ANAEROBIC TREATMENT PERFORMANCE OF CO-DIGESTED SLAUGHTERHOUSE WASTE

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Anaerobic Treatment Performance of Co-Digested Slaughterhouse Waste

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as the university supervisors.

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DEDICATION

This work is dedicated to my precious son, Prince Telewa, and to my wonderful family for their love and support throughout my studies. Our journey together has not only provided me with professional success, but it has also solidified my feelings for all of you. I appreciate it, and may God Almighty abundantly reward your generosity.

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ABBREVIATIONS AND ACRONYMS

ACoD	Anaerobic Co-Digestion
AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model 1
AMoD	Anaerobic Mono-Digestion
APHA	American Public Health Association
BMP	Biomethane Potential
BSI	Busia Sugar Industry
C	Carbon
CH_4	Methane
C/N	Carbon/ Nitrogen Ratio
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
DO	Dissolved Oxygen
FOG	Fats, Oils and Grease
GDP	Gross Domestic Product
H_2	Hydrogen
HRT	Hydraulic Retention Time

IWA	International Water Association
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LCFAs	Long Chain Fatty Acids
MPI	Meat Processing Industry
N	Nitrogen
N_2	Nitrogen gas
NH_3	Ammonia
NH_4^+-N	Ammonium nitrogen
O_2	Oxygen
OLR	Organic Loading Rate
SCOD	Soluble Chemical Oxygen Demand
STP	Standard Temperature and Pressure
SRT	Solids Retention Time
SHWW	Slaughterhouse Wastewater
SO_2	Sulphur Dioxide
SO_3	Sulphur Trioxide
SPI	Sugar Processing Industry
SPM	Sugar Press mud
TCD	Tons of Cane per Day

TS	Total Solids
VFAs	Volatile Fatty Acid
VS	Volatile Solids

ABSTRACT

Most underdeveloped countries struggle to find long-term solutions to safe and economical treatment and disposal of agricultural wastes. Slaughterhouse wastewater (SHWW) has a significant potential for biomethane (bioCH_4) yield when anaerobically treated. The procedure, however, is prone to failure. As a result, anaerobic codigestion (ACoD) is used to boost the efficacy of SHWW anaerobic monodigestion (AMoD). The main objective of this study was to assess the anaerobic treatment performance of SHWW co-digested with sugar press mud (SPM). The study used a biochemical methane potential (BMP) test to assess the bioCH_4 yield of SHWW codigested with SPM at varied mixing ratios. SPM boosted CH_4 yield and VS removal by 27% and 67%, respectively, at an optimum mixing ratio of 20%SHWW: 80%SPM. Furthermore, the influence of ACoD of SHWW with SPM on organic removal efficiency at various hydraulic retention times (HRTs) was investigated semi-continuously in lab-scale continuous stirred tank reactors (CSTRs) under mesophilic (37.0 ± 1.0 °C) conditions. At the optimal 15 days (d) HRT, ACoD increased CH_4 yield and VS removal by 69.1% and 62.4%, respectively. Further, the addition of SPM improved the stability of the AD process, as evidenced by a drop in ammonium nitrogen (NH_4^+-N) concentration. A modified Gompertz model was also used to determine the kinetics of organic degradation of SHWW and SPM. The study's kinetic analysis revealed that the rate of CH_4 yield increased by about 46% while the lag time was greatly reduced by approximately 87%. Therefore, use of SPM as a co-substrate improved the treatment performance of SHWW and recovery of bioCH_4. This would also help to increase the use of renewable energy sources in electricity generation, cutting greenhouse gas (GHG) emissions. The mesophilic temperature (37.0 ± 1.0 °C) was maintained throughout the study; therefore, it is recommended that investigations under thermophilic circumstances should be conducted in the future to investigate the effect of temperature on AD process stability.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The meat sector in the agro-processing industry has gained much interest as it massively contributes to industrial wastewater generation (e Silva et al., 2020). Automation of carcass dressing does use a lot of water, thereby producing high-strength effluent rich in protein and lipid content. Fortunately, such wastewater provides a good source of biodegradable organic matter, making it an excellent and cost-effective substrate for anaerobic digestion (AD). A critical issue, however, is the safe disposal of this wastewater, which is implicated in the development of disease-causing microbes, a serious environmental hazard, and a threat to human health (Salehiyoun et al., 2020). Regrettably, whenever it gets to the greener treatment of organic waste, modern slaughterhouses face challenge in treatment and disposal of wastes.

Slaughterhouse wastewater (SHWW) is indeed a growing threat to public health worldwide (Bustillo-Lecompte, Mehrvar & Quinones-Bolanos, 2016). As a matter of fact, a waste management strategy that is both economically and environmentally safe is required. Conventional treatment methods, on the other hand (incineration, landfill), have high initial investment costs and energy requirements and require highly skilled labour. Conversely, AD generates bioenergy, and the effluent serves as a substrate for nutrient recycling (Obi, Ugwuishiwu & Nwakaire, 2016).

Likewise, the sugar processing industry faces management challenges associated with the handling of the resultant sugar press mud (SPM). SPM is generated in substantial quantities, approximately at a rate of 0.01 to 0.07 tons per ton of ground sugarcane (Devia-Orjuela et al., 2019). Unfortunately, the SPM compost emits an obnoxious smell, creating a nuisance for residents proximate to the sugar factory. Moreover, toxic gases such as sulphur dioxide (SO_2) and sulfur trioxide (SO_3) emitted from burning briquettes made from SPM pollute the environment. Furthermore, when the briquettes are used in the boiler as a fuel, they form clinker (Rouf et al., 2010). Therefore, there is an urgent need to address the safety problems caused by both SHWW and SPM.

A large and growing body of literature has been published to date on the anaerobic mono-digestion (AMoD) of cattle slaughterhouse wastewater owing to its high protein and lipid content, which make it ideal for biomethanation (Schmidt et al., 2018; Musa et al., 2018; Selormey et al., 2021; Rhee et al., 2021). However, AMoD of SHWW is coupled with VFA accumulation and/or ammonia (NH_3) inhibition (Palatsi et al., 2011; Yenigün & Demirel, 2013). It is also associated with some operational hurdles such as sludge flotation, digester foaming, and pipe obstructions (Long et al., 2012). Besides that, due to an unbalanced carbon to nitrogen (C/N) ratio and a poor buffering capacity, digestion of SHWW as a single substrate is challenging in practice (Rhee et al., 2021).

SPM, on the other hand, consists of a complex lignocellulose material that hinders the hydrolysis of cellulosic carbohydrate. This clarifies why it exhibits a low bioavailability for bioconversion processes that further inhibits its efficiency. Moreover, the problem of fast acidification caused by SPM has an impact on the performance of biogas plants (Talha et al., 2017; Reyes et al., 2015; Rouf et al., 2010). Besides, SPM contains a high amount of ash, which may raise mud concentrations within the continuously stirred tank reactor (CSTR), resulting in a higher organic loading rate (OLR), which may inhibit the AD process (Talha et al., 2017). As a result, both wastes (SHWW and SPM) show potential as co-substrates for one another.

To address the challenges, associated with the AMoD of SHWW, authors have come up with various options. Mata-Alvarez et al. (2014), for instance, confirmed that incorporating a co-substrate would aid in countering such drawbacks. This is due to the fact that a co-substrate provides the necessary macro- and micronutrients, stabilizes pH, enhances buffer capacity and biodegradability, broadens the microbial community associated with the AD process, and increases CH_4 yield (González et al., 2017; Alvarez & Lide, 2008). SPM, in this opinion, is the best fit as a co-substrate since it has an ample buffering capacity and is also enriched with carbon sources, which can really augment the C/N ratio (Mugodo, Magama & Dhavu, 2017).

Nonetheless, ACoD of SHWW with SPM is an effective method for reducing environmental pollution and reclaiming clean energy. Also, the eco-system stands to gain when the effluent contains valuable nutrients such as nitrogen and organic carbon (Salehiyoun et al., 2020). Fortunately, substrate degradability, process stability, and digester efficiency can all be optimized by pre-treatment (González et al., 2020; Ripoll et al., 2022); two-stage AD systems (Meegoda et al., 2018; Sakarika et al., 2020); pH control by chemical alkali (Talha et al., 2017; Qamar et al., 2022); temperature selection (Meegoda et al., 2018; Rahman et al., 2022); and ACoD (Wu, 2007; Karki et al., 2021).

Several scholars have also evaluated the co-digestion of abattoir wastewater and some other substrates. Overall, the authors discovered that co-digestion of abattoir wastewater with other substrates outperformed AMoD. On the contrary, Monou et al. (2008) reported negative improvements for the ACoD of abattoir wastewater with potato processing wastewater. The authors attributed the findings to the abattoir wastewater's low pH and poor buffering capacity. Nonetheless, ACoD of SHWW with SPM is a possible solution that provides an effective remedy to environmental pollution while also maximizing energy recovery from such substrates.

1.2 Statement of the Problem

Most modern abattoirs in the developing world struggle to deal with SHWW in a sustainable manner. As a result, pollution and depletion of natural resources close to or around abattoirs has been a long-term environmental problem. For instance, the discharge of untreated SHWW into municipal sewerage systems can lead to soil and water contamination. Water bodies become overly nutrient-rich, resulting in excessive algae blooms. This will eventually lead to eutrophication, which will result in the deterioration of water quality and the depletion of dissolved oxygen (DO), endangering human and aquatic life. Additionally, it can lead to increased emissions of greenhouse gases (GHGs).

Similarly, the sugar industry produces massive amounts of environmentally hazardous byproducts. However, current SPM disposal methods are neither environmentally nor economically sustainable. For example, the SPM emits an obnoxious odour due to sugar and other organic constituents, causing air pollution. Furthermore, SPM is frequently used as a fuel, and when burnt in brick kilns, it results in the loss and waste of millions of tons of nutrients, ultimately degrading the environment. When burnt directly as briquettes, it produces clinkers and emits toxic gases SO_2 and SO_3, polluting the environment (Gupta et al., 2011). Another common application for SPM is fertilizer. Unfortunately, long-term input of untreated SPM into the soil, causes toxic substance accumulation, resulting in induced pollution. On the other hand, when freshly applied, it becomes toxic to plants due to rapid decomposition, releasing a large amount of heat and ammonia (Rouf et al., 2010).

Fortunately, the ACoD of SHWW with SPM provides an effective solution to these environmental threats, and the recovery of renewable bioenergy is critical to the economy. Despite extensive research on SHWW ACoD, only a few pieces of evidence have directly linked cattle SHWW to SPM in AD. A novel aspect of this study is the use of SPM, a good carbon source, as a co-substrate to boost and optimize the digestion of nitrogen-rich SHWW.

1.3 Objectives

1.3.1. Main Objective

The main objective of this study was to assess the anaerobic treatment performance of slaughterhouse wastewater co-digested with sugar press mud.

The specific objectives of this research were:

a) To evaluate the methane yield of slaughterhouse wastewater codigested with sugar press mud at various mixing proportions.
b) To determine the influence of co-digestion of slaughterhouse wastewater with sugar press mud on organic removal efficiency at various hydraulic retention times (HRTs).
c) To establish the kinetics of organic degradation of slaughterhouse wastewater and sugar press mud using a modified Gompertz model.

1.4 Research Questions

i. How does the ACoD of SHWW with sugar press mud at different mix ratios affect biomethane yield?
ii. What impacts do different HRTs have on organic matter removal efficiency?
iii. What are the kinetics of degradation predicted by a modified Gompertz model compare to the experimental results?

1.5 Justification of the Study

Without effective SHWW treatment, the environment is polluted, and both human and aquatic lives are endangered. ACoD of SHWW with SPM is a novel technique to counter problems associated with AMoD of SHWW. It also increases treatment efficiency and management of the two aforementioned agro wastes. Furthermore, reactor sharing can significantly enhance the plant's overall economics by boosting the amount of renewable energy recoverable as the digestate's agronomic quality is enriched with beneficial plant nutrients. Moreover, ACoD could be an alternative method of improving the economic diversion of agro wastes from the various sectors. The recovery of bioenergy could supplement the country's energy supply, with produced biogas helping to reduce GHG emissions and playing an important role in the energy transition from a fossil-based energy mix to an eco-friendly, low-carbon one. Consequently, this will reduce energy bills for the respective industries, while also creating a new revenue stream from the bottling and sale of bioCH_4. However, excess heat and power could be sold to the public grid. Figure 1.1

and 1.2 presents the schematic economic and environmental benefits of ACoD of the abovementioned agro wastes.

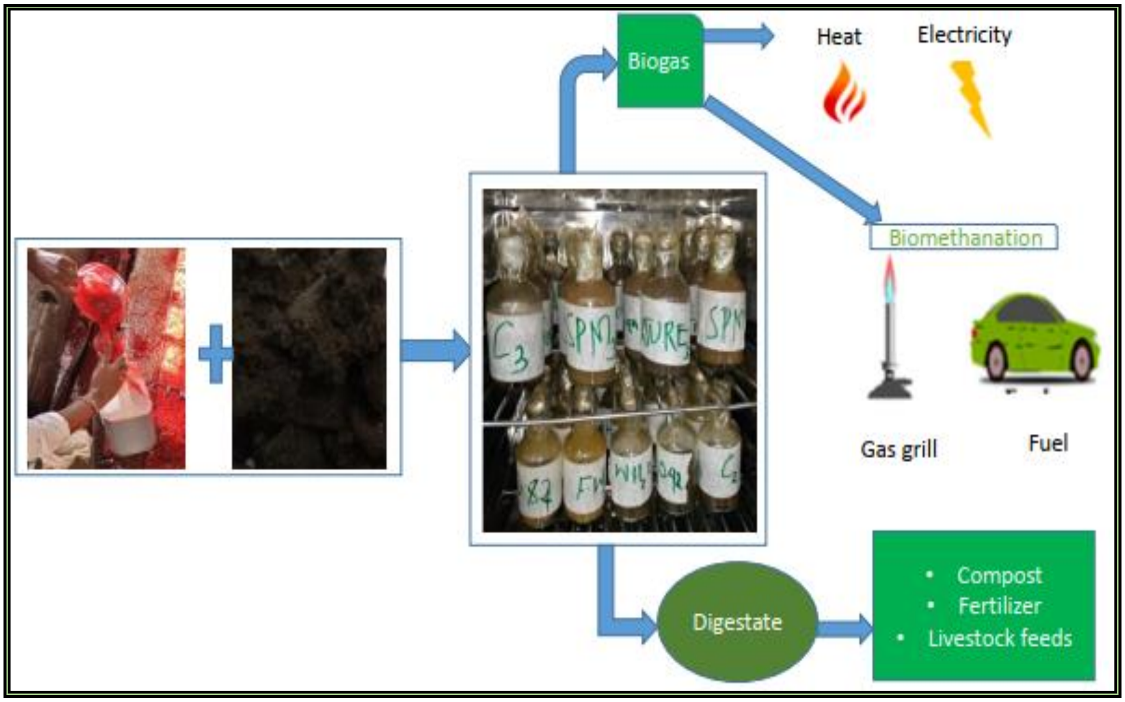

Figure 1.1: Graphical Presentation of Economic Benefits of ACoD of SHWW with SPM

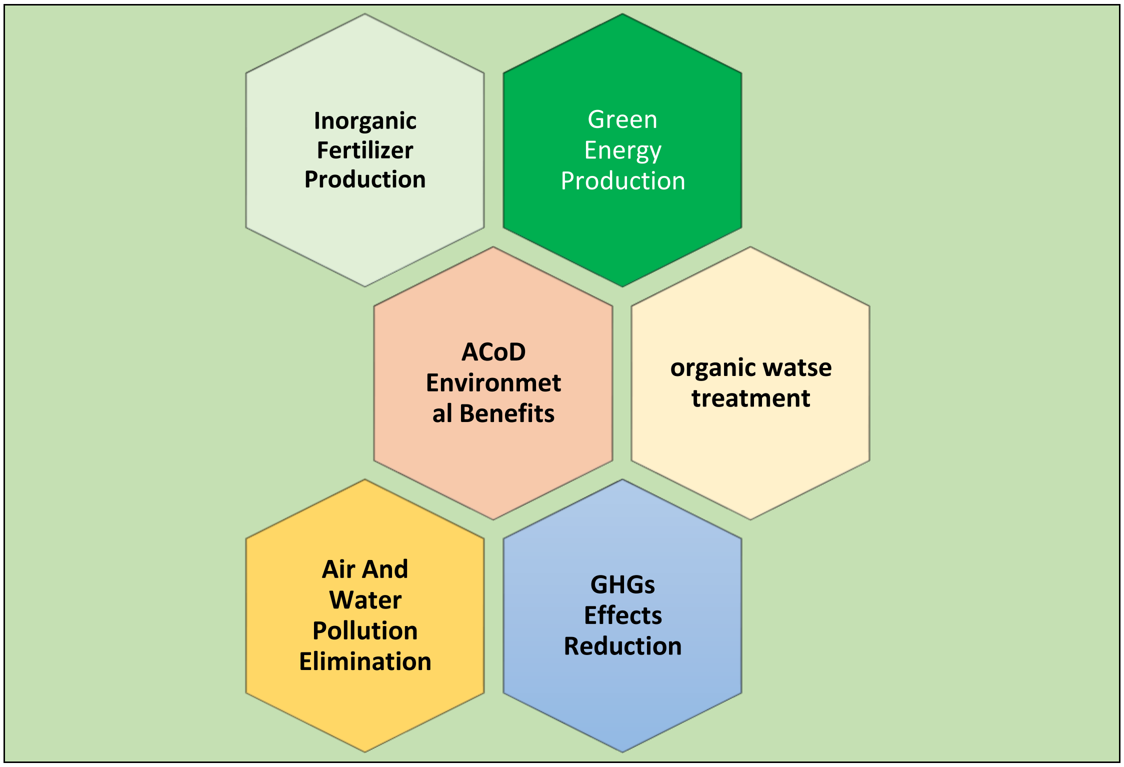

Figure 1.2: Environmental Benefits of ACoD of SHWW with SPM

1.6 Scope of the Study

The study was carried out at JKUAT Civil Engineering Environmental Laboratory, where 125mL and 1000mL serum bottles were designed and fabricated to resemble batch and CSTRs, respectively. The reactors were incubated at mesophilic conditions (37.0 ± 1.0 °C). This was to enable effective data collection, which would reflect the behavior of microorganisms under mesophilic conditions. The feedstock and digestate collected were characterized for pH, chemical oxygen demand (COD), total solids (TS), volatile solids (VS), and ammonium-nitrogen (NH_4^+-N).

