Co-digestion of kitchen waste and pig manure for enhanced biogas production

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DECLARATION

I, Ogochukwu Ruth Kanu-Uchenna, hereby declare that the dissertation titles "*Co-digestion of kitchen waste and pig manure for enhanced biogas production",* submitted in fulfilment of the requirements for the degree of Master of Engineering in Chemical Engineering, is my work, with sources of quotations pointed out in the work, and has not been previously submitted, either in full or in part, to any other academic institution.

Signed at the North-West University (Potchefstroom Campus).

Signature:

Date:

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ABSTRACT

Anaerobic co-digestion (ACoD) of kitchen waste (KW) and pig manure (PM) provides an effective means of treating organic waste and recovering biogas-based renewable energy. This study investigated the ACoD of KW and PM to enhance biogas production while recovering nutrients in a form suitable for agricultural reuse. Batch biochemical methane potential (BMP) tests were conducted at laboratory scale under mesophilic conditions (37 ± 2 °C) using six digesters configured with different KW:PM mixing ratios on a volatile solids (VS) basis: 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. All substrates were subjected to a combined mechanical (4-5 mm size reduction) and enzymatic pre-treatment to improve hydrolysis and substrate accessibility, while a well-adapted inoculum was employed to minimize lag phases and ensure process stability. Key process indicators, including daily and cumulative biomethane yields, pH, and ammonia levels, were monitored throughout a 28-day digestion period, and the final digestates were characterized for proximate composition, thermogravimetric behaviour, elemental profiles (ICP-MS), and agronomic quality.

The findings indicated that co-digestion improved methane production relative to mono-digestion of either substrate. Digesters with higher KW proportions (100:0, 80:20, and 60:40) exhibited short lag phases, pronounced early peaks in daily biomethane yield, and sustained methane generation during the exponential phase, leading to the highest cumulative biomethane yields, with the 100:0 and 80:20 reactors achieving final values of approximately 767 and 719 mL CH_4 g^{-1} VS added, respectively. In contrast, mixtures dominated by PM (20:80 and 0:100) produced lower cumulative yields (around 459 and 299 mL CH_4 g^{-1} VS), confirming that excessive manure content reduced substrate biodegradability and conversion efficiency. Nevertheless, all treatments maintained near-neutral pH, high biomethane fractions, and no inhibitory symptoms, indicating that the KW-PM combinations, supported by the buffering capacity of manure and the applied pre-treatment, provided stable digestion conditions across the tested ratios.

Digestate analyses revealed significant stabilization of organic matter, with thermogravimetric profiles indicating reduced volatile fractions relative to the feedstocks, and CHN data showing significant carbon depletion associated with methane formation. At the same time, total nitrogen in the digestate was conserved on a mass-percentage basis, with a high proportion present as ammonium-N, thereby enhancing the immediate plant-available nitrogen. Major plant nutrients

(N, P, K) were present at agronomically relevant levels, while potentially toxic elements such as Pb, Cd, Cr, Ni, As, and Hg occurred at very low concentrations, below analytical detection limits, and well within international guideline values for materials intended for land application. These findings demonstrate that the co-digestion of KW and PM, particularly at KW-rich mixing ratios of 80:20 and 60:40, results in high methane recovery and the production of a nutrient-rich, low-contaminant digestate suitable for use as an organic fertilizer. Although the 100:0 reactor achieved the highest cumulative methane yield, the 80:20 and 60:40 KW:PM ratios offered superior performance by combining high methane recovery with improved process stability, nutrient balance, buffering capacity, and potential relevance for small-scale systems. The study, therefore, informs the design of of small-scale KW-PM co-digestion system as a decentralized waste-to-energy and nutrient-recycling option for rural and peri-urban communities in South Africa and similar contexts.

Keywords: Anaerobic co-digestion; kitchen waste; pig manure; biogas production; methane yield; substrate pre-treatment; digestate; renewable energy; South Africa.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Term	Definition
AD	Anaerobic digestion
ACoD	Anaerobic co-digestion
AMPTS II	Automatic Methane Potential Testing System II
ASBR	Anaerobic sequencing batch reactor
CSTR	Continuous stirred tank reactor
DEFF	Department of Environment, Forestry and Fisheries (South Africa)
HRT	Hydraulic retention time
ICP-MS	Inductively coupled plasma–mass spectrometry
KW	Kitchen waste
LCFA	Long-chain fatty acid
OLR	Organic loading rate
PM	Pig manure
PFR	Plug flow reactor
SABIA	South African Biogas Industry Association
SRT	Solids retention time
TS	Total solids
TGA	Thermogravimetric analysis
VFA	Volatile fatty acid
VM	Volatile matter
VS	Volatile solids

LIST OF SYMBOLS

Symbol	Definition	Unit
C	Carbon content	% (w/w)
N	Nitrogen Content	% (w/w)
C:N	Carbon-to-nitrogen ratio	dimensionless ratio
BMP	Biochemical methane potential	mL CH_4 g^{-1} VS
CH_4	Methane	mL CH_4 g^{-1} VS
CO_2	Carbon dioxide	% (v/v)
H_2	Hydrogen gas	% (v/v)
H_2S	Hydrogen sulphide	mg m^{-3}
NH_3-N	Ammonia nitrogen concentration	mg L^{-1}
pH	Measure of acidity/alkalinity	dimensionless
TS	Total solids	g kg^{-1}
VS	Volatile solids	g kg^{-1}
HRT	Hydraulic retention time	days
SRT	Solids retention time	days
BOD	Biochemical oxygen demand	mg L^{-1}
COD	Chemical oxygen demand	mg L^{-1}
T	Temperature	^0C

CHAPTER 1 - INTRODUCTION

1.1 Background of the study

In recent decades, biogas has gained prominence as a key renewable energy option, produced through the anaerobic degradation of organic matter. Ani *et al.* (2024:331) demonstrated in their systematic review. Biogas is composed of methane and carbon dioxide, and serves as an alternative clean fuel that can lower net greenhouse gas emissions. Arslan *et al.* (2024:43) explained that biogas production from diverse substrates offers a sustainable pathway to replace fossil fuels, while Alengebawy *et al.* (2024:2643) highlighted that integrating biogas into energy portfolios decreases dependence on non-renewable sources and promotes circular economy. Ogunkeyede *et al.* (2024:61) added that valorising organic residues for biogas production mitigates environmental pollution and generates renewable energy. Adekunle and Okolie (2015:207) outlined the biochemical mechanisms underlying anaerobic digestion (AD) and elucidated biogas production. Organic matter used includes vegetable and animal waste, industrial organic waste, household biowaste, wastewater, and agricultural crops (Department of Forestry, Fisheries and the Environment, 2022). Typically, biogas consists of 35-75% methane, 25-65% carbon dioxide, 1-5% hydrogen, and trace amounts of water vapor, ammonia, hydrogen sulfide, and halides depending on substrates and environmental factors (Yentekakis and Goula, 2017:1). It can be used to generate electricity, heat, and fuel. It also supports waste and wastewater treatment and yields nutrient-rich digestate that can be used as fertilizer, making it a sustainable option that can aid climate change mitigation, strengthen energy security, and create employment opportunities in South Africa, although these broader economic outcomes lie beyond the direct scope of this study.

Enhancing biogas production is essential for expanding renewable energy generation and effective waste management. Sher *et al.* (2024:848) argued that optimizing operational parameters of anaerobic digesters can maximize methane yield, turning waste management practices into profitable, sustainable energy systems. Kunatsa *et al.* (2021:11) showed that adjusting substrate ratios, temperature, and pH improves biogas performance while reducing environmental impact. Khaliq *et al.* (2025:881) provided empirical evidence that optimized co-digestion strategies yield higher biogas production than mono-digestion. Similarly, Bidiko *et al.* (2025:02) reported that selecting suitable substrates and integrating co-digestion techniques enhances biodigester efficiency, reduces operational costs, and boosts energy recovery. AD is a biological process comprising hydrolysis, acidogenesis, acetogenesis, and methanogenesis, each of which is critical for converting organic matter into methane (Sher et al. 2024:842). Anacleto *et al.* (2024:2) demonstrated that substrate pre-treatment significantly improves biodegradability and accelerates digestion, thereby increasing methane yields, depending on the feedstock composition.

Co-digestion, the simultaneous digestion of multiple substrates, has emerged as a strategy to overcome the limitations of mono-digestion. Gyadi *et al.* (2024:4) observed that co-digestion balances the carbon-to-nitrogen ratio and dilutes inhibitors, producing synergistic effects that boost methane generation. Ostos et al. (2024:09) noted that it also enhances microbial diversity, stabilizing the process and optimizing yields. Mata-Alvarez *et al.* (2014:413) described anaerobic co-digestion (ACoD) as a process that improves system efficiency, energy production, and stability over mono-digestion. Mono-digestion of lignocellulosic materials is slow and yields low methane because their recalcitrant structure resists enzymatic attack and microbial degradation, making them less readily converted to biogas (Sawatdeenarunat *et al.* 2015:179; Karrabi *et al.* 2023:3), but adding co-substrates such as animal manure supplements nutrients and buffering capacity (Sun, L. 2015:24). Studies on co-digestion of wheat, rice, and oat straw with various manures report higher methane yields (200-400 Ml/Gvs) than mono-digestion (120-200 Ml/Gvs) due to complementary properties (Wang *et al.* 2012:79; Ye *et al.* 2013:2654; Lehtomaki *et al.* 2007:593; Rahman *et al*. 2023:6637; Akindele *et al.* 2019:273). Pre-treatment processes such as mechanical shredding and enzymatic hydrolysis further enhance the efficiency of anaerobic digestion. This study investigates the combined impact of KW and PM on biogas yield and aims to identify effective optimization methods for methane production. In South Africa, where about 15% of households lack electricity (Longe, 2020:2), abundant KW and PM offer opportunities for sustainable biogas-based energy solutions in rural areas.

There are four stages in the anaerobic co-digestion process, namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The advantage of this process is that all the stages are linked because a by-product of one step becomes the substrate for the next step, an all-in-one system (Kumar *et al.* 2024:35). Hydrolysis is where complex compounds like lipids, carbohydrates, proteins, and hemicellulose are broken down into water-soluble compounds like sugar and amino acids. Next is the acidogenesis (fermentation) stage, where acidogenic bacteria degrade amino acids, sugars, and lipids into volatile fatty acids, organic acids, carbon dioxide, and hydrogen gas.

The next stage is acetogenesis, where volatile fatty acids (acetic acid and butyric acid) are converted to carbon dioxide, hydrogen and acetate. The last stage is methanogenesis, in which methanogens, which are excellent anaerobes, convert acetic acid to methane and carbon dioxide. The final product of ACoD is biogas, which is composed of methane and carbon dioxide. The figure below illustrates the biochemical breakdown of the process.

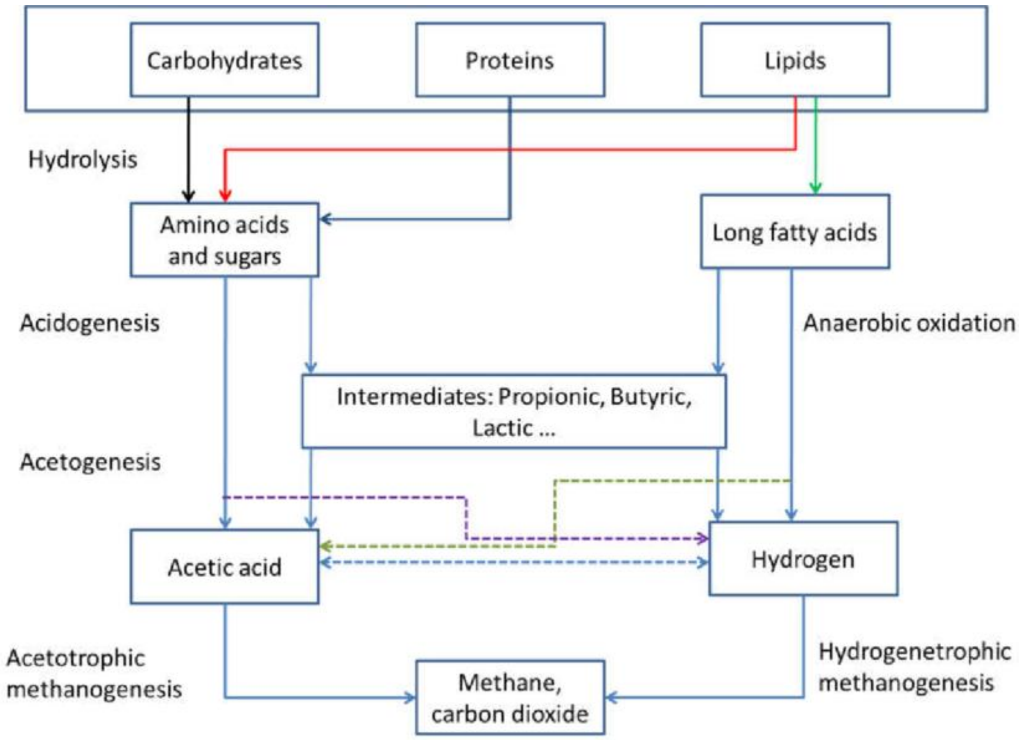

Figure 1.1: Schematic diagram showing anaerobic digestion (Kumar, 2024:37)

Among the myriad organic materials suitable for AD, KW and PM have attracted significant research interest due to their abundant availability and complementary characteristics. KW is rich in easily biodegradable compounds such as carbohydrates, proteins, and fats, which render it highly amenable to microbial degradation (Yang *et al.* 2024:2). In contrast, PM possesses a balanced composition of nutrients and a diverse microbial population that enhances the AD process through buffering capacity and micronutrient supply (Astals *et al.* 2012:63). Rahman *et al.* (2023:6636) observed that co-digesting organic waste and animal manure enhances biogas yield and mitigates challenges of mono-digestion, such as acidification and ammonia inhibition, largely due to the improved carbon-to-nitrogen (C:N) ratio. Furthermore, Baek *et al.* (2020:2) demonstrated that KW provides a high proportion of volatile solids, which are crucial for biogas production, and reduces inhibitory substances found in PM, thereby ensuring a more stable and efficient AD process. The economic viability of using food waste and animal manure is strengthened by their low or negative cost, underpinning their potential as valuable substrates for renewable energy (Ankathi, 2024:418).

