite, and it can be used in co-combustion systems. Energy-intensive thermal and thermochemical processes are not widely used in food waste treatment and energy recovery, particularly in developing countries, due to food waste's high moisture content and low thermal value (Rafey et al., 2020).

2.2.2 Biological/Biochemical energy recovery

Anaerobic digestion and fermentation utilize biological and biochemical reactions to recover energy from FW. FW is converted into biogas and bio-slurry in anaerobic digestion under anaerobic conditions facilitated by microbial action. Anaerobic digestion is the most viable technology for FW treatment (Karthikeyan et al., 2018). AD process represents a way of recycling nutrients, recovering energy, production of manure, and minimizing waste disposed at landfills. In the fermentation process, organic molecules in FW such as glucose are converted into alcohols (ethanol, a biofuel), acids, or gases by the action of micro-organisms in the absence of oxygen or any electron transport chain. Cafeteria FW, kitchen waste, fats from slaughterhouses, and non-edible waste oils have been utilized to produce biofuels (Prasoulas et al., 2020). Biodiesel is obtained from the transesterification of animal and vegetable oils. Considering the negative

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environmental impact, high energy demand, and cost of thermal/thermochemical processes, anaerobic digestion is preferred for recovering energy and nutrients from FW (Xu et al., 2018).

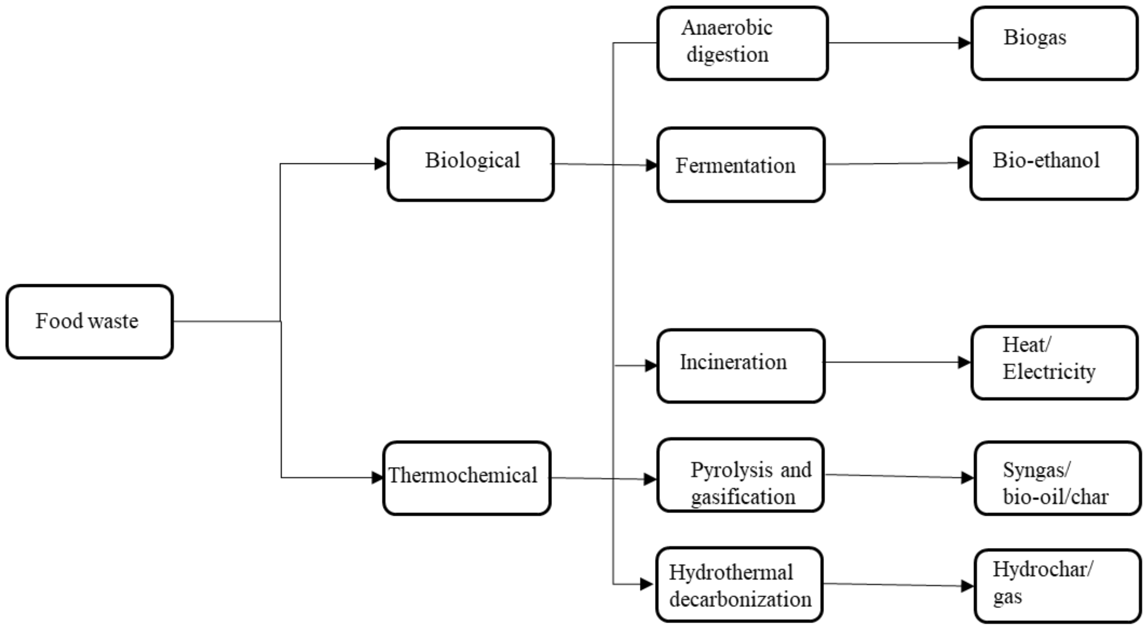

Figure 2.2 Summary of food waste-energy technologies (Pham et al., 2015)

2.3 Biochemistry of AD

AD is the process that involves the breakdown of organic matter by microorganisms in the absence of oxygen (Kuo & Dow, 2017). The by-products of AD are biogas, which is mainly methane (CH_4), carbon dioxide (CO_2), and the effluent called bio-slurry. AD occurs in four primary stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, as shown in Figure 2.3 (Adekunle & Okolie, 2015). FW provides an inexpensive substrate for AD and significantly improves biogas production relative to systems that convert manure or sewage sludge alone (Zhang et al., 2013).

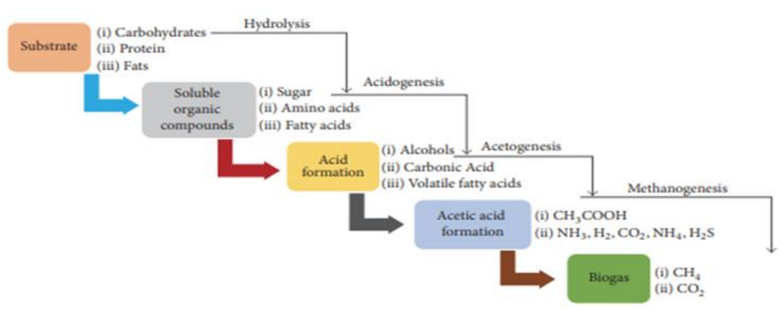

Figure 2.3 AD phases (Paritosh et al., 2017)

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During the hydrolysis stage, anaerobic bacteria convert organic matter (proteins, fats, and carbohydrates) into much simpler liquefied monomers and polymers (peptides, fatty acids, and saccharides) (Mata-Alvarez, 2005). The reaction is catalysed by enzymes called hydrolyses produced by the fermentative bacteria. Equation 1 shows how the reaction happens.

$$(C_6H_{10}O_5)n + nH_2O \rightarrow n(C_6H_{12}O_6) + nH_2 \qquad (2.1)$$

Acidogenic bacteria transform the end products of the first reaction into short-chain volatile fatty acids (VFAs), ketones, alcohols, hydrogen, and carbon dioxide. The main products of this stage are propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), acetic acid (CH_3COOH), and formic acid (HCOOH), lactic acid ($C_3H_6O_3$), ethanol (CH_3CH_2OH), and methanol (CH_3OH) (Fedailaine et al., 2015). The acidogenic process is summarized by Equation 2. Other by-products of the reaction are ammonia, carbon dioxide, hydrogen sulphide, and aldehydes.

$$left - right(C_6H_{12}0_6) \leftrightarrow 3n(CH_3COOH) \quad (2.2)$$

The end products of the acidogenesis process, VFAs (propionic acid, butyric acid) and alcohols, are converted into hydrogen, carbon dioxide, and acetic acid by acetogenic bacteria (Paritosh et al., 2017). In this process, the concentration of hydrogen is an essential factor as this reaction will only occur if the hydrogen partial pressure is low enough to hydrogen-scavenging. Hydrogen scavenging bacteria carry out this process. Equation 3 shows the acetogenic process.

$$CH_3COOH \rightarrow CH_4 + C0_2 \quad (2.3)$$

The hydrogen and acetic acid formed by acid formers will be converted into methane and carbon dioxide in the final stage. The hydrogenotrophic methanogens produce CH_4 using CO_2 as a carbon source and hydrogen as a reducing agent (Kondusamy & Kalamdhad, 2014).

$$O_2 + 4H_2 \rightarrow CH_4 + 2H_2O \ (2.4)$$

Types of feedstock

Biomass is used as the primary feedstock for biogas production (Kalac, 2011). The sources of biomass can be classed as primary and secondary. Primary biomass sources are plants grown for energy production such as sugar cane, sunflower and maize. Primary biomass resources undergo physical, chemical, or biological processing to produce secondary biomass resources(Sánchez et

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al., 2019). Biomass can be classified according to biomass properties such as TS, VS, C/N ratio, and BMP. Depending on the overall number of total solids in the feedstock, AD can also be categorized as either wet or dry digestion. In wet AD processes, the feedstock is slurred with the addition of water, and the total solid concentration is typically below 15% while in dry AD processes, the total solid concentration is higher (TS 20 – 40%) (Angelonidi & Smith, 2015). The most typical biomass used for AD includes agricultural residues, municipal solid waste and sewage sludge.

Agricultural waste is a widely used feedstock for AD. Animal waste and crop residues are the major constituents of agriculture waste. Animal waste is easily accessible and has high nitrogen content which is required for the anaerobe’s growth. Livestock and poultry waste, wastewater, feedlot runoff silage juices, bedding and feed all constitute animal waste. Factors such as type of species, breed, development stage, and amount and type of bedding and storage of the animals affect the methane potential of animal dung (Möller et al., 2004). The high ammonia concentration in animal waste has the potential of hindering the AD process (Rajagopal et al., 2013). Crop residues can be co-digested with animal waste to enhance methane yield. Numerous crops yield large amounts of wasted, stalks, straws and barks that could be used to produce biogas. Typically, crop residues have high lignocellulosic content. The biogas output of crop residues is higher than that of animal waste due to the high C/N ratio and high lignin content (Dar et al., 2021).

AD is widely utilized in the treatment of municipal solid waste (MSW) (Getahun et al., 2014). MSW contains a large fraction (over 50%) of biodegradable material (Demirbas, 2006). The large quantities of MSW being generated present the challenges which include the emission of greenhouse gases, contamination of water bodies by landfill leachate and limited municipal land availability (Zhu et al., 2010). The organic fraction of MSW is highly bio-degradable which makes it suitable for AD (Ghosh et al., 2020). The composition of MSW is affected by seasonal changes, geographical location, lifestyle and cultural variations and recycling habits (Kigozi et al., 2014). Pre-sorting of MSW before AD is required to remove inorganic materials. However, segregation and sorting of waste can increase the overall cost of AD of MSW. AD of MSW is associated with ammonia inhibition caused by the breakdown of protein-containing substances which can cause process instability. Challenges associated with AD of MSW can be resolved by co-digesting it

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with other feedstock such as rice straw and animal waste to optimize the C/N ratio (Ghosh et al., 2020).

The food processing industry generates a large amount of waste, which is typically rich in organic matter. Food processing waste includes dairy wastewater, slaughterhouse waste, and fruit juice manufacturing waste. AD of FW has fewer environmental impacts compared to incineration and landfilling (Mirmohamadsadeghi et al.,2019). AD of food processing waste can be hindered by the presence of inhibitors such as ammonia and VFAs. Ammonia and VFAS accumulate as a result of the breakdown of proteins and lipids (Chen et al., 2008). Fruits and vegetable waste can easily break down in an anaerobic digester since they typically have high VS and low TS. The quick hydrolysis of fruit and vegetable waste can potentially lead to acidification (Ji et al., 2017). As a result, the production of methane is inhibited and the efficiency of AD is affected (Ward et al., 2008). Inhibition of AD can be reduced by co-digestion, adjustment of the concentration of anaerobic inoculants, pre-treatment, of feedstock and active control of the reactor conditions which raise the C/N ratio (Ji et al., 2017; Liu et al., 2012).

Sludge is a by-product of the physical, chemical, and biological processes employed in municipal wastewater treatment plants (Di Capua et al., 2020). AD is regarded as a cost-effective and environmentally friendly method for processing large volumes of sludge. Sludge stabilization, improved sludge dewaterability, and the capacity to reduce and inactivate harmful microorganisms are other advantageous characteristics (Appels et al., 2008). The sludge composition has a significant impact on the amount of methane produced during AD. The main drawbacks of AD of sludge include low reaction rates due to the slow hydrolysis of bacterial aggregates, process, vulnerability, and accumulation of process inhibitors such, as ammonia, hydrogen sulphide and volatile silicon compounds (Di Capua et al., 2020). Challenges associated with AD of sludge can be eliminated by the use of feedstock pre-treatment methods (Liao et al., 2016).

2.3 AD products

2.3.1 Biogas

Biogas is composed of methane (50 trace carbon dioxide (2traces5%) and trace elements such as water vapour, particulate matter, and contaminants such as volatile organic compounds, sulphur compounds, siloxanes, and ammonia, as shown in Table 2.1 (Deepanraj et al., 2015; Vintilă et al.,

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2012). The composition of biogas is affected by substrate composition and process operating conditions. The presence of several trace compounds in raw biogas produced from AD may have adverse effects on beneficial uses (Amha et al., 2018).

Table 2.1 Composition of biogas (Vintilă et al., 2012)

Parameter	Content (%)
Methane	50 – 75
Carbon dioxide	25 – 45
Water vapour	2 – 7
Oxygen	<2
Nitrogen	<2
Ammonia	<1
Hydrogen	<1
Hydrogen sulphide	<1

Most of the carbon dioxide in an anaerobic digester is produced during the breakdown of large polymers (hydrolysis). CO_2 lowers the heating value of biogas and causes corrosion. A method such as water scrubbing can be utilized to get rid of CO_2 from the biogas. Removal of these trace elements is often done through biogas conditioning. Biogas conditioning removes hydrogen sulphide, siloxanes, water vapour, particulate matter, ammonia, and carbon dioxide (Chen et al., 2008). Hydrogen sulphide is produced in high concentrations when substrates such as silage, microalgae, and slaughterhouse wastes are used as feedstock. The substrates contain high sulphur and nitrogen content. Hydrogen sulphide is not desirable for the AD process because it inhibits the rate of methanogenesis. Hydrogen sulphide is removed from biogas by a process called biological desulphurization. Removal of these elements is crucial, especially when used in combined heat and power systems where these elements can cause corrosion (Al Seadi et al., 2008).