1.7 Limitations of the Study

The primary drawback of this research was insufficient mixing mechanisms for both batch and continuous reactors. Mixing enhances interaction between bacterial cells and solid substrates, thereby improving CH_4 production. This may have affected CH_4 production in the current study. Furthermore, the daily CH_4 production recorded could not be accurate due to a lack of an automated gas measuring mechanism for both batch and continuous experiments.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat Sector in Kenya

Livestock is a crucial sector in the Kenyan economy, accounting for approximately 12% of the Gross Domestic Product (GDP), 40% of the agricultural GDP, and employing approximately 50% of the agricultural labour force. Kenya's livestock population is estimated at 3,355,407 exotic cattle, 14,112,367 indigenous cattle, 17,129,606 sheep, 27,740,153 goats, 2,971,111 camels, 1,832,519 donkeys and 31,827,487 poultry, with red meat (meat and offal from cattle, sheep, goats, and camels) accounting for more than 80% of the total (Kenya Market Trust, 2019). From 1997 to 2030, annual per capita meat consumption in both developing and developed countries is expected to rise from 25.5–37 kg to 88–100 kg, respectively (Kenya Market Trust, 2019).

Cattle serve as the most significant source of red meat in Kenya, accounting for 77% of ruminant slaughter offtake (Kenya Market Trust, 2012). On a global scale, the livestock industry contributes to 17% and 33% of kilocalorie and protein intake, respectively, with production and consumption trends varying among industrialized and developing nations (Kenya Market Trust, 2014). Figure 2.1 shows the total cattle meat production in Kenya from 2010 to 2020 (Kamer, 2022). Production declined sharply in the year 2020, interrupting an upward trend observed since 2015. This could be attributed to disruptions in hotel and restaurant operations caused by the coronavirus (COVID-19) pandemic and the related containment measures implemented by the Kenyan government.

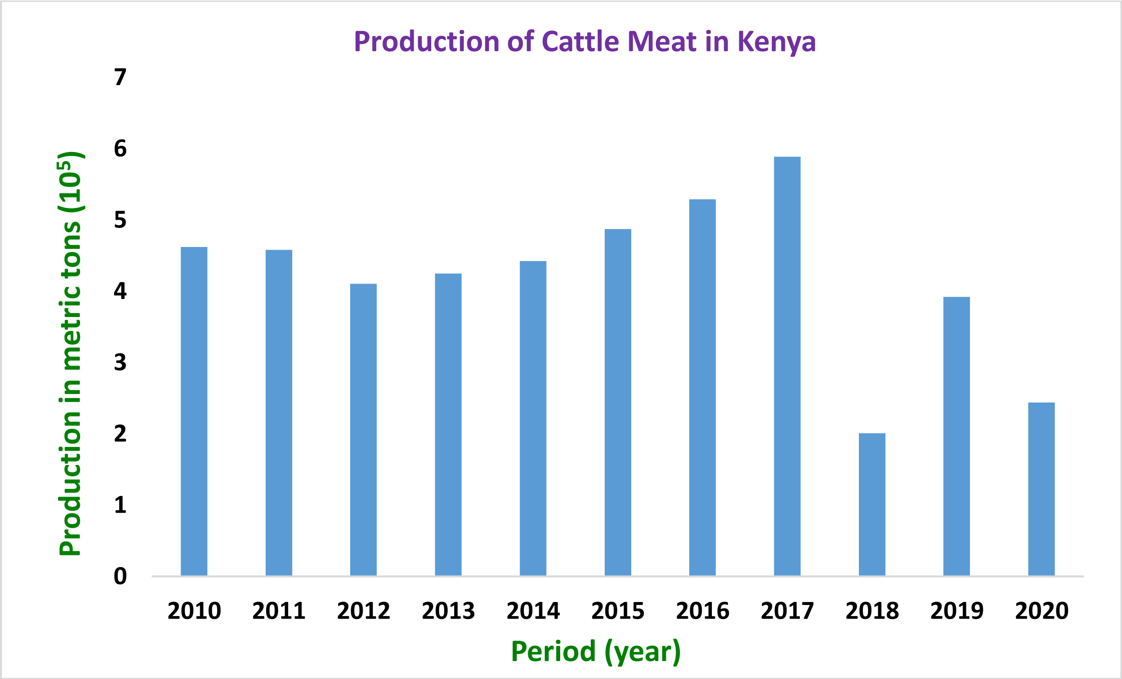

Figure 2.1: Cattle Meat Production in Kenya from 2010-2020

Slaughterhouse wastewater (SHWW) is a moderate-to-high strength effluent with approximately 45% soluble organics and 55% coarse suspended organics (Kabeyi & Olanrewaju, 2020). It is composed of cleaning water from all operational areas that come into contact with manure, carcasses, offal, blood, and waste meat. Moreover, organic matter in meat processing effluents primarily consists of faeces, gut contents, blood, carbohydrates in dissolved or colloidal forms, fat and protein byproducts (i.e., VFAs), and other organic nitrogen compounds (i.e., NH_3) (Reyes et al., 2015).

The COD concentration ranges from 18,000 mg/L to 43,000 mg/L (Bustillo-Lecompte et al., 2016; Musa et al., 2018), but the strength varies by industry and the number of animals slaughtered. The chemicals and detergents used to clean the abattoir facilities also have an impact on the COD concentration. Therefore, SHWW treatment prior to discharge needs to be done thoroughly to meet the prevailing standards. Or else, it may prevent light from reaching aquatic species, and the primary detrimental impact on natural water bodies such as rivers is deoxygenation, which promotes eutrophication and has an unappealing aesthetic value (Bustillo-Lecompte et al., 2016).

The occurrence of fat, oil, and grease (FOG) in SHWW portrays it as a potential source of bioCH_4 (Rhee et al., 2021). Unfortunately, its high tendency for rapid acidification makes SHWW a problematic waste (Musa et al., 2018). Furthermore, the recalcitrant nature of SHWW may cause a variety of operational issues such as foul odor, pipe clogging, bacterial cell surface adhesion, and scum layer formation (Long et al., 2012). These difficulties could jeopardize the smooth operation of their biogas plant.

There are various conventional treatment and disposal methods for SHWW, including landfilling, blood separation, skimming, incineration, screening, primary settlement, and composting. Consequently, these methods are costly, pose environmental challenges as pathways for potential GHGs to leak into the atmosphere, and lack focus on energy recovery (Mugodo et al., 2017, Selormey et al., 2021).

2.2 Sugar Sector in Kenya

Sugar cane (Saccharum Hybrids spp.) is among the world's largest cash crops with a global yield of 1.91 billion tons. Brazil is the largest producer in the world, followed by India, China, Thailand, Pakistan, and Mexico (Gunjal & Gunjal, 2021). In Africa, 33 out of 55 countries are engaged in sugar production, with Egypt and South Africa dominating the industry, accounting for 40% of the total production in Africa. They are closely followed by Sudan, Eswatini, Kenya, Morocco, Mauritius, Uganda, Ethiopia, Mozambique, Zambia, and Zimbabwe (Sugar Sub-Sector Profile, 2020). In regard to sugar cane production, Kenya was at 520,000 metric tons as at 2020 (Baraza, 2020). Figure 2.2 depicts the volume of sugarcane produced in Kenya between 2008 and 2019 (CEICdata.com, 2021).

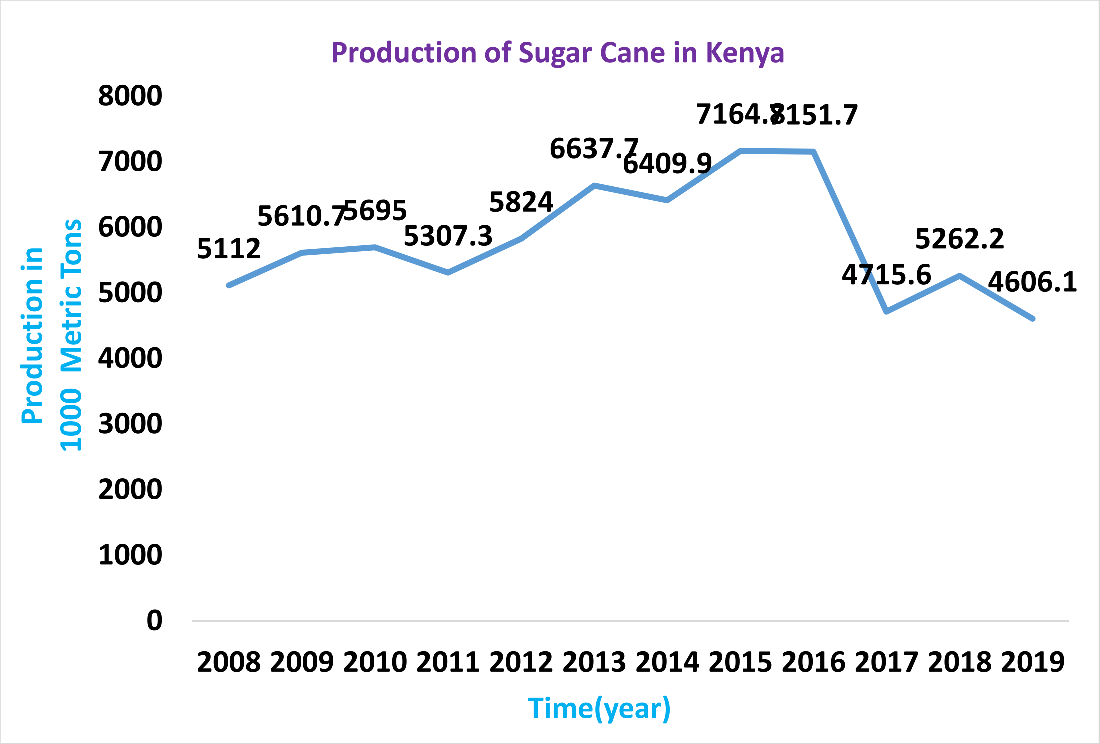

Figure 2.2: Production of Sugar Cane in Kenya

Source: CEICdata.com (2021)

Sugarcane represents one of Kenya's leading cash crops, with approximately 400,000 small-scale farmers supplying more than 90% of the milled cane, which provides income for inhabitants along the sugar belt zone. In Kenya, there are 16 sugar factories with a combined processing capacity of 56,800 tons of cane per day (TCD) (Sugar Sub-Sector Profile, 2020). There are 16 sugar industries in Kenya, with a total processing capability of 56,800 tons of cane per day (TCD) (Sugar Sub-Sector Profile, 2020). Table 2.1 shows a detailed description of the sugar-producing factories in Kenya.

Table 2.1: Sugar Factories and their Milling Capacity in Kenya

S/N	Sugar Company	County	Ownership	Capacity TCD	Status
1.	Miwani Sugar Company	Kisumu	Public	-	Under Receivership
2.	Ramisi Sugar	Kwale	Private	-	Closed
3.	Muhoroni	Kisumu	Public	2,200	Under Receivership
4.	Chemelil Sugar Company	Kisumu	Public	3,000	Milling
5.	Mumias Sugar Company	Kakamega	Public	8,400	Milling
6.	Nzoia Sugar Company	Bungoma	Public	3,000	Milling
7.	West Kenya Sugar Company	Kakamega	Private	4,200	Milling
8.	Sony Sugar Company	Migori	Public	3,000	Milling
9.	Soin Sugar Company	Kericho	Private	150	Closed
10.	Kibos Sugar & Allied Industries Limited	Kisumu	Private	3,500	Milling
11.	Butali Sugar Mill Limited	Kakamega	Private	3,000	Milling
12.	Transamara Sugar Company	Narok	Private	4,000	Milling
13.	Sukari Sugar Company	Homa Bay	Private	2,800	Milling
14.	Kwale International Sugar Company	Kwale	Private	3,300	Milling
15.	Ole Pito Sugar Company	Busia	Private	1,250	Milling
16.	Busia Sugar Industry	Busia	Private	1,500	Milling
TOTAL				**56,800**	

Source: Sugar Sub-Sector Profile, 2020

Sugar processing is associated with various value-added by-products, as depicted in Figure 2.3. Among them is SPM, a soft, spongy, lightweight, amorphous, dark brown to black material separated during the process of cane juice clarification by the sulphitation process. The clarification process separates the juice into clear juice that rises to the top and is redirected for sugar and mud extraction at the bottom. Just after that, the mud is filtered to separate the suspended matter, which includes insoluble salts and fine bagasse. According to Devia-Orjuela et al. (2019), SPM is produced at a rate of 0.01 to 0.07 tons per ton of ground sugarcane, resulting in an estimated 5,200–36,400 metric tons of SPM produced in Kenya according to 2020 sugarcane milling statistics.

SPM is either used as farm manure or mixed with vinasse in a drying and crystallizing process to produce bio-fertilizer. It can also be used to produce bioCH_4 or be used for metal absorption from wastewater (Gupta et al., 2011). However, improper management of SPM is hazardous to the environment and human life. For instance, SPM composts can be a source of blooming pathogenic fungi (Singh et al., 2019). Furthermore, when SPM is burned in the form of briquettes, it releases harmful SO_2 and SO_3 gases, which contaminate the air and contribute to ozone layer formation and depletion (Rouf et al., 2010).

SPM can be mixed with other organic fertilizers to improve the yield. Unfortunately, the resultant SPM affects human health when added directly to the soil, owing to the fast growth rate of disease-causing microbes (Diaz, 2016). The chemical composition of SPM generally depends on cane variety, soil condition, nutrients applied in the field, process of clarification adopted, temperature, precipitation aids, fineness of the filtration process, and other environmental factors (Reyes et al., 2015).

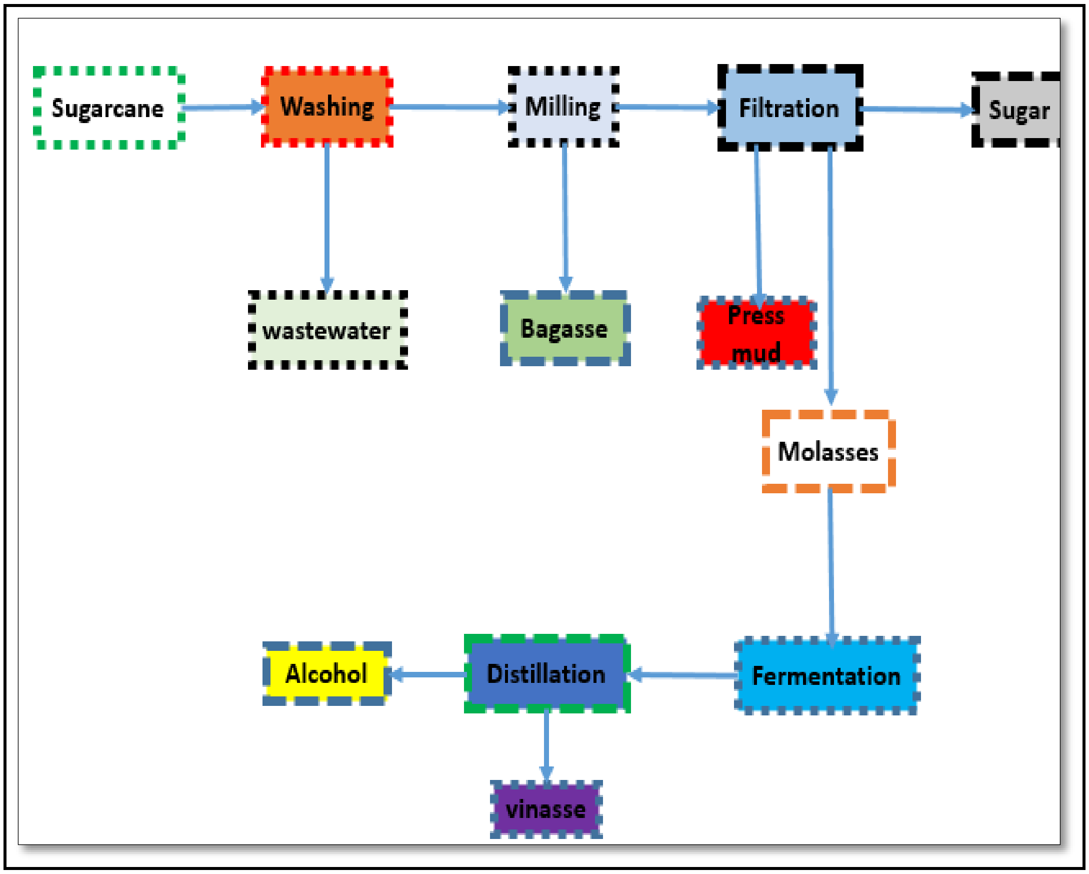

Figure 2.3: By-Products in Sugar Processing

Source: Bajpai (2017)

2.3 Anaerobic Digestion Process

In the AD process, the organic matter is broken down by a pool of microorganisms in the absence of oxygen (O_2) to form CH_4 and carbon dioxide (CO_2). Hydrolysis, acidogenesis, acetogenesis, and methanogenesis are the four master reactions involved in the AD process. In hydrolysis, complex organics such as carbohydrates, proteins, and FOG are broken down by acidogenic bacteria into sugars, amino acids, and LCFAs. Immediately, acidogenesis follows, converting the organics to acetic acid, H_2 and CO_2 by acetogenic bacteria. Acetogenesis closely follows; acetate and H_2 produced in these two reactions are substrates for methanogenic bacteria. Finally, CH_4 is produced by methanogenic bacteria in the last stage (Bajpai, 2017).