Recent studies confirm that integrating KW and PM yields higher biogas volumes and produces digestate with superior fertilizer properties (Liebetrau *et al.* 2021:7). Co-digestion enhances methanogenic performance by balancing the C:N ratio and improving microbial diversity (Tian *et al.* 20215:51; Kumar *et al.* 2024:38). The increased microbial diversity stabilizes the process and ensures resilience to feedstock variation (Kumar *et al.* 2024:38; Ostos *et al.* 2024:04). Akbay (2024:2) noted that digestate from co-digestion systems is richer in nutrients, contributing to sustainable agriculture and reduced dependence on chemical fertilizers (Nghiem *et al.* 2017:355).

Moreover, co-digestion systems can be integrated into decentralized waste management frameworks tailored to local waste generation, thereby reducing transport costs and emissions (Piadeh *et al.* 2024:12; U.S. Environmental Protection Agency, 2025:3). Considering South Africa's energy demand and organic waste potential, this study aims to optimize biogas production through the co-digestion of KW and PM. The findings will support sustainable energy development for rural communities and address environmental and waste management challenges.

1.2 Problem statement

South Africa faces significant energy and waste management challenges, as rapid urbanization and agricultural activity have intensified the accumulation of organic waste. KW and PM are generated in good quantities and possess complementary characteristics for AD, yet their efficient conversion to biomethane depends strongly on substrate balance and process conditions. Although previous studies have shown that co-digestion of food waste and animal manure can improve methane production and process stability, the reported optimum mixing ratios vary widely across feedstocks, reactor configurations, and operating conditions, making it difficult to identify a preferred ratio for KW-PM systems under specific conditions.

Moreso, while mechanical and enzymatic pre-treatment have each been reported to improve substrate biodegradability, limited information is available on their combined application to KW-

PM mixtures under mesophilic batch biomethane potential (BMP) conditions. The central problem addressed in this study is therefore the need to determine the KW:PM mixing ratio that gives the best BMP performance under controlled mesophilic conditions with combined mechanical and enzymatic pre-treatment, while maintaining acceptable process stability and producing a digestate with favourable nutrient characteristics.

1.3 Research objectives

The main objective of this study is to determine the optimum KW-PM mixing ratio for ACoD under mesophilic conditions following combined mechanical and enzymatic pre-treatment, using biomethane yield and process stability as the primary performance criteria.

1.3.1 The secondary objectives are to:

1. To evaluate the effect of different KW:PM mixing ratios on daily and cumulative biomethane yield under mesophilic BMP conditions.
2. To assess process stability across the tested mixtures using key operational indicators, including pH, ammonia concentration, and volatile solids (VS) reduction.
3. To examine the contribution of combined mechanical and enzymatic pre-treatment to substrate biodegradability and digestion performance within the selected KW-PM systems.
4. To characterise the resulting digestate in terms of selected physicochemical, elemental, and nutrient-related properties relevant to agricultural reuse.
5. To discuss the possible relevance of the findings for small-scale KW-PM biogas systems in South Africa as an implication of the technical results and supporting literature.

1.3.2 Research questions

1. What KW:PM mixing ratio gives the best BMP performance under mesophilic conditions after combined mechanical and enzymatic pre-treatment?
2. How do variations in KW:PM ratio influence process stability, as reflected by pH behaviour, ammonia concentration, and VS reduction during ACoD?
3. What are the physicochemical and nutrient-related characteristics of the digestate obtained from the tested KW-PM mixtures, and what do these indicate regarding its potential for agricultural reuse?

1.4 Research scope

This study is limited to the laboratory-scale experimental evaluation of ACoD of KW and PM under mesophilic conditions. This work focuses on the influence of substrate ratio and combined mechanical and enzymatic pre-treatment on BMP performance, process stability, and digestate quality. This research evaluates biogas production from co-digestion of KW and PM by analysing key operational parameters like temperature (mesophilic range of 35 °C), pH (optimal range of 6.8 -7.2), methane yield, and end-point ammonium-nitrogen concentration over a retention period of 28 days. The investigation considered six KW:PM mixing ratios on a VS basis, namely 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100, under controlled mesophilic conditions using batch digestion tests. Key performance indicators included daily and cumulative biomethane yield, pH, ammonia concentration, VS reduction, and selected digestate characteristics obtained through physicochemical, elemental, thermogravimetric, and nutrient-related analyses.

1.5 Significance of the study

This research contributes to the expanding body of knowledge on biogas production, particularly in South Africa, where there is a pressing demand for sustainable energy options. The outcomes have the potential to strengthen energy security by promoting biogas as a renewable source that can complement conventional electricity systems, and may help the development of small- and medium-scale biogas plants, thereby benefiting policymakers, researchers, and stakeholders in both the renewable energy and waste management sectors.

The study also provides useful information on digestate quality, thereby extending the assessment of KW-PM co-digestion beyond energy recovery alone to include nutrient recovery and possible agricultural reuse. In this respect, the work supports the broader concept of resource recovery from organic waste and contributes to understanding how waste treatment, renewable energy generation, and nutrient recycling can be linked within one system

1.6 Research methodology

This study adopted a quantitative experimental research design, combining literature review, laboratory investigation, and analytical characterisation. The core of this study consist of laboratory-scale BMP tests conducted under mesophilic conditions to evaluate the effect of varying KW:PM ratios on BMP performance after combined mechanical and enzymatic pre-treatment. Substrate preparation, digestion monitoring, and digestate characterisation were undertaken to generate data on methane production, process stability, and post-digestion material quality. The experimental findings were then interpreted with reference to relevant literature in order to explain observed trends and to discuss the wider technical relevance of the results.

1.7 Gap Statement

Previous studies show that co-digesting KW and PM can improve methane production by balancing nutrients, reducing acidification, and enhancing process stability. However, the optimum mixing ratio varies across studies, and limited research has examined combined mechanical and enzymatic pre-treatment under mesophilic conditions, particularly in South Africa. This study therefore evaluates different KW-PM ratios to identify the best balance between biomethane yield, process stability, and digestate quality.

1.8 Structure of the thesis

Chapter 1 introduces the study and outlines the background, problem statement, justification, aims and objectives, scope and significance. Chapter 2 provides an in-depth review of relevant literature. Chapter 3 describes the experimental materials, research methods, and analytical procedures. Chapter 4 reports and interprets the findings, and Chapter 5 summarizes the study's main conclusions and offers recommendations for future work.

1.9 Summary of the chapter

In summary, the study promotes the optimum co-digestion KW-PM ratio as a sustainable and efficient method for biogas production. This approach also offers advantages in biogas generation, waste management and nutrient recycling for agricultural applications.

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CHAPTER 2 - LITERATURE REVIEW

2.1 Introduction

This chapter reviews the literature relevant to the anaerobic co-digestion (ACoD) of kitchen waste (KW) and pig manure (PM), focusing on substrate characteristics, operating conditions, co-digestion performance, pre-treatment methods, and digestate quality. The review examines the key factors influencing KW-PM optimisation under mesophilic conditions, including substrate ratio, methane yield, process stability, and pre-treatment. The chapter also compares findings from previous KW-PM related studies to identify differences in optimum mixing ratios, operating conditions, and performance, thereby establishing the research gap addressed in this study.

2.2 Purpose of the literature review

This literature review serves several key purposes. Firstly, it aims to provide an in-depth overview of the existing knowledge on biogas production through the anaerobic co-digestion of KW and PM. This includes exploring the fundamental principles of anaerobic digestion (AD), the advantages of co-digestion, and the specific characteristics of KW and PM as substrates. Secondly, the review seeks to identify gaps in current research, including optimal co-digestion mix ratios, the influence of pre-treatment methods, and the specific challenges and opportunities posed by the South African context. Highlighting these gaps, the review underscores the need to evaluate the optimal mix ratio for biogas production from KW and PM. Finally, the review aims to establish a strong theoretical foundation for the research by synthesizing the current understanding and providing context for the experimental investigation.

2.3 Scope of the review

This chapter encompasses a systematic analysis of existing literature on AD, co-digestion, and their applications in renewable energy generation. Specifically, it focuses on the physicochemical properties of KW and PM and their implications for biogas yield, as well as the digestate obtained after digestion is completed. This considers the digestate as a biofertilizer for farming. The review also examines the influence of various factors, such as temperature, pH, and microbial community composition, on the anaerobic digestion process. Furthermore, it examines pre-treatment methods employed to enhance biogas production from these substrates. While the review draws on global research, it places particular emphasis on studies conducted in South Africa or relevant to its context, given the region's specific waste management practices and environmental conditions.

2.4 Structure of the chapter

This chapter is structured to provide a logical and coherent progression of information. It starts by reviewing the anaerobic digestion pathway, highlighting the biochemical stages and the key operating and environmental conditions that govern process efficiency (University of Maryland Extension, 2014:1). Following this, the chapter analyses co-digestion as a technique to improve biogas production, highlighting its advantages over single-substrate digestion and discussing the synergistic effects achieved by combining different substrates. Subsequently, the review explores the characteristics of KW and PM as feedstocks for biogas production, examining their composition, biodegradability, and potential inhibitory substances. Also, the chapter examines various pre-treatment methods employed to enhance biogas yield from KW and PM, including physical and enzymatic/biological pre-treatments, and assesses their effectiveness in improving substrate digestibility. Finally, the chapter investigates the properties of digestate for use as fertilizer.

2.5 Anaerobic digestion process

AD is a biological process in which microorganisms degrade organic matter in the absence of oxygen to produce biogas and digestate. The process occurs is in four linked stages: hydrolysis, in which complex polymers are broken down into soluble compounds; acidogenesis, where these intermediates are converted into volatile fatty acids and related products; acetogenesis, which produces acetate, hydrogen, and carbon dioxide; and methanogenesis, where methanogenic archaea convert acetate and hydrogen/carbon dioxide into methane. Although these stages are fundamental to all AD systems, the present review is concerned less with general AD theory than with how substrate composition, operating conditions, and pre-treatment affect methane yield and stability in KW-PM co-digestion. Figure 2.1 gives a schematic layout of a typical anaerobic digestion process.

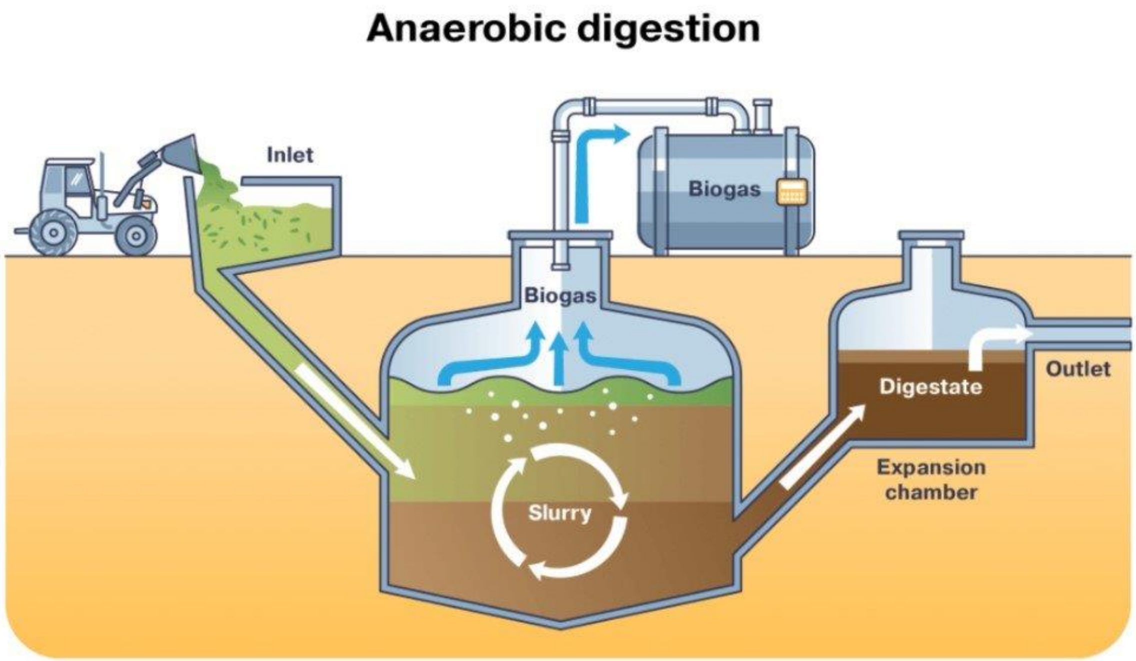

Figure 2.1: Schematic layout of anaerobic digestion process (Southern Scientific Services, 2024)

2.5.1 Factors affecting anaerobic digestion

Several operational factors influence AD efficiency, including temperature, retention time, organic loading rate, and reactor design. Most biogas plants operate under mesophilic conditions around 30-40 °C (Jameel *et al.* 2024:9;Shoukat *et al.* 2025:4), which support stable microbial communities and require less energy input than thermophilic operation. Retention time and organic loading rate must be balanced to allow sufficient substrate-microbe contact without overloading the system (Pan *et al.* 2021:8; Wei *et al.* 2019:4; Moen *et al.* 2003:6;), and typical mesophilic digesters achieve effective performance at HRTs of 20-30 days and moderate OLRs (Zhang *et al.* 2023:7). Different digester configurations (e.g., fixed-dome, CSTR, plug-flow) offer various trade-offs in mixing and solids handling (Kenpro Foundation, 2022:18; Sharma and Arya, 2017:76; Banerjee *et al.* 2022:4), but this study focuses on laboratory-scale BMP reactors under controlled mesophilic conditions

2.6 Substrates characterization

2.6.1 Kitchen waste: composition, biodegradability, and limitations

KW generally has a high C/N ratio, indicating dominance of carbon-rich components such as carbohydrates and lipids, with a lower proportion of nitrogen (Salangsang *et al.* 2022:2), lead to ammonia inhibition at excessive concentrations (Salangsang *et al.* 2022:2). Co-digestion, in which two or more substrates are digested together, provides a means of adjusting the overall C/N ratio. When KW and PM are co-digested, the carbon-rich KW offsets the nitrogen-rich PM, yielding a more balanced C/N ratio in the digester. This synergistic effect can enhance biogas production by providing the microbial community with the necessary nutrients in appropriate proportions. The balanced C/N ratio in co-digestion can also mitigate the risk of ammonia inhibition often associated with digesting animal manure alone (Rahman *et al.* 2023:6636)

Biodegradability, the extent to which microorganisms can break down organic matter, is another critical factor influencing biogas production in AD. Highly biodegradable substrates are more readily hydrolysed and converted into biogas, while less biodegradable materials require more time and effort from the microbial community. The biodegradability of both KW and PM varies depending on their specific composition (Somsila *et al.* 2021:124).

KW, due to its diverse composition, exhibits a range of biodegradability. Components like fruit and vegetable scraps are generally highly biodegradable, readily undergoing hydrolysis and acidification. However, more fibrous fractions degrade more slowly and may require extended retention times to achieve effective digestion (Tedesco *et al.* 2025:01).