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2.3.2 Bio-slurry

Bio-slurry obtained from the biogas plant may be considered a source of manure as it contains considerable amounts of both macro and micro-nutrients (Bonten et al., 2014). The quality of bio-slurry as a fertilizer is dependent on the concentration of nitrogen, phosphorus, potassium, calcium, magnesium, and the ratio between nutrients in the bio-slurry (nitrogen/phosphorus or nitrogen/potassium) (Mukhuba et al., 2018). According to Burton et al. (2009), the typical bio-slurry composition is cellulose, lignin, biomass sludge, and inorganic components. The nutrient composition of bio-slurry as a fertilizer depends on the substrate and the hydraulic retention time (HRT). Bio-slurry can be used as it is produced or processed into marketable bio-fertilizers with improved quality and transportability (Al Seadi et al., 2013).

2.4 Factors that affect the biogas production process

AD of FW is affected by various factors. The process primarily depends upon the composition of the substrate, temperature, pH, carbon-nitrogen ratio (C/N), organic loading rate (OLR), inhibitory factors, HRT, and nutrient concentration. It is crucial to maintain optimum conditions to properly break down the organic compounds (Fisgativa et al., 2016).

Fisgativa et al. (2016) stated that biogas production is affected by the physicochemical properties of the substrate. Physicochemical characteristics of FW are variable due to many factors which include differences in sources, processing and handling methods, eating habits, culture, climate, and seasons (Meng et al., 2015). Despite the variation in the physicochemical properties, FW is rich in carbohydrates, proteins, lipids, and minerals, which are easily degradable and make it an ideal substrate for biogas production. FW composition also affects bio-digester operational parameters such as temperature and retention time. AD can be enhanced by optimizing the physicochemical properties of FW through co-digestion, pre-treatment or, the addition of nutrients (Carlsson et al., 2012).

Saraswat et al. (2019) stated that temperature plays a critical role in methane production. AD microbes operate under different temperature ranges which are psychrophilic, mesophilic, and thermophilic. Psychrophiles operate within 10 – 20°C with an optimum at 25°C. Mesophiles operate at temperatures within 20 – 45°C with an optimum at 35°C. Thermophiles operate at temperatures within 50 – 65°C with an optimum of 55 °C (Dobre et al., 2014; Kothari et al., 2014;

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Náthia-Neves et al., 2018). The relative growth rate of methanogens under the different temperature conditions is shown in Figure 2. Under the psychrophilic temperature range, microbial activity is slower than in the mesophilic temperature range (Lettinga et al., 2001). The thermophilic temperature range favours faster biochemical reactions, lower retention times, higher death rates, of pathogens and increased solubility compared to psychrophilic and mesophilic temperature ranges (Kim et al., 2006; Tian et al., 2018). However, the major drawbacks of thermophilic temperatures are the higher energy requirement and process instability (Vanegas & Bartlett, 2013). According to Tian et al. (2018), thermophiles are more sensitive to temperature changes of ±10°C while mesophiles can endure temperature changes of ±30°C. The AD process is more stable and less susceptible to environmental fluctuations when operating within the mesophilic temperature range (Mao et al., 2015). The activity of mesophiles is inhibited more by ammonia accumulation than that of thermophiles (Wang et al., 2016). A study by Zamanzadeh et al. (2016) showed that the thermophilic temperature range favoured the formation of VFAs, which decreased biogas production and stability. Mesophilic temperature conditions are more commonly used for AD than thermophilic temperature conditions because of higher process stability and lower energy cost (Kothari et al., 2014; Tufaner & Avsar, 2016). Temperature is a significant factor to consider for the anaerobic microbial culture, as the conversion of acetic acid to methane is predominantly influenced by temperature changes in the environment (Önen et al., 2019).

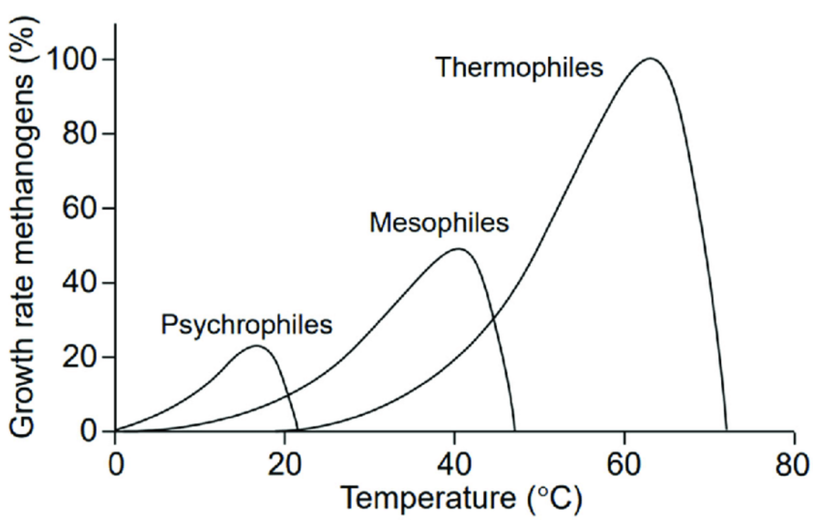

Figure 2.4 Growth rate of methanogenic bacteria with temperature (Lettinga et al., 2001)

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The C/N ratio of the substrate affects biogas production. Carbon is used as an energy source for micro-organism activity and nitrogen is required for microbial growth. Rocamora et al. (2020) reported that an optimal C/N ratio of $20 - 30$ is required for a stable AD process. A low C/N ratio leads to the formation of process inhibitors such as VFAs and ammonia, limiting biogas production and leading to process failure (Tanimu et al., 2014). Co-digestion is used to correct the C/N ratio. Agricultural waste is co-digested with FW to optimize the C/N ratio. Animal manure is an excellent co-substrate because of its high buffer capacity and a wide variety of nutrients which prevents the formation of process inhibitors (Mata-Alvareza et al., 2014). A study by Wang et al. (2014) showed that an increase in C/N ratios reduced the negative effects of ammonia and maximum methane potentials of the substrates were achieved with C/N ratios of 25 and 30 at 35°C and 55°C, respectively. Beniche et al. (2021) obtained optimum biodegradability and methane yield during the co-digestion of FW and agricultural wastes at $C/N = 45$. The optimum C/N is dependent upon substrate physicochemical properties.

Methanogenic bacteria thrive best under neutral to slightly alkaline conditions and are an inhibited at pH lower than 6 (Mao et al., 2015). Latif et al. (2017) reported that the optimum AD process occurs in the pH range between 7 and 8.5. Acidic conditions are toxic to methanogenic bacteria. Most FWs have acidic pH values, which will consume the digester alkalinity and negatively impact the biogas production process (Cerón-Vivas et al., 2019). A temperature shift promotes the build-up of VFAs, which lowers the pH.

HRT depends on the substrate type, mixing rate, and agitation (Gaby et al., 2017). An increase in retention time leads to higher VS reduction, higher required digester volume, and higher acclimation to pH variations and toxic compounds. A study conducted by Lui et al. (2018) showed that a higher methane yield was produced from a fixed HRT than from a varying HRT. Shi et al. (2017) study showed that higher methane yields from the AD of wheat straw were produced at higher HRT. The average retention time of organic substrates in a bio-digester operating under mesophilic conditions (20°C − 45°C) is between 15 and 30 days (Mirmohamadsadeghi et al., 2019). HRT is affected by temperature (Al Seadi et al., 2008), as indicated in Table 2.2.

Table 2.2 Thermal stage and minimum retention time (Al Seadi et al., 2008)

Thermal stage	Temperature (°C)	Minimum retention time (days)

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Psychrophilic	< 20	70 − 80
Mesophilic	20 – 40	30 − 40
Thermophilic	50 – 65	15 − 20

AD is significantly affected by the OLR. OLR affects the microbial community involved in the AD process thus biogas production. A study by Lui et al. (2017) revealed that FW is a desirable substrate with a high-bearing OLR under thermophilic conditions. Maintaining an optimum loading rate prevents the accumulation of excess VFAs (Aslanzadeh et al., 2014). The OLR has a linear relationship with biogas production up to a certain point while beyond that point, process inhibition due to VFAs accumulation is expected. High OLRs could cause the failure of the AD process. According to Nagao et al. (2012), stable digestion of FW is attained when the OLR is between 0.5 kg and 2 kg of total VS/m^3/d. Babaei & Shayegan (2011) reported that VS degradation and biogas yield decreased with OLR.

Despite being required in small amounts, nutrients play a pivotal role in biogas production. Macro-nutrients such as carbon, oxygen, sulphur, nitrogen, phosphorus, and calcium, and trace elements such as iron, manganese, zinc, and molybdenum are required for microbial growth (Ahmed et al., 2015). An adequate amount of nutrients is necessary for a stable AD process. According to Zayed & Winter (2000), a high concentration of trace metals has an inhibitory effect on the growth of microorganisms. Carbon and nitrogen are required for energy and the building of microbes' cell wall structure (Chiu & Lo, 2016). Small quantities of micro-nutrients such as calcium, sodium, potassium, magnesium, and chlorine are required for the microbe's activity (Paritosh et al., 2017).

Materials in FW such as heavy metals, antibiotics, VFAs, and detergents may have an inhibitory effect on biogas production. Ammonia is an essential substance in AD since it is necessary for bacterial growth. However, high concentration levels might lead to the inhibition of methane Shahbaz et al. (2020) reported that the AD process can be negatively affected by the production and build-up of VFAs, which can result in inhibition and impair the production of biogas, causing a delay in the process. A pH reduction is what causes VFAs to suppress the function of methanogens, which could make acid-sensitive enzymes inactive. Heavy metal concentrations may limit methanogenic activity, and the extent of the inhibition is dependent on a variety of

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variables, including the total metal concentration, the chemical forms of the metals, pH, and redox potential (Mudhoo & Kumar, 2013).

Mono-digestion of FW is associated with process instability which results from insufficient trace elements which regulate enzyme reactions (Mehariya et al., 2018). Challenges associated with mono-digestion are eliminated by co-digestion, which is feeding two or more substrates in the biogas reactor (Jiang et al., 2018). Xu et al. (2017) study showed that co-digestion of FW with other substrates helps maintain process stability by balancing the C/N ratio and providing trace minerals. The standard method is to mix FW with substrates such as animal manure and sewage sludge (Chiu and Lo 2016).

FW pre-treatment methods are employed to enhance the biogas production process (Deepanraj et al., 2017). Pre-treatment facilitates the digestion process by improving the substrate's surface properties for better microbial interactions, reducing the toxic compounds, and improving the hydrolysis rate kinetics for proteins and lipids (Arelli et al., 2020). Pre-treatment methods aim to improve hydrolysis and facilitate solubilisation (Menon et al., 2016). Pre-treatment also increases the accessibility of hardly accessible compounds and increases substrate solubility. Pre-treatment can be classified into various categories, such as mechanical, chemical, thermal, and biological pre-treatments (Kondusamy & Kalamdhad, 2014).

The types of AD reactors include batch-type, semi-continuous, single-stage, and multi-stage. A batch reactor is fed with a fixed amount of substrate for a certain HRT before it is emptied. A semi-continuous reactor is fed with feedstock on an interval basis and digested slurry is removed in stages. In a one-stage AD reactor, all the AD process stages occur in one reactor. Since they do not share the same ideal environmental conditions, multi-stage reactors separate the hydrolysis/acidification operations from the acetogenesis/methanogenesis processes (Janesch et al., 2021). A multi-stage system has improved process stability relative to one-stage systems (Kim et al., 2008). Multi-stage AD systems are also capable of treating an increased load of substrates and higher methane yields compared to single-stage AD systems (Chatterjee & Mazumder, 2019). However, multi-stage digesters are more expensive to build and maintain compared to single-stage systems. AD reactors can be further classified as wet or dry types. Wet AD reactors utilize substrates with a TS content of 16% or less, while dry AD reactors utilize substrates with over 22% TS content (De Gioannis et al., 2008). The dry reactor technology is widely used for AD of

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MSW due to the high TS content. Most animal wastes have TS content below 16% and require less pre-treatment which is typically a reduction of particle size. In a study conducted by Angelonidi & Smith (2015), wet AD plants produced higher biogas yields per tonne of waste treated compared to dry AD plants. However, dry AD plants offered advantages which include greater flexibility over the type of substrate accepted, shorter retention times and reduced water use (Forster-Carneiro et al., 2008).