The methanogenic bacteria are extremely sensitive to changes in temperature and pH, and are vulnerable to small amounts of oxygen (Hejnfelt & Angelidaki, 2009). Inhibition of CH_4 production occurs through the accumulation of H_2 and VFAs in the reactor produced during acidogenesis. However, a symbiotic relationship exists between the acidogens, acetogens, and methanogens. The methanogens maintain the digester environment by consuming the H_2 produced. Hydrogen-scavenging bacteria function as CH_4-producing bacteria (Al Seadi et al., 2008). Furthermore, methanogens are the slowest-growing organisms, making them crucial in the AD process (Manser, 2015). Consequently, anaerobic treatment must meet methanogen requirements for efficient carbonaceous pollutant removal (COD). The schematic AD process is shown in Figure 2.4.

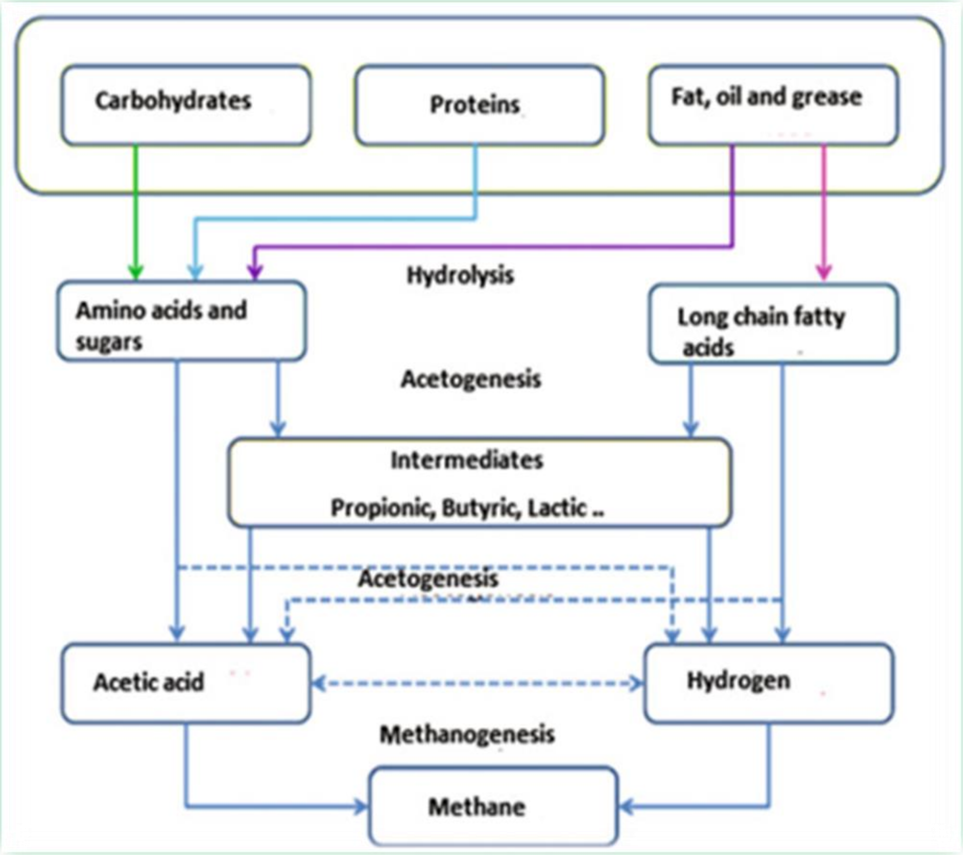

Figure 2.4: Anaerobic Digestion Process

Source: Bajpai (2017)

It is worth noting that AD is regarded as a valuable solution for handling degradable organic wastes since it accrues several benefits depending on the type of waste. In essence, this enables the reduction of solids through cost-effective and sustainable solid waste management and disposal. Moreover, pollution is controlled by stabilizing the organic solids prior to disposal, thereby avoiding uncontrolled decomposition, which can result in land, water, and air pollution. Furthermore, occupational hazards and disease transmission to humans and animals are reduced by pasteurizing waste for pathogen reduction prior to disposal on agricultural land. Besides that, agronomic values are enriched by both recycled nutrients and the organic fertilizer produced from digestate (Mugodo et al., 2017, Salehiyoun et al., 2019).

2.4 Anaerobic Co-Digestion

ACoD is the simultaneous fermentation of two or more substrates in a homogenous mixing proportion for CH_4 production. AMoD of SHWW is usually problematic in practice due to the presence of inhibitory compounds (Rhee et al., 2021). Also, the AMoD of SPM leads to rapid acidification and slow hydrolysis of cellulosic carbohydrates. This explains its low efficiency in bioavailability for bioconversion processes and thus low CH_4 production (Talha et al., 2017). The ACoD of these two organic biomasses helps in remediating the problems encountered during their AMoD.

ACoD enhances CH_4 production by establishing a positive synergism in the digestion medium, supplying and balancing nutrients necessary for the digestion process, and increasing buffer capacity due to a faster hydrolysis rate (Wu, 2010). In addition, a more versatile and robust microbial community is enhanced with a wider tolerance of operational conditions (Gonzalez et al., 2017). Moreover, a final sludge with the potential to be used as a bio-fertilizer is obtained (Salehiyoun et al., 2020). Also, co-digestion effectively utilizes wastes with high inhibiting components when co-digested with well-performing substrates. For instance, ACoD has been reported to minimize NH_3 inhibition through balancing of the C/N ratio by a co-substrate (Pratima & Ale, 2015; Reyes et al., 2015). The use of co-substrates could perhaps significantly improve the plant's overall economics due to equipment sharing and the utilization of available free space. Hence, this results in better digester performance with higher CH_4 yields. Some of the pros and cons of co-digestion are summarized in Table 2.2.

Table 2.2: Advantages and Disadvantages of ACoD

Pros	Cons
• Improved microbial stability	• Increased digester effluent COD
• Better nutrient balance and digestion	• Additional pre-treatment requirements
• Final sludge can be used as a soil conditioner	• Hygienisation requirements
• Existing infrastructure can be used	• Lack of knowledge about optimized mix ratio
• Reduced greenhouse gas emissions	• Requirement of wastewater treatment
• Renewable biomass disposable for digestion in agriculture generation	• Land usage restrictions for digestate
• Creates diversion opportunities (like usage of organic fraction of landfills)	• Additional mixing requirements
• Additional CH_4 collection	• High utilization degree required
• Equalization of particulate, floating, settling,	• Decreasing availability and rates
• acidifying, etc. wastes, through dilution by manure or sewage sludge	• Economically critical dependent on crop costs and yield
• Possible gate fees for waste treatment	

Source: Akunna (2018)

SHWW can be mixed with a wide variety of organic substrates in AD. In particular, SHWW has been previously co-digested with other organic wastes like poultry droppings, food waste, fruit and vegetable waste, etc. (Bouallagui et al., 2009; Porselvam et al., 2017; Rahman et al., 2022). However, SHWW has an unbalanced C/N ratio and low buffering capacity (Rhee et al., 2021). Therefore, the C/N ratio gets balanced when SHWW, which has enough nitrogen content, is mixed with SPM, which is rich in carbon (Bella and Rao, 2021). Furthermore, SPM has a higher buffering capacity and sufficient alkalinity, which aid in the maintenance of a stable pH in the presence of methanogens, and they contribute to a diverse range of nutrients for the growth of microorganisms (Mugodo et al., 2017).

Additionally, availability and quality of feedstocks, as well as the cost of obtaining, transferring, preparing (or pre-treating), and storing the extra feedstock, should all be considered in the ACoD. Consequently, ACoD can improve CH_4 production where the main feedstock source is in limited supply, thereby making single-feedstock digestion unsustainable. This is also applicable to AD firms located in geographically remote areas where the transportation cost of the main feedstock could be problematic and other types of locally available feedstock are supplemented. Therefore, the stable year-round operation of anaerobic digesters treating substrates that are seasonal by nature or during crop rotation can be secured (Akunna, 2018).

Furthermore, the chemical structure of co-digestion feedstocks should always be known as it aids in the maintenance of a proper balance of several parameters such as the C/N ratio, inhibitors, pH, micro- and macronutrients, and degree of biodegradability (Bella & Rao, 2021). Thus, rather than selecting mixture ratios at random, it is preferable to use an optimized ratio to achieve a higher CH_4 yield. Before selecting substrates for co-digestion, their composition should be thoroughly examined in previously published works. Overall, the most important benefits of an ideal co-substrate for SHWW include improved pH control, increased micro- and macronutrient supply, and decreased inhibitory effects of toxic compounds (Bella & Rao, 2021). Table 2.3 depicts previous works related to the ACoD of abattoir wastewater.

Table 2.3: Performance of ACoD of Slaughterhouse Wastes with Different Substrates

Source	Co-substrate	Operation Conditions	Improvements	Comments
Salehiyoun et al. (2019)	SHWW + WMS	Batch and CSTR; 37±1°C; HRT 18d, 13.5d, 11d; OLR 1.5 kg VS/m^3 d	50% CH_4 increase	For batch runs, increase in OLR (1.5-2 kg VS/m^3 d), increased both TVFA and NH_4^+-N concentration and a drop in pH, causing process inhibition.
Latifi et al. (2019)	Poultry SHWW + sewage sludge	Batch; 34±1°C; HRT 50d, 42d	63% VS removal; 88% COD reduction	Increasing TS and decreasing ISR led to accumulation of VFAs and high NH_3 concentrations hence lower CH_4 yields.
Rahman et al. (2022)	Poultry droppings + SPM	Batch and CSTR; 20-45 °C; HRT 20d	CH_4 increased by 8 and 29% in contrast to AMoD of PM and PD, respectively	The maximum CH_4 yields were found at 35–45 °C but yields were not found to be significantly.
Sounni et al. (2021)	OMW + SHWW	Batch and ASBR; 37±1°C; OLR 10 g COD/L/day; HRT 20d	Reactor degraded 10 g COD/L/day	At 5–6 g COD/L/day, biogas yield dropped, probably due to digester overloading and microbial pathway disruption.
Bayr et al. (2012)	SHWW + rendering plant	CSTR; 35 and 55±1°C; 1.0 and 1.5kg VS/m^3 day OLRs; 50 d HRT	262–572 mL CH_4/g VS added	1.5 kg VS/m3 d OLR, was unstable after operation of 1.5 HRT at thermophilic, due to accumulating NH_3, VFAs and probably also LCFAs
Panizio et al. (2020)	SHWW + OFI	CSTR; 38±1°C; OLR 64 g VS L^{-1} day^{-1}	57% (v/v) CH_4 increase	Inhibition of the biogas production process was observed in other reactors.

Bouallagui et al. (2009)	FVW + AWW	Single-stage ASBR; 35 and 55±1°C;20 d, 10d HRT; 2.56 g VS l^{-1} day^{-1} OLR	75% more CH_4 yield	At 10 days HRT results showed a decrease of biogas production rate for AW and AW + FVW digestion processes due to the high amount of free NH_3 at high OLR.
Monou et al. (2008)	AWW + PPWW and/or RPS	Batch; mesophilic temp; HRT 22 d	72% VS removal and 35 mL average daily CH_4 yield; 32% max CH_4	ACoD with AWW did not improve the digestion process due to poor buffering and low pH
Hailu et al. (2020)	Cattle AWW + FVW	Unstirred two-staged ASBR; 38±0.2 °C;	70.26% more CH_4 yield; 57.11%VS reduction	The relative reduced biogas production in some of the reactors was due to high FAN, fat floatation, and inadequate substrate–bacteria contacts.

SHWW- Slaughterhouse Wastewater; SPM- Sugar Press Mud; AWW- Abattoir Wastewater; FVW- Fruit and Vegetable Waste; waste mixed sludge (WMS); olive mill Wastewaters (OMW); PM-Poultry Droppings; SHWs-Slaughterhouse Wastes; OFI-Opuntia fícus-indica; PPWW-Potato Processing Wastewater; RPS-Raw Pig Slurry; CSTR-Continuously Stirred Tank Reactor; OLR-Organic Loading Rate; HRT-Hydraulic Retention Time; VS-volatile solids; ASBR- Anaerobic Sequencing Batch Reactors; ISR-inoculum-substrate ratio

2.5 Anaerobic Digestion Operation Efficiency Parameters

Co-digestion may not improve digestion on its own; it is dependent on variables such as pH and alkalinity, process temperature, waste organic content and biodegradability, type of start-up inoculum, organic loading rate (OLR), solids and hydraulic retention times, nutrient balance, inhibitor presence, and reactor mixing (Yenigün et al., 2013; Liu et al., 2017; Akunna, 2018; Rahman et al., 2022).

2.5.1. Temperature

The rate of digestion in the bioreactor is extremely sensitive to temperature. Microbial species are temperature-specific; therefore, they respond differently to abrupt changes in temperature (Van et al., 2020). Accordingly, microorganisms play a crucial part as indicators in the stability and failure of AD processes (Wang, 2021). Microbial species can grow and multiply in four temperature ranges: psychrophilic (20 °C), mesophilic (25–40 °C), thermophilic (45–60 °C), and hyperthermophilic (90–100 °C) (Manyi-Loh, 2013). Temperature influences not only the choice of structure of microbial communities inside reactors, but also the metabolic activities of bacteria, hydrolysis process kinetics, mass transfer, gas solubility in a digester, settleability of biological solids, thermodynamic equilibrium of biochemical reactions, metal bioavailability, and ultimately CH_4 yield (Panigrahi & Dubey, 2019; Van et al., 2020; Rahman et al., 2022).

Indeed, the two most important temperature ranges for CH_4 yield are mesophilic and thermophilic. Therefore, the decision to use either a mesophilic or a thermophilic temperature range depends on the net economic gain that each can provide. Nonetheless, most commercial plants operate at mid-mesophilic (35–37 °C) temperatures (Wandera et al., 2018). However, thermophilic fermentation is associated with several potential advantages. These include: a greater breakdown rate of organic solids; an improvement of solid-liquid separation; a higher metabolic rate; increased reaction rates, leading to the possibility of higher loading rates in addition to increased CH_4 production; a high specific growth rate of microbes; low HRT; a higher degree of pathogen deactivation; and a better hygienic effect (minimization of bacterial and viral pathogen accumulation) (Yenigun et al., 2013; Diaz, 2016; Liu et al., 2017; Meegoda et al., 2018; Panigrahi & Dubey, 2019). The bioreactor operates under autothermal conditions during thermophilic digestion, and the biogas produced can serve as a heat source as well as power generator (Amani et al., 2010).

However, there are a number of notable microbiological characteristics associated with thermophilic AD that may reduce reactor efficiency. Such features entail low bacterial growth, high endogenous death rates, a lack of diversity, and a much higher sensitivity to sudden temperature changes (Amani et al., 2010). In addition, the high rate of acidogenesis in thermophilic processes causes propionic acid accumulation in the bioreactor, which may limit methanogenic operation (Panigrahi & Dubey, 2019). Some other sources of worry in thermophilic AD are high energy demand and process instability, which may have a deleterious impact on energy balance and the overall AD process, respectively (Panigrahi & Dubey, 2019; Van et al., 2020). However, thermophilic systems are more susceptible to VFA accumulation than mesophilic AD, probably owing to variations in cell membrane structure. This inhibits methanogenesis and potentially decreases the pH-buffer system (Amani et al., 2010; Wang, 2015).

Nevertheless, mesophilic AD remains appealing because it uses less energy than thermophilic AD (Diaz, 2013; Meegoda et al., 2018). Even though mesophilic AD thrives at a reduced temperature, it lasts much longer and produces less CH_4. On the contrary, Wandera et al. (2018) recorded a higher CH_4 yield in mesophilic AD than in thermophilic AD for nitrogen-rich wastes. According to González-Fernández and Garca-Encina (2009), this is due to the fact that thermophilic AD operates at higher NH_3 levels, inhibiting methanogenesis and thus resulting in poor process stability.Furthermore, mesophilic AD seems to be able to sustain higher OLR (Bayr, 2012). Psychrophilic temperatures, on the other hand, are seldomly utilized because of the slow rate of biodegradation (Akunna, 2018). A few bioreactors are temperature-dependent and do not require heating; however, they frequently experience seasonal fluctuations in yield (Meegoda et al., 2018).

The potential benefits of thermophilic AD (i.e. higher hydrolysis and conversion rates) and mesophilic AD (i.e. higher process stability and effluent quality) are coupled in temperature-phased AD systems (Panigrahi & Dubey, 2019). This configuration employs a mesophilic digester as a polishing stage, thereby avoiding the drawbacks of the thermophilic process. This has been demonstrated to be a safe and efficient method of sludge stabilization that attains higher bioconversion rates and CH_4 yield than existing mesophilic AD systems (Amani et al., 2010; Panigrahi & Dubey, 2019).

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2.5.2. Organic Loading Rate

Organic loading rate (OLR) defines the correlation between the organic matter treatment rate and digester size, expressed as weight or organic matter in terms of COD or VS (or TS) added per volume of the reactor per day (Akunna, 2018). The amount of organic material served to a bioreactor in a day in continuous digesters is measured as OLR. Therefore, it is used to size digesters based on substrate and operating temperature (Wu, 2010).

The higher the OLR a system can efficiently treat, the higher the waste treatment capacity and CH_4 production, and thus the higher the system's economic viability. However, overburdening a bioreactor may cause problems if the feedstock is rapidly hydrolyzed and acidified, resulting in an overabundance of VFAs because of low levels of essential microbes that can possibly suppress methanogenesis and disrupt the AD process (Meegoda et al., 2018). Elevated OLR into the digester is coupled with system failure due to inhibitory compound formation, nutrient imbalance, acid buildup, and microorganism shock (Bella & Rao, 2021).

Moreover, significant and sudden changes in OLRs can alter the equilibrium of acidogenesis and methanogenesis in AD systems. Interestingly, low-performing reactors encourage the formation of microbial consortiums that are ideally equipped to deal with severe organic loading (Amani et al. 2010). Therefore, optimum OLR is crucial, especially when dealing with SPM, which is high in ash content. For instance, at higher OLR, SPM may result in elevated concentrations of mud within the CSTR that can possibly hinder the AD process (Liu et al., 2012; Talha et al., 2017). Nevertheless, high-rate digesters usually withstand high OLR and NH_3 concentrations that could exist without signs of system failure because bacteria could be acclimated to NH_3 if the system was fed at a slowly increasing concentration (Yenigun et al., 2013).