2.6.2 Pig manure: characteristics and buffering properties

PM is characterised by a low C/N ratio, indicating a relatively high nitrogen content, largely present as ammonia. While elevated nitrogen levels can be advantageous by supplying essential nutrients for microbial activity, excessive ammonia concentrations may inhibit methanogenic microorganisms and negatively affect anaerobic digestion (Salangsang *et al.* 2022:2). The risk of ammonia inhibition underscores the need for careful management of nitrogen levels during digestion. Furthermore, pig manure contains a mix of readily biodegradable substances, such as proteins and fats, alongside more resistant components, such as fibrous tissue, which can slow degradation. This heterogeneous composition affects the overall biodegradability and digestion kinetics, often necessitating co-digestion or pre-treatment strategies to achieve optimal methane yields.

2.6.3 Synergistic rationale for co-digestion (C/N balance, nutrient complementarity)

The AD process converts organic material into biogas through a series of biochemical reactions, producing a gas mixture dominated by methane (CH_4) and carbon dioxide (CO_2). Although mono-digestion, the processing of a single feedstock, has been widely used for producing biogas, its performance is frequently limited by nutrient imbalances, process fluctuatons, and inhibitory compounds (Luo and Pradhan, 2024:1). To enhance biogas yield and overall process efficiency, researchers have explored the co-digestion of multiple substrates, a method that combines different organic materials to improve microbial synergy, nutrient balance, and methane generation (Luo and Pradhan, 2024:3).

The combination of KW and PM has been widely studied because this mixture offers high biogas potential with improved process stability (Tian *et al.* 2015:51). KW is rich in carbohydrates, lipids, and proteins, providing an energy-rich substrate for AD. However, its mono-digestion process often leads to rapid acidification and microbial inhibition due to excessive VFA production. PM, on the other hand, contains ammonia, trace elements, and buffering compounds that help stabilize the pH and prevent process failure (Zhu *et al.* 2022:4).

Recent research has shown that co-digesting KW and PM yields notable synergistic benefits. Li *et al.* (2013:2086), for example, observed that co-digestion increased methane production by up to 35% relative to KW mono-digestion, an improvement linked to a more balanced microbial community, enhanced hydrolysis, and more stable VFA utilisation.

Moreover, co-digestion systems typically harbour a more diverse microbial community, which enhances substrate utilisation and overall metabolic efficiency (Luo and Pradhan, 2024:3. In such systems, the high-fat fraction of KW supplies long-chain fatty acids (LCFAs), which are slowly degraded by methanogens, while PM provides essential buffering agents that prevent the accumulation of inhibitory compounds.

Furthermore, co-digestion enhances biogas composition by increasing methane purity. A comparative study by found that mono-digestion of KW produced biogas with a 52-55% methane content, whereas co-digestion with PM increased the methane content to 60-65%, making it a more viable fuel source (Misganaw *et al.* 2013:303).

Numerous studies have shown that co-digestion can raise biogas production by approximately 20-50% relative to mono-digestion (Kriswantoro *et al.* 2024:07). For example, Ofon *et al.* (2024:89) found that combining food waste with animal manure led to higher methane yields, which they attributed to achieving a more favourable carbon-to-nitrogen (C/N) ratio (Ofon *et al.* 2024:84).

Mono-digestion of food waste is prone to rapid acidification that suppresses microbial activity, whereas digesting animal manure alone often results in excessive ammonia levels that are harmful to methanogens. Co-digesting these substrates mitigates both problems: the carbon-rich food waste offsets the nitrogen-rich manure, producing a more balanced environment that enhances biogas output and improves process stability (Abbas *et al.* 2023:2).

Comparative studies on biogas yields have shown that co-digestion improves the degradation of volatile solids (VS), thereby increasing the fraction of substrate converted to biogas. In addition, combining high-energy feedstocks such as food waste or crop residues with lower-energy co-substrates like manure enhances the biogas production rate by supplying more readily degradable organic matter while maintaining process stability (Varol and Ugurlu, 2017:402).

2.7 Co-digestion for improved biogas production

2.7.1 Mechanism and process synergy

Co-digestion offers several advantages compared with single-substrate digestion (Karki *et al.* 2021:2). Co-digestion improves nutrient balance, creating favourable conditions for microbial activity and enhanced biogas production. It also dilutes inhibitory compounds, increases substrate biodegradability, and supports more efficient digestion. In addition, co-digestion can improve process economics by increasing organic loading capacity. Diverting organic waste from landfills further reduces methane emissions, contributing to greenhouse gas mitigation and more sustainable waste management. However, through co-digestion, these greenhouse gas emissions can be captured and converted into renewable energy, reducing environmental impact (Bidiko *et al.* 2025:02). Furthermore, co-digestion helps manage waste more effectively by diverting organic materials from landfills, reducing leachate formation, and minimizing pollution risks (Morelli *et al.* 2020:227).

2.7.2 Mixing ratios

Several studies have explored how co-digestion influences biogas production. Tian *et al.* (2023:3), for example, investigated the anaerobic co-digestion (ACoD) of PM and rice straw in batch reactors using different mixing ratios. They reported that co-digestion increased methane generation, with PM-to-rice straw ratios of 1:1, 1:5, and 1:10 yielding cumulative methane productions that were 44.21%, 87.15%, and 62.47% higher, respectively, than the values calculated from the corresponding mono-digestion tests. The optimal ratio was determined to be 1:5, resulting in a more balanced nutrient composition and significantly enhancing the synergistic effect. In a similar study, Tian *et al.* (2015:52) examined the ACoD of KW and PM under mesophilic conditions. The researchers tested seven different total solids ratios of PM to KW: 1:0, 5:1, 3:1, 1:1, 1:3, 1:5, and 0:1. The findings indicated that co-digestion enhanced methane production compared to the solo digestion of either substrate. The optimal mixing ratio was found to be 1:1, providing a balanced nutrient environment conducive to microbial activity.

Co-digestion of KW and PM provides significant benefits over mono-digestion, with a more balanced nutrient profile, stronger buffering, and a richer microbial community in the digester. KW is rich in readily biodegradable carbohydrates, which enhance microbial activity, while PM provides essential nitrogen sources that support microbial growth and maintain system stability (Tian *et al.* 2015:51). The complementary nature of these feedstocks ensures a more efficient AD process, yielding higher biogas output and more stable performance than mono-digestion. Moreover, co-digestion strategies can help to mitigate common challenges associated with individual feedstocks, such as ammonia inhibition from high-nitrogen waste or acidification due to excessive carbohydrate content (Lu *et al*. 2017:3).

The ratio of KW to PM influences both the stability of AD and the resulting biogas yield. Optimal mixing ratios can enhance microbial activity, improve nutrient balance, and prevent acid accumulation, which can hinder biogas production (Rahman *et al.* 2023:6638). For instance, a well-balanced C:N ratio is essential for maintaining microbial health; if the ratio is too high in carbon or nitrogen, it can lead to suboptimal digestion conditions (Zhang *et al.* 2022:10). Studies have shown that different substrate ratios lead to varying levels of methane production. For example, Kriswantoro *et al.* (2024:09) reported that a 1:1 mixing ratio of Napier grass to food waste maximised methane yield, achieving good biodegradability and a biogas production of 1,161.33 mL/g VS. This optimal ratio not only provided stable digestion performance but also minimized the lag phase associated with AD. The stability of the AD process is tied to the chosen substrate ratios, underscoring the importance of optimizing these parameters for effective biogas production.

Numerous experimental studies have been conducted to identify suitable mixing ratios for co-digesting KW and PM. For example, Dennehy *et al.* (2018:535) reported that a feedstock mixture containing 60:40 (on a VS basis) of food waste increased methane yield. Likewise, Molinuevo-

Salces *et al.* (2012:37) reported that a 50:50 mixture on a dry-weight basis, with vegetable processing waste, boosted methane production by up to three times compared with the mono-digestion of PM alone. These findings highlight that incorporating a higher proportion of KW helps balance the C:N ratio, reduces ammonia inhibition, and thereby enhances biogas generation efficiency (Hanum *et al.* 2022:9). Santaweesuk *et al.* (2025:4) reported that co-digestion of PM with PM/Napier grass at around 1:1 ratio often produces the highest methane yields due to balanced nutrient content and stable ammonia concentrations, preventing inhibition while enhancing biogas efficiency. This finding aligns with other studies indicating that co-digestion yields higher biogas production efficiency than mono-digestion.

Variation in reported outcomes is linked to differences in feedstock characteristics, process settings, and reactor configurations. For example, while some studies found optimal results at a 1:1 ratio, others reported better performance at ratios such as 1:5 or 1:3 (Yen *et al.* 2017:7). Such discrepancies emphasise that optimal mixing ratios depend on site-specific conditions and the nature of the substrates. In addition, factors such as operating temperature, pH, and total solids concentration strongly influence biogas yields.

Numerous co-digestion studies involving food waste and manure report improved methane yields at intermediate mixing ratios, although the exact optimum varies with feedstock and reactor conditions (Kerklaan, 2025:7; Orhorhoro and Erameh, 2021:58).

2.7.3 Challenges and inhibitions in co-digestion systems

Challenges and inhibitory factors in co-digestion systems primarily arise from substrate characteristics and prevailing process conditions. PM, for example, has high nitrogen content, and the resulting ammonia build-up at elevated concentrations can be toxic to methanogenic microbes, leading to lower methane output (Tian *et al.* 2015:51). By contrast, KW is dominated by easily degradable organics, so VFAs can build up quickly, causing acidification and a decline in pH, ultimately suppressing the methanogenic activity (Tian *et al.* 2015:51). Maintaining the pH within an optimal range (6.5-8.0) is therefore essential for process stability. Moreover, high total solids concentrations may impede microbial metabolism, thereby reducing biogas production efficiency (Almomani and Bhosale, 2020:5). Given the variability in substrate characteristics and operational parameters, it is crucial to carefully balance feedstock ratios and environmental conditions to prevent inhibition and sustain consistent performance. Continuous monitoring and adjustment of these factors are vital to maintaining stable, long-term biogas generation in co-digestion processes.

2.8 Biogas yield and composition

2.8.1 Factors influencing biogas volume

2.8.1.1 Substrate properties

The biochemical composition of the feedstock significantly influences the resulting biogas yield. Substrates with high levels of carbohydrates, proteins, and lipids typically generate larger biogas volumes because they are more easily biodegradable (Anacleto *et al.* 2024:5). For instance, a study by Xu *et al.* (2022:44) found that paper waste, which primarily consists of carbohydrates, was less readily degradable and produced lower methane yields than food waste, which is rich in proteins and lipids. When a 4:1 ratio of food waste to paper waste was used, greater organic degradation and better biogas production were observed. In contrast, adjusting the ratio to 1:1 reduced methane yield by up to 15.5%. Additionally, thermophilic digestion was shown to enhance both substrate breakdown and process stability compared with mesophilic conditions, indicating greater efficiency and resilience in handling varying substrate compositions. In co-digestion processes, substrates rich in proteins and lipids, such as KW, exhibit greater degradability and methane production potential than carbohydrate-dominated materials (Liu *et al.* 2022:9). This highlights substrate composition as a key determinant of AD efficiency.

2.8.1.2 Operational conditions

Biogas production is highly sensitive to temperature, pH, hydraulic retention time (HRT), and organic loading rate (OLR). For instance, a review by Ngabala and Emmanuel, (2024:9) highlighted that maintaining mesophilic temperatures helps support stable microbial communities, which are conducive to efficient biogas production. In addition, the study highlighted that maintaining a pH between 6.5 and 8.5 is crucial for optimal methanogenic activity and maximum methane production. The authors also noted that the interaction between HRT and OLR is key to maintaining process stability and improving gas yields.

2.8.1.3 Feedstock characteristics

Co-digestion significantly influences the composition of biogas produced from mixed substrates. Combining different feedstocks promotes greater microbial diversity and a wider range of metabolic routes, which in turn leads to higher methane production and changes in gas composition. A detailed study (Mousa *et al.* 2016:117) found that co-digestion of KW and poultry manure resulted in notably higher cumulative biogas production and methane content than digesting each substrate alone. Specifically, when the digester operated with an approximate 2:1 ratio of KW to poultry manure, it achieved the highest biogas yield and methane concentration, reaching up to 48% methane under mesophilic conditions. A researcher attributed this enhanced performance to the synergistic effects of co-digestion, including a more balanced nutrient profile, improved buffering capacity, and greater stability within the digester. These factors collectively fostered optimal microbial activity and promoted more efficient metabolic pathways. (Rahman *et al.* 2021:6643).

2.8.1.4 Case studies from South Africa

A study conducted in South Africa by Sebola *et al.* (2015:592) provided insights into the biogas production potential of PM and other substrates under AD. The study assessed several substrates for AD, namely cow dung, PM, chicken manure, and sewage waste, under mesophilic conditions at 35 °C. Each digester was prepared with 100 g of substrate mixed with 800 ml of water, giving a substrate-to-inoculum ratio of 1:8, and operated in 1-liter batch reactors over a six-day period. Initial pH values ranged from 6.6 to 7.6, falling within the optimal window for methanogen activity. The substrates' chemical composition varied, with C/N ratios of 26.2 for cow dung, 17.6 for PM, 8.1 for chicken manure, and 8.6 for sewage waste. Volatile solids content spanned 45-58%, while moisture content ranged from 7% to 34% on a dry basis. Since the experiments used batch reactors, neither the OLR nor the HRT was externally controlled; however, biogas was collected daily under standard conditions.

The results showed that reducing the particle size of the substrates increased biogas production and methane yield. The finest particle size tested (25 µm) produced the highest methane volumes: 574 mL for cow dung, 552 mL for PM, 483 mL for chicken manure, and 416 mL for sewage waste. These yields represented improvements of 3-30% compared to larger particle sizes. Overall, cow dung and PM exhibited the most stable digestion and the highest methane recovery, which the authors attributed to their favourable C/N ratios, moisture content, and greater microbial accessibility (Sebola *et al.* 2015:15).

2.9 Pretreatment technologies

2.9.1 Mechanical pretreatment

Mechanical pre-treatment reduces particle size and alters the structural integrity of biomass. The resulting increase in surface area enhances microbial and enzymatic contact, leading to a faster hydrolysis during AD. Common mechanical methods include milling, grinding, and ultrasonication.

Several studies have confirmed the effectiveness of mechanical pre-treatment. For instance, comminution of vegetable waste mixed with sewage sludge reduced fermentation time (Rodriguez *et al.* 2017:158). Similarly, grinding and milling have been shown to enhance the surface morphology of substrates, significantly influencing AD (Aworanti *et al.* 2023:06).