The basic requirements of AD reactors include a continuous organic load rate, a short HRT, and the capacity to produce the maximum volume of methane. Reactor shape must take into consideration mixing and heat loss. Digested material from an actively running digester is used to seed a new reactor, reducing the start-up time (Budiyono et al., 2009). Microbial populations available in rumen fluid have been used as seeding material in AD, often to increase the production of fatty acids from lignocellulosic feedstocks (Aragaw et al., 2013).

2.5 Biomethane chemical potential

BMP is defined as the maximum volume of methane produced per gram of volatile substrate (Esposito, 2012). It indicates the biodegradability of a substance and its methane production potential. BMP assays are widely used to determine organic substances' methane potential and biodegradability. The methods use the same principle but differ in experimental setups and experimental conditions (Chiang & Maria, 2011). Filer et al. (2019) and Koch et al. (2015) reported that there is no accepted standard procedure for determining the BMP of organic substances. Methods of BMP tests are either experimental or theoretical. The experimental methods vary in setups and operating conditions because of the difference in the physicochemical properties of substrates (Rodriguez-Chiang, 2011). Experimental methods used to determine the BMP of substrates include conventional BMP tests, Automatic BMP test, and spectroscopy.

2.5.1 Experimental methods of determining BMP

BMP tests are often configured manually. However, the manual experimental setup takes time and effort, and there is a chance that daily gas measurements will be inaccurate. Pham et al. (2013) stated that the most widely used methods for determining BMP include the German standard procedure VDI 4630, the Möller method, and the Hansen method. The methods are similar, but

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they vary in operational parameters. Temperature is a crucial aspect to consider when evaluating the methane potential of substrates, as the anaerobic degradation of organic substrates is temperature-dependent (Chen et al., 2008). BMP tests typically involve conducting experiments at either mesophilic (35 ± 2 °C) or thermophilic (55 ± 2 °C) temperatures, with the help of equipment such as a water bath, incubator, or thermally-controlled chamber to maintain the desired temperature.

The VDI method utilizes thermophilic (55°C) or mesophilic conditions (37°C), and a buffer solution is used (Holliger et al., 2016). The Möller method is conducted under mesophilic conditions (37°C), and no buffer solution is added. The Hansen method is conducted under thermophilic conditions (55°C) (Jingura & Kamusoko, 2017). Conventional BMP tests involve mixing substrate and inoculum under specified operating conditions, and the biogas/methane produced is measured by manometric or volumetric techniques (Esposito, 2012). Amodeo et al. (2020) compared manometric, gravimetric, and automated volumetric BMP tests and concluded that they were slight differences in the biomethane yield values. Gas chromatography and gas analysers are used to determine the composition of biogas or by using a carbon dioxide scrubbing solution such as sodium hydroxide (NaOH). Conventional setups for the BMP test were more time- and labour-intensive compared with the automated apparatus.

In BMP tests, the amount of methane produced is typically measured using either manometric or volumetric techniques, which involve liquid displacement methods and pressure transducers or manometers. Other tools, such as glass syringes and Tedlar bags, can also be used. The liquid displacement method is commonly used due to its low cost, durability, and ease of setup and maintenance. However, the selection of the barrier solution for absorbing carbon dioxide can be a drawback. Typically, an alkaline solution is used, and regardless of the measurement method, the results must be corrected to standard temperature and pressure conditions to facilitate comparison between different BMP tests.

Several factors, including inoculum source, substrate concentration, inoculum-to-substrate ratio (ISR), temperature, reactor size, and pH can impact BMP test results. The inoculum source and its adaptation to the test substrate are crucial factors in BMP assessment. Common sources of inoculum include sludge from anaerobic digesters used in wastewater treatment, animal manure, and the organic fraction of municipal solid waste or food waste (Holliger et al., 2016). Holliger et

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al. (2016) recommended inoculum from an active and stable anaerobic digester. The inoculum may contain nutrients required for AD which limits the need for additional nutrient supplements. Nutrients promote microbial growth and activity, which enhances biogas production (Romero-Güiza et al., 2016). Koch et al. (2017) study showed that the specific methane production rate of the substrates was influenced by the choice of the inoculum, especially for sewage sludge, F, W and cellulose. The VDI 4630 (2016) protocol recommends inoculum to be pre-incubated for 2 to 5 days at the same temperature as the active digester prior BMP test, to reduce background biogas production from the inoculum. The endogenous methane production from blank assays is subtracted to obtain the methane contribution of the substrate (Meng et al., 2015).

The pH, substrate concentration, and ISR are critical in the experimental determination of BMP. The optimal pH range for AD is 6.8 to 7.5 (Ward et al., 2008). The pH must be maintained within the range to ensure that the BMP test comes to completion. During AD, intermediate products such as VFAs are formed, which can potentially lead to a decrease in the pH of the system and have an impact on the activity of methanogens (Jiang et al., 2018). A high concentration of substrate in anaerobic digestion can hinder proper mixing and cause the build-up of VFAs, resulting in a decrease in pH and inhibiting the production of biogas (Alzate et al., 2012). While the digestion of FW may be susceptible to VFA accumulation due to its composition and high biodegradability, other substrates may have buffering capacities, which reduces the potential of VFA accumulation (Kafle et al., 2014). As a result, the optimal ISR ratio may vary depending on the type of substrates under investigation. An overloading of the system brought on by a low ISR may result in the accumulation of VFAs, inhibition, and reduced methane yields (Angelidaki & Sanders, 2004). For highly degradable substrates like FW, a minimum ISR of 2 is advised; however, for less degradable substrates like lignocellulosic waste, an ISR of 1 may be employed (Holliger et al., 2016).

According to Holliger et al. (2016), large reactors (500 to 2000 mL) are better suited for heterogeneous substrates while small reactor sizes (100 mL) are good for homogeneous materials. Meng et al. (2018) used 500 mL serum bottles and 20 L reactors under the same experimental circumstances to study the impact of reactor size on the methane potential of pig urine and rice straw. The study demonstrated a 27% increase in methane yield for the same substrate digested under the same experimental circumstances in 20 mL compared to a 500 mL bottle. A straightforward daily manual mixing is sufficient to achieve maximal interaction between

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substrates and the microorganisms due to the very small reactor size utilized for the BMP testing (Holliger et al., 2016). Due to the impact of headspace pressure and composition, headspace volume impacts both manometric and volumetric gas measurement setups (Himanshu et al., 2017). Magnetic stirring, manual shaking, or orbital shaking at rotations per minute can all be used to mix substances in BMP tests. Due to the uneven distribution of substrates, ineffective mixing may result in poor biogas production, whereas high-intensity mixing disrupts syntrophic interactions of the microbial consortia and subsequently encourages VFA accumulation, which lowers biogas production (Amani et al., 2010).

The spectroscopy method is an emerging technique used to determine the BMP of substrates (Grewan, 2020). The technique determines the ultraviolet, visible, and infrared radiation's absorption, transmission, diffusion, or fluorescence. Atomic spectroscopy, which measures compounds in the gaseous phase following volatilization, and molecular spectroscopy, which measures substances directly in liquids, are examples of spectroscopic techniques (Hülsemann et al., 2020). The Fourier transform mid-infrared spectroscopy and near-infrared spectroscopy are typical examples of infrared (IR) spectroscopic devices (Jingura & Kamusoko, 2017). For determining the BMP of solid waste, IR has shown to be a quick and accurate method (Lesteur et al., 2011).

2.5.2 Automatic test

The Automatic Methane Potential Test System II is a widely used procedure that provides automated measurement of ultra-low biogas flow using tipping buckets (Kleinheinz & Hernandez, 2016). The device supports 15 bottles with a volume of 500 mL. The methane generated is measured directly using a liquid displacement and buoyancy method. Carbon dioxide and trace elements are removed before measuring methane yield. The AMPTS II has a carbon dioxide absorption unit that uses a 3 M NaOH solution. The AMPTS II is the preferred method since it takes less time and provides higher qualitative and quantitative data than conventional BMP tests (Shi et al., 2017). Despite the precision offered by automated systems, manual BMP tests are still widely used due to their cost-effectiveness and ability to produce comparable results. A study conducted by (Wang et al., 2014) showed that the methane yields of cellulose obtained from conventional and automatic experimental setups were comparable. However, the methane yield obtained from the automated apparatus showed greater precision.

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2.5.3 Theoretical test

Theoretically, BMP can be estimated from the elemental chemical composition and its chemical oxygen demand (COD) (Nielfa et al., 2014). The methods assume complete degradation of the substance and that the micro-organism's utilization of the substrate as energy is negligible. Buswell's formula (Equation 2.5) is used to calculate the theoretical methane potential using known values of its elements carbon (C), hydrogen (H), oxygen (O), sulphur (S), and nitrogen (N).

$$C_aH_bO_cD_d + \left(\frac{4a-b-2c+3d}{4}\right)H_2O \rightarrow \left(\frac{4a-b-2c+3d}{4}\right) CH_4 + \left(\frac{4a-b+2c+3d}{8}\right)CO_2 + dNH_3 \quad 2.5$$

The component composition method involves the use of carbohydrates, fat, and protein to calculate theoretical methane potential. The general formula 0.42, 0.52 and 1.01 Nm^3 CH_4/kg VS can be produced from carbohydrates ($C_6H_{10}O_5$), proteins ($C_5H_7O_2N$) and lipids ($C_{57}H_{104}O_6$) (Forgács, 2012). COD gives information about the amount of organic matter in a substrate. The information can be used to estimate methane yield by using the fact that one mole of methane requires two moles of oxygen for complete oxidation. Each gram thus corresponds to 4 grams of COD (Wang et al., 2010).

2.6 Discussion of the reviewed literature

Large quantities of FW are being generated in the food supply chain, from the production stage to the consumption stage. A more significant percentage of the FW is generated at the consumption stage from canteens, restaurants, and food outlets. The generated FW is disposed of at landfills, where it decomposes to produce methane and leachate, which are detrimental to the environment. The disposal of FW also has financial implications that involve transportation costs and costs involved in food production. The land resource for landfills is also slowly running out due to population growth and urbanization. The challenges associated with FW disposal at landfills can be mitigated using AD.

AD can be used to recover energy and nutrients contained in organic matter. The organic matter decomposes to produce biogas, a renewable energy source, and bio-slurry, rich in readily available nutrients. Though widespread in developed countries, AD utilization in FW treatment is still limited due to economic, technical, and social factors. While numerous studies at South African universities show that large quantities of FW are being generated, there is a lack of literature on

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its potential for energy and manure generation. Data on the energy potential of FW is essential for planning, designing, and sizing AD facilities. This study sought to characterize and determine the energy and manure production potential of the solid FW generated by the University of Venda canteen.

2.7 Conclusion based on the reviewed literature

The literature reveals that a large proportion of FW generated at academic institutions is disposed of at landfills where it poses serious threats to the environment and human and animal life. FW disposal at landfills represents a loss of a valuable energy resource and resources used in food production. AD has the potential to mitigate environmental and economic implications associated with landfilling. Though it is a widespread and proven affordable technology for FW treatment and valorisation, its use in South Africa is still limited due to the lack of adequate data on FW physicochemical traits, energy, and manure production potential. BMP and composition of FW form the baseline for the planning, design, and sizing of biogas plants. The study sought to contribute to the body of knowledge on canteen FW composition, its energy and manure generation potential.

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CHAPTER THREE

EFFECT OF TEMPERATURE AND CO-DIGESTION OF FW AND CD AT PREDETERMINED RATIOS ON BIOGAS YIELD AND QUALITY

Abstract

The generation of large quantities of FW is receiving increasing attention worldwide because of concerns associated with its disposals, such as greenhouse gases emission and groundwater pollution. While AD of FW is a proven technology for waste management, its use has not yet been sufficiently explored. This study investigated the influence of mixing ratios of FW and CD and temperature on biogas yield, methane content, and biomethane potential (BMP) of the substrates. FW and CD mixed on a wet mass basis at ratios 0:100, 25:75, 50:50, 75:25 and 100:0 (w/w) were batch incubated at 35 ± 1 °C and 55 ± 1 °C, using a 2 x 5 full factorial experimental design. Biogas yield and methane content were monitored daily. Experimental results were subjected to ANOVA, and the means were separated using Fisher's LSD test at a 5% level of significance. The obtained results showed that the temperature and substrate mixing ratios significantly ($p < 0.05$) affected biogas yield, methane content, and BMP. The highest biogas yield and BMP were 4903.33 ± 38.84 mL and 301.879 ± 2.07 mLCH_4/g VS at 35°C, and 7151.67 ± 11.55 mL and 401.88 ± 1.98 mLCH_4/g VS at 55 °C. The lowest biogas and BMP yields were 5301.67 ± 62.51 and 328.278 ± 4.265 mLCH_4/g VS at 55 °C, and 3291.67 ± 81.45 mL and 328.28 ± 4.26 mLCH_4/g VS, respectively, for mono-digestion of FW at 55 °C and 35 °C. Overall, co-digestion of FW and CD produced higher biogas yield and BMP than mono-digestion of the substrates at both test temperatures. These findings can be used to size heated and unheated biogas plants using co-digested FW and CD substrates.