Besides these effects, OLR is a critical factor in the proper operation of circulating and feeding pumps. Pump wear and tear may be accelerated by solid contents. The operating solids retention time (SRT) at industrial level is typically higher (nearly double) than at laboratory level. Perhaps the reason is to alleviate inhibition issues (Panigrahi & Dubey, 2019). Previously, it was shown that anaerobic digesters operating at a higher OLR experienced a decrease in pH, COD, and CH_4 production rates (Escudero et al., 2014; Musa et al., 2018).

Furthermore, abrupt changes in OLR induce microbial population shifts, with CH_4 yields reverting to normal rates upon establishing tolerance to higher loading rates (Meegoda et al., 2018). It has been envisioned that enhanced diversification of methanogens results in better digester efficiency and resistance to overloading after an initial instance of overloading (Meegoda et al., 2018).

2.5.3. Solids and Hydraulic Retention Time

The retention time, defined as the mean time that the feedstock is retained in the digester, governs the design and operation of the anaerobic digester. The retention time must be sufficient to guarantee the accomplishment of all fermentation steps for which the digester is responsible. Because the feedstock components in a wet reactor are frequently differentiated into liquid and solid material properties, the retention time is classified into SRT and HRT.

The SRT is the average time the substrate spends in the digester. This is an essential factor that verifies the extent of LCFA accumulation and thus aids in prolonging lipid conversion, which tends to affect methanogenic activity. SRT is directly affected by microbial growth levels and the rate at which excess microbial biomass is removed from the digester. Methanogens, for example, grow at a slower rate compared to any other microbial group in AD. As a result, slow-growing microbes that aid in the degradation of LCFAs benefit from high biomass SRT (Panigrahi & Dubey, 2019).

Elevated SRT also mitigates the effects of shock loadings by providing adequate buffering capacity and encouraging microbial adaptation to inhibitory compounds (Meegoda et al., 2018). Some studies have found that longer SRTs of 30 days or more result in better effluent quality, higher reactor efficiency, and increased CH_4 production (Talha et al., 2017). Moreover, temperature influences biodegradation rate, microbial regeneration time, and SRT. As a result, thermophilic digesters typically have a shorter SRT than mesophilic digesters (Akunna, 2018). Hydraulic retention time (HRT) is the average time that soluble feedstock remains in a reactor and is calculated as the quotient of reactor volume, m^3, V, and average reactor feeding rate, m^3/day, Q, as simplified by Equation 2.1.

$$\theta = \frac{V}{Q} \tag{2.1}$$

To allow for operation stability, the HRT should be kept at a value that is roughly two times greater than the generation time of the slowest microbial growth, i.e., methanogens

(Amani et al., 2010). Nevertheless, the HRT may vary due to substrate modifications or because of temperature variations. As a result, the ideal value depends on technology, process details, temperature, and waste composition (Amani et al., 2010). To eliminate dead zones, the HRT should be maintained for a sufficient amount of time. For instance, short HRT means a slow growth rate of methanogens, which can cause washout of important microbes and accumulation of VFAs that inhibit methanogens, thereby permitting process instability (Wandera et al., 2018). Moreover, shorter HRT will also aid in the reduction of operational costs and reactor size. As a result, it is critical to determine whether the only disadvantage of using shorter HRTs is the microorganisms' regeneration time (Akunna, 2018). Lowering HRT gradually at regular intervals while maintaining constant substrate concentration promotes microbial consortium growth, whereas abruptly lowering HRT causes biomass washout (Meegoda et al., 2018).

Acidogenic and methanogenic microbes, for example, grow at various rates. In comparison to acidogenic microbes, methanogenic bacteria grow quickly. Therefore, controlling the growth period of both kinds of microbes in single-stage digesters is critical. That's because the acidogenic microbes like lower pH but shorter HRT, which are both harmful to methanogens (Amani et al., 2010; Bella & Rao, 2021).

Several studies have recommended longer HRT for the fermentation of lignocellulosic biomass (Nwokolo et al., 2019) to provide adequate time for the recalcitrant structure to hydrolyze. Similarly, Talha et al. (2017) discovered that 15 days of HRT was best for SPM AD. Mesophilic digestion can usually be completed in 15–30 days. However, the optimum benefit is discovered in bioreactors with a low OLR and an elevated HRT (Meegoda et al., 2018). All of these explanations prove that HRT effects degradation performance, CH_4 yield, and the distribution of microbial consortiums.

2.5.4. pH and Alkalinity

A solution's pH value, or hydrogen ion level, suggests whether it is acidic or basic. The pH condition has a significant impact on the process stability (Amani et al., 2010). The pH value is an excellent predictor of the fermentation process in the reactor. Microorganisms are normally sensitive to certain pH environments. Acidogenic, acetogenic, and methanogenic microbes, in particular, prefer different pH ranges for growth and development. Slow-growing methanogens, for example, are extremely sensitive to pH (7.5-8.5); however, fast-growing acidogens grow in an optimum pH range of 5.2-6.5, while acetogens are generally less sensitive and can function in a pH range of 6.6-7.6. (Bella & Rao, 2021). However, previous research indicates that a neutral pH of around 6.7–7.6 is favourable for the efficient operation of an anaerobic digester, which is achieved through the buffering ability of different components within the digester (Akunna, 2018).

Acids will accumulate as fermentative microbes grow faster than methanogens, reducing the pH. AD in two stages is an ideal solution. Even when using a two-stage anaerobic digester, if the pH is not regulated properly, the volume of gas generated is minimal. Occasionally restoring pH to an optimal level does not reinstate reactor stability unless reseeding is performed. Inadequate buffering capacity will also result in poor yield (Meegoda et al., 2018; Sakarika et al., 2020). To address low pH in the system, feeding could be suspended to allow the methanogens time to minimize VFA concentrations, or alkali chemicals could be added to control pH when it falls below 6.5 to provide additional buffering capacity (Talha et al., 2017; Qamar et al., 2017).

Since lime is cheap and readily available, it is popularly used to adjust the pH. However, excessive lime addition does not help raise pH because it produces insoluble sodium bicarbonate, which is ineffective at neutralizing excess VFAs (Bella & Rao, 2021). Furthermore, salt toxicity reduces bacterial activity (Yenigun et al., 2013). Alternatively, codigestion in a suitable proportion has proven to prevent a drastic decrease in pH (Karki et al., 2021; González et al., 2017; Wu, 2007). Furthermore, Pratima and Ale (2015) reported that slaughterhouse waste was able to buffer itself and prevent acidification during digestion due to its adequate alkalinity.

Therefore, because of these distinct microbial requirements, pH and OLR need special control in a one-phase bioreactor. At low or high OLR, VFAs accumulates due to the slower methanogenesis rate, resulting in an acidic pH in the digester. Feedstock composition is a critical factor in the digester's performance since nitrogenous feedstock emits ammonium hydroxide during methanogenesis, causing an increase in the pH of the digestion media, and hence resulting in an alkaline pH in the digester (Hutňan et al., 2010).

Furthermore, lipid-rich effluents such as SHWW boost acidogens growth in anaerobic environments, resulting in increased VFA generation and accumulation and a rapid pH drop. The combination of this pH drops and low bicarbonate alkalinity causes acid inhibition of methanogens, resulting in reactor breakdown (Palatsi et al., 2011). Furthermore, an increase in pH above 8.5 results in VFAs accumulation, dropping the pH, which inhibits methanogenesis. Accordingly, under an optimal pH range, alkalinity aids in the control of potential VFA accumulation and improves reactor stability (Madsen et al., 2011).

2.5.5. Start –Up Inoculum

The start-up or restart period of an anaerobic reactor is typically the most crucial stage, and its effectiveness is determined by the origin of the microbes, the size of the inoculum, and the initial operation mode. The start-up times, quantity of inoculum needed, and fermentation efficiency will vary based on the type of inoculum used (Amani et al., 2010).

Seeding is necessary in the AD process because it plays an important role in initiating reactions and degrading organic solid particles. An adequate number of active microbial populations ought to be present in the seeding sludge. As a result, active anaerobic bioreactors, especially those that treat similar kinds of waste, could be a good source of inoculum. In general, digested material from a pre-existing reactor or ruminant manure (cow dung slurry) could be used as seed for digested feedstocks (Amani et al., 2010). Anaerobic reactors are typically started with heavy seeding (at least 10% of the reactor volume or waste VS) or by keeping the waste pH between 6.8 and 7.2 to promote the natural growth of a relevant microbial population, resulting in both shorter and longer (up to 30 days) start-up times.

The amount and type of the active methanogenesis inside the digester greatly affect the AD process. During the start-up period, the lag-phase is reduced by inoculating anaerobic digesters with active anaerobic microorganisms. Moreover, the kind of feedstock and operating conditions of the source affect the microbial characteristics of the inoculum (Akunna, 2018). To avoid byproduct inhibition, an optimal mixture of inoculum and substrate based on VS content rather than weight or volume is required (Panigrahi & Dubey, 2019). A low inoculum-to-feed ratio may favor acidogens over methanogens, resulting in a low pH. In the event that this happens, recovery depends on system alkalinity. Where alkalinity is poor, for example, a chemical buffer may be introduced to the feedstock to prevent system breakdown (Qamar et al., 2022). Furthermore, fresh feedstock is usually pre-inoculated with some of the digested residues in batch and plug-flow reactors, whereas for dry sludge, AD inoculum-to-feed ratios greater than 10 are recommended during start-up (Akunna, 2018).

The mixing intensity and OLR have a strong connection during startup. If elevated OLRs are required during digester startup, using minimally mixed conditions will result in a faster start-up and better long-term efficiency. Nonetheless, there is no significant relationship between OLR and the abundance of the individual microbes in minimally mixed systems, implying that minimal mixing promotes balanced degradation for a wider range of OLRs during startup (Amani et al., 2010). Therefore, the bacterial population must include a sufficient number of methanogens.

2.5.6. Toxicity and Inhibition

Compounds in waste that are poisonous to microorganisms can disrupt AD processes. A substance's toxic effects on microorganisms are determined by its nature, concentration, and the extent to which the process has become acclimated to the substance (Karki et al., 2021). Toxicity can be reduced by lowering the pH and temperature, diluting with water, and/or increasing waste (Akunna, 2018). Inhibition is typically characterized by a reduction in the microbial population and CH_4 yield, the absence of H_2, the accumulation of VFAs, and a decrease in pH (Amani et al., 2010). NH_3 and hydrogen sulfide are the most common microbial inhibitors (H_2S).

Overall, NH_3 is a gas toxic to microbes, animals, and humans. Free NH_3 (1700 mg/L) is the most toxic because it can pass through a cell membrane, causing a proton imbalance and potassium deficiency (Yenigun et al., 2013). Ionic NH_4^+ is less toxic; a concentration of around 5000 mg/L affects acidogens and decreases the activity of methanogens (Palatsi et al., 2011). An increase in pH and temperature increases the level of NH_3 toxicity due to a higher ratio of free NH_3 to its ionized (NH_4^+) form (Amani et al., 2010). Consequently, pH control by alkali treatment is necessary in the AD system to stimulate bacterial growth and ensure process optimization. However, high salt content can severely inhibit microbial growth (Lazor et al., 2010). Salt accumulation is harmful to microbes because of the excessive increase in osmotic pressure caused by water flow across the cell membrane, which can result in cell death (Akunna, 2018).

At a pH of 7.0, sulfides are present as H_2S, which is odorous, harmful to humans, animals, and microorganisms, and corrosive to metals. Sulfate is less toxic to methanogens. H_2S, on the other hand, is known to diffuse into a cell membrane and denature native proteins via sulfide and disulfide cross-linking between polypeptide chains (Amani et al., 2010). Sulfate-reducing bacteria survive in the presence of sulfates and sulfides, which inhibit methanogenesis (Akunna, 2018). Sulfide inhibition, on the other hand, can be reduced by diluting the influent waste, adding iron salt to the treatment system to precipitate sulfide from solution, stripping the reactor liquid or scrubbing and recirculating the reactor biogas, and biological treatment of the reactor biogas.

2.5.7. Nutrient Availability and Balance

Carbon (N) provides energy to anaerobic bacteria and is naturally abundant in organic wastes, whereas nitrogen (N) increases the microbial population and is regarded as the limiting nutrient for the AD process (Rhee et al., 2021). The C/N ratio indicates the NH_4^+ -N released, the VFAs accumulated within the reactor, and the substrate's nutrient concentration (Panigrahi & Dubey, 2019). The C/N ratio is a key factor of the AD process because a value that is too low or too high will either slow down or stop the process entirely (Bouallagui et al., 2009). For instance, if N is abundant, methanogens will consume it quickly, and decreased bacterial growth due to N deficiency results in low biogas yield. Conversely, low N value results in NH_3 inhibition (Palatsi et al., 2011).

Generally, wastes rich in protein have a low C/N ratio, whereas lignocellulosic biomasses, particularly SPM, have a high C/N ratio due to their high C content (Wu, 2007). SHWW's low C/N ratio influences a high protein solubilisation rate, resulting in high NH_3-N and VFA concentrations within a system (Rhee et al., 2021). A C/N ratio of 20:1-30:1 has been reported in the literature to be suitable for successful bacterial growth in an AD system (Mata-Alvarez et al. 2014). Because these ratios are not always available, they should be mixed with other suitable substrates. It is usually preferable to combine waste with low and extreme C/N ratios to achieve optimal C/N ratios. Feedstock with a high C/N ratio is advised.

2.5.8. Biodegradability of Organic Waste

The recalcitrant structures of lignin, cellulose, and hemicellulose that are not easily hydrolyzed by enzymes may limit biodegradability. Lignin has been proven to be the most important factor limiting lignocellulosic material biodegradability (Nwokolo et al., 2020). Prior treatment or pretreatment may be required for these compounds (Meegoda et al., 2018; Kamusoko et al., 2019; González et al., 2020; Ripoll et al., 2022).

Hydrolysis of particulate biodegradable organic matter is a relatively slow biological reaction for some compounds, and it may be the process-limiting step in the treatment of high solid wastes. Such wastes typically necessitate longer retention times in order to achieve high levels of treatment and CH_4 production. In contrast, if the organic constituents are predominantly soluble in nature, high levels of biodegradation can be achieved with shorter retention times (Akunna, 2018).

2.5.9. Mixing

Proper mixing aids in the blending of the mixture, allowing for uniform distribution of substrates, microbes, and enzymes; complete waste stabilization; prevention of scum formation; avoidance of grit particle deposition; chemical uniformity; increased CH_4 production and more efficient pathogen destruction; and prevention of thermal gradients in the digester (Bella & Rao, 2021). Only proper mixing allows for sufficient interaction between the microbes and the essential minerals. Violent mixing, on the other hand, causes shear stress on the cell walls and disturbs slow-growing microbes (Amani et al., 2010). Furthermore, the effects of combining duration and intensity on AD performance are contradictory. This suggests that excessive and continuous mixing may have a negative impact on digester stability, altering the prevailing desirable conditions in some cases. This suggests that excessive and continuous mixing may have a negative impact on digester stability, altering favorable reactor environments such as operating pH, moisture levels, and so on (Bella & Rao, 2021).

Another critical consideration is the mode of mixing. Mixing techniques include mechanical agitation, pumped circulation, and gas circulation (Panigrahi & Dubey, 2019). Pumped circulation has some drawbacks, such as rag clogging, impeller grit wear, and bearing breakdowns. Gas circulation is an effective mixing technique that reduces scum buildup and granule disintegration while also improving dewaterability (Bella & Rao, 2021). In terms of energy efficiency in different mixing regimes, gas mixers consume more energy than mechanical recirculation of digester contents. When the mixing techniques are compared, mechanical mixing is found to be the most cost-effective, followed by gas circulation, and finally pumped circulation (Panigrahi & Dubey, 2019).

Continuous mixing, intermittent mixing, and minimal mixing are some of the mixing strategies available (Amani et al. 2010). Continuous mixing has been proven to produce more CH_4 than unmixed conditions. A non-mixed reactor configuration encourages tight microbial community proximity, which improves microbial interactions and VFA degradation (Hailu, 2020).

2.6 Kinetic Modelling

Despite the growing popularity of AD, thorough and practical modeling of these systems remains a work in progress. The kinetics of an AD process provide critical details for the analysis, design, and operation of a digestion system. Kinetic models calculate the cumulative CH_4 production potential, hydrolysis rate, lag time, and maximum CH_4 yield (Kafle & Kim, 2012; Kafle & Chen, 2016; Wandera et al., 2018). The ideal kinetic model should be chosen not just to estimate the performance of specific digesters, but also to accurately assess the metabolic pathways and factors underlying substrate AD (Pramanik et al., 2019). As a result, an appropriate model for system augmentation and long-term AD operation is required (Nguyen et al., 2019; Rahman et al., 2022). Nonetheless, each of the kinetic models has distinct advantages. Various kinetic models have been employed to fit the experimental cumulative yield in both batch and continuous digesters.

2.6.1. Chen and Hashimoto Model

Chen and Hashimoto used this model, shown in Equation 2.2, to simulate cattle manure fermentation in a completely mixed system without solid reuse.

$$F_{(t)} = F_O . (1 - \frac{K}{R_{max} .\ O - 1 + K}) \qquad (2.2)$$

Where:

$F_{(t)}$ = the cumulative CH_4 production ($mLCH_4/gVS$),

F_O = the CH_4 production potential ($mLCH_4/gVS$),

R_{max} = the maximum specific rate of CH_4 production ($mLCH_4/gVS/h$)

k = the kinetic parameter dimensionless.

Because the Chen and Hashimoto model best fits both continuous and batch experimental data, it is often utilized to estimate the volumetric CH_4 production rate in the AD system. The retention time (O) is critical in assessing AD activities (Kafle & Kim, 2013; Kafle et al., 2016). This model was successful in forecasting CH_4 yield from pig manure (Pham et al., 2014). However, in the kinetic study of AD of the solid fraction of piggery slurries, this model proved inadequate to represent the actual situation (Andara & Esteban, 1999).