However, the benefits of mechanical pre-treatment must be weighed against its energy requirements. The energy input for processes like milling can be substantial and, if not optimized, may offset gains in biogas yield. Therefore, it is essential to balance the energy demand of pre-treatment against the energy recovered as biogas.

2.9.2 Enzymatic pretreatment

Enzymatic pre-treatment involves the application of enzymes to break down complex polymers into more easily digestible forms. The process enhances the hydrolysis of cellulose and hemicellulose into fermentable sugars, thereby improving subsequent biogas formation by microorganisms. Enzymatic pre-treatment is often favoured for its specificity and gentle operating conditions, which preserve the integrity of the desired products.

Dinca *et al.* (2024:910) indicated that enzymatic pre-treatment can significantly enhance biogas yields. For example, the application of cellulases and hemi-cellulases to lignocellulosic substrates has been shown to degrade insoluble cellulose and increase methane production. The effectiveness of this approach depends on enzyme concentration, incubation time, and the physicochemical characteristics of the biomass.

However, the use of enzymatic pre-treatment is constrained by high enzyme costs and limited enzyme activity. Enzymes can be expensive, and their activity may be inhibited by certain compounds present in the biomass. To overcome this issue, recent research studies have investigated the potential of fermented cow dung for producing lignocellulolytic enzymes as a cost-effective substitute for commercial enzymes (Raymond *et al.* 2015:7). Cow dung consists of rich, diverse microbes like bacteria and fungi capable of hydrolytic enzyme secretion during fermentation (Behera and Ray, 2020:1). During controlled fermentation of cow dung, the activity of the microorganisms speeds up, which results in an endogenous production of enzymes (e.g., cellulase, lipase, amylase, and proteases) (Vijayaraghavan *et al*. 2015:115). The enzymes degrade the lignocellulosic biomass and other complex organics in a feedstock such as KW or PM, thereby simulating commercial enzymatic pre-treatment (Zhou *et al.* 2023:226).

The use of fermented cow dung as a pre-treatment stage complies with the circular economy and waste-to-resource concept by using locally available resources (HomeBiogas, 2024). It not only decreases dependence on industrial inputs but also helps to reduce operational costs and improve the scalability of biogas systems in rural areas. Furthermore, cow dung can be fermented on a small scale on-site using simple technology, making it particularly suitable for smallholder households and community-level biogas applications (Shaibur *et al.* 2021:2).

2.9.3 Combined pretreatment strategies and research gaps

Combining mechanical and enzymatic pre-treatments can synergistically enhance biogas production. Mechanical treatments can break down the biomass structure, enlarge the available surface area, and enhance the accessibility of internal polymers to enzymatic action (Maurya *et al.* 2015:599). Subsequently, enzymatic pre-treatment can more effectively hydrolyse these polymers into fermentable sugars (Pal *et al.* 2016:1). This integrated approach enhances hydrolysis efficiency and biogas generation beyond what is achievable by either method alone. This suggests that optimizing the sequence and conditions of combined pre-treatments can lead to more efficient biogas production processes.

In South Africa, investigations into the co-digestion of KW and PM are still relatively few. Only a handful of local studies have explored combined KW-PM systems, with most South African research emphasizing the effects of particle size and broader food-waste co-digestion rather than focusing specifically on KW-PM mixtures (Sebola *et al.* 2015:13; Awosusi *et al.* 2021:794). In contrast, more comprehensive investigations into KW-PM ratios and temperature effects have been conducted in China and Bangladesh, with findings indicating increased methane yields but differing optimal substrate mixes (Tian *et al.* 2015:52; Rahman *et al.* 2023:6637). The absence of locally focused research constrains the development of tailored recommendations for substrate ratios, process management, and digestate utilization in South Africa. As a result, there is a clear need for targeted local trials, supported by appropriate policy measures and farmer training initiatives.

2.9.4 The need for more research on pretreatment techniques and their cost-effectiveness

A review of the current literature highlights a significant gap in research on optimizing pre-treatment techniques for the co-digestion of KW and PM. While thermal (Li *et al.* 2022:03; Jo *et al.* 2024:33), mechanical, biological (Tran *et al.* 2021:458), and enzymatic (Vasmara *et al.* 2023:3) pre-treatments have been studied, there is limited understanding of how combining these methods might synergistically enhance the performance of KW-PM blends. Investigating such integrated approaches is crucial, as they have the potential to increase substrate accessibility and boost biogas yields during co-digestion. Without focused research in this area, the benefits of combined pre-treatments for KW and PM may remain largely untapped.

In addition to technical considerations, a thorough assessment of the economic feasibility of these pre-treatment strategies is necessary. Beyond evaluating technical performance, it is important to analyse both capital and operational costs to assess cost-effectiveness in practical settings (Sebola and Tesfasgiorgis, 2015:81). Economic evaluations will provide valuable guidance for stakeholders and policymakers, informing investment decisions and supporting the adoption of scalable, sustainable biogas solutions. Addressing these research gaps will help bridge the divide between laboratory innovation and commercial deployment, ultimately advancing renewable energy objectives.

2.10 Process performance and evaluation

2.10.1 Indicators: moisture content, total solids (TS), and volatile solids (VS)

2.10.1.1 Moisture content

The performance of the AD process is influenced by physicochemical conditions, biogas yield, and system stability. Among these factors, the moisture content plays a critical role in substrate degradation, mass transfer efficiency, and microbial metabolism (Kumar *et al.* 2024:40). KW and PM have distinct moisture contents, which affect their biodegradability, digestion kinetics, and suitability as co-substrates in biogas production.

KW is highly degradable and energy-rich, with a moisture content of 60% to 90%, depending on its composition. While its high-water content facilitates microbial uptake, excessive moisture dilutes the OLR, reducing biogas production efficiency (Mrosso *et al.* 2023:4). On the contrary, PM has a moderate moisture content (70-80%), which helps maintain steady microbial activity and provides buffering capacity in AD systems (Sebola *et al.* 2015:15). Co-digesting KW with PM can optimize moisture levels, organic matter degradation, and biogas (biomethane) yield, thereby enhancing process stability and efficiency.

2.10.1.2 Total solids (TS) and volatile solids (VS)

The total solids (TS) of substrates consist of organic and inorganic fractions, with the organic fraction comprising volatile solids (VS), which are decomposed by anaerobic microorganisms. The VS fraction typically includes carbohydrates, proteins, fats, and other biodegradable compounds, which directly influence biogas yields. On the contrary, the inorganic fraction consists of non-degradable materials such as minerals and ash, which do not contribute to methane production (Orhorhoro *et al.* 2017:132). This indicates that high-VS substrates yield higher methane production, whereas low-VS substrates yield lower biogas yields. Apparently, TS and VS are critical parameters that influence biomass degradation and methane yield, with optimal TS levels promoting effective microbial metabolism and stable digestion conditions (Ahmad *et al.* 2024:7).

The TS and VS are essential for understanding how well substrates perform during ACoD. TS shows the amount of dry material and influences how the digester is designed and operated, while VS represents the organic portion that microbes can break down to produce methane. KW with an 85-96% VS composition and animal manure with a 72-93% VS composition and 5-9% TS composition are ideal for co-digestion. Maintaining the TS between 5-15% supports efficient gas production of about 278-292 NmL CH_4/g VS, whereas higher TS levels can cause acid buildup and reduce the biogas yield. Regular monitoring of TS is essential for process balance, stability, and high biogas productivity (Odejobi *et al.* 2021:5521).

2.10.1.3 Biochemical oxygen demand and chemical oxygen demand as indicators of degradability

The biochemical oxygen demand (BOD) is an important measure used to assess how easily microorganisms can break down organic matter in a substrate. It measures the amount of oxygen required by microorganisms to decompose organic compounds under aerobic conditions. A higher BOD value shows that the substrate has more biodegradable organic material, which often translates into greater potential for biogas production. For example, maize silage and grass showed higher BOD-related activity and produced more methane, making BOD a useful early indicator of how well a substrate will perform before conducting longer, more complex tests like the biochemical methane potential (BMP) (Suchowska-Kisielewicz and Jędrczak, 2019:2).

The chemical oxygen demand (COD) shows how much oxygen is needed to chemically break down all organic substances in a sample, including those that are not easily biodegradable. It provides an overall picture of the organic strength and potential energy content of a substrate. Substrates such as maize silage, with a COD of around 1884 g/kg dry matter, showed higher degradability than chicken manure, with 1156 g/kg dry matter. Strong correlations ($R^2 = 0.85$) between COD reduction and methane production confirm that COD values are a dependable indicator of how much organic matter can be converted to biogas. Combining COD with BOD provides a more complete view of the organic load and its biodegradability in AD. While COD reflects the total organic content, using it alongside BOD offers clearer insight into both the quantity and the quality of biodegradable material (Suchowska-Kisielewicz and Jędrczak, 2019:2). BOD and COD are closely linked to biogas production, as they indicate how much organic matter available for microbial digestion. The BOD/COD ratio is an important metric for assessing substrate biodegradability and methane yield potential. A comprehensive review of biodegradability indicators confirms that BOD/COD ratios above 0.5 indicate the presence of readily biodegradable organic matter, optimal for anaerobic digestion. Ratios below 0.3 point to poor biodegradability and limited methane potential **(**Suchowska-Kisielewicz and Jędrczak, 2019:2).

2.11 Challenges and causes of non-improvement in biogas production

Although limited studies have reported the joint impact of co-digestion and mechanical-enzymatic pre-treatment, some research has found that biogas production can be stagnated or decrease.

An imbalanced carbon-nitrogen ratio often leads to ammonia accumulation or excessive acidification, both of which inhibit methanogens and lower methane yields (Wang *et al*. 2014:2; Salangsang *et al.* 2022:2). Likewise, overdoing mechanical pre-treatment can cause the release of phenolic compounds and long-chain fatty acids that inhibit microbial pathways (Fan *et al.* 2022:2). Zhang *et al.* (2025:1) suggested that higher proportions of KW in a co-digestion system build up VFAs, which further reduce methane output.

2.12 Collection and storage of produced biogas (pilot scale)

2.12.1 Gas collection techniques

Laboratory experiments typically employ small batch digesters fitted with gas-tight sampling ports and water-displacement systems to measure biogas production. A comprehensive study validated various techniques for monitoring biogas volume, such as liquid replacement systems (LRS) and continuous liquid replacement systems (CLRS), both of which connect to reactors using gas-tight seals to prevent leaks. These setups enable precise measurement by tracking the volume of displaced liquid as biogas accumulates, ensuring reliable, accurate data collection without significant gas loss (Pham *et al.* 2013:867).

The integrity of these gas-tight systems is crucial, as even minor leaks can lead to underestimation of biogas yields. To analyse the gas composition, researchers often use methods like gas chromatography or wet-chemistry approaches, in which carbon dioxide is absorbed into alkaline solutions to determine the methane content. According to Pham *et al.* (2013:871), properly sealed water-displacement setups can serve as an economical and accurate substitute for high-cost equipment such as gas chromatographs, especially in laboratory-scale AD experiments.

Fixed-dome and floating-drum digesters are highly regarded for their reliability and low operating costs, making them particularly valuable in developing countries. Fixed-dome digesters feature a closed, dome-shaped structure without moving parts, which contributes to their exceptional durability and lifespan, often exceeding 20 years. Their underground installation offers protection from physical damage and temperature fluctuations, making them highly suitable for rural and challenging environments. However, constructing these digesters requires skilled labour to ensure they are gas-tight and to prevent methane leaks (Budiman, 2020:85).

On the other hand, floating-drum digesters use a movable gas holder, typically a metal drum, which allows them to maintain steady gas pressure and simplifies gas volume measurement. Both digester designs enable daily monitoring and biogas storage, supporting effective management and oversight of production. These benefits are consistent with findings by Budiman (2020:85), who highlights the systems' durability, effectiveness in preventing contamination, and user-friendly operation, especially in rural settings.

2.12.2 Gas storage chambers

Pilot-scale research confirms the effectiveness of the three main biogas storage types discussed.

Flexible gas bags, typically made from PVC or Tedlar films, are widely adopted for their cost-effectiveness and compressive storage capabilities, making them ideal for short-term use. These bags are lightweight, flexible, and easy to install, which supports small- to medium-scale biogas systems. Tedlar (PVF) is widely used for gas sampling bags and analytical sampling (short volumes, low pressures), not for bulk storage (U.S. Environmental Protection Agency, 2025).

Floating drum collectors (Figure 2.2) represent a more traditional approach to biogas storage. Their design features a movable drum that visibly indicates gas volume and helps maintain relatively constant pressure during extraction. However, the steel drums are prone to rust and corrosion over time, necessitating regular maintenance (Energypedia, 2015:2).

Pressurized steel tanks provide compact storage solutions for high-pressure biogas or upgraded biomethane. Their robust design and ability to withstand high pressures make them suitable for long-term or large-scale storage applications. These tanks are especially appropriate for storing upgraded biomethane, enabling its transport and subsequent use as vehicle fuel or for injection into natural gas grids (Deublein and Steinhauser, 2011:330).

Overall, these findings align with existing claims regarding the benefits, material properties, and usage scenarios of these three storage solutions on the pilot scale.

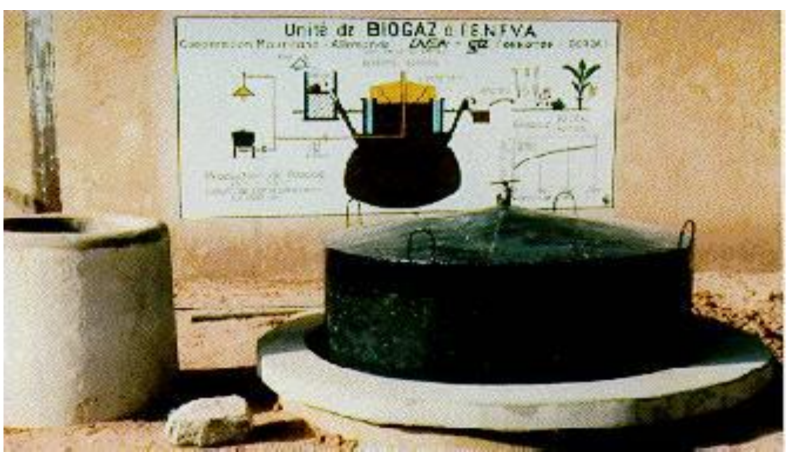

Figure 2.2: Floating drum biogas plant (Energypedia, 2015:2)

2.13 Digestate utilization as fertilizer

The nutrient-rich digestate produced by AD is considered a valuable fertilizer that can enhance soil health and increase crop productivity. Research (Doyeni *et al.* 2021:2) indicates that digestates derived from animal wastes, such as pig, chicken, and cow manure, consistently applied over several years, lead to increased soil nutrient levels, particularly phosphorus and potassium, compared with the use of synthetic nitrogen fertilizers. These improvements are reflected in higher crop yields and better grain quality, highlighting improvements in nitrogen use efficiency (NUE) and overall nutrient availability. Digestate provides ammonium-rich nitrogen that plants can readily absorb, improving nutrient uptake. However, while long-term application can enhance soil chemistry and crop performance, the effects vary with substrate type and environmental conditions. Careful management of the digestate, focusing on application timing, dosage, and split-fertilization strategies, is essential for maximizing agronomic benefits and supporting environmental sustainability.