Keywords: AD; biochemical methane potential, co-digestion, FW, waste management

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3.1 Introduction

Global economic development and population growth have contributed to an alarming increase in the amount of FW produced, mainly in institutional (school canteens), industrial (food processing factories), and residential and commercial sectors (restaurants) (Morone et al., 2019). Melikoglu et al. (2013) reported that globally, FW production will increase by 44% between 2013 and 2025. A study by De Lange & Nahman (2015) estimated that the total amount of FW generated in South Africa was 12.6 x 10^6 tonnes per year, of which 90% is disposed at landfills. As a result of the large volumes of FW being generated, municipal services are under increasing pressure to use alternative disposal methods (Sebola et al., 2014). FW decomposes to form methane which contributes to global warming and leachate contaminates groundwater (Costa et al., 2019). Converting FW into energy and bio-fertilizer through AD has great potential to reduce the negative impacts of FW disposal in landfills (Capson-Tojo et al., 2016).

AD is a proven technology for treating organic waste such as sewage sludge, animal manure, and agro-processing waste (Liu & Lv, 2016). AD of FW is increasingly considered viable for recovering energy and nutrients from FW due to its higher organic matter compared to sewage and animal manure (Franqueto et al., 2020). However, mono-digestion of FW is associated with digester instability due to the formation of VFAs and ammonia, which inhibits the activity of methanogens (Chiu & Lo, 2016). Co-digestion of FW with other substrates is considered an effective method to overcome challenges associated with mono-digestion of FW. Morales-Polo et al. (2018) reported that co-digestion of FW with other substrates increases alkalinity, and process stability, reduces the formation of process inhibitors such VFAs, and increases methane yield. Co-digestion of FW with various substrates such as animal manure, sewage sludge, rice husks and maize husks have been used to optimize the C/N ratio and stabilize AD (Jabeen et al., 2015; Mata-Alvarez et al., 2014).

Factors that affect the AD process include reactor design, operational conditions, and substrate characteristics (Cioabla et al., 2012). Operational parameters such as temperature, OLR, retention time and mixing must be monitored and maintained within optimum ranges for maximum production of biogas (Lohani & Havukainen, 2018). Among the several operational conditions ration sure is the most important parameter since it influences the activity rate, process stability, and microbial activity (Gaby et al., 2017). Substrate characteristics such as VS content, C/N, C O

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,,D and substances such as ammonia, hydrogen sulphide and heavy metals either enhance or inhibit the AD process (Jingura & Kamusoko, 2017). Temperature and substrate characteristics are critical parameters that significantly influence AD performance.

AD microbes operate under different temperature ranges which are psychrophilic (10 – 20°C), mesophilic (20 – 45°C), and thermophilic (50 – 65°C) (Náthia-Neves et al., 2018). Thermophilic temperatures favour faster biochemical reactions, lower retention times, higher death rates of pathogens, and increased solubility compared to psychrophilic and mesophilic temperatures (Kim et al., 2006). However, the major drawbacks of thermophilic temperatures are the higher energy requirement and process instability (Vanegas & Bartlett, 2013). The mesophilic temperature range provides higher stability of the process and lower sensitivity to environmental changes (Mao et al., 2015). Mesophilic temperatures are more commonly used for AD than thermophilic temperatures because of higher process stability and lower energy cost (Tufaner & Avsar, 2016).

Several studies (Ashekuzzaman & Poulsen, 2010; Morales-Polo et al., 2018; Qin et al., 2016) have established that substrate properties affect biogas production and microbial activity. Nwokolo et al. (2020) reported that the substrate properties influence biogas yield, methane content, biodegradability, and degradation kinetics. Substrate characteristics can be optimized by pre-treatment methods such as mechanical, thermal, chemical, and biological pre-treatments (Kondusamy & Kalamdhad, 2014). FW is increasingly being considered a potential substrate for AD because of its high biodegradability and ever-increasing volumes being generated (Zhang et al., 2014). However, insufficient knowledge about the source-specific physicochemical properties and energy potential of FW limits its use as a substrate for AD in South Africa. The objective of this study was to characterize FW from a typical South African university food canteen and evaluate the effect of mixing FW and CD on biogas yield, methane content, and BMP at 35°C and 55°C.

3.2 Materials and Methods

3.2.1 Sample collection and preparation

FW was collected from the University of Venda canteen after meals for five consecutive days. The FW was manually sorted to remove inorganic materials and mixed on a clean surface. To improve homogeneity, the FW was mashed and mixed using a kitchen blender. CD was collected from the

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University of Venda's School of Agriculture Experimental Farm. The CD was mixed with water and inorganic materials such as pebbles were removed. The prepared FW and CD samples were stored at 4 °C and -20 °C for shorter and longer periods, respectively. Compositional changes due to water loss and changes in VS content are minimal at 4 °C for shorter storage periods (less than 7 days) and at -20 °C for longer periods (greater than one month) (Holliger et al., 2016).

Anaerobic sludge was used as inoculum to provide the initial microbial population for the AD experiment. The anaerobic sludge was collected from a laboratory AD set up of CD incubated under test temperatures. Holliger et al. (2016) recommended the use of anaerobic sludge from biogas plants using animal manure as the main substrate due to the diversity of anaerobic microbes. The anaerobic sludge was degassed till insignificant biogas was produced. Anaerobic sludge was stored at ambient temperature for a shorter period of storage and 4 °C for a longer period of storage before digestion.

3.2 Experimental design and data analysis

A 2 x 5 factorial completely randomized experimental design was used to assess the influence of substrate mixing ratios and temperature on the yield of biogas, methane content, and quality of bio-slurry. The substrate mixing ratio had five levels which are co-digestion mixtures of food waste and cow dung (FW/CD) at ratios 100:0, 75:25, 50:50, 25:7,5 and 0:100 (w/w) and two predetermined temperature levels set 35 ℃ and 55 ℃. The volume of biogas produced, and methane content was monitored and measured during the experiment. Figure 3.1 shows a schematic diagram of the experimental design showing the factors, their levels, and the replications.

Experimental results were subjected to statistical analysis using the Minitab 19 (Minitab, 2020) statistical software using the generalized linear model (GLM). ANOVA for a 2 x 5 factorial experiment at a 5% level of significance was used to assess the effects of substrate mixing ratios and temperature on the biogas yield, methane content, and BMP. Where a significant ANOVA result was obtained, the mean separation was done using the Fisher's LSD test at a 5% level of significance.

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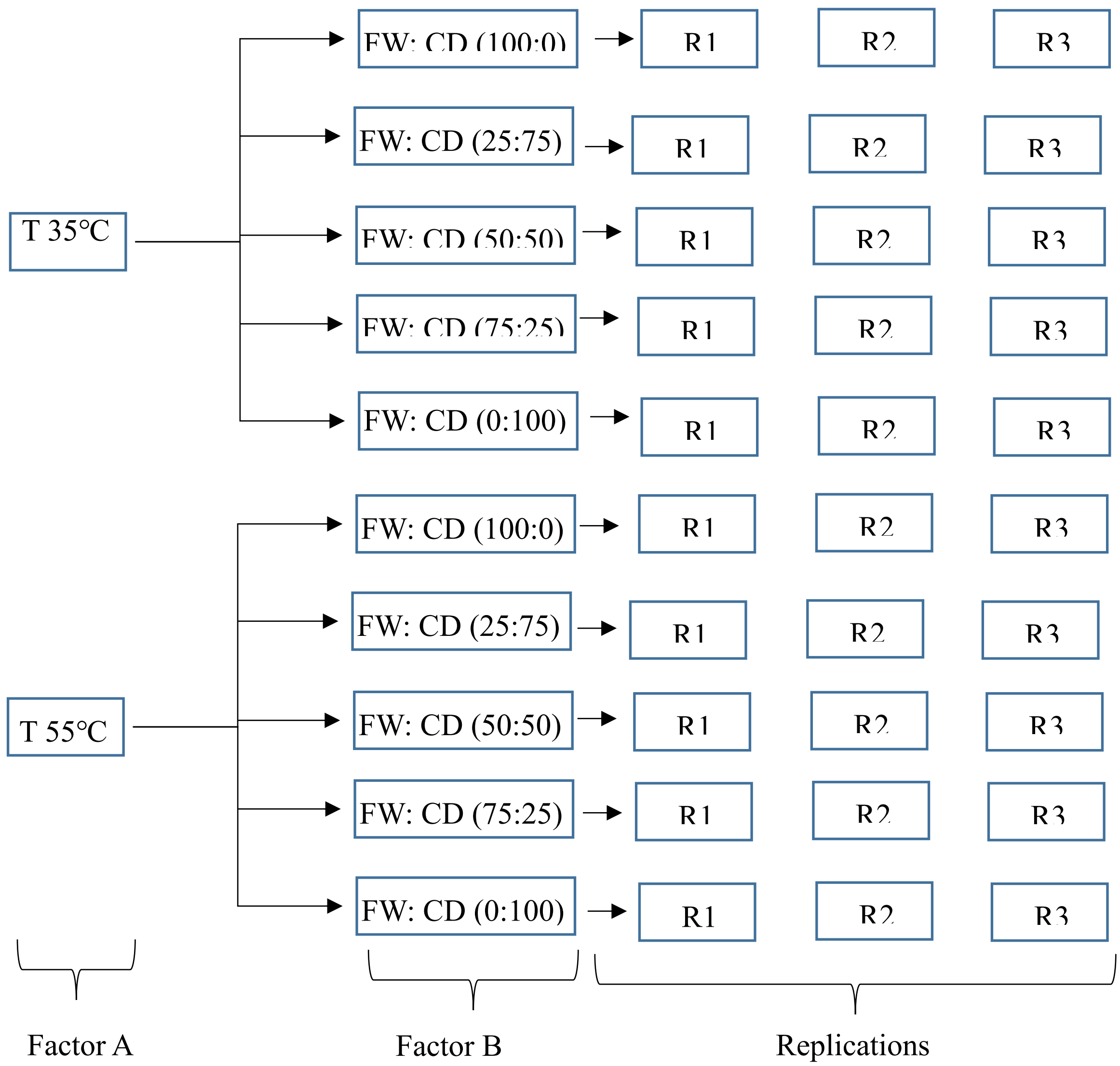

Figure 3.1 Schematic presentation of 2 factorial experimental design (Factor A = Temperature at two levels of 35 °C and 55 °C, and Factor B = five substrate mixing ratio (w/w)

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3.3 Experimental procedure

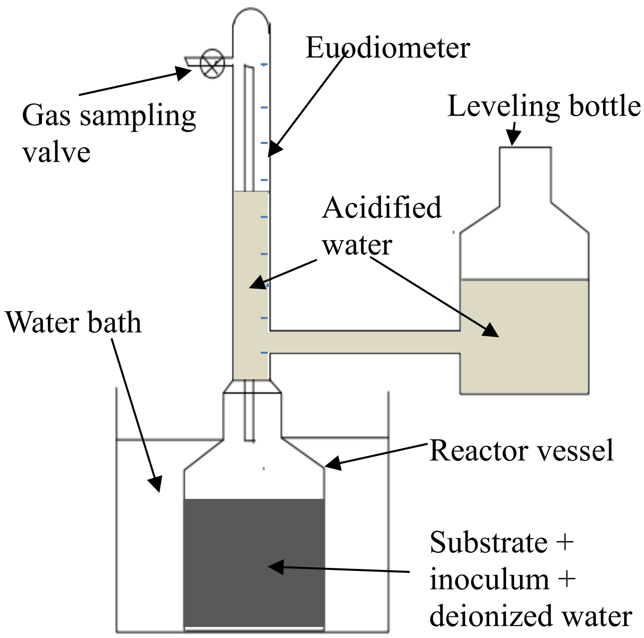

Figure 3.2 Schematic diagram for the experimental set up

The AD batch experiments were conducted using 500 mL volume Duran glass vessels (reactor) coupled to the 500 mL eudiometer tube. The reactors were filled with the substrate sample and inoculum at an inoculum/substrate ratio = 2 on VS basis as recommended by VDI 4630 protocol (VDI 4630, 2016). The amount of substrate was calculated according to Equation 1. Deionized water was added to achieve 70% of the total volume of the reactor vessel. The headspace of the reactor/eudiometer setup was flashed with nitrogen gas for 3 min after the addition of inoculum and substrate to create anaerobic conditions. A blank essay of inoculum mixed with deionized water was subjected to AD to determine its background methane. The biogas produced from the blank assay was subtracted from the biogas produced from the substrate assay at every sampling time. A positive control assay of CD mixed with inoculum at inoculum/substrate ratio (ISR) 2 was maintained at both test temperatures which are 35 °C and 5 °C using water bath.