2.6.2. Transfer Function Model

A transfer function model (Equation 2.3) may be employed to determine the CH_4 potential and maximum CH_4 production rate as well as lag phase (λ), which is a significant factor for assessing the efficiency of AD processes (Zhang et al., 2015). Furthermore, mostly in the presence of potentially harmful substances or recalcitrant biomass, a transfer function model could provide good fitting performance (Bohutskyi et al., 2018).

$$F_{(t)} = F_O \left\{ 1 - \exp\left[\frac{-R_{max}(t - \lambda)}{F_O} \right] \right\} \tag{2.3}$$

Where:

$F_{(t)}$ = the cumulative CH_4 production ($mLCH_4/gVS$),

F_O = the CH_4 production potential ($mLCH_4/gVS$),

R_{max} = the maximum specific rate of CH_4 production ($mLCH_4/gVS/h$),

λ = the lag phase period (h),

t = the incubation time (h).

2.6.3. Cone Model

The cone model presented in Equation 2.4 is an estimation technique capable of determining the CH_4 production rate and maximum cumulative CH_4.

$$F_{(t)} = \left(1 - \frac{F_O}{1+(K_t)^{-n}}\right) t \tag{2.4}$$

Where:

$F_{(t)}$ = the cumulative CH_4 production (mLCH$_4$/gVS),

F_O = the CH_4 production potential (mLCH$_4$/gVS),

k = the kinetic parameter dimensionless,

n = the shape factor,

t = the incubation time (h).

Furthermore, the model can predict the CH_4 yield trend based on the shape constant. Its sigmoidal shape curve may adequately define the lag period, exponential stage, and steady phase of the AD process (Nguyen et al., 2019). Several studies have found that the cone model is ideal for ACoD (Zhen et al., 2015; Zhang et al., 2019).

2.6.4. First- Order Kinetic Model

The basic model often used to describe the AD of recalcitrant biomass is a first-order kinetic model (Equation 2.5).

$$Y = A.(1 - \exp[-K_t]) \tag{2.5}$$

Where:

Y = the cumulative CH_4 production (mLCH$_4$/gVS),

A = the CH_4 production potential (mLCH$_4$/gVS),

k = the kinetic parameter dimensionless,

t = the incubation time (h).

A first-order kinetic model provides a simple foundation for comparing the efficiency of stable processes in real-world situations. This model provides more details on the hydrolysis rate constant and assumes that hydrolysis governs the whole process and also that substrate availability is the determining factor (Zhang et al., 2015). As a result, the model can evaluate the hydrolysis rate (Zhen et al., 2015). The model, however, does not forecast the optimum conditions for biological processes, lag phases, or system breakdowns, but instead only investigates the exponential phase of CH_4 production (Kafle and Chen, 2016). Several authors have successfully applied a first-order kinetic model to simulate the CH_4 digestion of various biomass (Gonzalez et al., 2014; Kafle & Chen, 2016; Wandera et al., 2018; Zhang et al., 2019). Nguyen et al. (2019) found that the cone model fit the exploratory data more accurately than the first-order kinetic model.

2.6.5. Logistic Model

The logistic model (Equation 2.6) presumes that the rate of yield is equal to the CH_4 volume already produced, as well as the highest rate of production and potential of yield, and it can fit the initial dramatic growth before attaining a steady state (Wang et al., 2021).

$$F_{(t)} = \frac{F_O}{1+\exp\left\{\frac{4.R_{max}.(\lambda-t)}{F_O}+2\right\}} \tag{2.6}$$

Where:

F (t) = the cumulative CH_4 production (mLCH_4/gVS),
F_O = the CH_4 production potential (mLCH_4/gVS),
R_{max} = the maximum specific rate of CH_4 production (mLCH_4/gVS/h),
λ = the lag phase period (h),

t = the incubation time (h)

In addition to the specific and cumulative yield, the period of the lag phase (λ) is a key determinant of AD performance. The logistic kinetic model, on the other hand, is one of the complex models that is frequently used to evaluate. The logistic function model is appropriate for an initial exponential rise and final stabilization at maximum output, assuming that the rate of yield is equal to the amount of CH_4 already generated (Wang et al., 2021). Similarly, the logistic model perfectly fits the practical CH_4 production in cattle manure AD (Wang et al., 2021). The logistic function also emerged as the model with the best match in terms of CH_4 production and the lag phase in the AD of food waste (Parra-Orobio, Donoso-Bravo, & Torres-Lozada, 2017). On the contrary, the Logistic model did not fit experimental data for AD of both sludge and wastewater recycled pulp and paper (Bakraoui et al., 2020).

2.6.6. Modified Gompertz Model

In a batch experimental setup, the modified Gompertz model (Equation 2.7) could accurately simulate the accumulation of CH_4 from AD, assuming that CH_4 yield is roughly equal to bacterial metabolism (Kafle & Kim, 2013).

$$F_{(t)} = F_o . \exp\left\{-\exp\left[\frac{R_{max} . e}{F_o}(\lambda - t)\right] + 1\right\} \tag{2.7}$$

Where:

F (t) = the cumulative CH_4 production ($mLCH_4/gVS$),

F_O = the CH_4 production potential ($mLCH_4/gVS$),

R_{max} = the maximum specific rate of CH_4 production ($mLCH_4/gVS/h$),

λ = the lag phase period (h),

t = the incubation time (h)

This model is able to accurately predict the lag time in CH_4 production data. Lag phase is a crucial factor in assessing AD efficiency since it reflects the shortest time required to generate CH_4. The model also employs a classic sigmoidal curve, which is commonly used to define microbial development, and CH_4 yield is thought to be a function of microbial development (Kafle & Kim, 2013). This kinetic model also provides the CH_4 yield potential and maximum CH_4 production rate, which are both crucial for assessing the AD process. These parameters are critical in AD modeling for recalcitrant organic feedstocks like lignocellulosic materials (Bohutskyi et al., 2018). This model is preferred because it has been frequently used in recent years to explain and estimate the kinetics of yield in AD processes (Nguyen et al., 2019; Wandera, 2018; Bohutskyi et al., 2018).

2.6.7. Anaerobic Digestion Model 1 (ADM 1)

The innovation of Anaerobic Digestion Model 1 (ADM1) was a great step in kinetic modeling (Sun et al., 2021). This well-organized model includes numerous stages, with at least 26 dynamic state variables, and a plethora of parameters for defining biochemical and physicochemical processes (Ozgun, 2019). Even though the ADM1 model reflects the complexity of anaerobic processes, practical applications for modeling and control are tricky to use. Identifying model variables in real-world operating conditions is also difficult. The ADM1 model also falls short of depicting all of the complex events that occur in an anaerobic digester; thus, it must be expanded to include additional phenomena (Sun et al., 2021).

2.7 Summary of Literature and Research Gap

Comprehensive studies have been done on the ACoD of SHWW (Rahman et al., 2022; Sounni et al., 2021; Hailu et al., 2020; Salehiyoun et al., 2020; Latifi et al., 2019; Panizio et al., 2019; Monou et al., 2008). They have evaluated performance of the ACoD of abattoir wastewater with different organic substrates based on different OLRs, temperature conditions, reactor mixing, pH, and reactor configuration. However, they did not explore more on ideal mix ratio and the optimum HRT for ACoD of SHWW. Therefore, the influence of various mixing proportions in the ACoD of SHWW and SPM on CH_4 production and organic removal was determined in this study.

2.8 Conceptual Framework

The conceptualization of the dependent and independent variables in the anaerobic treatment performance of SHWW process are well illustrated in Figure 2.5.

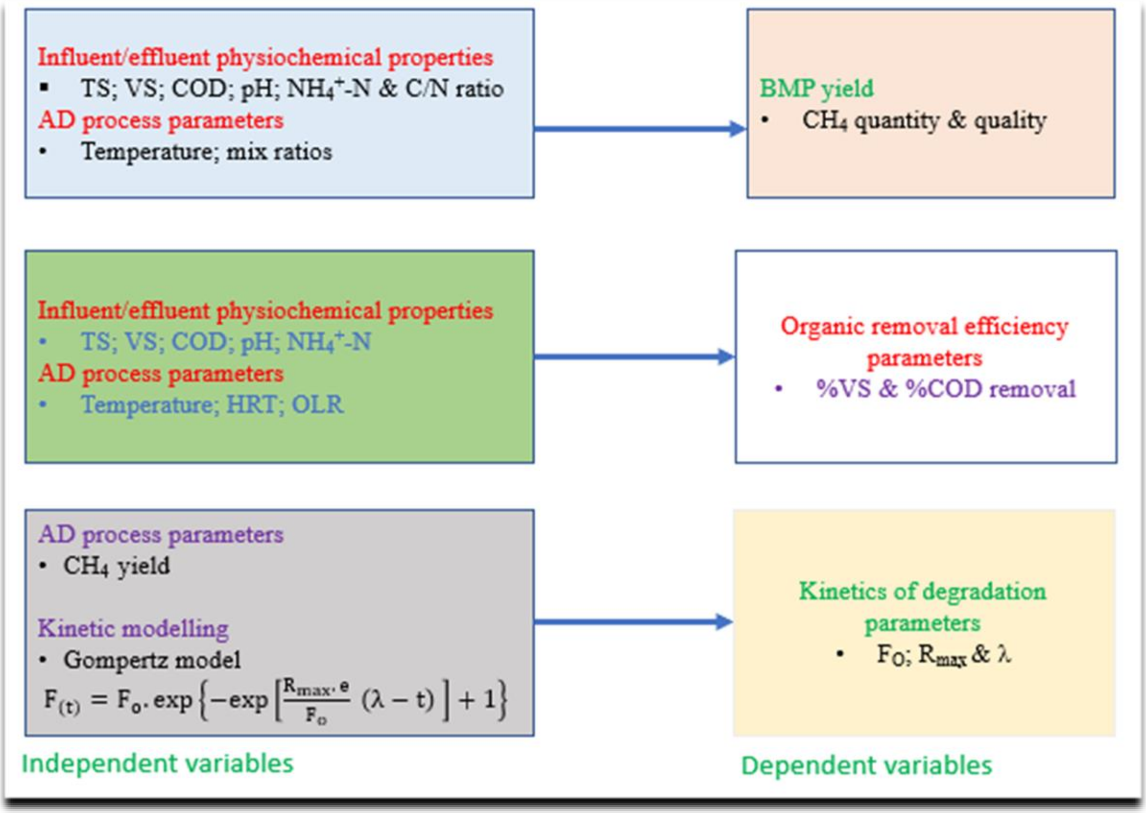

Figure 2.5: Conceptual Framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

This study was a quantitative research strategy that took the form of an experimental design and was concerned with systematic manipulation of percentages of SHWW and SPM in determining the best mix ratio. For the purpose of this study, the physio-chemical analysis of these mixes was determined. Moreover, BMP of these mixes was determined under batch experiment and the treatment efficiency of the best mix determined under continuous experiment at different HRTs. Also, the BMP data was simulated using Gompertz model to determine kinetic parameters using the statistical SPSS software (IBM SPSS Statistics 17 (2008)). Descriptive statistical tools such as mean, standard deviation, root mean square error (RMSE) and the coefficient of determination (R^2), were also employed for each reactor to compare the accuracy of the studied model. On the other hand, one-way ANOVA was used as inferential test set at 0.05 alpha levels of significance.

3.2 Seed Sludge and Substrates

The SHWW samples were collected from a cattle abattoir in the outskirts of Juja town in Kiambu County, Kenya. While, the SPM was collected from Busia Sugar Industry (BSI), in Busia County located in western Kenya. SHWW samples were tapped at the discharge drain before contamination. Samples of SPM were collected while in their fresh state directly from the production line, packed and transported in a cool box to JKUAT, within 24 hours. In the laboratory, the SPM was pre-processed to reduce the particle size and increase surface area for ease of feeding and further, fasten the biodegradation process.

For easy feeding into the reactors, SPM was sieved through 0.42-mm sieve and added to distilled water to 6% total solids (TS). Mixture of 10% distilled water and 90% anaerobic sludge obtained from an active mesophilic (37 °C) biogas digester treating dairy manure was used as inoculum. Until feeding, all the feedstocks were labeled, sealed, and refrigerated at 4 °C to minimize undesirable fermentation processes. Table 4.1 under results and discussion section summarizes the raw SHWW, SPM, mixed feedstocks and inoculum physicochemical characteristics, from the analyses undertaken.

3.3 Evaluation of the Methane Yield at Various Mixing Proportions

One of the most well-known methods for assessing the CH_4 potential, and biodegradability of wastewater and waste biomass is the BMP test (Filer, Ding & Sheng Chang, 2019). The influence of SPM addition as a co-substrate on the performance of AD of SHWW was studied by mixing the SHWW with the SPM in different mixing ratios as recommended by previous studies (Salehiyoun et al., 2020; Panizio et al., 2020). These sequential mixing ratios were represented by R1; R2; R3; R4; R5, R6 and R7, respectively (Table 3.1). These proportions were tested in batch experiment under mesophilic condition (37±1.0 °C) for 66 days with the intention of looking for the mixing ratio with the optimum performance.

In the current study, BMP tests were prepared according to the procedure used by Wandera et al. (2018). The batch assays were conducted using 125mL glass digesters with a working volume of 80mL sealed with rubber stoppers. The blending ratios between SPM and SHWW and the substrate to inoculum ratio on a VS basis used in this study are presented in Table 3.1. SPM was sieved to a particle size of 0.42mm and dissolved with distilled water to achieve the target TS concentration of 6%, on a wet weight basis and then fed into the digesters after fully mixing. Control and each mix ratio were conducted in triplicate.

Before commencement, all reactors were purged with nitrogen gas for around 5 minutes to get rid of air from the headspace and to help ensure an anaerobic environment. These lab-scale digesters were subsequently placed into a lab incubator (Model SV-05E/09E/23E, Lab Companion, Isuzu Seisakusho Co., Ltd., Japan) at a mesophilic temperature of 37.0 ± 1.0 ^{o}C. The digesters were shaken manually twice every day for about 1-2 minutes. Figure 3.1 provides a breakdown of the BMP test method used in the current study.

Table 3.1: Mix proportion of the substrates (SHWW and SPM)

Reactor ID	Mix Ratio	AD Type	Operation mode	Remarks
R1	100%SHWW+0%SPM	AMoD	Batch	Test digester
R2	80%SHWW+20%SPM	ACoD	Batch	Test digester
R3	60%SHWW+40%SPM	ACoD	Batch	Test digester
R4	50%SHWW+50%SPM	ACoD	Batch	Test digester
R5	40%SHWW+60%SPM	ACoD	Batch	Test digester
R6	20%SHWW+80%SPM	ACoD	Batch; Continous	Test digester
R7	0%SHWW+100%SPM	AMoD	Batch	Test digester
R8	90%Inoculum+10%Water	AMoD	Batch; Continous	Control digester

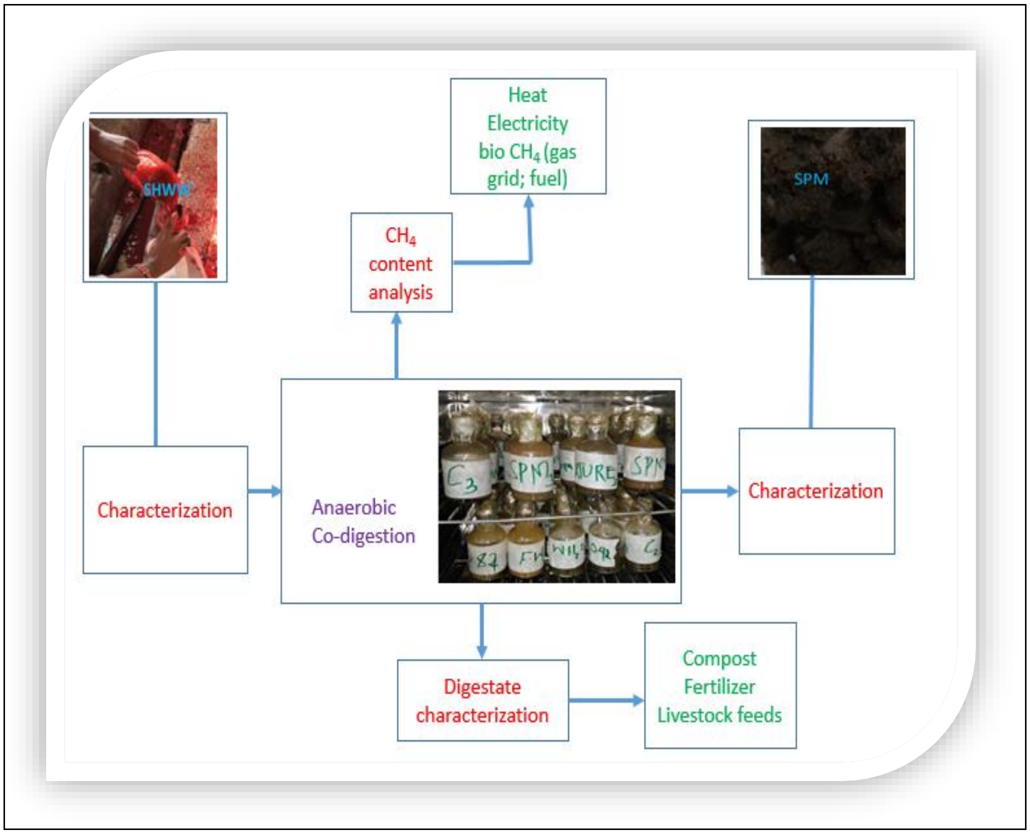

Figure 3.1: The Schematic Illustration of the Batch Experimental Set-up

3.3.1. Analytical Techniques

Before feeding into the respective reactors, the homogenized inoculum and substrates were characterized in triplicate and the resultant mean values recorded accordingly. The TS and VS were determined according to Standard Methods for Examination of Water and Wastewater (APHA, 2017). For COD analysis, the closed reflux technique was used. The pH readings were taken from the samples directly via a portable pH meter (pH3210, Germany). The Nessler method was used to measure $NH_4^{+-}N$ concentration and was determined using a Shimadzu UV-VIS-1800 spectrophotometer (DR 2500, Hach, USA). The pre-processed feedstocks (SHWW and SPM) were characterized for Carbon (%C), hydrogen (%H), Oxygen (O %) and nitrogen (%N) contents using elemental analyzer (EA 1112 Flash CHNS/O-analyzer). The composition and volume of the biogas were measured using gas analyzer (Geotechnical instrument (UK) Ltd, S/N: BM14068) and airtight syringe, respectively.