Conducting a thorough physicochemical analysis of the digestate is crucial for determining its suitability and safety for use as an agricultural soil amendment. Essential parameters to be assessed include pH, moisture content, TS, VS, as well as BOD and COD. The concentrations of nitrogen, phosphorus, and potassium are also measured, as these values provide important information about nutrient availability and potential environmental impacts. In addition to these chemical assessments, ensuring microbial safety is equally important. This is typically achieved through culture-based techniques or molecular assays, which help confirm the absence of pathogens and other harmful contaminants. Together, these analyses ensure that the digestate applied to farmland is both effective for crop production and compliant with environmental regulations (Vitti *et al.* 2021:3).

The nutrient composition of digestate depends heavily on the substrate ratio and the process conditions used during ACoD. Research indicates that the digestate derived from KW and PM co-digestion is particularly rich in plant-available nitrogen and phosphorus, underscoring its value as a biofertilizer alternative (Khatun *et al.* 2025:6). However, the actual nutrient content can vary considerably with feedstocks types, digestion parameters, and operational practices employed (Möller and Müller, 2012:1). Consequently, it is essential to analyse each batch of digestate before applying it to agricultural land to ensure its nutrient profile is appropriate and to optimize fertilization outcomes.

2.14 Environmental and socio-economic implications of biogas production

AD has been widely reported to reduce greenhouse gas emissions from organic waste, divert material from landfills, and produce renewable biogas that can complement conventional energy systems. These broader environmental and socio-economic benefits provide important context for KW-PM co-digestion, but this study contributes specifically evidence on methane yield, process stability, and digestate quality under mesophilic conditions rather than direct measurement of large-scale sustainability or economic impacts (Kumar *et al.* 2025:28378).

2.15 Research gaps and future research directions

2.15.1 Pilot-scale studies to validate lab-scale findings

Bridging the gap between laboratory research and real-world implementation relies heavily on pilot-scale studies, offering a means to validate ACoD processes under practical conditions. A recent project exemplified this approach by focusing on pilot-scale ACoD specifically designed for the red meat industry (Tessele Consultants, 2025:5). The initiative involved operating two fully automated 400-liter digesters, which served as a controlled platform to assess real feedstock mixtures, track biogas production, and evaluate operational suitability within an on-site environment. By addressing practical considerations such as mixing, temperature regulation, and safety compliance at this scale, researchers resolved key challenges prior to commercial deployment. This strategy not only reduces investment risk but also enhances confidence among stakeholders, considering larger-scale adoption

Despite recent progress, significant research gaps remain in the ACoD of KW and PM. One key area warranting further investigation is the development of pre-treatment strategies tailored to KW-PM mixtures, which could improve substrate biodegradability and boost biogas yields. Furthermore, there is a notable shortage of full-scale operational data on KW-PM co-digestion systems in South Africa, limiting the availability of practical recommendations and the transfer of technology to local stakeholders. Economic assessments that combine capital and operational expenditures with biogas-related benefits remain limited, making rigorous cost-benefit assessments difficult. Addressing these gaps through focused research, pilot-scale trials, and integrated assessments will be vital for optimizing anaerobic digestion performance, facilitating broader technology adoption, and guiding effective policy development.

2.16 Exploration of novel pre-treatment methods

Recent publications (Szaja and Bartkowska, 2024:2; Shi *et al.* 2025:2) highlight the considerable potential of novel pre-treatment methods specifically tailored for ACoD to boost biogas production while lowering energy requirements. Physical pre-treatments such as ultrasonic and microwave irradiation are gaining attention for their effectiveness in improving substrate digestibility. These techniques work by disrupting cell walls and enhancing hydrolysis, thereby facilitating more efficient digestion. On the biochemical front, using enzymes such as amylases, lipases, and proteinase K as pre-treatments has significantly improved methane yields by accelerating the hydrolysis of complex polymers. In studies on co-digesting food waste with bioplastics, enzymatic pre-treatment increased methane output by over 60%, with proteinase K especially effective in enhancing the physical degradation of the feedstocks (Atchala *et al.* 2025:12). Research highlights the importance of optimizing pre-treatment strategies for specific substrate combinations to achieve maximum methane yields and economic viability. A recent meta-analysis by Anacleto *et al.* (2024:2) found that the effectiveness of pre-treatment methods in AD is strongly depends on the chemical composition of the substrates, whether they are lignocellulosic, protein-rich, or lipid-rich. The study demonstrates that tailoring pre-treatment approaches, such as using enzymatic methods for protein-dominated substrates, can significantly boost methane production. These findings highlight the importance of designing pre-treatment protocols optimized for specific substrate types, thereby enhancing biogas output (Anacleto *et al.* 2024:2).

Research on co-digesting PM and food waste suggests that multi-step pre-treatment can create synergistic benefits. For example, thermal-enzymatic pre-treatment has been reported to enhance hydrolysis and increase biogas yields beyond either approach alone. Additional studies that focus on optimizing feedstock ratios and operational parameters further support the idea that aligning pre-treatment strategies with specific substrate characteristics and employing combined pre-treatments can sustainably improve methane production and process stability. These findings underscore the value of continued research into the customization and integration of pre-treatment methods to achieve economically viable biogas production (Bermúdez-Penabad *et al.* 2024:208).

2.17 Policy implications for adopting co-digestion technology in South Africa

Recent publications underscore the importance of establishing strong policy frameworks to promote the adoption of co-digestion in South Africa. In 2022 (Grewan and Troi, 2023:4), the South African Biogas Industry Association (SABIA) introduced a National Biogas Strategy outlining measures such as diverting organic waste from landfills, setting recycling targets, supporting tariff structures, and providing incentives for agricultural biogas initiatives. The strategy stresses collaboration among government, industry, and academia to accelerate waste-to-energy deployment. Additional recommendations include subsidies and standards for digestate utilization to create a conducive environment for the sustainable uptake of biogas.

Government initiatives, like the National Waste Management Strategy 2020 and recently introduced tax incentives, are central to promoting renewable energy and investment in AD systems (DEFF, 2020:14). Numerous reports and guidelines further call for policies that support research funding, capacity building, and technology transfer to local communities and farmers. These interventions are essential for bridging gaps among research, industry, and society, enabling sustainable waste management and advancing national renewable energy goals (DEFF, 2020:22). Table 2.1 summarises the literature's engagement with these policy, technical, and institutional dimensions.

Table 2.1: Summary on co-digestion, pretreatment, and outcomes relevant to KW and PM

Author(s) & Year	**Substrate(s) / Feedstock**	**Pretreatment / Method**	**Outcome / Key Findings**	**Relevance to Present Study**
Deressa *et al.* (2015)	Fruit & vegetable wastes + manure	Batch digestion, gas collection	Identified flexible gas bags, floating drums, and steel tanks as effective storage	Guides pilot-scale gas handling in South Africa context
Sebola *et al.* (2015)	Food waste oils + manure	Digestate analysis	Recommended sampling and nutrient testing protocols	Basis for digestate fertilizer assessment
Sarteshnizi *et al.* (2018)	Biogas from various wastes	Combustion & calorific analysis	Reported CH_4 range of 50–75% with heating values ~22–25 MJ/m³	Informs heating impact evaluation of KW+PM
Kumar *et al.* (2019)	Lignocellulosic biomass	Enzymatic pre-treatment	Enhanced hydrolysis, but	Warns about overdosing

			high enzyme load caused VFA surge	enzymes in KW+PM digestion
Almomani *et al.* (2021)	Food waste + sewage sludge	Co-digestion (C/N ratio control)	Imbalanced C/N caused ammonia inhibition, lowering methane yields	Highlights the importance of balancing KW and PM ratios
Ogunkeyede *et al.* (2021)	Mixed substrates	Mechanical pre-treatments (ball milling, ultrasonics)	High energy input sometimes outweighed methane gain	Relevant for evaluating pre-treatment cost-benefit
Tsapekos *et al.* (2022)	Agricultural residues	Enzymatic pre-treatment	Improved methane yields, but excessive treatment caused inhibition	Supports careful enzyme optimization
Osuji *et al.* (2024)	KW + PM	Co-digestion	High nutrient recovery in digestate, suitable for organic fertilizer	Validates digestate use from KW+PM systems
Zhang *et al.* (2014/2024)	Kitchen waste	Co-digestion, observation of VFA accumulation	Excess KW increased VFAs, suppressed methane generation	Demonstrates risks of substrate imbalance in KW/PM

While Table 2.1 provides a broader contextual overview of policy, technical, and pre-treatment themes relevant to KW-PM systems, Table 2.2 summarises the key experimental studies, highlighting the substrate ratios, operating conditions, and performance outcomes most relevant to this research. These findings provide a clear basis for the optimisation focus of the present study.

Table 2.2: Comparative synthesis of KW-PM related co-digestion studies

Study	**Substrates and ratio**	**Reactor/operating mode**	**Temperature**	**Pre-treatment**	**Methane/biogas yield**	**Stability indicators**
Tian et al. (2015)	PM:KW TS ratios 1:0, 5:1, 3:1, 1:1, 1:3, 1:5, 0:1	Single-stage batch AD (BMP-type)	35 ± 1 °C (mesophilic	Standard substrate preparation	At PM:KW = 1:1: methane yield 409.5 mL CH_4 g^{-1} VS; biodegradability 85.03%	Technical digestion times T_{80} for the seven ratios: 15, 21, 22, 27, 49, 62, 61 days
Li et al. (2013) - rice straw + KW + PM	KW + corn stover + chicken manure	Single-stage batch AD (BMP-type)	37 °C (mesophilic)	Mechanical cutting and mixing	Mono BMPs: KW 725 mL g^{-1} VS (683 mL g^{-1} VS specific), CS 470/214, CM 617/291	Biodegradability: KW 94%, CS 45%, CM 47%
Misganaw et al. (2013)	Organic KW + cattle manure, ratios 100:0, 0:100,	Single-stage batch AD (BMP-type)	37 °C (mesophilic)	None	Highest methane yield 14,653.5 mL g^{-1} VS at 75% OKW + 25% CM	Co-digestion: 1.01-1.84× more biogas than pure CM or pure OKW; 75% CM mixture

	25:75, 50:50, 75:25					reduced cumulative methane by 23% relative to 25% CM
Varol and Ugurlu (2017)	Dairy manure + maize silage	DM:MS = 4:1 (w/w) in co-digestion; DM alone also tested	Continuous AD in CSTR and hybrid anaerobic reactor (HR)	None	Co-digestion improved volatile solids degradation and biogas production rate	Stability improved by nutrient complementarity and buffering from co-substrates

Previous studies show that co-digesting food waste and manure improves methane production, but the optimum mixing ratio remains uncertain. While food waste increases methane potential, excessive amounts may cause acidification. Manure enhances buffering and nutrient balance but can reduce biodegradability at high levels. These differences highlight the need to evaluate KW-PM ratios under mesophilic conditions with combined mechanical and enzymatic pre-treatment in this study.

2.18 Gap statement

Previous studies have shown that co-digesting kitchen or food waste with manure can improve methane production, enhance process stability, and increase nutrient recovery. However, the reported optimum substrate ratios and methane yields vary considerably, and process stability differs across studies. In addition, hardly any study evaluated KW-PM mixtures under using combined mechanical and enzymatic pre-treatment, while also assessing digestate quality. This study addresses these gaps by comparing six KW:PM ratios under controlled laboratory conditions to identify the mixture that provides the best balance between biomethane yield, process stability, and digestate quality.

2.19 Summary of the chapter

This chapter reviewed the literature on the ACoD of KW and PM, with emphasis on substrate characteristics, operating conditions, pre-treatment methods, and digestate quality. This literature shows that co-digestion can improve methane production and process stability, although the optimum substrate ratio and performance vary across different feedstocks, reactor systems, and operating conditions. It also highlights that pre-treatment can enhance biodegradability and methane recovery, but its effectiveness depends on the substrate and treatment approach used. These findings provide the foundation for Chapter 3, which describes the experimental evaluation of different KW-PM mixtures under mesophilic conditions using combined mechanical and enzymatic pre-treatment.

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CHAPTER 3 - EXPERIMENTAL PROCEDURES

3.1 Introduction

This chapter details the experimental approaches adopted to examine the anaerobic co-digestion (ACoD) of kitchen waste (KW) and pig manure (PM) to enhance biogas production. The chapter outlines the systematic preparation of substrates, detailing both the mechanical and enzymatic pre-treatment processes applied to improve substrate biodegradability. It further describes the configuration of the ACoD system, emphasizing the operational parameters, including mesophilic temperature control, substrate-to-inoculum ratios, and gas measurement procedures, which were carefully maintained to ensure stable and efficient digestion.

Beyond biogas quantification, the chapter also presents the analytical techniques utilized to evaluate both the raw substrates and the resulting digestate. Digestate characterization encompassed proximate composition, thermogravimetric analysis (TGA), elemental profiling by Inductively Coupled Plasma-Mass Spectromy (ICP-MS), and measurements of ammonia content and pH. These analyses were conducted not only to assess the degree of substrate degradation but also to assess the nutrient content and chemical stability of the resulting digestate. Such information is crucial for determining its potential suitability as a bio-fertilizer, as well-digested effluent rich in nitrogen, carbon, and essential minerals, while low in toxic elements, can serve as an effective and sustainable soil amendment.

Furthermore, to ensure the scientific rigour, reproducibility, and reliability of the experimental findings, this chapter also details the data collection and statistical analysis procedures implemented. By providing a logically structured methodological framework that spans substrate preparation, pre-treatment, digestion, biogas monitoring, and digestate evaluation, this chapter establishes the foundational approach for examining both the biogas production potential and the post-digestion agronomic value of KW-PM co-digestion systems.