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$$M_{sub} = \frac{M_{in}.VS_{in}}{2.VS_{sub}} \tag{3.1}$$

where: M_{in} = mass (g) of the inoculum,

M_{sub} = mass (g) of the substrate,

VS_{in} = the volatile solids content (%) in the inoculum

VS_{sub} = volatile solids content (%) in the substrate.

The reactors were shaken by hand twice a day to enhance homogeneity and prevent the formation of intermediate substances such as VFAs. The volume of biogas generated was measured every 24 h by measuring liquid displaced in a eudiometer tube. The experiment was terminated when the volume of biogas produced during three consecutive days was less than 0.5% of the accumulated volume of biogas as recommended by the (VDI 4630. (2016). A portable biogas analyser (GeoTech Biogas5000, UK) was used to determine the amount of methane and carbon dioxide in the biogas stream (Appendix 1).

3.4 Determination of the quantity and quality of biogas

The volume of biogas produced was recorded every 24 h during the experiment period by reading the displacement of the level of the barrier solution in the eudiometer tube. To minimize the dissolution of carbon dioxide, acidified water was used as a barrier solution. The volume of biogas produced was measured at atmospheric pressure according to the method prescribed in the ISO standards (ISO/DIS 14853, 1999). A portable biogas analyser was used to measure methane content. The biogas analyser was connected to the gas sampling port of the eudiometer by an airtight tube. An airtight tube was fitted with a filter to prevent water vapour from reaching the biogas analyser. The cumulative biogas yield was calculated using Equation 3.2. Methane yield and specific methane yield were calculated using Equations 3.3 and 3.4 respectively (Xu et al., 2021).

$$\text{CBY (mL)} = \sum_{n=1}^{n} \text{DBYn} \tag{3.2}$$

$$\text{DMY (mL)} = \text{DBY (mL)} \times \text{CH4 (\%)} \tag{3.3}$$

$$\text{SMY} \left(\frac{\text{mL}}{\text{g VS}}\right) = \frac{\sum \text{DMY (mL)}}{\text{g VS of substrate added}} \tag{3.4}$$

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where: CBY = Cumulative biogas yield in milliliters (mL),

DMY= daily methane yield in milliliters (mL),

SMY = specific methane yield milliliters per gram of volatile solids (mL/g VS),

n = corresponding day during AD.

3.5 Determination of moisture content, TS and VS

The TS and VS were determined using standard methods (APHA, 2005). A mass of 10g of each of the samples was heated in an oven at 105 °C for 24 h. The dry weight of the samples was measured till it showed constant weight. Moisture content (MC) and TS were calculated using Equations 3.5 and 3.6, respectively (APHA, 2005)

Approximately 2g of the dried samples were placed and burnt in a muffle furnace at a temperature of 550 °C for 240 min. After the furnace was turned off, the samples were left in the furnace to cool for 120 min. The samples were further cooled in a desiccator for an additional 120 min. The final weights of the samples were measured using a precision balance. The VS content was calculated using Equations 3.7.

$$\mathrm{MC} = \frac{(\mathrm{M3} - \mathrm{M1})\mathrm{X}100}{\mathrm{M3} - \mathrm{M2}} \qquad (3.5)$$

$$\%\mathrm{TS} = \frac{(\mathrm{M1} - \mathrm{M2})\mathrm{X}100}{\mathrm{M3} - \mathrm{M2}} \qquad (3.6)$$

$$\%\mathrm{VS} = \frac{(\mathrm{M1} - \mathrm{M4})\mathrm{X}100}{\mathrm{M1} - \mathrm{M2}} \qquad (3.7)$$

where: M1 = mass (g) of the dried sample + crucible

M2 = mass (g) of the crucible

M3 = mass (g) of the wet sample + crucible and

M4 = mass (g) of the residue + crucible after ignition

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3.6 Determination of the elemental composition of the samples

The total carbon, hydrogen, and total nitrogen composition of the samples were determined using a CHNS-O elemental analyser (Elementar, Vario EL cube, Germany).

3.7 RESULTS AND DISCUSSION

3.7.1 Physicochemical characteristics of the samples

Table 3.1 shows the physicochemical characteristics of the substrates used in this study. FW: CD (100:0) had the highest MC (80.51%) and FW: CD (25:75) had the least MC (71.99%). MC increased with the increase of FW proportion in the co-digestion mixture. According to James et al. (2006), 68 – 80% substrate MC is required for optimum biogas production. Substrates used in this study had sufficient MC favourable for AD. FW: CD (25:75) had the highest TS% and FW: CD (100:0) had the least TS%. FW: CD (100:0) had the highest VS/TS% (97.60%), indicating the high biodegradability of FW, and cow manure had the lowest VS/TS% (77.31%). Zhai et al. (2015) reported similar characteristics for kitchen waste and cow manure respectively. The VS/TS% of the co-digestion mixtures increased with the increase of FW proportion. The VS/TS% of all substrates used in this study are favourable for biogas production. Several studies reported that pH significantly affects the AD process. Mei & Peng (2016) reported that pH influences the growth of AD microbes and affects the dissociation of compounds such as organic acid, which facilitates the AD process. Lee et al. (2020) reported that methanogens perform well in a pH range of 6.7 − 7.4. Similarly, Weiland (2010) reported that biogas production is severely inhibited when the pH falls below 6.0 or rises above 8.5. In this study, the substrates' pH values were in the range of 6.3 − 7.93 which is suitable for the AD process.

The C/N ratio is a critical factor in the AD process. FW: CD (75:25) had the highest C/N ratio of (21.634/1) and FW: CD (25:75) had the lowest C/N ratio of (17.598/1). The C/N of FW: CD (50:50) had a lower C/N ratio than FW: CD (75:25) because the lower proportion of CD has less nitrogen content. Low C/N ratios can result in the accumulation of total ammonia nitrogen and VFAs which inhibit biogas production. High C/N ratio results in the rapid consumption of nitrogen by methanogens which results in low biogas production (Yan et al., 2015). In this study, the maximum biogas yield was obtained from FW: CN (75:25) which had a C/N ratio of 921.634/1). Dai et al. (2016) obtained maximum biogas yield at a C/N of (17/1) for anaerobic co-digestion of

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waste-activated sludge and ryegrass. Romano and Zhang (2008) recommended that the C/N ratio be maintained at 15/1 for the co-digestion of onion juice and digested sludge. However, Li et al. (2011) recommended an operating C/N ratio range of 20/1 to 30/1 with an optimal ratio of 25/1 for anaerobic bacterial growth in an AD system. The optimal C/N ratio varies with the type of feedstock to be digested.

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Table 3.1 Physicochemical characteristics of substrates used in this study.

Parameter	Anaerobic sludge (inoculum)	FW: CD 0:100	FW: CD 25:75	FW: CD 50:50	FW: CD 75:25	FW: CD 100:0
MC (%)	95.40 ± 0.001	76.07 ± 0.002	71.99 ± 0.02	76.67 ± 0.02	77.31 ± 0.01	80.51 ± 0.01
TS (%)	4.60 ± 0.01	23.93 ± 0.02	28.00 ± 0.02	23.33 ± 0.02	22.70 ± 0.01	19.49 ± 0.01
VS/TS (%)	71.23 ± 0.02	77.31 ± 0.01	80.32 ± 0.04	87.79 ± 0.01	90.38 ± 0.02	97.60 ± 004
pH	7.44 ± 0.02	6.94 ± 0.01	7.7 ± 0.02	6.77 ± 0.03	7.93 ± 0.02	6.3 ± 0.02
C (%)	-	44,19	42,35	39,42	42,62	34,542
H (%)	-	6,645	6,088	5,571	6,448	4,690
N (%)	-	2,175	2,251	2,24	1,97	1.73
C/N	-	20,317	18,813	17,598	21,634	19,967

MC = Methane content, TS = Total solids, VS = Volatile Solids, FW: CD = Food waste/Cow dung mixing ratio (w/w). Values in the table are means of triplicates plus standard deviation.

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3.7.2 Effect of temperature and co-digestion on biogas yield, biogas methane content, and BMP

The total biogas yield, biogas methane content, and BMP obtained in this study are presented in Table 3.2. Temperature, mixing ratios and the interaction between temperature and mixing ratios significantly affected ($p < 0.05$) the total biogas yield. The study results show that significantly higher biogas yields ($p < 0.05$) were produced at 55 °C than at 35 °C. Further, for all FW: CD mixing ratios, the HRT for 55 °C was 35 days while for 35 °C was 40 days. The results are consistent with the findings of Yamashiro et al. (2013) who found that thermophilic temperature conditions (55 °C) produced higher biogas yield than mesophilic temperature conditions (37 °C) when dairy cow manure and highly concentrated food processing waste were co-digested. As reported by Weiland (2010) and Bouallagui et al. (2004), the growth rate of methanogens is higher at thermophilic temperatures than at mesophilic temperatures which accelerate the AD process. The present study indicates that 55 °C is the optimum temperature for anaerobic co-digestion of FW and CD.

FW: CD mixing ratio affected the total biogas yield, and the highest total biogas yield was obtained for the mixing ratio of FW: CD of 75:25 for both 55 °C (7151.67 ± 11.55 mL) and 35 °C (4903.33 ± 38.84 mL) as shown in Table 3.2, accepted as the optimum FW: CD ratio for co-digestion of FW and CD. The result is consistent with the findings of Aragaw et al. (2013) who also obtained maximum biogas yield from co-digestion of 75% kitchen waste and 25% CD. Total biogas yields for mono-digestion of FW and CD were 5301.67 ± 62.51 mL and 5901.67± 75.22 mL, and 3786.67 ± 128.49 mL and 3291.67 ± 81.44 mL, for 55 °C and 35 °C, respectively. The results show that co-digestion mixtures had higher total biogas yields than mono-substrates at both 55 °C and 35 °C. A study by Pax et al. (2020) showed that thermophilic co-digestion of sewage sludge/bio-waste mixtures enhanced biogas yields by 45 to 50% over mesophilic co-digestion. In this study, increasing the FW portion in the co-digestion mixture increased the total biogas yield up to the mixing ratio of FW: CD of 75:25 and thereafter decreased the digestion of CD only for both test temperatures (Table 3.2). The increase in biogas yield can be attributed to an enhanced C/N ratio by co-digestion (El-Mashad & Zhang, 2010). The results indicated that biogas yield can be enhanced by co-digestion.

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Temperature, mixing ratios, and the interaction between temperature and mixing ratios significantly affected ($p < 0.05$) the average biogas methane content. The average methane content range at 35 °C was 57.82 – 64.76% which is higher relative to the 53.09 – 59.02% average methane content at 55 °C (Table 3.2). A similar methane content range (55 − 65%) was also reported by Malik et al. (2020) for co-digestion of FW and CD. The higher methane content at 35 °C may be attributed to the stability of the AD system at mesophilic temperatures (Vanegas & Bartlett, 2013). It was observed that the average biogas methane content of co-digestion mixtures was higher than that of mono-substrates. At 35 °C, the highest average biogas methane (64.76%) contained was obtained from FW: CD, (75:25) and the lowest (57.82%) was from mono-digestion of CD. This agrees with the findings of Oladejo et al. (2020) who obtained the highest methane content (64.6%) from anaerobic co-digestion of FW and pig dung and the lowest (54.0%) from mono-digestion of FW-only. This can be attributed to an increase in the buffer capacity of the AD process by mixing FW and CD. The results show that average biogas methane content is affected by temperature and co-digestion.