The daily produced CH_4 volume from each reactor was measured using a gas-tight syringe, and then converted to the volume under standard temperature and pressure (STP, 0 ^oC and 101 kPa). The biogas content was analyzed for CH4 (CH_4, %), and carbon dioxide (CO_2, %) using a gas chromatography (GC 7890 A, Agilent, Santa Clara, CA 95051, USA) fitted with a thermal conductivity detector, and a stainless-steel column (13803-U, Sigma–Aldrich, Saint Louis, MO, USA). The splitless inlet, oven, and TCD detector temperatures were all kept at 60, 70, and 200 oC, respectively. The CH_4 and CO_2 were measured by a dual wavelength infrared cell with a reference channel. The certified gases CH_4 (60, 15.01%) and CO2 (40, 15.01%) were used to calibrate the gas analyzer. Argon gas was used as the carrier gas in the GC, while nitrogen was used as the makeup gas. The GC was calibrated using standard gases consisting of CH_4 (60%) and CO2 (40%) on a volume basis (v/v).

3.3.2. Analysis of the Substrate's Bio-energy Conversion Capacity

The Theoretical CH_4 Potential (TMP) of the feedstock (SPM and SHWW) was estimated using Boyles (modified Buswell) Equations (3.1 and 3.2), depending on the elemental composition (Meegoda et al., 2018; Ugwu & Enweremadu, 2019; Nwokolo et al., 2020). The prediction of TMP was founded on the following assumptions: the conditions for microbial and substrate digestion are ideal; mixing was completed, and the temperature was maintained at a constant.

The elemental analysis was undertaken to determine the percentages of the following key elements: C, H, O, and N. The measured output from the batch reactor was biogas, whose composition (CH_4 and CO_2) was tested.

$$\mathrm{C_nH_aO_bN_c} + \left(\mathrm{n} - \frac{\mathrm{a}}{4} - \frac{\mathrm{b}}{4} + \frac{3\mathrm{c}}{4}\right)\mathrm{H_2O} \rightarrow \left(\frac{\mathrm{n}}{2} + \frac{\mathrm{a}}{8} - \frac{\mathrm{b}}{4} - \frac{3\mathrm{c}}{8}\right)\mathrm{CH_4} + \left(\frac{\mathrm{n}}{2} - \frac{\mathrm{b}}{4} + \frac{3\mathrm{c}}{8}\right)\mathrm{CO_2} + \mathrm{cNH_3} \qquad Eq. 3.1$$

$$\mathrm{TMP}\left(\frac{\mathrm{mL\ CH_4}}{\mathrm{gVS}}\right) = \frac{22.4 * 1000 * \left(\frac{\mathrm{n}}{2} + \frac{\mathrm{a}}{8} - \frac{\mathrm{b}}{4} - \frac{3\mathrm{c}}{8}\right)}{12\mathrm{n} + \mathrm{a} + 16\mathrm{b} + 14\mathrm{c}} \qquad Eq. 3.2$$

Where: $C_aH_bO_cN_d$ is the chemical formula for the substrates derived experimentally; a, b, c, d are the atomic masses of carbon (12), hydrogen (1), oxygen (16), and nitrogen (14), respectively; TMP is the theoretical CH_4 potential at standard temperature and pressure (STP)

Equation 3.3 was also used to calculate the synergistic effect of co-digestion of SHWW with SPM (Bohutskyi et al., 2018).

$$\text{Increase in CH}_4\text{ yield} = \frac{\text{BMP}_{\text{co-digestion}}}{\text{BMP}_{\text{SPM}} * \%\text{SPM} + \text{BMP}_{\text{SHWW}} * \%\text{SHWW}} \qquad \text{Eq. 3.3}$$

Where: $\text{BMP}_{\text{co-digestion}}$ is the bioCH_4 potential of the co-digestion sample (mL CH_4 g VS^{-1}); BMP_{SPM} is the experimental bioCH_4 potential measured in the AD of SPM (SPM: SHWW 100: 0 ratio) (mL CH_4 g VS^{-1}); %SPM is the percentage of SPM in the ratio; BMP_{SHWW} is the experimental bioCH_4 potential recorded in the AD of SHWW (SPM: SHWW 0:100 ratio) (mL CH_4 g VS^{-1}); and %SHWW is the percentage of SHWW in the ratio.

When the increase in CH_4 yield is > 1, a synergistic effect (S) occurs; = 1, no synergistic effect (N); <1, the effect is antagonistic (A) (Bohutskyi et al., 2018).
Equation 3.4 was also used to calculate the CH_4 yield (mL/g TS_{added}) (Bamba et al., 2021).

$$\text{Methane yield } = \frac{\text{BMP}_{\text{cum}}}{\text{TS}_{\text{added}}} \qquad \text{Eq. 3.4}$$

Where BMP_{cum} is the cumulative CH_4 yield (mL) and TS_{added} is the weight (g) of total volatile solids fed to the digester. Also, the removal efficiency of COD, VS, and TS were calculated using Equation 3.5.

$$\text{Removal efficiency}(\%) = \frac{G_i - G_f}{G_i} \qquad \text{Eq. 3.5}$$

Where G_i and G_f represent the initial and final concentrations of parameters, respectively.

3.4 Effect of Co-Digestion on Organic Removal Efficiency at Various HRTs

The continuous AD experiment was run in semi-continuous mode with CSTRs with a working volume of 800 mL. Each digester was made entirely of glass, sealed with an airtight polyethylene cap, and wrapped in parafilm. Two ports were installed at the top of each digester, one for feeding and sampling and the other as a biogas outlet from the reactor headspace to biogas collector bags. Figure 3.2 depicts a schematic illustration of the CSTR experimental setup.

The experimental setup consisted of a control reactor (with inoculum only) and three paired test digesters (with 20%SHWW: 80%SPM) mix. The 80% SPM: 20% SHWW mixture was used since it presented the optimum CH_4 potential, based on results obtained in batch experiments.

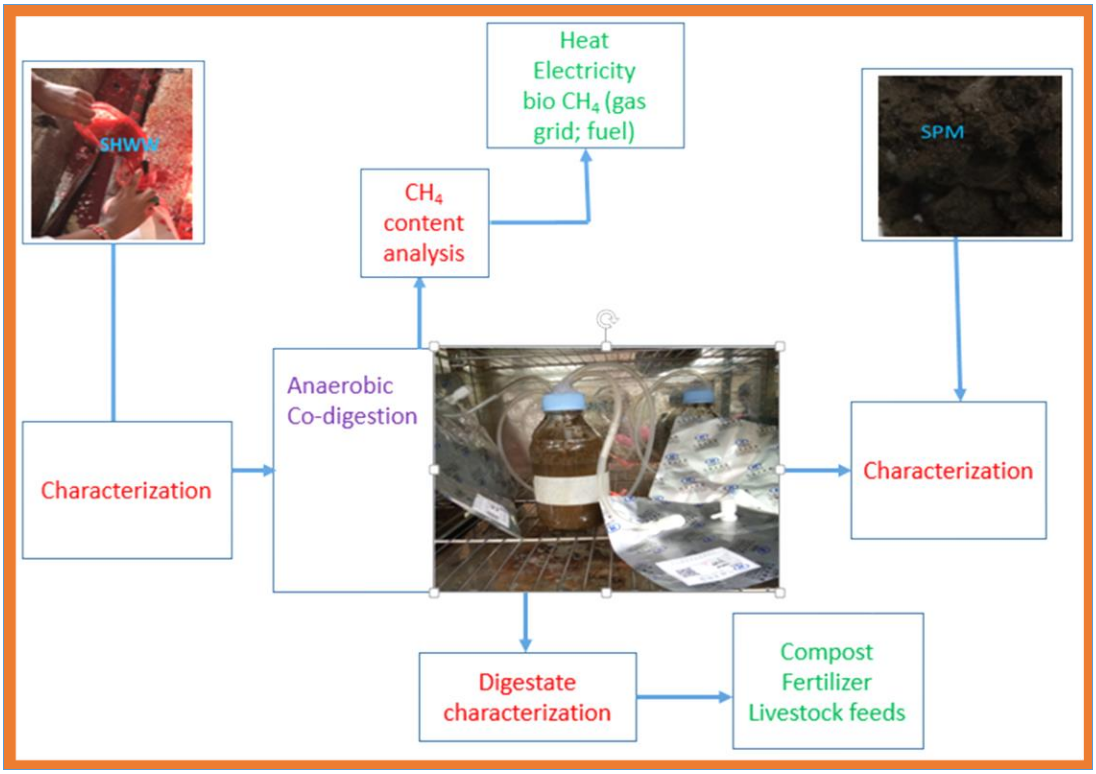

Figure 3.2: The Schematic Illustration of the CSTR Experimental Set-up

Control digesters aid in comparing the advantages and disadvantages of co-digestion for AD process stability and performance. The study result was used to evaluate the influence of the addition of co-substrate (SPM) on the degradation and CH_4 production of the SHWW. It also provided the optimum HRT with the best performance measured by the highest reduction of VS and CH_4 yield.

The 20%SHWW: 80%SPM mix was tested in a semi-continuous reactor with the control at HRTs of 15, 10, and 5 days as recommended by Talha et al. (2017) for AD of lignocellulose biomass, i.e., SPM, and at corresponding organic loading rates (OLR) of

2.9, 4.3, and 8.6 g VS. Ld-1, respectively. In order to satisfy HRT for 15, 10, and 5 days, 53.33, 80, and 160 mL of digested sludge (Table 3.2) were drained and the same volume of feed sludge was fed to the reactors every day, respectively, for five days, that is, from Monday through Friday. On Mondays and Fridays, the reactors were fed double the daily volume to compensate for the lack of feed during the weekend.

Table 3.2: Operating Conditions and Parameters during CSTR Experiment

Days	OLR ($gVSL^{-1}$ d^{-1})	HRT (days)	SPM: SHWW mix ratio	Flow rate (ml/day)	Temperature
1-45	2.9	15	80:20	53.33	37±1.0 °C
46-75	4.3	10	80:20	80	37±1.0 °C
76-90	8.6	5	80:20	160	37±1.0 °C

Initially inoculum was acclimated for about 10 days until no gas production was observed; then reactor was fed, started at an HRT of 15 days and operated at a constant HRT until the fluctuation of effluent properties and biogas production was within ±10%. After sufficient data collection, loading and effective volume alterations, the conditions were switched to 10 days HRT and later to HRT 5 days in the same reactor to avoid any lag period in terms of biogas production or other parameters for HRT 10 and 5. Every digester headspace was purged for roughly 2-3 minutes with 99.9% pure nitrogen.

Mixtures were prepared daily from the stored substrates, maintaining the weight ratio of each waste in the mixture and completing the final volume with distilled water to obtain a TS content of around 6%. The produced biogas was collected in 1.0 L biogas collector bags and measured on a daily basis using 100mL glass syringe while methane contents was measured by GC using syringe sample, process biochemistry parameters like pH, TS, VS, NH_4^+-N concentration and COD were measured at each three days interval.

3.5 The Kinetic Modelling

Several authors have modelled the batch experiment data (Kafle & Kim, 2012; Kafle & Chen, 2016; Wandera et al., 2018), and the results have been found to produce reasonable predictions of full-scale behaviour. Kinetic modelling is widely used in predicting CH_4 yields, establishing key parameters for reactor design, and optimizing the performance of AD process.

For simulating CH_4 accumulation data with a lag phase (λ), which is a critical factor in determining AD efficiency, the modified Gompertz model is preferable (Kafle & Chen, 2016). Besides that, where toxic substances or bio-resilient organic compounds exist, this kinetic model is more suitable (Bohutskyi et al., 2018). A modified Gompertz model, expressed by Equation 2.7, was integrated into the model to simulate the batch experimental data (Kafle & Kim, 2013; Wandera et al., 2018). The predicted CH_4 yield and the constants F_0, R_{max} and λ were determined by a non-linear least-square regression analysis conducted in the SPSS program (IBM SPSS Statistics 17 (2008)). The predicted CH_4 yield was plotted against the measured CH_4 yield using MS Excel 2013.

3.5.1. Statistical Evaluation

To evaluate the model, statistical indicators, namely, root mean square error (RMSE) and the coefficient of determination (R^2) were calculated for each reactor to compare the accuracy of the studied model. RMSE, given by Equation 2.8, is interpreted as the standard deviation between the predicted and measured values with a lower RMSE indicating a better fit (Kafle & Kim, 2012). Whereas R^2 is also known as the goodness-of-fit-index, which was determined using SPSS v.17 software.

$$\mathrm{RMSE} = \left(\frac{1}{n}\sum_{m=1}^{n}\left(\frac{d_m}{Y_m}\right)^2\right)^{\frac{1}{2}} \qquad (2.8)$$

Where:

Y = the measured value of CH_4 volume (mL/g VS),

d = the difference between the experimental and predicted value of CH_4 volume,

n = the number of measurements and m is the mth measurements.

Furthermore, to compare the effect of mixing ratio in each reactor, differences in experimental data among results obtained were evaluated using one-way analysis of variance (ANOVA) in Microsoft Office Excel. The results are regarded as significant only if the p-value is lower than 0.05 (i.e., $p < 0.05$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of ACoD on Biomethane Production

The present study demonstrated that the CH_4 yield of the SPM80%:20%SHWW mix ratio was much greater than the AMoD of SHWW and SPM, respectively, even though the difference for the other mix ratios was relatively small. In fact, the CH_4 yield of SPM80%:20%SHWW (478.40 mL CH_4/g VS) was about 27% and 59% higher than in SHWW and SPM, respectively (Figure 4.1). The mix ratio influences the characteristics of each substrate; hence, improved CH_4 yield could be obtained through self-buffering of the digestion medium (Reyes et al., 2015).

Generally, SPM allowed for nutrient balance, thus balancing the C/N ratio in the process. This highlights the effectiveness of co-digesting SHWW with a carbon-rich waste like SPM. Furthermore, ACoD allowed for increased organic matter content, implying that SHWW can be combined in such fractions with SPM. As a result, this would eventually impact the environment positively, leading to cost reduction during biogas plant operation. Bohutskyi et al. (2018) and Reyes et al. (2015) discovered comparable results. The current findings are consistent with those of other studies of a similar nature (Talha et al., 2017; Rahman et al., 2022).

Historically, animal manure has been an important raw material, particularly for biogas production. Similarly, Kafle and Chen et al. (2016) investigated the biogas potential of various animal manures in batch experiments, including dairy (295 mL CH_4/g VS), horse (222 mL CH_4/g VS), goat (242 mL CH_4/g VS), chicken (425 mL CH_4/g VS), and swine manure (495 mL CH_4/g VS).

Elsewhere, according to Wang et al. (2012), dairy manure produced 437.3 mL CH_4/g VS of biogas, while chicken manure produced 311.4 mL CH_4/g VS of biogas. It should be noted that the amount of biogas produced by AD varies depending on the type of manure used (species, breed, age, body weight, feed, etc.). Also, the difference in organic matter, carbohydrate, and fat content influences its specific chemical and physical composition.

Furthermore, Ma et al. (2020) discovered that swine, cattle, and poultry manure produced 238 mL CH_4/g VS, 314.7 mL CH_4/g VS, and mL CH_4/g VS of biogas, respectively. In comparison to these studies, our substrates can adequately replace animal manure as a major feedstock in our biogas digesters. Figure 4.1 presents the total CH_4 yield over the course of the study.

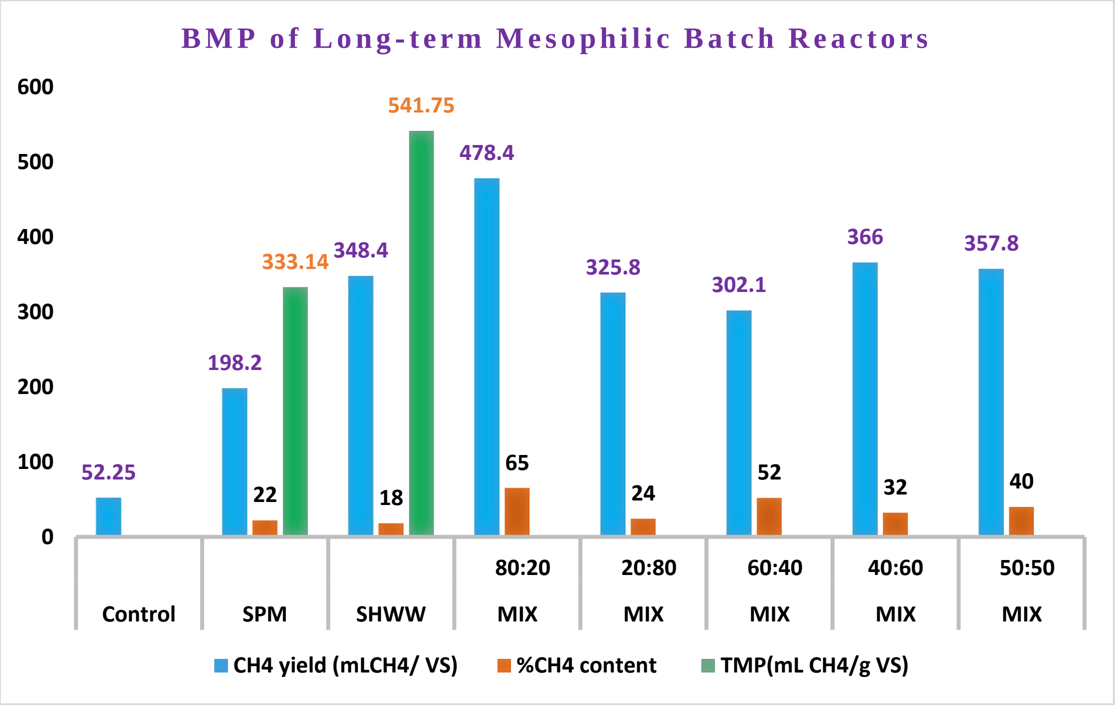

Figure 4:1: BMP of Long-Term Mesophilic Batch Reactors

In relation to cumulative specific CH_4 yield, the bioreactors analyzed were in order of: 80%SPM:20%SHWW>40%SPM:60%SHWW>50%SPM:50%SHWW>100%SHWW>20%SPM:80%SHWW>60%SPM:40%SHWW>100%SPM>control. Additionally, a one-way ANOVA test was performed to compare the effect of different mixing ratios on CH_4 production. The test however, revealed that there was no statistically significant difference on CH_4 yield for mixtures of 60%SPM:40%SHWW, 50%SPM:50%SHWW, and

20%SPM:80%SHWW (p =1.000-0.884). This suggests that codigesting two or more substrates is not a guarantee to achieve higher CH_4 yields than the AMoD of substrates in the mix. In fact, the AD process in biodigesters SPM20%:80% SHWW, SPM60%:40%SHWW, and SPM was inefficient. Very little performance efficiency was attained in terms of organic matter degradability and CH_4 production.