3.2 Research design

A quantitative experimental research design was employed to assess how varying substrate ratios and pre-treatment methods influence biogas production performance. This design enabled controlled manipulation of the independent variables, specifically, the ratio of KW and PM, as well as the type of pre-treatment applied (mechanical, enzymatic, and combined mechanical and enzymatic pre-treatment methods), while allowing for precise measurement of key outcomes such as cumulative biogas yield, methane concentration, and rates of substrate degradation.

In addition to biogas measurements, the research design incorporated digestate analysis to assess substrate transformation during digestion and evaluate the agronomic potential of the resulting effluent. Digestate samples obtained at the end of the digestion were subjected to proximate analysis, TGA, elemental analysis by ICP-MS, and measurements of ammonia concentration and pH. These analyses provided valuable insights into the extent of organic matter stabilization and nutrient enrichment, as well as the potential inhibitory elements that could affect downstream applications.

Including digestate evaluation as an integral component of the research design was essential for determining whether the final product is suitable for use as an organic fertilizer or soil conditioner. The nutrient composition (including nitrogen, phosphorus, and potassium), ash content, and mineral profile were systematically compared against established agronomic guidelines to assess whether the digestate meets desirable standards for safe and effective soil amendment. By integrating both energy recovery through biogas production and resource recovery through digestate quality assessment, the research design ensured a holistic and sustainable evaluation of the co-digestion process.

Overall, this design provided a robust methodological framework for analysing not only the efficiency of biogas generation but also the broader environmental and agricultural benefits of KW-PM co-digestion systems.

3.2.1 Experimental design justification

Six KW mixing ratios (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100 on a volatile solids ((VS) basis) were selected to provide a gradual transition from KW to PM under identical mesophilic conditions. This design enabled direct comparison of mono-digestion and co-digestion while identifying the substrate ratio that best balanced methane production and process stability.

The selected ratios were guided by previous studies showing that mixed food waste with manure systems generally produce more methane than mono-digestion (Tian *et al.* 2015:2; Hu *et al.* 2019:4647), although the optimum ratio differs with substrate type, reactor design, and operating conditions. Having both mono-digestion controls and four intermediate mixtures provided a broad assessment of the shift from carbon-rich KW to nutrient-rich PM, allowing evaluation of how substrate balance influences methane yield and process stability.

3.2.2 Justification of digestion period

The BMP tests were conducted for 28 days at 37 ± 2 °C, a duration commonly used for mesophilic batch digestion studies. Previous BMP guidelines indicate that 20-30 days is generally sufficient to capture most methane production while maintaining practical testing periods (Angelidaki *et al.* 2009:928). A 28-day digestion period therefore provided a consistent basis for comparing methane yields and process stability across the different KW ratios.

3.2.3 Inoculum adaptation

A well-adapted inoculum was employed to minimise start-up lag phases and improve the reliability of BMP measurements across treatments. In BMP testing, it is widely recognised that inoculum acclimatisation to anaerobic conditions and organic substrates is critical for achieving rapid onset of methane production and stable digestion behaviour. Following established practice, the inoculum used in this study was taken from an active AD system (Angelidaki *et al.* 2007:7) and stored under conditions that preserved anaerobic microbial activity prior to use, ensuring that methanogens and supporting microbial communities were already functioning when the KW:PM mixtures were introduced. This approach helped ensure that differences in BMP were mainly due to the KW:PM mixing ratios rather than the inoculum adapting to the new substrates, while remaining consistent with recommended inoculum selection guidelines.

3.2.4 Justification for mechanical and enzymatic pre-treatment

Combined mechanical and enzymatic pre-treatment was applied to the KW-PM mixtures before digestion to improve hydrolysis and make the substrates more accessible to microorganisms. The substrates were first reduced to a particle size of approximately 4-5 mm using a kitchen blender and ball mill, increasing surface area and improving mixing. Previous studies have shown that mechanical size reduction enhances enzymatic hydrolysis and increases substrate availability, which can improve methane production when used as part of an effective pre-treatment strategy (Hu *et al*. 2018:4647; Karrupiah and Azariah, 2019:4).

Enzymatic pre-treatment was carried out using a low-cost enzyme solution produced by fermenting cow dung with water and honey for 21 days at 34-38 °C. The fermented liquid was then stored at 4 °C and 100 mL was added to each AMPTS II reactor together with the inoculum and substrate mixture. Previous studies have shown that fermented cow dung can enhance hydrolysis and improve methane production (Kessy *et al.* 2026: 322).

Applying the same mechanical and enzymatic pre-treatment ensured that differences in performance were primarily due to the KW:PM mixing ratios rather than pre-treatment conditions.

3.2.5 Replication strategy and measurement level

Each KW:PM mixing ratio was tested in duplicate under the same mesophilic conditions to improve the reliability and repeatability of the results. Duplicate reactors were used because the study was conducted under controlled laboratory conditions with limited reactor capacity, with the primary aim of comparing methane production across the different substrate ratios. Mono-digestion of KW and PM was also included as reference treatments to provide a baseline for evaluating the performance of the co-digestion mixtures.

Analytical measurements were replicated where appropriate to improve the reliability of the results. Substrate proximate analysis and digestate characterisation, including total solids (TS), ammonia, pH, CHN, TGA, and ICP-MS, were performed in triplicate, and the mean values were reported. BMP was monitored daily throughout the 28-day test, while methane content was determined using gas chromatography. Daily and cumulative biomethane yields were calculated as the average of the duplicate reactors for each treatment.

NOTE: Biological replication refers to independently operated digesters at the same substrate ratio, whereas analytical replication refers to repeated laboratory determinations carried out on the same collected sample.

3.2.6 Defining optimal performance

Optimal performance was determined using several indicators rather than cumulative biomethane yield alone. The preferred KW:PM ratio was selected based on biomethane yield and daily production trends, lag phase, process stability (pH, ammonia concentration and VS reduction), the buffering and nutrient contribution of PM, digestate quality for agricultural reuse, and practical considerations related to feedstock handling.

Cumulative biomethane yield was the main indicator of energy performance, but the highest-yielding treatment was not necessarily considered the preferred condition. Although the 100:0 KW treatment produced the highest yield, the 80:20 KW:PM ratio was considered the preferred mixture because it combined high biomethane recovery with process stability, improved substrate balance, suitable digestate quality, and practical feedstock management.

The preferred KW:PM ratio was determined from measured biomethane, process stability, substrate, and digestate indicators without numerical weighting. Practical and economic considerations were literature-based, as pilot-scale validation and techno-economic assessment were not conducted.

3.3 Ethical considerations

This study adhered to established laboratory safety protocols for the safe handling of biological materials, chemical reagents, and high-temperature equipment:

i. KW, PM, and digestate samples were handled with appropriate personal protective equipment, including gloves, laboratory coats, and face masks, to minimize the risk of pathogen exposure to researchers.
ii. AMPTS II systems and anaerobic digesters were operated in well-ventilated laboratory spaces to prevent the accumulation of potentially hazardous gases such as methane and hydrogen sulfide.
iii. High-temperature equipment, including ovens, muffle furnaces, and thermogravimetric analysers (TGA), was operated exclusively by trained personnel familiar with proper handling procedures and safety precautions.
iv. Acid digestion procedures for ICP-MS analysis were conducted within fume hoods, and researchers wore appropriate personal protective equipment to prevent exposure to corrosive acids and harmful fumes.
v. All waste materials generated during the experiments were properly autoclaved or chemically disinfected before disposal, in accordance with North-West University biosafety guidelines and waste management protocols.

This research did not involve direct experimentation on human or animal subjects and, therefore, received a “no risk” research ethics committee letter of decision.

3.3.1 Hazard and operability (HAZOP) and laboratory safety considerations

A Hazard and Operability (HAZOP) assessment was undertaken to identify and evaluate potential risks throughout the experimental work, encompassing substrate handling, pre-treatment operations, ACoD processes, gas collection systems, and digestate handling. This assessment examined deviations from normal operating conditions that could pose risks to personnel safety, compromise equipment integrity, or affect the reliability of experimental results. Various hazard categories were considered, including biological, chemical, thermal, and gas and digestate-related risks. Based on this evaluation, appropriate preventive measures were established and maintained throughout the experimental procedure to ensure safe laboratory operations (Mocellin *et al*, 2022:3461; United States Environmental Protection Agency (EPA), 2014:1). Table 3.1 presents the identified hazards and their corresponding control measures.

Table 3.1: Hazard and operability (HAZOP) assessment for experimental procedures

Process stage	**Potential hazard**	**Possible cause**	**Consequence**	**Control/safety measures**
Substrate collection (KW, PM)	Exposure to pathogens	Manual sorting of KW and PM	Health risk to personnel (eye/skin/gastrointestinal contamination)	Use of gloves, lab coats, face masks, hand washing, well-ventilated areas and surface disinfection
Substrate sorting and shredding	Physical injury	Sharp objects (glass, metals and plastics), and moving blender blades	Cuts, abrasions, equipment damage	Hand-sort substrates with tools/tongs, remove all non-biodegradable materials, inspect blender and

				ball mill for damage before use
Mechanical pre-treatment (blending, milling)	Equipment overload/mechanical hazards	Excessive substrate loading/operation of the blender and ball mill	Entanglement, equipment damage, overheating	Ensure equipment lids are secured, controlled batch sizes, equipment rest periods, follow manufacturer's SOPs
Oven drying (105 °C)	Thermal hazard	Hot surfaces and crucibles	Burns	Heat-resistant gloves, cooling in desiccator before handling
Enzymatic pre-treatment (cow dung fermentation)	Gas buildup during fermentation	Anaerobic microbial activity	Pressure buildup, container rupture, odour exposure	Loose-fitted lids, work in ventilated areas, label container: "Biological - Fermenting cow dung - pathogen

				risk" with date
Enzymatic handling	High microbial load in enzyme solution	Concentrated and active microbial culture after fermentation	Infection risk during decanting and transfer	Wear PPE when handling fermented liquid, decant slowly to minimize splashing and aerosol formation - Store at 4 °C in clearly labelled containers
Anaerobic digestion	Biogas generation (CH_4, CO_2, H_2S, trace gases)	Gas leakage from digesters during mesophilic digestion	Flammability/explosion (CH_4), toxicity (H_2S), asphyxiation (CO_2 displacement)	Airtight seals/tubes, leak testing, smoking prohibition, well-ventilated lab space, and label digesters.
Anaerobic digestion	Over-pressurization of digesters/gas lines	Blocked gas lines, bottle seal failure	Sudden gas release, bottle rupture	Regular inspection of tubing and gas outlets.

				In case of leak (odour, hiss): stop agitation, ventilate area immediately, troubleshoot only after gas dispersed
Anaerobic digestion	Temperature deviation from 37 ± 2 °C	Water bath malfunction, sensor drift	Inhibition of methanogenesis, VFA accumulation, and process failure	Calibrate AMPTS II temperature sensors, monitor continuously; set alarm for deviations >2 °C, check water bath daily; maintain level and heating function
CO_2 absorption (NaOH)	Chemical exposure	Corrosive NaOH solution	Chemical burns	Gloves, eye protection, spill containment

Gas chromatography	Compressed gas cylinders	GC operation, N_2 flushing of digesters	Pressure hazards, asphyxiation, fire (H_2)	Secure cylinders upright with chains/straps, trained personnel only, ensure GC lab is well ventilated
Acid digestion (ICP-MS)	Chemical hazard	Nitric acid and hydrogen peroxide	Corrosive burns, fumes	Fume hood use, acid-resistant PPE
High-temperature analysis (TGA)	Thermal/electrical/ fire hazard	GC-TCD, ICP-MS, TGA high-voltage components (900 °C)	Burns, equipment damage, electric shock, fire	Only trained operators, controlled access, report unusual noises, smells, or error messages immediately
Digestate handling	Exposure to digestate pathogens	Sampling, filtration, and grinding for proximate/ICP-MS/TGA analysis	Health risk (infection, respiratory irritation, skin contact with NH_3)	Wear PPE, treat digestate as biohazardous, handle digestate in a well-

				ventilated area, disinfect waste before disposal
Digestate handling	Acid digestion (ICP-MS)	Microwave digestion in high-pressure vessels	Corrosive acid burns, pressure hazards, toxic NO_x fumes	Wear acid-resistant PPE, conduct acid digestion only in certified fume hood, follow microwave digester SOP; never open vessels while hot or pressurized, store acid waste in labelled hazardous waste containers for approved disposal

Digestate handling	Heavy metal exposure	ICP-MS analysis of digestate	Chronic toxicity, environmental contamination	Use pipettes and closed containers. dispose of metal-containing solutions as hazardous waste, Use procedural blanks and CRMs (Accustandard) for QC

3.3.2 Key safety rules adhered to during the study

Beyond the hazard identification and mitigation measures detailed in the HAZOP assessment, a comprehensive set of laboratory safety protocols was rigorously followed throughout the experimental program. These protocols were designed to minimize risks arising from biological materials, chemical reagents, high-temperature equipment, biogas production, and digestate handling during the anaerobic digestion (AD) process. Adherence to these established safety procedures served multiple purposes: protecting personnel from potential hazards, preserving the integrity of experimental equipment, and ensuring the reliability and reproducibility of the data collected (Cascardo *et al*, 202:227; United States Environmental Protection Agency (EPA), 2014:2). The principal safety protocols observed during this study are outlined below.

3.3.3 General laboratory conduct

i. All experimental work involving substrates, digestate, or chemicals was performed with appropriate personal protective equipment, which includes gloves, a laboratory coat, closed shoes, and safety glasses) to minimize exposure to biological and chemical hazards.

ii. Procedures involving biohazardous materials, corrosive chemicals, and biogas-generating setups were carried out in well-ventilated areas or under fume hoods to reduce inhalation risks.

iii. After each procedure, hands were washed thoroughly with soap and water, and work surfaces were disinfected using 70% ethanol or an appropriate disinfectant to limit microbial contamination.

iv. Eating, drinking, and smoking were strictly prohibited in the laboratory to prevent inadvertent ingestion of contaminants.

3.3.4 Biological hazards (KW, PM, inoculum, digestate, fermented cow dung)

i. KW, PM, inoculum, digestate, and fermented cow dung were all treated as potentially infectious materials, and direct contact with skin and mucous membranes was avoided.

ii. Aerosol generation during grinding, sieving, and blending was minimized by performing these operations in a fume hood or with local exhaust ventilation.

iii. All biological containers (substrates, inoculum, digestate, enzyme solutions) were clearly labelled with content, date, and relevant hazard warnings.

iv. Biological waste, including residual substrates, inoculum, digestate, and contaminated disposables, was autoclaved or chemically disinfected before disposal in accordance with North-West University biosafety and waste-management protocols.