BMP is used to evaluate the methane potential and biodegradability of organic matter (Jingura & Kamusoko, 2017). Temperature, mixing ratios, and the interaction between temperature and mixing ratios significantly affected ($p < 0.05$) the BMP of the substrates. The highest BMP (401.89 ± 1.98 mLCH4/g VS) was obtained from FW: CD mixing ratio of 75:25 (w/w) at 55 °C and the lowest BMP (211.56 ± 4.30 mLCH4/g VS) was obtained from mono-digestion of food waste at 35 °C. BMP values of all co-digestion mixtures were higher than for pure CD and FW. Superior BMP values were obtained at 55 °C relative to 35 °C. The results obtained in this study are consistent with the findings of Rattanapan et al. (2019) on anaerobic co-digestion of FW and domestic wastewater who reported higher BMP values at 55 °C relative to 3tives5 °C. BMP increased with an increasing proportion of FW in the co-digestion mixture, with maximum BMP obtained for the FD: CD (75:25) mixing ratio (Table 2). The BMP of 0:100 FW: CD ratio at 35 °C and 55 °C was higher than that of 100:0 FW: CD ratios, which were 211.56 mLCH4/g VS at 35 °C and 328 mLCH4/g VS at 55 °C, respectively, at 236.69 mLCH4/g VS and 354.16 mLCH4/g VS. The results obtained in this study for the BMP of mono-digested CD are comparable to those of Pax et al. (2020) whose BMP of 302, 228, and 241 L/kg VS for the AD of fine and coarse and unscreened CD respectively. The results of this study indicate that the BMP of the substrates can be enhanced by co-digestion.

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Table 3.2 Effect of temperature and FW/CD mixing ratio on biogas yield, methane content, and BMP

SR – FW: CD (w/w)	T (°C)	RT (days)	Total biogas yield (mL)	Average biogas methane content (%)	BMP ($mLCH_4$/g VS)
0:100	55	35	5301.67^{e} ± 62.51	55.52^{g} ± 0.21	328.278^{c} ± 4.265
25:75	55	35	6865.00^{b} ± 137.48	53.09^{h} ± 0.36	324.238^{c} ± 7.005
50:50	55	35	6256.33^{c} ± 63.11	59.02^{e} ± 0.03	360.638^{b} ± 3.831
75:25	55	35	7151.67^{a} ± 11.55	57.64^{f} ± 0.36	401.887^{a} ± 1.981
100:0 (control)	55	35	5901.67^{d} ± 75.22	58.68^{e} ± 0.07	354.156^{b} ± 3.333
0:100	35	40	3786.67^{h} ± 128.49	57.82^{f} ± 0.81	211.563^{g} ± 4.303
25:75	35	40	4308.33^{g} ± 74.89	63.14^{e} ± 0.07	248.066^{e} ± 2.935
50:50	35	40	4816.67^{f} ± 53.92	62.46^{c} ± 0.46	293.736^{d} ± 5.422
75:25	35	40	4903.33^{f} ± 38.84	64.76^{a} ± 0.38	301.879^{d} ± 2.07
100:0 (control)	35	40	3291.67^{i} ± 81.44	60.92^{d} ± 0.28	236.686^{f} ± 10.207
Treatment and interactions				P-value	
SR			0.000	0.000	0.000
T°C			0.000	0.000	0.000
SR x T°C			0.000	0.000	0.000

FW: CD = Food waste/cow dung mixing ratio (w/w), T°C = test temperature, SR = Substrate mixing ratio, FW: CD (0:100 (w/w)) = control treatment, BMP = biochemical methane potential. The values presented in the table are mean of triplicates plus the standard deviation. Column means with the same superscripts are not significantly different according to Fisher's LSD test ($p > 0.05$).

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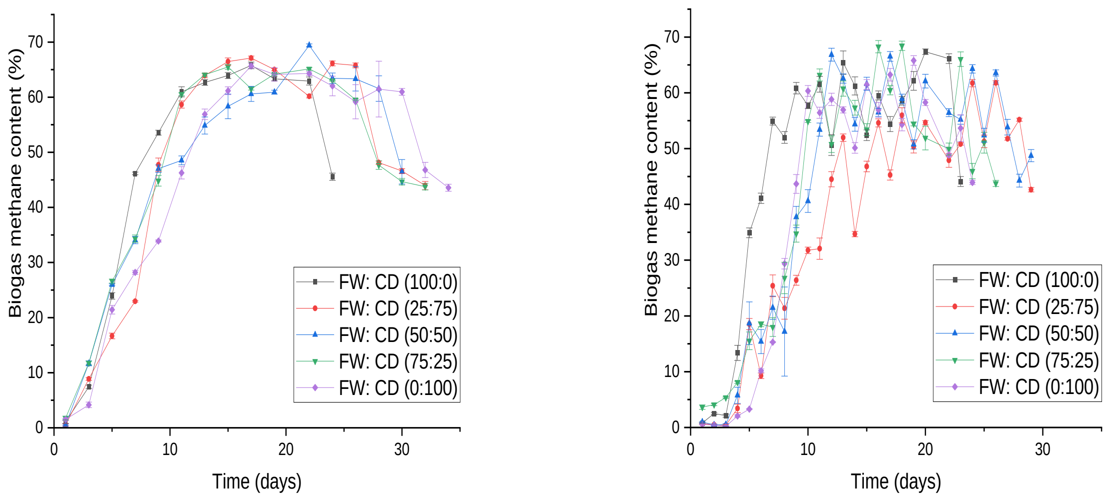
Figure 3.3 shows the daily variation of biogas methane content under experimental conditions used in this study. Figure 3.3a shows that methane content increased steadily from day 1 to about day 15 and then fluctuated between 45− 65%. At 55 °C, the methane content increased steadily from day 1 to day 10 and thereafter fluctuated between 40 − 65%. At 55 °C, a lag phase in methane content was observed from day 1 – day 3 (Figure 3.3b). The delay in the increase of methane content may be due to the slow adaptation of the microbes (Koch et al., 2015).



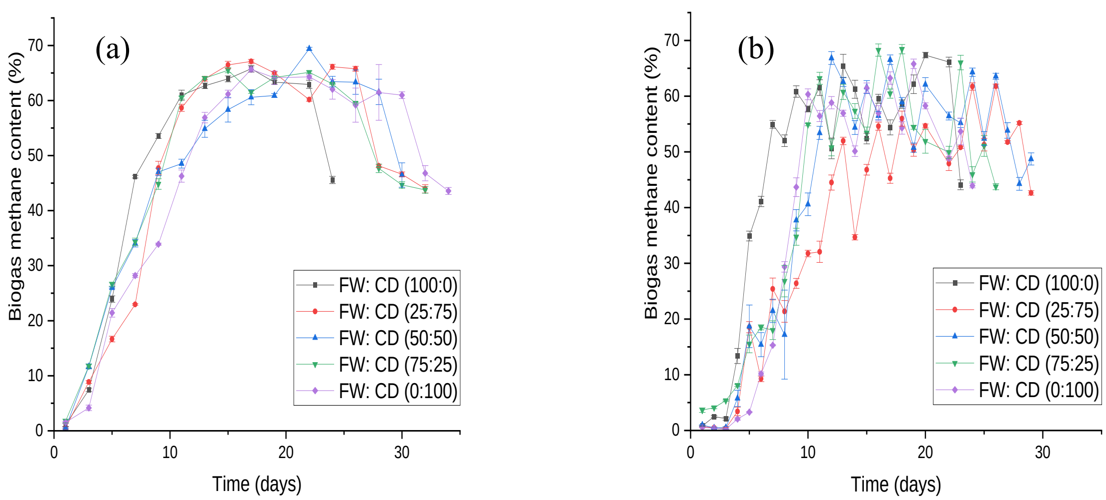


Figure 3.3 Daily variations of biogas methane content (a) at 35°C and (b) at 55°C

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Presented in Figure 3.4 are the daily variations of the biogas yield and biogas accumulation for the experimental conditions used in this study. At 35 °C, biogas yield increased steadily from day 1 to day 25 and gradually level off (Figure 3.3b) while at 55 °C, biogas production increased steadily from day 1 to day 20 and levelled off (Figure 3.2d). Figure 3.2a shows that FW: CD ratios of 100:0, 50:50 and 75:25 reached peak biogas yields on day 1. FW: CD (25:75) and FW: CD (100:0) reached peaks on days 15 and 19 respectively. The delay in reaching peak production may be due to the presence of cellulose in CD which breaks down slowly (Koch et al., 2015).

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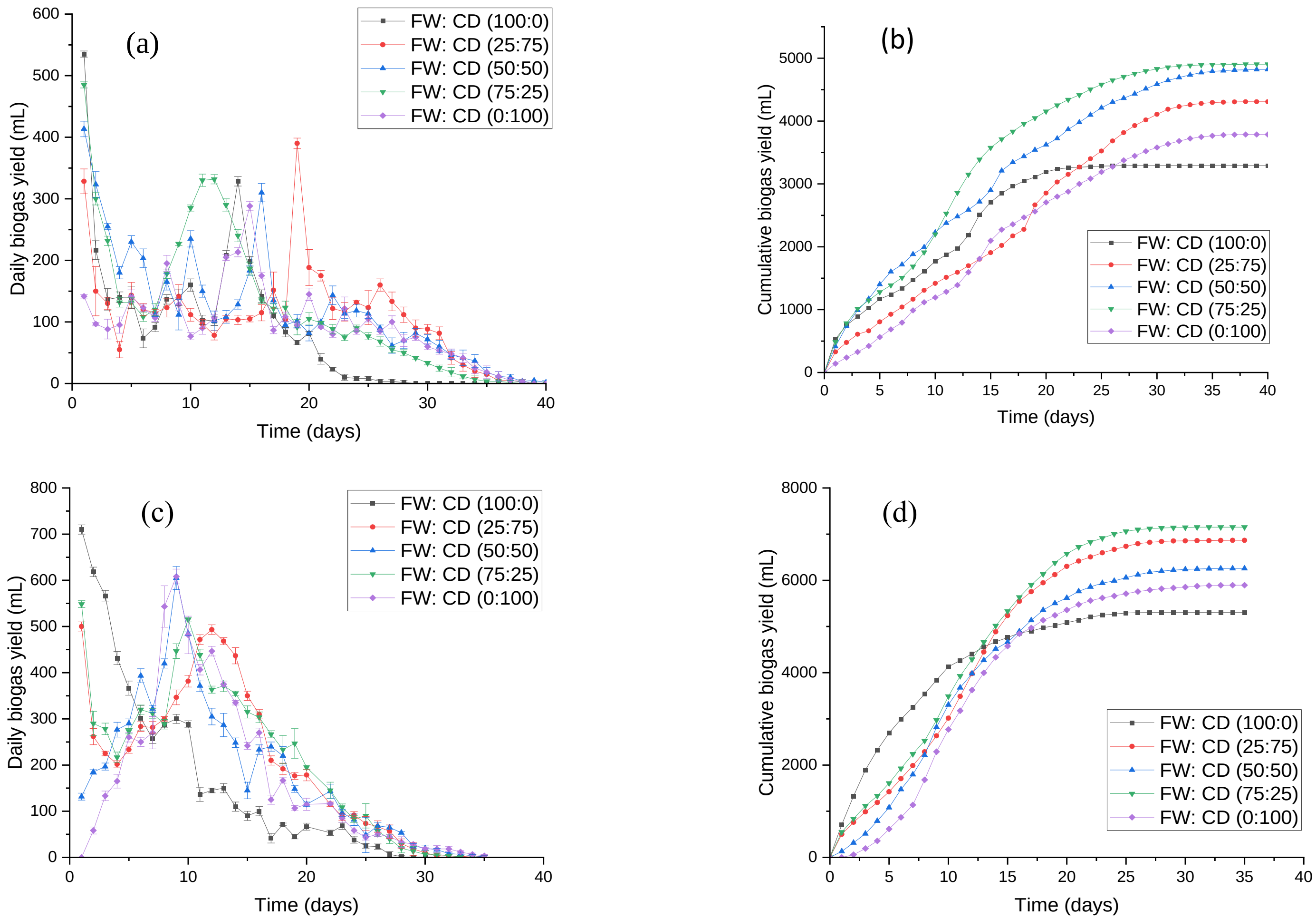

Figure 3.4 Daily variations of biogas yield (a) and accumulation (b) at 35 °C and daily variations of biogas yield (c) and accumulation (d) at 55 °C

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3.8 Conclusion

The results showed that temperature and co-digestion had a significant effect on total biogas yield, biogas methane content, and BMP ($p<0.05$). The biogas yield and BMP of the substrates were higher at 55℃ than at 35℃. Total biogas yield and BMP increased with the increase of FW proportion in the co-digestion mixture. The highest biogas yield and BMP were obtained when FW and CD were co-digested at a mixing ratio of 75:25 (w/w) at 55℃. All co-digestion mixtures of FW and CD produced greater levels of total biogas yield, methane content and BMP than the mono-digestion of the substrates.