In this regard, the high NH_4^+-N concentrations (6887 mg l^{-1}) at the start of the experiment in 20%SPM:80% SHWW probably hindered the methanogens within the reactor, resulting in low CH_4 yield. Furthermore, protein degradation in SHWW is depicted by relatively high NH_4^+-N concentrations (6407 mg l^{-1}) that imply the possibility of NH_3 inhibition during AD. However, SPM, has the lowest NH_4^+-N concentrations (1300 mg l^{-1}), hence a suitable co-substrate. A study by Yenigün & Demirel (2013) observed that high concentrations of free NH_3 above 55 mg L^{-1} diffuse across cell membranes, causing microbial destruction and disrupting the entire AD process. The free NH_3 toxicity could be mitigated through the ACoD of SHWW with agricultural wastes high in carbon, like SPM. Furthermore, a good substrate mix stabilizes the C/N ratio and optimizes the mixture's buffering capacity, resulting in increased reactor efficiency (Reyes et al., 2015).

In addition, a one-way ANOVA test revealed that there was a statistically significant difference on CH_4 yield between 40%SPM: 60%SHWW ($p = 0.001$) and 80% SPM:20% SHWW ($p = 0.000$). However, our findings suggest the existence of an ideal ratio of SHWW: SPM at which ACoD of these feedstocks is simple. Interestingly, when the proportion was set at 80%SPM:20%SHWW, there was an enormous improvement in the CH_4 yield. Hence, 80% SPM:20% SHWW is the ratio for optimal CH_4 yield during ACoD of SPM and SHWW.

Furthermore, as shown in Figure 4.1 and 4.2, AD of SHWW yielded a net CH_4 production of 348.40 mL CH_4/g VS, which is significantly higher than that of SPM. This confirms that the functional microorganism activities in SHWW digestion favor the entire digestion process. This high CH_4 yield can also be attributed to the high protein and lipid content of

SHWW, which is readily available (Borowski, 2015; Selormey et al., 2021; Guo et al., 2021).

SPM resulted in a low CH_4 yield because of its high lignin content, it is possible that not all of the carbon in SPM is bio-available for microbial degradation as a result of lignocellulose substrate. Lignocellulosic biomass (LB) is an abundant and renewable resource from plants mainly composed of polysaccharides (cellulose and hemicelluloses) and an aromatic polymer (lignin). As a consequence, ACoD of SHWW with SPM stimulates methanogenic activity. This, however, comes at the expense of the digestion of SPM. Fortunately, the huge difference in the C/N ratios can be brought into the optimal range by blending SHWW and SPM in varying proportions. In addition, the low BMP presented in SPM in concurrent with the findings from other similar studies of low yields from lignocellulosic agro-wastes (Reyes et al., 2015; Talha et al., 2017). This is due to the slow hydrolysis of complex carbohydrates in lignocellulosic biomass, which requires long contact times to be hydrolyzed by hydrolytic microbes. Furthermore, Talha et al. (2017) observed that SPM's low CH_4 yield was due to its high ash content, probably related to cane variety with varying lignin content, soil conditions, and other environmental factors.

Besides that, previous findings regarding SPM digestion point to the presence of process inhibitor factors (Porselvam et al., 2017). Indeed, feedstock with a high C/N ratio has a low buffering capacity and generates an excess of VFAs during digestion, resulting in a pH drop (Rhee et al., 2021). At the commencement of acidogenesis, pH is most likely to drop before actually rising as the VFAs degrades to produce CH_4. In that regard, VFAs accumulation can probably cause process instability as it can reflect a kinetic uncoupling between the acidogenesis and acetogenesis phases of the AD process (Porselvam et al., 2017). Herein, the inhibition reported in SPM is most inclined to this attribute.

Overall, current results also confirm that the presence of SPM impacted the CH_4 quality. SPM digestion produced CH_4 with a low average CH_4 content (22%) when compared to the 80% SPM: 20% SHWW mix proportion. The study findings shows that a mix ratio of

SPM80% with 20% SHWW resulted in a 65% increase in CH_4 content. However, an increase in SHWW portions (100%, 80%, and 60%) resulted in a decrease in average CH_4 content of only 18%, 24%, and 32%, respectively.

In particular, SHWW and SPM ACoD effectively improved biogas yield. These observations are consistent with the results of González et al. (2020), who assessed the synergetic, economic, and environmental feasibility of the ACoD of mechanically pretreated (sieving) SPM. This study showed that both the environmental and energetic profiles as well as the profitability of CH_4 yield could be enhanced when the ACoD of SPM is considered. Even though several studies have been made about the efficiency of ACoD of agrowastes for biofuels, this study focuses on both its treatment and CH_4 production. Furthermore, treatment performance differs depending on location, and such factors as temperature which can be customized for industrial usability.

Moreover, TMP recorded a higher maximum value from SHWW (541.75 mL CH_4/g-VS) compared with SPM (333.14 mL CH_4/g-VS) (Figure 4.1). This may be explained by the more energy-rich lipids and proteins in SHWW than in SPM. In view of this finding, mono-digestion of both substrates showed relatively low TMP. This could be due to recalcitrant matter in SPM and SHWW or/and reduced methanogen activity because of an unbalanced C/N ratio (Bohutskyi et al., 2018; Rhee et al., 2021). With respect to SPM, low bioconversion is in line with the degradability of lignocellulosic biomasses (González et al., 2014; Talha et al., 2017). Accordingly, this study concurs with other previous studies that agro-wastes pose digestibility difficulties during AD (Palatsi et al., 2011; Yenigün & Demirel, 2013). Figure 4.2 shows the cumulative CH_4 yield in the various mix proportions.

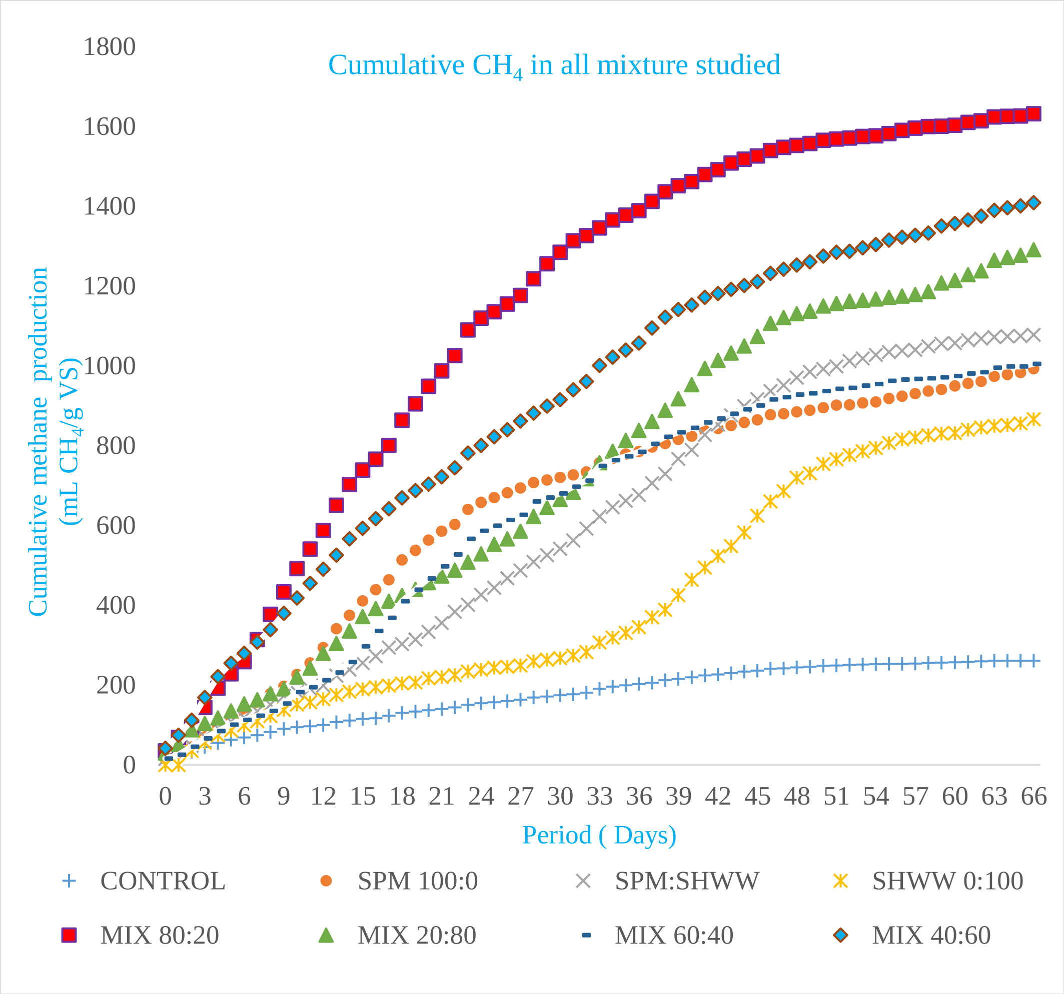

Figure 4.2: Cumulative CH_4 Yield in the Investigated Mix Proportions

4.1.1. Seed and Substrates Characteristics

The performance and stability of the biodigestion process are highly influenced by substrate characteristic, operating parameters, and an array of different microbial groups, and their functions (Hailu et al., 2020). Results of the average values (mean ± SD) of physicochemical characteristics of the raw substrates and fresh sludge are presented in Table 4.1. The VS/TS% of SHWW and SPM was 90 and 91%, respectively, which was found to be more suitable for AD, as previously reported by Jeung et al. (2019). Additionally, SHWW showed a high organic matter content expressed by the concentration of COD (16 gL−1), TS (3.5%) and VS

(3.2%). Ultimately, the organic matter content of SHWW was remarkably higher than previously observed by Hernández-Fydrych et al. (2019) and Bouallagui et al. (2009), possibly due to solid separation prior to sampling and the fact that SHWW composition varies with meat type, daily rate of processing, and butchering operational processes (washing, cutting, etc.) (Salehiyoun et al., 2020). The sampled SHWW, in particular, contained blood, which resulted in a high COD concentration. However, the organic matter content was enriched when SHWW was mixed in different proportions with SPM and diluted to 6% TS (Table 4.1). Therefore, combining substrates makes a good feedstock for AD studies.

The C/N ratio is a crucial element for AD. The low C/N ratio (9.65) exhibited in SHWW is similar to prior findings (Rhee et al., 2021). According to Bouallagui et al. (2009), low C/N ratios is a result of elevated nitrogen content, resulting in NH_3 accumulation and, as a result, pH values that adversely impact methanogens responsible for CH_4 production functional microorganisms. On the contrary, a high C/N ratio means that methanogens are quickly utilizing nitrogen in a way that results in a deficiency in the AD process, resulting in a drop in CH_4 yield. Therefore, a stable C/N ratio must be maintained to maximize CH_4 generation and process stability. The best C/N ratio, according to previous studies, range from 20 to 30 (Talha, 2017). This is efficient for functional microbial metabolic activities and adequate to sustain system operation and satisfy nutrient and energy needs for cell growth (Mata-Alvarez et al. 2014). Consequently, substrates rich in C/N ratios have a low buffering capacity and are associated with high volatile fatty acids (VFA) accumulation during digestion. Substrates low in C/N ratios, on the other hand, have a high buffering capacity potential. As a result, elevated NH_3 concentrations during the digestion process limit microorganism growth. Consequently, SHWW is a problematic substrate for biogas plants due to perceived NH_3 inhibition and an unbalanced C/N ratio (Rhee et al., 2021).

pH is a vital factor in AD. Therefore, the ideal pH for methanogenesis ranges from 6.5 to 8.2 (Porselvam et al., 2017). In that regard, the current investigation recorded pH values of 8.06 and 5.41 for SHWW and SPM, respectively. However, the activity of methanogenic microorganisms involved in the digestion process decreases at a higher or lower pH. This emphasizes the importance of ACoD in optimizing reactor buffer capacity and AD efficiency. The pH level for SHWW remained above 8.0 for almost the entire process due to the relatively higher NH_4^+-N concentration (6407 mg/L) (Table 4.1), which was caused by the degradation of the proteins in SHWW. Bayr et al. (2012) also reported similar scenarios during the AD of rendering plant and slaughterhouse waste under mesophilic and thermophilic conditions. These findings imply the possibility of NH_3 inhibition during the AD of the explored substrates, potentially inhibiting methanogenesis.

In contrast, the SPM reactor had a low initial pH because of its acidic nature. Consequently, it did not recover fully despite having a C/N ratio (26.23) well within the allowable threshold. This implies that the buffering capacity of the system was insufficient to keep a pH level within the satisfactory limits for AD. Moreover, the C/N ratio of SPM (26.23) is in concurrent with previous studies (Alvarez & Liden, 2008; Talha et al., 2017). Previous studies have reported that ACoD improved the C/N ratio of the digestate and minimized the toxicity of nitrogen in the form of NH_3 (Reyes et al., 2015; Pratima & Ale, 2015). However, the lignocellulose nature of SPM limits its biodegradability, therefore hindering the AD process. During the anaerobic co-digestion of SPM with food, Cárdenas-Cleves et al. (2018) observed similar results. According to Qamar et al. (2022), pH adjustment through alkali treatment can maintain the stability of the process. As for the 80% SPM: 20% SHWW mix ratio, the initial pH significantly was elevated from 7.2 to 8.10 in the effluent due to the consumption of VFAs, thus indicating the presence of a buffer effect that maintained optimal AD conditions.

Table 4.1: Characterization of feedstocks and the inoculum (Mean ± SD)

Parameter	SPM	SHWW	Control	MIX 80:20	MIX 20:80	MIX 60:40	MIX 40:60	MIX 50:50
TS (%)	6.3±0.3	3.5±0.3	7.1±0.3	6.2±1.5	6.1±0.4	5.98±.2	5.89±0.8	6.3±0.6
	(5.1±0.3)	(2.4±0.3)	(3.1±0.3)	(4.5±1.5)	(5.7±0.4)	(5.8±0.2)	(5.6±0.8)	(5.2±0.6)
VS (%)	5.7±0.6	3.2± 0.3	6.3±0.3	4.3±0.8	5.0±0.3	4.2±1.1	4.9±0.6	3.8±0.5
	(2.5±0.6)	(1.5±0.3)	(2.4±0.3)	(1.4±0.8)	(2.5±0.3)	(2.4±1.1)	(2.0±0.6)	(1.8±0.5)
(%)VS/TS	90	91	90	70	82	70	83	60
(%)VS removal	60	53	62	67	50	43	59	52
PH	5.41±0	8.06±0	7.2±0	7.2±0	7.3±0	7.1±0	7.2±0	7.3±0
	(7.76±0)	(8.34±0)	(7.69±0)	(8.10±0)	(8.25±0)	(8.21±0)	(8.23±0)	(8.20±0)
TCOD (g l^{-1})	7.36±0	16±0.1	15.0±0.1	10.8±0.1	12.2±0.3	11.8±0.1	14.6±0	15.6±0
	(5.16±9)	(12±6.1)	(11.0±9.1)	(8.3±6.1)	(9.5±9.3)	(8.3±8.1)	(11.4±7)	(12.7±4)
(%)COD removal	30	25	27	23	22	30	22	16
NH_4^+-N (mg l^{-1})	1300±3.3	6407±5.5	1097 ±8.7	5521±7.3	6887±9.7	6463±4.6	6323±8.3	6671±6.5
	(1205±0.3)	(4208±0.5)	(674±0.7)	(2426±0.3)	(2151±0.7)	(3841±0.6)	(4560±0.3)	(4500±0.5)
C (%)	27.28±0.2	32.62±0.1						
H (%)	16.51±0.6	17.8±0.7						
O (%)	1.37±0.7	2.45±0.4						
N (%)	1.04±0.4	3.38±0.3						
C/N ratio	26.23	9.65						

Notes: n=3; the first values refer to the substrates and mixtures before AD; the second values in brackets refer to the respective digestate after AD.

Total and soluble COD, TS, and VS values showed the presence of a high content of organic matter in the studied substrates (Table 4.1). According to Monou et al. (2008), VS biodegradability strongly depends on original TS concentrations. With 53% VS destruction for SHWW, an 80:20 SPM: SHWW mix ratio yielded a 67% VS breakdown.

The ACoD of SHWW with SPM was believed to have enhanced the biodegradability of the mixed medium. For instance, the large concentration of VS (57%) in the SPM as a co-substrate led to increased VS degradation. This could be attributed to the digestion medium's synergistic effect and improved digestibility. However, a high VS breakdown in conjunction with a low maximum CH_4 content, on the other hand, denotes an imbalance between acidogenesis and methanogenesis. This is caused by low pH and nutrient deficiency (Porselvam et al., 2017).

During the trials, COD levels relatively declined (16-30%) due to the anaerobic breakdown process in all digesters. Overall, COD degradation was not attractive even for an 80% SPM: 20% SHWW reactor. One such scenario may imply that supplementary treatment is necessary immediately after AD so that the effluent can be unloaded into the surroundings in compliance with the applicable standards. Therefore, AD is regarded as a practical treatment method for SHWW due to its high COD and pathogen inactivation efficiency. Moreover, odour issues associated with abattoir effluents are limited (Panizio et al., 2019).