3.3.5 Chemical hazards (NaOH, HNO_3, H_2O_2, heavy metals)

i. The 3 M NaOH solution used for CO_2 absorption was prepared only in a fume hood by slowly adding NaOH pellets to water, while wearing gloves, goggles, and a lab coat, to control the exothermic reaction and prevent splashes.

ii. Acid digestion for ICP-MS, using nitric acid and hydrogen peroxide in a microwave vessel, was carried out inside a certified fume hood with acid-resistant gloves, a lab coat, and a face shield to avoid exposure to corrosive reagents and fumes.

iii. Microwave digestion vessels were never opened while hot or under pressure, and manufacturer standard operating procedures were strictly followed to manage pressure hazards.

iv. Chemical wastes (acid digests, NaOH, and metal-containing solutions) were collected in clearly labelled hazardous-waste containers and disposed of through approved institutional channels.

3.3.6 Thermal hazards (ovens, muffle furnace, TGA)

i. Ovens, muffle furnaces, and the thermogravimetric analyser were operated only by trained and authorized personnel, following the respective equipment standard operating procedures.

ii. Heat-resistant gloves and tongs were used when handling hot crucibles or samples, and sufficient cooling time was allowed before weighing in the desiccator to prevent burns and thermal shock.

iii. Combustible materials were kept away from thermal equipment, and adequate clearance and ventilation around ovens and furnaces were maintained to reduce fire risk.

3.3.7 Gas hazards (CH_4, CO_2, H_2S, N_2)

i. AMPTS II digesters were operated in a well-ventilated space, away from open flames, hot surfaces, and sparks, and smoking was strictly prohibited near the experimental setup.

ii. Gas tubing and connections were inspected daily for kinks, blockages, or leaks, and NaOH scrubber bottles were kept stable and properly connected without being over sealed to prevent over-pressurization.

iii. All digesters were labelled with substrate ratio, inoculum mass, start date, and the hazard warning "Biogas - methane - flammable; H_2S present" to indicate process risk.

iv. Compressed gas cylinders (such as nitrogen and carrier gases) were secured upright with chains, fitted with appropriate regulators, and routinely checked for leaks.

3.3.8 Mechanical and electrical hazards

i. Guards and lids were always ensured to be in place before operating the blender, ball mill, and no attempt was made to reach into moving machinery.

ii. Operation of GC-TCD, ICP-MS, TGA, and the AMPTS II system was restricted to trained personnel who followed manufacturer manuals and institutional safety policies.

3.3.9 Quality control and standards

i. GC-TCD, pH meter, ICP-MS, Automatic Methane Potential Testing System II (AMPTS II) sensors, and the analytical balance were calibrated before each batch of measurements to ensure accuracy and traceability.

ii. Procedural blanks and certified reference materials (Accustandard) were run alongside ICP-MS and ammonia analyses to verify analytical accuracy and detect contamination or instrumental drift.

iii. To ensure reliable data and strong statistics, all analyses were carried out in triplicate.

3.3.10 Emergency response

i. Laboratory personnel were aware of where eyewash stations, safety showers, fire extinguishers, spill kits, and first-aid kits were located and how to use them correctly.

ii. All incidents, including spills, injuries, and equipment malfunctions, were reported promptly to the supervisor and safety officer in accordance with institutional requirements.

3.4 Materials and equipment

3.4.1 Substrate collection and preparation

KW was sourced from cafeterias in town around the North-West University Potchefstroom campus community, with the collected material consisting mainly of Irish potato peels, with 15% from pastries, fruits, and vegetables. To ensure high microbial activity, fresh PM was obtained directly from a local piggery. Both substrates were carefully hand-sorted (Figure 3.1) to remove plastics and other non-biodegradable contaminants. The KW was then shredded to a particle size of 4-5 mm using a kitchen blender and further grinded with a ball miller, while the PM was sieved to achieve uniformity. To minimise premature decomposition, all prepared samples were stored at 4 °C in airtight containers.

Figure 3.1: Sorting substrates to remove non-biodegradable contaminants

3.4.2 Enzymes

A laboratory-prepared fermented cow dung extract served as the enzymatic agent for pre-treatment in this study. The enzyme solution was generated by naturally fermenting fresh cow dung with a small amount of honey, which acted as a carbohydrate (sugar) source to promote microbial activity. This mixture was allowed to ferment anaerobically for 21 days at ambient temperatures ranging from 34 to 38 °C. Throughout the fermentation process, indigenous microorganisms present in the cow dung produced a variety of hydrolytic enzymes, such as cellulases, proteases, and amylases, which enhance substrate decomposition. After fermentation was complete, the liquid fraction was separated by decantation, pouring off the lighter layer and allowing the heavier component to settle. This was then stored at 4 °C until needed. This bio-fermented enzyme extract was subsequently used as the enzymatic pre-treatment medium for the KW-PM mixtures.

The use of fermented cow dung as a natural enzyme source offers notable benefits for research on biogas production. This approach offers an economical and sustainable alternative to commercial enzymes, making it suitable for low-resource and rural environments (Hamzah *et al.* 2023:2). Cow dung contains a diverse microbial community with bacteria capable of degrading cellulose, starches, and proteins, making it a nutrient-rich substrate for enzyme-producing microbes. In this context, honey may serve as a readily available carbon source that enhances microbial growth, supports fermentation activity, and boosts enzyme production by improving nutrient availability, although direct studies on honey-supplemented cow-dung fermentation remain limited (Alaerjani *et al.* 2022:5). Altogether, this strategy supports the broader objective of developing affordable, locally adaptable technologies for renewable energy generation via AD.

3.4.3 Equipment

Major equipment used in the study included:

- Automatic Methane Potential Testing System II (AMPTS II)
- Gas chromatograph (GC-TCD)
- Digital pH meter
- Analytical balance (0.001 g precision)
- Oven (105 °C)
- Muffle furnace (550 ºC)
- Desiccator, thermometer, and thermostatic water bath
- Kitchen blender (1000W)
- Fritsch pulverisette 6 planetary ball miller
- SDT Q600 TGA instrument
- Micro-wave digester
- Inductively Coupled Plasma (ICP-MS) spectrometer

All equipment were calibrated before use to maintain measurement accuracy.

3.5 Pre-treatment methods and procedures

Pre-treatment plays a crucial role in improving both biodegradability and biogas yield during AD. In this study, combined mechanical and enzymatic pre-treatment strategies were applied to break down complex organic materials in KW and PM, thereby increasing their accessibility to microorganisms and enhancing overall biogas production efficiency.

3.5.1 Mechanical pre-treatment

The substrates, collected fresh (KW (consisting of 85% potato peels and 15% mix of papaya, sandwich, carrots, mushrooms, and cauliflower) + PM), were first oven-dried independently at 65 ºC for 5 days. This was done to remove about 85% of the moisture content in the substrates. Afterward, the substrates were subjected to mechanical size reduction in the laboratory using a kitchen blender, resulting in particle sizes of 4-5mm. This process significantly enhanced the surface area exposed to microbial enzymatic activity, thereby promoting more efficient degradation. Additionally, the improved uniformity of the substrate particles facilitated better mixing and increased contact with anaerobic microorganisms within the digester, ultimately optimizing the conditions for effective bioconversion.

3.5.2 Enzymatic pre-treatment

In this investigation, a novel enzymatic pretreatment was used, involving a naturally derived enzyme source produced by fermenting cow dung in the laboratory. Fresh cow dung was blended with water and honey and fermented anaerobically for 21 days at 34-38 °C to encourage endogenous production of hydrolytic enzymes such as cellulases, amylases, proteases, and lipases. After fermentation, the liquid extract was separated by decantation, stored at 4 °C, and added at a dose of 100 mL per AMPTS II reactor at the start of each BMP run, together with 400 g of inoculum and the KW-PM substrate mixture to give a total working volume of 500 mL. These dosage were selected based on literature reports that fermented cow dung provides a low-cost source of ligno-cellulolytic enzymes capable of enhancing hydrolysis and subsequent methane production.

Employing laboratory-fermented cow dung as an enzyme source presents multiple advantages. This approach enriches microbial diversity by introducing a broad spectrum of naturally occurring enzymes and beneficial microorganisms, thereby enhancing the degradation of lignocellulosic substrates more efficiently than conventional single-enzyme additives. It is also economically advantageous, as it relies on accessible raw materials and reduces the need for costly commercial enzymes. Furthermore, this method achieved greater sustainability by repurposing organic waste into valuable bio-resources and reducing reliance on synthetic inputs. Notably, its practicality extends to rural and resource-limited areas, making it a viable option for small-scale biogas producers seeking to optimize digestion efficiency with locally available, environmentally responsible resources.

3.5.3 Combined mechanical and enzymatic pre-treatment

The integration of mechanical and enzymatic pre-treatment methods offered a synergistic approach to enhancing the biodegradation of organic substrates. Mechanical size reduction using a kitchen blender increased the available surface area and improved substrate homogeneity, creating optimal conditions for subsequent enzymatic action. When combined with the application of laboratory-fermented cow dung as a natural enzyme source, the pre-treated substrates benefited from both improved physical accessibility and enhanced enzymatic activity. This dual approach promoted faster degradation of complex organic compounds, thereby increasing hydrolysis efficiency and improving substrate conversion inside the digester (Hu *et al.* 2018:4648). Collectively, integrating these methods enhanced bioconversion efficiency, thereby supporting higher yields AD processes.

This pre-treatment strategy increased substrate bioavailability, thereby providing favorable conditions for microbial activity during AD. Together, the mechanical and enzymatic treatments worked synergistically to break down complex organic materials into simpler, more readily utilizable fractions (Karrupiah and Azariah, 2019:12). As a result, the biogas production efficiency improved, with higher yields observed in subsequent digestion experiments. This integrated pre-treatment approach optimized organic-to-energy conversion and demonstrated practical benefits for overall process performance.

3.6 Experimental setup

3.6.1 Digester design and configuration

The ACoD system was primarily designed to maintain an airtight environment in which microorganisms could decompose biodegradable substrates under anaerobic conditions, producing biomethane, carbon dioxide, and trace gases such as oxygen, hydrogen sulfide, and hydrogen. The apparatus provided optimal conditions for ACoD by promoting the conversion of volatile organic acids into biomethane and other gaseous byproducts, while enabling efficient gas collection. Its structural integrity was assessed to ensure acceptable performance at minimal cost.

3.7 Inoculum preparation

For this research, the inoculum used was stabilized, anaerobically digested cow dung sourced from the operational digester of a nearby biogas facility. The inoculum had a VS concentration of 2.82 g VS L^{-1} and, to preserve microbial viability prior to use, it was stored at 4 °C in a well-ventilated laboratory environment. In each BMP reactor, the inoculum was mixed with the substrates at an inoculum-to-substrate ratio of approximately 2:1 on a VS basis**,** which is within the range recommended for BMP assays to prevent overloading and maintain stable methanogenic activity. Before substrate loading, the inoculum was degassed by incubation under anaerobic mesophilic conditions at 37 °C for 10 days to eliminate residual biogas production and stabilise background microbial activity. This procedure ensured that methane measured during the BMP tests was primarily derived from the added KW-PM substrates rather than from the inoculum itself, in line with standard BMP practice using digested sludge inocula under mesophilic conditions.

3.8 Proximate analysis of substrates

The substrates used in this study were subjected to proximate composition analysis in line with the requirements of South African National Standard (SANS) 5925. Each substrate was systematically analysed for TS, volatile solids (VS), moisture content (MC), and elemental composition, including nitrogen, carbon, hydrogen, and oxygen. All measurements were carried out in triplicate to improve analytical reliability, and the mean values obtained were documented and used for reporting.

3.8.1 Estimation of moisture content (MC) and total solids (TS)

The mass of the samples after the moisture was dried off is referred to as the TS of the substrate. These values were obtained as detailed below and are expressed as percentages of the total mass.

Method: Initially, the oven was preheated to 105 °C, and the empty crucibles were weighed and their weights recorded. Approximately 2 g of each substrate (KW and PM) was then added to the crucibles, which were reweighed and placed in the oven. The samples were dried for 24 hours until a constant mass was reached, after which the crucibles were removed, allowed to cool in a desiccator for about seven minutes, and weighed again. The TS and MC of the substrates were subsequently calculated using the equations 3.1 & 3.2, with results expressed as percentages:

$$\textbf{Total Solids \% (TS)} = \frac{W_3 - W_0}{W_1 - W_0} \times 100 \qquad \textbf{3.1}$$

$$\textbf{Moisture Content \% (MC)}=\frac{W_1-W_3}{W_1-W_0}\times 100 \quad \textbf{3.2}$$

Where:

- W_0 = Empty crucible weight
- W_1 = Crucible weight + Sample weight
- W_3 = Crucible weight + Oven dried sample weight

3.8.2 Estimation of Volatile Solids (VS) and Ash Content

Method: After weighing the moisture-dried samples, the crucibles were transferred into a furnace and pre-heated to 800 °C a minimum of 6hrs. At this period, the sample has already converted to white ash. The sample-filled crucible was allowed to cool to 100 °C in the open before being placed into a desiccator to reach room temperature. Equation 3.4 illustrates the calculation of the percentage of ash content. The proportion of digestible biological matter in the substrate and inoculum is VS. This established the sample required for ACoD to produce biomethane. The percentages of ash content and volatile solids in the sample were determined using the following formulas:

$$\textbf{Percentage Ash \%}=\frac{\text{Ash weight}}{\text{Weight of sample taken}}\times 100 \quad \textbf{3.3}$$

$$\textbf{Volatile Solids \%}=\frac{\text{Weight of sample taken-Ash weight}}{\text{Weight of sample taken}}\times 100 \quad \textbf{3.4}$$

Where:

- Ash weight = weight of the residue remaining after combustion
- Weight of sample taken = initial weight of the sample analysed

3.9 Anaerobic digestion experimental setup

In accordance with the European standard method VDI 4630, a laboratory-scale batch experiment was conducted to evaluate the potential of ACoD of KW and PM to enhance biogas production using the AMPTS II, as illustrated in Figure 3.2 (Oechsner *et al.* 2020:95). Experiments were performed at a mesophilic temperature of 37 ± 2 °C. The AMPTS II system enabled automatic monitoring and control of substrate mixing and gas production, with a measurement precision of 0.1 mL following carbon dioxide absorption using a NaOH solution.