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CHAPTER FOUR

EFFECT OF SUBSTRATE TYPE AND TEMPERATURE ON PHYSICOCHEMICAL CHARACTERISTICS OF BIO-SLURRY

Abstract

Population growth has led to the extensive use of chemical fertilizers to meet the rising food demand. The overreliance on chemical fertilizers is detrimental to the environment, animals, and humans. This has led to increased attention to an alternative environmentally friendly source of nutrients for plants. Anaerobic digestion (AD) is a proven method that can be used to recover nutrients from organic matter. In this study, bio-slurries from the AD of FW and CD subjected to 35℃ and 55℃ were evaluated as potential bio-fertilizers by characterizing their nutrient composition. A 2 x 2 factorial design experiment was used to determine the influence of substrate type and temperature on the nutrient composition of bio-slurry. Nitrogen concentration was determined using ion chromatography. Potassium, phosphorous, calcium, zinc, copper,

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magnesium, and manganese were determined using an x-ray fluorescence instrument. The results were subjected to analysis of ANOVA at a 5% level of significance. The means were separated using Fisher's LSD test at a 5% level of significance. Bio-slurry obtained from the AD of the substrates at 55℃ had significantly higher ($p < 0.05$) NPK concentrations than bio-slurry obtained from AD at 35℃. The nitrogen (N), phosphorous (P) and potassium (K) concentrations of the bio-slurry from the AD of CD at 55 ℃ were 2485.33 mg/kg, 1.61% and 1.25%. Bio-slurry obtained from AD of FW at 55 ℃ had nitrogen (N), phosphorous (P) and potassium (K) concentration of 0.2441%, 1.067% and 0.859% respectively. The findings of this study will be used to advise farmers on the application amount and rate of bio-slurry based on crop nutrient requirements.

Keywords: Bio-slurry, nutrient composition, characterization, fertilizer, FW, CD, manurial value

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4.1 Introduction

Ever-increasing food demand due to population growth has led to excessive utilization of chemical fertilizers (Ali et al., 2021; Smith & Siciliano, 2015; Wang et al., 2018). Excessive use of chemical fertilizers has negative environmental implications such as the emission of greenhouse gases, degradation of soil fertility, air and groundwater pollution (Chadini et al., 2019; Savci, 2012). Besides being costly, chemical fertilizers have high toxicity and low biodegradability (Funes-Pinter et al., 2020; Gupta et al., 2015). To counteract the negative impacts of chemical fertilizers, the use of organic fertilizers such as animal manure, crop ,residues and bio-slurry has been proposed in numerous studies (Bonten et al., 2014; Chaka et al., 2020; Oyedeji et al., 2014; Wang et al., 2019). Bio-slurry, a co-product of AD of organic substances has been considered good manure as it contains considerable amounts of macro and micro-nutrients (Muhmood et al., 2014).

The quality of bio-slurry as a fertilizer depends on the content of nitrogen, phosphorus, potassium, calcium, and magnesium, N/,P and N/K ratios (Musse et al., 2020; Wagaw, 2016). According to Shahariar et al. (2013), bio-slurry contains 20 − 30% more nutrients than conventional manures such as animal manure, farmyard manure and compost. Kumar et al. (2022) reported that heavy metal concentrations in bio-slurry are typically low. Numerous heavy metals such as Mn, Cu, Ni, Zn, Cr, and Cd are toxic to the environment when present in high concentrations (Coelho et al., 2018; Pichhode & Nikhil, 2016). The microbial composition of bio-slurry also aids to improve soil fertility and health (Rafiuddin et al., 2018). Unlike chemical fertilizers, bio-slurry does not have a definite amount of nutrients. Bio-slurry physicochemical parameters can be optimized by changing AD process parameters and composting (Chaka et al., 2020; Bonten et al., 2014). Bio-slurry can be used in wet form (slurry) or dried form (compost). The benefit of using dried form bio-slurry includes increased volume and better handling however its major drawback is the loss of nutrients such as ammonia which is a source of nitrogen (Kumar et al., 2022).

Several studies (Ali et al., 2021; Haile & Ayalew, 2018; Khanafi et al., 2018; Rahman et al., 2011) have demonstrated that the appropriate application of bio-slurry can improve soil fertility, increase plant growth and yield of crops. However, the excessive application of bio-slurry causes pollution of water due to excessive nutrient input and heavy metal accumulation in the soil (Zhang et al., 2021). Xu et al. (2019) reported the lower carbon bio-slurry application reduced soil microbial

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activity. The effect of bio-slurry on soil properties depends on application amount and aging time. Therefore, it is necessary to determine the nutrient composition of bio-slurry to recommend an appropriate bio-slurry application rate.

The use of bio-slurry to improve soil fertility is not yet widespread due to variability in nutrient composition which makes it difficult to establish fertilizer application rates. The nutrient composition of bio-slurry depends on the type of substrate utilized, HRT, pre-treatment, and post-treatment methods utilized including storage after AD (Coelho et al., 2018). Several studies (Funes-Pinter et al., 2020; Mukhtar et al., 2022; Mukhuba et al., 2018) conducted on bio-slurries from the AD of poultry manure, dairy manure, and agricultural residue established that bio-slurry is rich in mineral elements like N, P, Ca, Zn, Mg, S, Fe, Cu, Co, Mn and can be used as a fertilizer. With the increase in the utilization of FW as a substrate for AD due to its high biodegradability (Bong et al., 2018), the use of its bio-slurry as a fertilizer is not well explored. This study aimed to determine and compare the nutrient compositions of bio-slurry obtained from the AD of FW and CD at 35°C and 55°C

4.2 Materials and Methods

4.2.1 Sample collection and preparation

FW and CD bio-slurry samples were collected after the termination of the AD experiments conducted at 35°C and 55°C. The samples were filtered and stored under room temperatures before proximate analysis.

4.2.2 Experimental design and data analysis

A 2 x 2 factorial experimental design was used to assess the effect of substrate type and temperature on the quality of bio-slurry. Bio-slurry was collected after the termination of AD experiments of FW and CD incubated at 35 °C and 55 °C. Figure 4.1 shows a schematic diagram of the experimental design showing the factors, their levels, and the replications.

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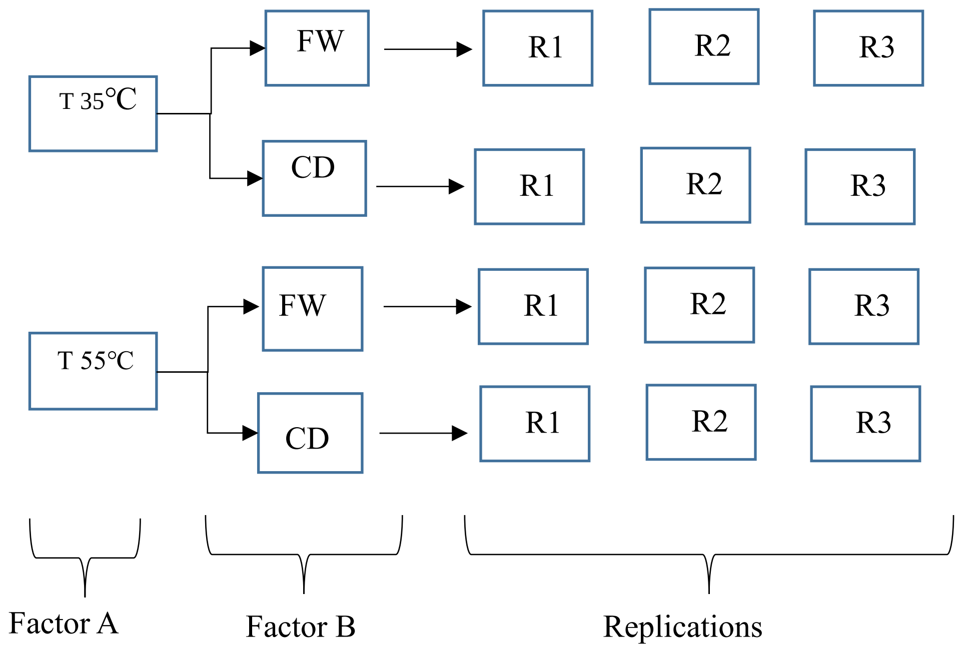

Figure 4.1 Schematic presentation of the experimental design with 2 factors (Factor A= Temperature and Factor B = substrate and three replicates

Experimental data were obtained in triplicates and reported as mean. The data were subjected to statistical analysis using the Minitab 19 (Minitab, 2020) statistical software using the GLM. ANOVA for a 2 x 2 factorial experiment at a 5% level of significance was used to assess the effects of substrate type and temperature on the quality of bio-slurry. Where a significant ANOVA result was obtained, the mean separation was done using the Fisher's LSD test at a 5% level of significance.

4.2.3 Determination of TS and volatile content

The TS and VS content of the samples was determined using the APHA method mentioned in Section 3.5.

4.2.4 Determination of pH and electrical conductivity

The pH of the samples was measured using a portable pH meter (Thermo Fisher Scientific Orion, South Africa). Electrical conductivity was measured using a multi-parameter portable instrument (MultiLab IDS YSI, USA)

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4.2.5 Determination of bio-slurry nutrient composition

Nitrogen was determined using an ion chromatography instrument (850 Professional IC Metrohm, Switzerland) using AOAC method 978.01. A mass of 0.5 g of the samples was digested using 10 mL HNO_3 and 4mL $HClO_4$ by heating the mixture at 200°C for 240 min in a fume cupboard. After digestion, an aliquot of bio-slurry was allowed to cool at room temperature. The cooled aliquot was filtered with 0.4 μ m Whatman filter paper then with a 0.2 μ m syringe filter and diluted using deionized water before injecting it into the ion chromatography system. A standard stock solution of sodium ions (1000 mg/L) was prepared by adding anhydrous NaCl salt into deionized water. Nitrate and Sulphate stock solutions containing 1000 mg/L were purchased from Sigma-Aldrich (Johannesburg, South Africa). Mixed standard solutions of 1.00, 2.00, 3.00, 4.00, 5.00 and 0.20, 0.40, 0.60, 0.80 and 1.00 mg/L were used to calibrate the ion chromatograph.

Potassium, phosphorus, iron, manganese, calcium, magnesium, and copper concentrations were determined using an X-ray fluorescence instrument. The air-dried samples were ground and sieved through a 2 mm sieve. A mass of 5 g of the sample was placed in a benchtop x-ray fluorescence (XRF) spectrometer (Bruker Nano GmbH, Germany) to determine its nutrient composition.

4.3 Results and Discussion

4.3.1 Effect of substrate type and temperature on physical properties of bio-slurry

Table 4.1 shows the physical properties of CD and FW bio-slurry obtained in this study. The study result shows that temperature, substrate type, and the interaction between substrate type and temperature significantly affected TS%, E, C, and pH. The highest TS% was obtained from bio-slurry obtained from the AD of FW at 35 °C and the least was obtained from CD bio-slurry from a reactor that was operating at 55 °C. The TS% of FW and CD bio-slurries was lower than that of undigested FW and CD. The lower TS% content in FW and CD bio-slurries in comparison to their respective undigested substrates was expected since anaerobic microbes use organic matter during the AD process (Möller & Müller, 2012). The study results show that the interaction between temperature and substrate did not significantly affect ($p < 0.05$) the VS/TS%. The VS/TS% of the bio-slurries were in the range of 72.53 − 74.38%. The FW and CD bio-slurries had VS/TS% within the 72.53 –75.79%. The remaining VS/TS% can be attributed to organic matter content made up of substances such as VFAs and lignocellulose material (Parra-Orobio et al, 2012).

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Table 4.1 Effect of temperature and substrate type on physical characteristics of bio-slurry

S	T	%TS	%VS/TS	EC (mS/cm)	pH
CD	55	$6.49^{c} \pm 0.4$	$72.53^{a} \pm 1.0$	$7.52^{b} \pm 0.14$	$8.08^{a} \pm 0.24$
CD	35	$9.46^{a} \pm 0.47$	$73.58^{a} \pm 3.1$	$4.60^{a} \pm 0.025$	$8.1^{a} \pm 0.25$
FW	55	$9.08^{a} \pm 0.2$	$74.38^{a} \pm 0.54$	$7.34^{d} \pm 0.04$	$4.6^{b} \pm 0.07$
FW	35	$10.62^{b} \pm 0.8$	$75.79^{a} \pm 1.3$	$4.63^{c} \pm 0.053$	$5.4^{c} \pm 0.1$
Treatment and interactions					
S		0.001	0.144	0.000	0.000
T		0.000	0.356	0.000	0.013
S*T		0.085	0.889	0.000	0.017

FW = food waste, CD = cow dung, EC = electrical conductivity, %TS =Total solids and %VS = Volatile Solids. The values presented in the table are the mean of triplicates plus the standard deviation. Column means with the same superscripts are not significantly different according to Fisher's LSD test ($p > 0.05$).