4.2 Treatment Performance on the Organic Removal Efficiency

The performance of the co-digestion processes was evaluated in terms of gas (quality and quantity) production and VS reduction for the different hydraulic retention time (HRTs) monitored as presented in Table 4.2. The highest biogas yield was obtained in 15 d HRT with an average value of 350.8 mL/g-VS. However, the biogas composition remained constant throughout the HRT tests (at about 50–65% of CH_4 and 50–35% of CO2). Increasing the HRT increased CH_4 yield probably due to the sufficient retention time to allow for enhanced substrate degradation by and reduced wash-out of functional microorganisms (bacteria and archaea). However, the values observed were much lower than those reported by other authors on the AD of SHW (Cuetos et al., 2013; Panizio et al., 2020).

The addition of the SPM as co-substrate to SHWW AD enhanced the biogas yield by 69.1% at the optimum HRT of 15 d. Moreover, the addition of carbon-rich co-substrate (SPM) to nitrogenous substrate SHWW) could have led to more suitable C/N ratio. Furthermore, an increase in the OLR resulted in a decrease in biogas production (Table 4.2). This is attributed to increasing OLR resulting in reduced hydraulic retention time, accumulation of VFAs, and possibility of wash-out of functional microorganisms (bacteria and archaea) which results into low density of microorganism density per unit volume of bioreactor (Hailu et al., 2020). Biogas production is influenced by the production of inhibitory intermediate digestion intermediates i.e., compounds such VFAs during the digestion process (Palatsi et al., 2011).

For instance, reactors operating at 5 d HRT exhibited the lowest biogas potential of about 255.36 mL/g-VS, signifying possible inhibition from intermediate products such VFAs which are toxic to methanogens and possibility of wash-out of functional microorganisms (bacteria and archaea) resulting from short hydraulic retention time. This could also be due to the highest ammonium nitrogen (NH_4^+-N) concentration (8333 mg/l) as shown in Table 4.2. Progressive increase in HRT, lead to sufficient contact time with microorganisms and reduce wash-out of functional microorganisms (bacteria and archaea). As a result, methane production is improved due to balanced C: N ratio that in turn cause a reduction in NH_4^+-N concentration.

Furthermore, it could be attributed to either nutrient deficiency or insufficient contact between microorganisms and substrate due to inadequate contact time; VFA accumulation resulting into lowering pH level in the bioreactor, consequently, inhibiting methanogenesis process. Similarly, Hejnfelt & Angelidaki (2009) reported HRT of less than 3 days to be very low in completely mixed systems as it could cause washout of functional microorganisms (bacteria and archaea), as methanogens are assumed to have longer generation times of several days. Therefore, in this work, the ACoD of SPM and SHWW under semi-continuous operation presented possible inhibitory problems. This is similar to what Cuetos et al. (2013) reported during the ACoD of maize and poultry blood.

Table 4.2: Performance of CSTRs at different HRTs

	Parameter	Unit			15 HRT	10 HRT	5 HRT
Duration		**Days**			1 to 45	46 to 75	76 to 90
OLR		**mL/d**			53.3	80	160
			Control	**Influent**	**Effluent**	**Effluent**	**Effluent**
Removal efficiency	**pH**		7.79±0.0	7.34±0.0	8.09±0.	7.96±0.1	7.82±0.1
	TS	**%**	72.18±0.1	69.31±0.1	40.96±2.3	56.47±1.4	62.23±4.5
	VS	**%**	69.91±0.2	42.3±0.3	15.9±4.1	19.50±3.2	23.11±6.2
	COD	**g/L**	15±0	32.96±0	8.64±2.4	10.24±4.1	16.64±3.0
	NH_4^+-N	**mg/L**	6162±2.3	9387±3.1	5334±2.0	6763±1.3	8333±1.1
Gas Yield	**CH_4 Yield**	**mL/g-VS**	108.4±3.0		350.8±3.3	305.76±5.1	255.36±4.0

Volatile solids' (VS) reduction is a measure of organic matter utilization in the AD process and used to monitor digester's performance. During the AD process, VS are degraded to a certain extent and converted into biogas. The degree of stabilization is often expressed as the percent reduction in VS ((Hailu et al., 2020). The average VS reduction of reactors at 5 d HRT, 10 d HRT and 15 d HRT was 45.4%, 53.9%, and 62.4%, respectively. This VS reduction was found almost consistent with the biogas production rate.

It was further established, that 15 d HRT recorded the highest VS reduction of about 62.4%, which clearly shows a good condition of the AD process indicating that the decomposition of biodegradable organic matter was better at longer HRT. VS conversion to biogas in the other reactors operating at 10 and 5 d HRT, respectively, was hindered probably due to the generation of inhibitory substances and shorter hydraulic retention time. Nonetheless, the increased VS removal efficiency in overall experiment indicates an exponential growth of functional microorganisms which in turn yielded favourable results. Longer HRT particularly in high lipid wastewater, promote scum reduction, forming the potential of a system and better VS reduction (Rahman et al., 2022). Therefore, in present study, performance of reactor at HRT of 15 d was found to be very much efficient at retaining biomass. Furthermore, higher HRT will require a larger digester volume, increasing the overall operational costs (Talha et al., 2017).

AD process stability depends on the buffering capacity of the digester contents. Alkalinity is an important parameter that measures bioreactors buffering capability to neutralize the increased acid from the acidogenesis. High alkalinity values indicates that the methanogenic digesters have a greater capacity to resist pH changes (Yang et al., 2018). pH is an important parameter in the AD process. Consequently, in the current investigation, the pH level of each digester was stabilized in the range of 7.82 and 8.09, reflecting a stable system. A massive pH change was not experienced throughout the experiment due to the good buffering capacity achieved through codigestion.

According to previous studies, a neutral pH of around 6.7–7.6 is preferred for the effective operation of an anaerobic reactor (Bouallagui, 2009). In this study, the pH of the reactors remained within the working range (7.82–8.09) for all HRTs. Also, NH_4^+-N is very toxic to methanogenic bacteria and inhibits their growth when its concentration is within the inhibition level (Selormey et al., 2021; Guo et al., 2021). However, in this study, NH_4^+-N values were much lower than toxic limits reported in previous studies when digestion of nitrogenous wastes (Cuetos et al., 2013). The NH_4^+-N level of 5334mg/l at 15 d HRT increased to 6763 mg/l at10 d HRT, and finally to 8833 mg/l at 5 d HRT. Moreover, reactors which operated at shorter HRT of 5 d experienced a high level of NH_4^+-N levels attributed to the degradation of the nitrogenous organics in the SHWW. Consequently, a reduced rate of CH_4 production in reactors operating at 10 d HRT and 5 d HRT was observed due to the accumulation of a relatively high level of NH_4^+-N concentration (Table 4.2).

4.3 Kinetic Modelling of parameters of Degradation

To describe the AD kinetics, modified Gompertz model (Equation 2.7), was fitted to CH_4 gas produced in this study. This model links the rate of CH_4 production and microbial activity (Gnaoui et al., 2020). The lag phase in CH_4 yield is accurately predicted by the modified Gompertz model. This is critical for modeling the AD of recalcitrant biodegradable feedstocks like lignocellulosic materials (Kafle & Kim, 2013; Kafle & Chen, 2016). The optimum proportion (80% SPM:20% SHWW) exhibited the shortest λ, with the highest R_{max}, which was almost double compared to the AMoD of SPM and SHWW (Table 4.3). This demonstrates that the 80%SPM: 20%SHWW mixture was readily biodegradable and contained more AD efficient functional microbial consortia.

Table 4.3: Modified Gompertz Kinetic Modeling

Parameter	**Control**	**SPM**	**MIX 50:50**	**SHWW**	**MIX 80:20**	**MIX 20:80**	**MIX 60:40**	**MIX 40:60**
P_o (mL CH_4/g VS)	289.34	949.06	1299.57	887	1631.63	1478.96	1019.73	1464.39
R_{max} (mL CH_4/g VS/d)	5.23	29.31	23.09	32.54	50.25	26.05	28.11	30.31
λ(d)	6.10	1.63	4.98	6.12	0.78	2.73	3.86	2.88
R^2	0.993	0.994	0.995	0.995	0.999	0.995	0.999	0.995
RSME	5.75	23.05	25.42	49.44	11.55	27.43	17.58	28.48

The λ for AMoD of both SHWW and SPM was estimated to be relatively longer (6 d) compared to the mixed ratios. Thus, the high content of difficult biodegradable compounds could be the reason for the longer lag times in our study. The unbalanced C/N ratio can also be blamed for the long λ in SHWW. In addition, the perceived inhibition due to free NH_3 and the rapid hydrolysis nature of SHWW reduce the rate of degradation (Bohutskyi et al., 2018). Similarly, Cárdenas-Cleves et al. (2018) reported an overall reduction of λ during ACoD of SPM with food waste.

The simulation of the reactor performance also demonstrated that ACoD improved Rmax by between 77–90%, and between 82–84% for AMoD of SHWW and SPM, respectively. At the same time, the Rmax values for varying mix proportions were within a narrow range (Table 4.3). The implication of this finding is that all the mixed ratios had comparable effects on boosting methanogenic activity, despite the difference in their total optimum CH_4 yield. Nonetheless, the study findings indicate that co-digestion improved AD by boosting Rmax while significantly reducing λ. These improvements are critical for improving system economics, because both variables (Rmax and λ) imply lower digester volumes and, as a result, lower capital investment and higher energy output from large-scale continuous AD systems (Bohutskyi et al., 2018).

The model simulated the experimental data with high accuracy and adaptability for both AMoD and ACoD, as presented in Table 4.3. The model's gradient shape accurately portrayed AD's lag, exponential, and stationary stages (Figure 4.3). Moreover, the simulated theoretical cumulative CH_4 data was consistent with the experimental values, implying that the suggested model represents an excellent fit. Accordingly, the co-efficient of determination (R^2) improved as the SPM percentage increased. R^2 was at its maximum value when the SPM was 80 and 60%, and 0.995 when the SPM ratio was 50, 40, and 20%, respectively.

Nevertheless, the R^2 values of the modified Gompertz model were high (0.993–0.999), which makes the model more adequate in our case. Similarly, Kafle & Chen (2016) observed a lower R^2 value (0.994) for energy crops. Elsewhere, Srivastava & Chakma (2021) reported efficient fitting (R^2>0.99) for observed CH_4 production. Consequently, BMP is more significantly affected in plants' biomass since the cell membranes of their feedstock are shielded by a complex lignocellulosic material. In contrast to the present findings, Xu et al. (2014) found a quite weak correlation (R^2 = 0.334) while using lignocellulosic biomass.

Also, the statistical indicator RMSE presented in Table 4.3 was used to evaluate if the model prediction fits with the experimental data. The lower RMSE (11.55) and higher R^2 value (0.999) were calculated for the 80%SPM: 20%SHWW mixture followed by the 60%SPM: 40%SHWW mixture (RMSE = 11.54, R^2 = 0.999). Consequently, according to the kinetic analysis results (deviation between simulated and experimental CH_4 yield and statistical indicators), the 80% SPM: 20% SHWW mixture was found to be the best mix proportion for ACoD of SHWW and SPM.

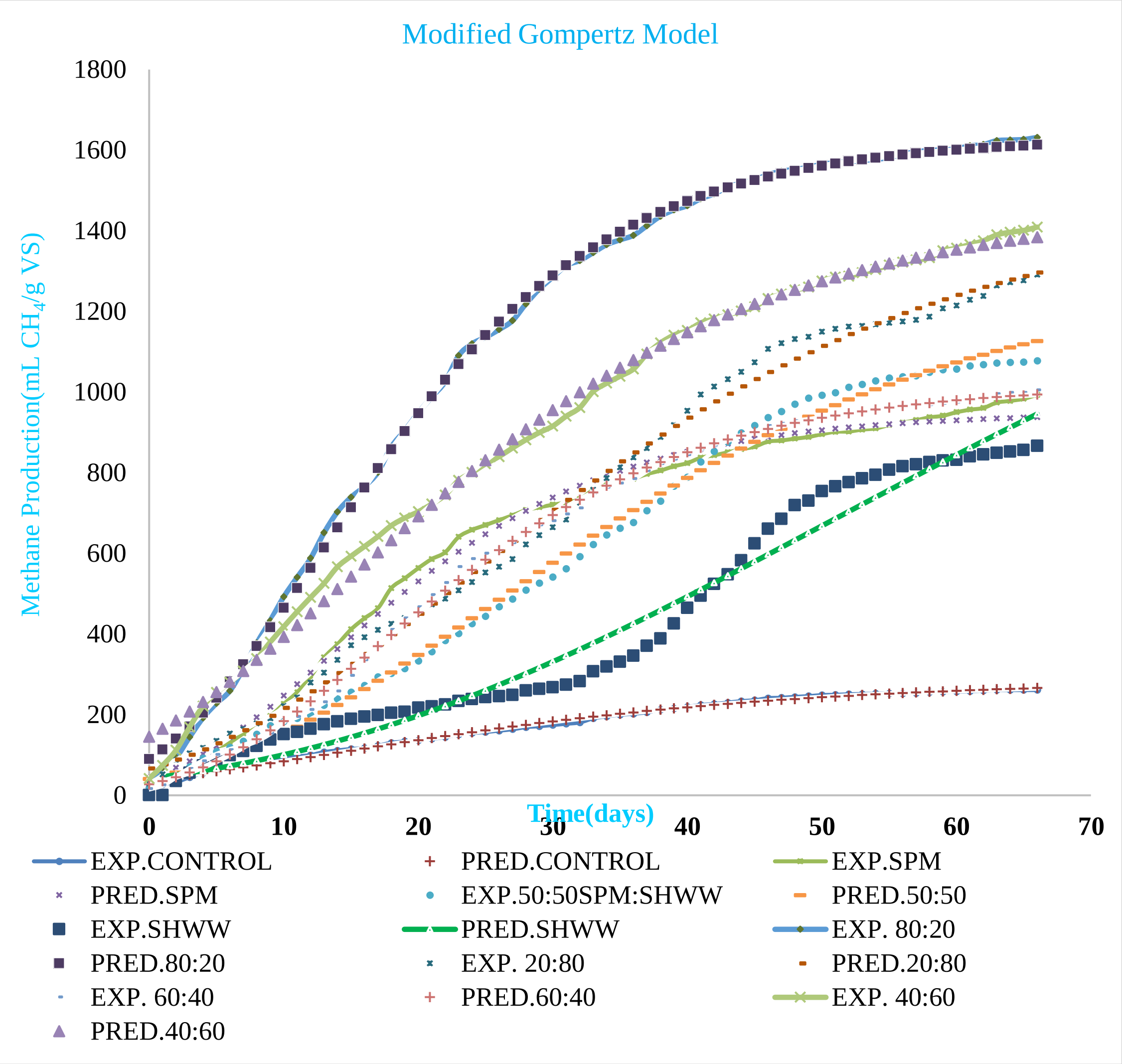

Figure 4.3: Experimental and simulation lines of cumulative CH_4 production from different substrate mix proportions

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The focus of this study was to evaluate the anaerobic treatment performance of slaughterhouse wastewater co-digested with sugar press mud in mesophilic conditions, with the intent of enhancing treatment efficiency and management of agro wastes. The following are the study's key findings:

I. Slaughterhouse wastewater (SHWW) co-digestion with sugar press mud (SPM) at an optimal mix ratio of 20%SHWW: 80%SPM enhanced methane yield and volatile solids (VS) removal by approximately 27% and 67%, respectively. The methane yield is comparable to one obtained from such substrates as cow dung. Therefore, using slaughterhouse wastewater with sugar press mud for methane production is an effective treatment of the wastes as well as for bio methane recovery.

II. The operation of continuous stirred tank reactors (CSTRs) at optimum 15 d hydraulic retention time (HRT) enhanced the methane yield to a maximum of 69.1%, and volatile solids (VS) removal to a maximum of 62.4%. This greatly improved treatment performance and process stability.

III. The modified Gompertz model revealed that methane production rate was enhanced by 46%, while lag time was reduced by 87% upon codigestion of Slaughterhouse wastewater with sugar press mud. Moreover, the model perfectly fit the experimental data with high R^2 values of the range 0.993–0.999, which makes the model sufficiently accurate for predicting the system performance of anaerobic codigestion of slaughterhouse wastewater with sugar press mud.

5.2 Recommendations

5.2.1. Recommendations

I. Slaughterhouse wastewater co-digestion with sugar press mud at an optimal mix ratio of 20%SHWW: 80%SPM enhanced methane yield by approximately 27% and volatile solids (VS) removal by about 67%. Therefore, the study recommends the substitute use of slaughterhouse wastewater with sugar press mud in biomethane production and treatment of organic wastes.

II. The hydraulic retention time of 15 days is recommended for operating the field scale anaerobic digesters with continuous feeding, to maximize the organic removal efficiency from the system and to prevent the washout of the biomass.

III. The study also recommends the use of modified Gompertz model for kinetics of organic degradation prediction as it better fitted the experimental results for anaerobic codigestion of slaughterhouse wastewater with sugar press mud.

5.2.2. Areas of further research

I. Further research is needed to assess the industrial applicability of the produced methane due to the limitations of the current study.

II. Future studies could shed more light on the economic implications of hydraulic retention times longer than 15 days under thermophilic conditions on overall process performance and reactor scale-up.

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APPENDICES

Appendix I: SHWW collection at Juja Slaughterhouse

Juja Slaughterhouse wastewater drains

Appendix II: Preparation of fresh SPM

Fresh SPM sieved to 42mm particle size

Appendix III: Setting up the batch reactors

Flashing the Batch Bottles with N_2 Gas

Appendix IV: The Incubation of batch reactors

Batch bottles incubated at mesophilic temperature (37.0 ± 1.0 °C)

Appendix V: The CSTRs experiment

Setting up the CSTRs at different HRTs

Appendix VI: The incubation of CSTRs

Fabricated CSTRs incubated at mesophilic temperature (37.0 ± 1.0 °C)