The pH values of CD bio-slurries from a reactor operating at 35 °C and 55 °C were 8.1 and 8.08. The pH range of the bio-slurries was comparable to the one reported by Gaur and Suthar (2017). Higher pH values in CD bio-slurries obtained in this study can be attributed to the stability of the AD process (Xie et al., 2015). The pH values of FW bio-slurries were 5.4 and 4.6 at 55 °C and 35 °C respectively. The lower pH value can be attributed to an accumulation of VFAs during AD of FW. The EC range for CD bio-slurries was in the range of 4.17 − 7.37 mS/cm. EC values for dairy cow manure bio-slurry reported by Mukhtar et al. (2022 were within the 840 - 1532 mS/cm range which is higher than the ones reported in this study. The difference in EC values can be attributed to the differences in the number of free ions, salinity level, and physical properties of the bio-slurry (Voelkner et al., 2015).

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4.3.2 Effect of substrate type and temperature on chemical properties of bio-slurry

Table 4.2 shows the results for the effect of temperature and substrate type on the chemical characteristics of bio-slurry. Substrate type, temperature, and the interaction between temperature and mixing ratios significantly affected the concentration of N, P, and K in bio-slurry. In this study, higher concentrations of N, P, and K were obtained for bio-slurry collected from the AD of FW and CD at 55 °C relative to 35 °C. Similar findings were reported by Labatut et al. (2014) who found higher N concentration and pH values of bio-slurries at thermophilic temperatures relative to mesophilic temperatures. The higher N concentration can be attributed to the effective conversion of organic matter to ammonium nitrogen through mineralization during AD at thermophilic temperature (Kumar et al., 2022). Further, Labatut et al. (2014) reported that thermophilic AD produces more organically stable bio-slurry with higher digestibility and mineralization compared to mesophilic AD. A study conducted by Varma et al. (2021) showed a higher concentration of N and P in CD bio-slurries than reported in this study. The difference in concentrations can be attributed to the difference in physical, chemical, and microbial activity (Xie et al., 2015)

Temperature and the interaction between temperature and substrate type did not significantly affect $p > 0.05$) the concentration of Cu and S in the bio-slurries. The substrate type, temperature, and the interactions between substrate type and temperature significantly affected ($p < 0.05$) the concentrations of Mg, Zn, Fe, Mn, and Ca in the bio-slurries. The concentration of Ca in CD bio-slurries was significantly higher ($p < 0.05$) than in FW bio-slurries. According to Mata-Alvarez et al. (2014), nutrient composition and quality of bio-slurry are depended on the type of substrate used for AD. The concentration of heavy metals in CD bio-slurry obtained in this study was higher than the finding of Mukhuba et al. (2018). The difference can be attributed to the difference in physicochemical characteristics of the substrates and AD conditions (Fang, 2010).

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Table 4.2 Effect of temperature and substrate type on chemical characteristics of bio-slurry

S	T	N (%)	K (%)	P (%)	Mg (%)	Zn (%)	Cu (%)	Fe (%)	Mn (%)	Ca (%)	S (%)
CD	55	$0.2485^{a} \pm 0.001$	$1.250^{a} \pm 0.001$	$1.610^{a} \pm 0.01$	$1.663^{a} \pm 0.05$	$0.109^{a} \pm 0.001$	$0.015^{a} \pm 0.000$	$1.31^{b} \pm 0.001$	$0.063^{a} \pm 0.000$	$4.204^{b} \pm 0.001$	$0.7676^{a} \pm 0.015$
CD	35	0.2482 ± 0.000	$1.140^{b} \pm 0.000$	$1.149^{b} \pm 0.01$	$1.346^{b} \pm 0.000$	$0.112^{b} \pm 0.000$	$0.015^{a} \pm 0.000$	$1.452^{a} \pm 0.001$	$0.064^{a} \pm 0.001$	$4.813^{a} \pm 0.003$	$0.7715^{a} \pm 0.012$
FW	55	$0.2441^{c} \pm 0.000$	$1.067^{d} \pm 0.0002$	$0.859^{d} \pm 0.0002$	0.916d ± 0.000	$0.006^{c} \pm 0.000$	$0.011^{b} \pm 0.002$	$0.078^{c} \pm 0.01$	$0.003^{b} \pm 0.001$	$0.736^{c} \pm 0.003$	$0.6235^{b} \pm 0.016$
FW	35	$0.2404^{b} \pm 0.000$	$0.605^{c} \pm 0.001$	$0.583^{c} \pm 0.001$	$0.837^{c} \pm 0.000$	$0.006^{c} \pm 0.000$	$0.012^{ab} \pm 0.002$	$0.137^{d} \pm 0.001$	$0.007^{c} \pm 0.002$	$0.832^{d} \pm 0.001$	$0.6298^{b} \pm 0.008$
Treatment and interactions p-Value											
S		0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000
T		0.000	0.000	0.000	0.000	0.01	0.631	0.000	0.020	0.000	0.516
S*T		0.000	0.000	0.000	0.000	0.03	0.631	0.000	0.122	0.000	0.871

FW = food waste, CD = cow dung, EC = electrical conductivity, %TS =Total solids content and %VS = Volatile Solids. The values presented in the table are the mean of triplicates plus the standard deviation. Column means with the same superscripts are not significantly different according to Fisher's LSD test ($p > 0.05$).

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4.4 Conclusion

The study investigated the effect of substrate type and temperature on the nutrient composition of bio-slurry. The NPK concentration after the AD process at 55 ºC was significantly higher ($p < 0.05$) relative to 35 ºC. However, the heavy metal concentration was higher in bio-slurry obtained from AD at 35 ºC than at 55 ºC. The pH of CD bio-slurries was higher than that of undigested CD. Due to the potential accumulation of VFAs, the pH of FW bio-slurry was lower than undigested FW. The CD bio-slurry can be used to neutralize acidic soils, improve soil fertility, and soil structure. The study established that CD bio-slurry has a better manurial value than FW bio-slurry.

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CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The objectives of this study were to evaluate the potential of canteen FW for biogas and manure production. The study investigated the effect of temperature and co-digestion mixing ratios on biogas yield and methane content. The study further analysed the physicochemical characteristics of FW and CD.

Based on the experimental results, the following conclusions were made:

(a) The first part of the study investigated the effect of co-digestion of FW and CD and temperature on biogas yield, methane content and BMP. Temperature, mixing ratios and the interaction of temperature and mixing ratio significantly affected ($p < 0.05$) the total biogas yield, biogas methane content and BMP. Co-digestion of FW and CD resulted in higher biogas yield than mono-digestion of the substrates. The results showed that the total biogas yield increased with increasing FW proportion in the AD co-digestion of FW and CD. Temperature had a significant effect on total biogas yield, biogas methane content, and BMP. The biogas yield and BMP of the substrates were higher at 55°C than at 35°C. However, the higher energy requirement is the major drawback of operating the digester at the optimum thermophilic methanogens' temperature range. The highest biogas yield and BMP were obtained when FW and CD were co-digested at a mixing ratio of 75:25 (w/w) at 55°C. From this study, co-digestion of FW and CD increased total biogas yield, biogas methane content and BMP with the increasing proportion of FW in the co-digestion substrate. All AD co-digestion mixtures of FW and CD produced greater levels of increased total biogas yield, methane content and BM than the mono-digestion of the substrates. This study recommends a mixing ratio of FW and cow manure of 75:25 (w/w) for the sizing and feeding of domestic and commercial biogas plants.

(b) The second part of the study assessed the effect of substrate type and temperature on the physicochemical characteristics of bio-slurry. Temperature, substrate type and the interaction of temperature and mixing ratio significantly affected ($p < 0.05$) the

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concentrations of N, P, K, Mg, Zn, Fe, Mn and Ca. Temperature and the interaction between temperature and substrate type did not affect ($p < 0.05$) the concentration of Cu and S in the bio-slurries. The results show that higher N, P and K concentrations were obtained at 55 °C relative to 35 °C. Substrate type, temperature and the interaction of substrate type and temperature significantly affected the physicochemical characteristics of bio-slurry. Bio-slurry can also be used to neutralize acidic soils, improve soil fertility, and soil structure.

5.2 Recommendations for further research

(a) The design of the reactors limited the monitoring of pH which is a critical factor that affects AD. AD of FW was inhibited due to the possible formation of process inhibitors such VFAs which lower the pH. Further research on the AD process can be done under monitored or adjusted pH levels.

(b) This study did not identify the optimum FW and CD mixing ratios as indicated by lower biogas yield, methane content and BMP of mono-digestion of CD for both 35° and 55° compared to the maximum values at co-digestion of FW: CD (75:25). Hence, further research is necessary to identify the optimum FW and CD mixing ratios.

(c) The study determined the effect of substrate type and temperature on nutrient composition of pure FW and CD bio-slurries. Further studies are needed to determine the effect of co-digestion on the physicochemical characteristics of bio-slurry.

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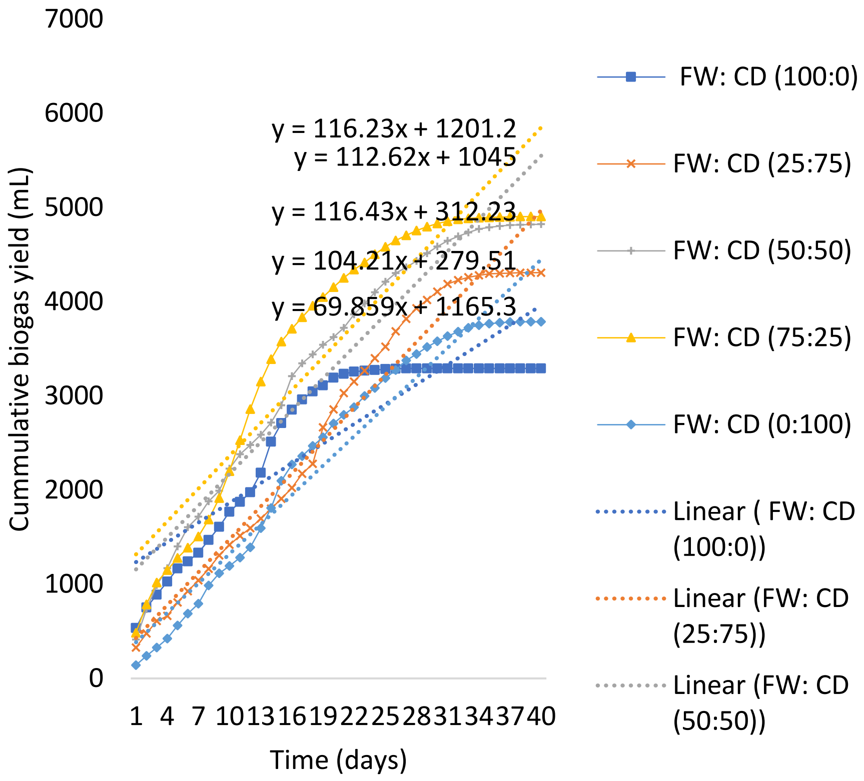
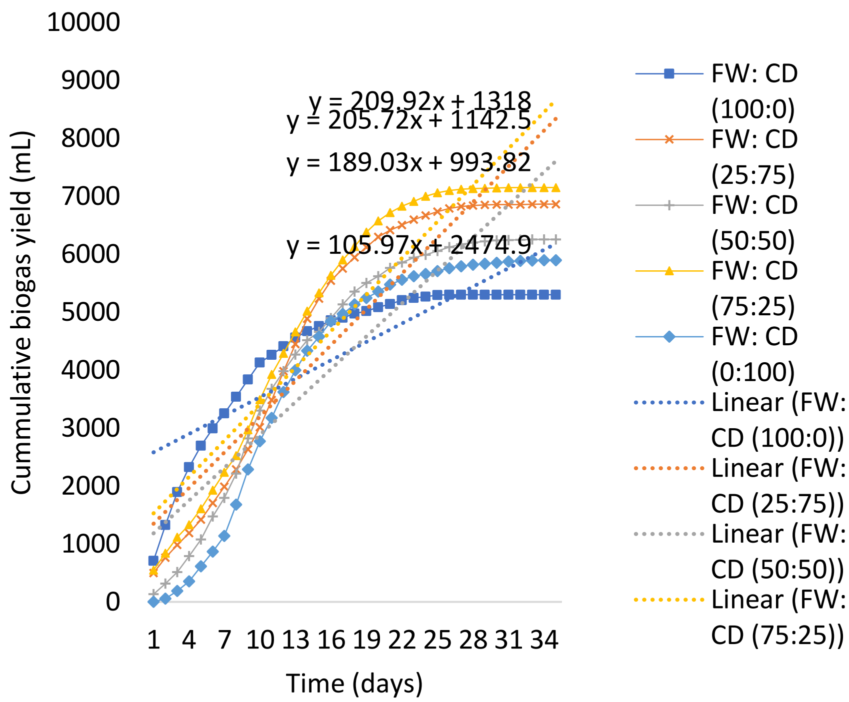
APPENDIX



Appendix A 1 Cumulative biogas yield against time at 35ºC



Appendix A 2 Cumulative biogas yield at 55ºC

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