Figure 3.2: Automatic methane potential test system II (AMPTS II)

The digestion experiment was conducted in twelve 500 mL Schott Duran digester bottles (model 10,093435, Germany), each equipped with a mechanical mixing motor, a carbon dioxide absorption unit, a gas volume measurement system, and a bioprocess incubation module. In line with the VDI 4630 specification, 400 g of stabilised inoculum was loaded into each bottle of the AMPTS II system. Prior to substrate addition, the inoculum was digested on its own to release any residual biogas. The substrate dosage for each reactor was then calculated using the volatile solids (VS) content of both the inoculum and the substrate, as defined by Equation 3.5.

Digester bottles labelled A, B, C, D, E and F were operated in batch mode with varying mixing ratios, as detailed in Table 3.1. These ratios, adapted from Tian *et al.* (2015:52) with slight modification, established mono-digestion baselines and enabled evaluation of co-digestion synergy under balanced nutrient conditions.

$$M_s = \frac{MiCi}{2Cs} \qquad \textbf{3.5}$$

$$\text{Concentration (\%)} = \frac{TS\ x\ VS}{100} \quad \textbf{3.6}$$

Where:

- M_s is the mass of the substrate (g),
- M_i is the mass of inoculums (g),
- C_i is the concentration of inoculums (%),
- Cs is the concentration of substrates (%).

The ratios of the different digesters used in the present experiment are shown in Table 3.2

Table 3.2: Ratio of different digesters

Digester	**Mixture proportion**
A	100% KW
B	80% KW and 20% PM
C	60% KW and 40% PM
D	40% KW and 60% PM
E	20% KW and 80% PM
F	100% PM

The experiment was replicated twice, and the average values were taken and recorded.

Physicochemical and analytical measurements, including TS, VS, CHN, and ICP-MS analyses, were carried out in triplicate to improve the reliability of the results and minimise measurement variability, whereas, the BMP experiments were performed in duplicate for each treatment because of limitations in reactor availability and laboratory resources. Although this experimental design was adequate for comparing methane production among the different substrate mixtures, the use of duplicate BMP tests may have reduced the ability to detect small differences between treatments, which should be considered during interpretation.

Prior to initiating the AMPTS II, the following parameters were programmed into the system: 10% carbon dioxide as the flush gas concentration, 60 seconds of agitation, and 60 seconds of rest. Throughout the digestion process, the mixing speed was maintained at 80%. Each digester's headspace was set at 100 mL, and a biomethane concentration of 60% was assumed. To ensure anaerobic conditions, nitrogen was used to flush the digesters and eliminate residual oxygen. For carbon dioxide removal, 100 mL screw-cap bottles containing 3 M NaOH were used in the absorption unit. Flexible tubing was employed to connect the digester bottles to the carbon dioxide absorption unit, while the second tube led the absorption unit to the gas volume-measuring device, which quantified the biomethane produced. Gas chromatography was employed to analyse gas yield and determine the amount of biomethane generated by the system.

The experiment was terminated on day 28, when the daily gas production dropped to less than 1% of the cumulative yield for three consecutive days, in accordance with the International Water Association Task Group (IWA) Anaerobic Biodegradation Activity and Inhibition (ABAI) guidelines (Angelidaki *et al.* 2007:7).

Each experimental condition was performed in duplicate due to reactor availability, with close monitoring to ensure the statistical consistency, validity, reproducibility, and reliability of the data obtained. Control experiments consisted of mono-digestion of KW and PM individually under the same operating conditions, serving as baselines for comparing co-digestion performance, synergistic interactions, and improvements in biogas yield and process stability.

3.10 Proximate analysis of digestate

Proximate analysis of the digestates employed in this investigation was conducted following the Certified Reference Materials for each element from Accustandard (Molecules Analytical Lab Solutions Pvt Ltd, 2026:1). The digestates underwent comprehensive characterization for total solids (TS), ammonia (NH_3), pH and elemental constituents, namely: macro nutrients – nitrogen, phosphorus, potassium, calcium, magnesium and sulphur: micro nutrients – iron, copper, zinc and manganese: heavy metals – lead, cadmium, chromium, nickel, arsenic and mercury. To ensure analytical precision, all measurements were conducted in triplicate, with mean values calculated and reported (Figure 3.3).

3.10.1 Estimation of moisture content (MC) and total solids (TS)

Method: The oven was set to 105 °C, and the masses of clean and empty crucibles were first recorded. Approximately 2 g of each digestate sample was then placed into the crucibles, which were weighed again and transferred to the oven. The samples were dried for 24 hours, or until their mass remained constant, after which the crucibles were removed and placed in a desiccator for about seven minutes to cool to room temperature before the final weighing. For elemental analysis of nitrogen, carbon, hydrogen, and oxygen, the oven-dried digestate samples were subsequently ground with a mortar and pestle to obtain a uniformly fine powder. This was then sieved through

Figure 3.3: Mortar grinding digestate for CHN and TGA analysis

75 micron sieve and 300 micron sieves for CHN and TGA analyses, respectively.

The percentage of TS and MC in the digestate was determined using equations 3.7 and 3.8, respectively.:

$$\textbf{Total Solids \% (TS)}=\frac{W_3\text{-}W_0}{W_1\text{-}W_0}\times 100 \qquad \textbf{3.7}$$

$$\textbf{Moisture Content \% (MC)}=\frac{W_1\text{-}W_3}{W_1\text{-}W_0}\times 100 \qquad \textbf{3.8}$$

Where:

- W_0 = Empty crucible weight
- W_1 = Crucible weight + Sample weight
- W_3 = Crucible weight + Oven-dried sample weight

3.10.2 Estimation of volatile solids (VS) and ash content

The determination of VS and ash content was initially intended to follow the SANS 5925 ignition procedure, which involves heating oven-dried samples in a high-temperature muffle furnace (800 °C) using porcelain crucibles. However, practical challenges arose during implementation: the porcelain crucibles available in the laboratory at the time were unsuitable for high-temperature combustion, and the mass of the dried sample was extremely small (approximately 0.1 g). Working with such minimal quantities introduced considerable uncertainty, as the measurements became susceptible to limitations in balance sensitivity, particle loss during transfer, and the minimal ash residue remaining after ignition. Under these conditions, the standard gravimetric approach for VS and ash determination proved impractical.

To overcome these challenges, thermogravimetric analysis (TGA) was employed as an alternative method. TGA offers distinct advantages for small sample masses, providing continuous mass-loss data across a controlled temperature range and enabling accurate determination of MC, volatile matter, and residual ash. Consequently, the VS and ash content values reported in this study were derived from TGA measurements rather than the conventional SANS 5925 combustion procedure.

3.10.3 Sample preparation

Representative samples of the liquid digestate were oven-dried, homogenized by grinding in a mortar and pestle (Figure 3.3), then sieved with a < 300 µm mesh before analysis. Approximately 5 mg of each sample was carefully weighed into a clean alumina TGA crucible to ensure rapid heat transfer and minimize internal temperature gradients during analysis. Given the exceptional sensitivity of TGA to micro-scale samples, additional sieving was deemed unnecessary.

Method: Thermogravimetric analysis was performed using an SDT Q600 TGA instrument. The TGA method involved a temperature ramp from room temperature to 900 °C at a rate of 10 °C/min under a nitrogen, N_2 atmosphere (100 mL/min). Upon reaching 900 °C, the atmosphere was switched to carbon dioxide (CO_2 100 mL/min), and the temperature was held until complete conversion of the sample was achieved. This procedure was repeated for all samples (1-6), and the resulting data were compiled for further analysis.

3.10.4 Total suspended solids using EPA method 160.2

Gravimetric analysis is the most commonly applied technique for determining total suspended solids in water. In this quantitative approach, the mass of residues retained on glass-fibre or cellulose-ester membrane filters is measured after filtration. The method can be used for a wide range of water types, including drinking water, surface and saline waters, as well as domestic and industrial wastewater. Its practical working range for total suspended solids determination extends from about 4 mg/L up to 20,000 mg/L.

Preparation of glass fibre filter disc

1. The glass fibre filter was placed on the membrane filtration unit with the wrinkled side facing upwards.
2. The filter was rinsed by applying a vacuum while three successive 20 mL portions of distilled water were passed through the disc.
3. The vacuum was maintained after the water had drained in order to remove any residual moisture from the filter.
4. After rinsing, the filter was removed from the apparatus using tongs and dried in an oven at 103-105 °C for 1 hour.
5. The dried filter was then placed in a desiccator and stored until it was needed.
6. The drying step was repeated until a constant mass (weight loss less than 0.5 mg) was achieved, and the filter was weighed immediately before use.

3.10.5 Selection of sample volume

For a 4.7-cm-diameter filter, a sample volume of 100 mL was initially used for filtration. If the mass of the retained residue was below 1.0 mg, the sample volume was increased to ensure that at least 1.0 mg of residue was collected. When filters of other diameters were employed, filtration began with a sample volume corresponding to 7 mL of water per square centimetre of filter area. In each case, the collected residue mass was adjusted to be proportional to the 1.0 mg target, ensuring sufficient suspended solids for reliable measurement.

3.10.6 Filtration process

1. The filtration setup was assembled, and suction was applied to the unit.
2. The filter was moistened with a small volume of distilled water to ensure firm contact with the fritted support.
3. The sample was vigorously shaken, and the required volume was accurately transferred onto the filter using a graduated cylinder.

4. The vacuum application continued to remove all traces of water after the sample had passed through.
5. While suction was maintained, the graduated cylinder was rinsed, and its contents were passed through the filter, and any non-filterable material adhering to the walls of the funnel was washed down with three portions of distilled water, allowing complete drainage between each rinse.
6. After the final wash, vacuum was continued for a short period to ensure that all residual water was removed from the filter.
7. The filter was then carefully removed from the filtration support, dried in an oven at 103-105 °C for at least one hour, cooled in a desiccator, and subsequently weighed.
8. This drying and weighing sequence was repeated until a constant mass was obtained, defined as a weight loss of less than 0.5 mg between successive measurements
9. The non-filterable residue was calculated.

The formula for calculating the total suspended solids is:

$$\textbf{TSS (mg/L)}=\frac{(\text{C-D})\times 1000}{\text{V}} \qquad \textbf{3.9}$$

Where TSS is total suspended solids, C and D are weights of filter and filter + residue, respectively, and V is the sample volume in millilitres.

3.11 Elemental analysis (ICP-MS)

The digestate was analysed at the Eco-Analytica Laboratory of North-West University, Potchefstroom Campus. Prior to analysis, semi-solid digestate samples were subjected to acid digestion (Figure 4.4) in high-pressure digestion vessels using a mixture of nitric acid and hydrogen peroxide. The addition of hydrogen peroxide was necessary to reduce the high carbon content observed in the samples. The digestion vessels were then placed in a microwave digester programmed according to sample-specific parameters to ensure complete dissolution of the matrix.

Figure 3.4: Digestate before and after acid digestion in the ICP-MS lab

Following digestion, element concentrations in the resulting liquid samples were determined using an Agilent 7900 inductively coupled plasma mass spectrometer equipped with an X-lens configuration (Figure 3.5). Quantification was achieved through continuous inline internal standard addition (Agilent Internal Standard Mix) and external calibration using certified reference materials for each element (Accustandard). Procedural blanks and quality control (QC) standards were analysed with each batch to ensure analytical accuracy, and recoveries maintained between 90% and 110% for all reported elements.

3.11.1 Ammonia (NH_3) analysis

Ammonia concentrations were measured using the automated EasyChem 200 discrete analyser, following the manufacturer's protocol. Briefly, samples were filtered through a 25 mm, 0.45 µm nylon syringe filter, and the resulting filtrate was analysed for ammonia content.

3.11.2 pH measurement

The pH of each sample was measured using a calibrated HANNA HI2002-02 pH meter. Calibration was performed at pH 4.01, 7.00, and 9.01 using NIST-traceable certified calibration standards. Quality control was implemented every 10 samples by verifying the pH with reference standards (pH 4 and pH 9) from a second source to ensure measurement accuracy and instrument reliability.

Figure 3.4: Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) with autosampler and dedicated control software (Agilent Technologies, USA)

3.12 Data analysis

Data analysis was conducted to assess how different substrate mixing ratios and pre-treatment methods influenced biogas production, digestion stability, and the extent of substrate degradation. The following analytical procedures were performed:

3.12.1 Biogas yield and methane content

Daily biogas volumes were determined using the water displacement technique, and cumulative biogas yields were subsequently computed for each digester. The methane content was determined by gas chromatography (GC-TCD), and the daily methane yield was calculated as follows:

$$\textbf{Methane Yield (mL/g VS)} = \frac{\text{Total Methane Produced (mL)}}{\text{Volatile Solids Added (g)}} \qquad \textbf{3.10}$$

The biogas and methane yields were reported on a VS basis to enable comparison across different substrates and mixtures.

3.12.2 Substrate degradation and volatile solids reduction

VS destruction was calculated using the following formula:

$$\textbf{VS Reduction (\%)} = \frac{VS_{initial} - VS_{digestate}}{VS_{initial}} \times 100 \qquad \textbf{3.11}$$

This indicator was used to assess the efficiency of digestion under each mixing ratio.

3.13 Statistical analysis

Each treatment was tested in duplicate, therefore, the results were interpreted mainly through descriptive analysis. Mean values, standard deviations, tables, and graphs were used to compare biomethane production, process stability, and digestate characteristics across the different KW:PM mixing ratios. This approach allowed clear identification of performance trends while recognising that the limited number of biological replicates did not support strong statistical inference.

3.14 Software and tools

Microsoft Excel served as the primary tool for organizing raw data, performing calculations, and generating graphs and tables summarizing the experimental results. Spreadsheet functions were used to compute mean values, standard deviations, and other descriptive statistics, and to produce the plots presented in Chapter 4 for visual comparison of treatment performance.

3.15 Quality assurance and quality control

The validity and reliability of the experimental results were supported by implementing several quality management measures:

- All analyses were conducted in triplicate (duplicate for the ACoD) to account for experimental variability, and average values with standard deviations were calculated and reported for each parameter measured.
- Equipment calibration procedures were rigorously performed for all analytical instruments, including GC-TCD, pH meter, ICP-MS, and AMPTS II sensors, before each batch of tests to ensure measurement accuracy and consistency.
- Procedural blanks and certified reference standards were systematically analysed alongside samples during ICP-MS and ammonia testing to verify analytical accuracy and detect potential contamination or drift.
- TGA crucibles were thoroughly cleaned, dried in a desiccator, and reweighed prior to each experimental run to ensure mass accuracy and eliminate any residual material that could affect results.
- Duplicate digesters were operated simultaneously for each experimental condition, and any deviations greater than 10% between replicates triggered immediate repeat runs to verify data integrity and identify potential experimental errors.
- Temperature in all digesters was monitored continuously using calibrated sensors to maintain strict mesophilic conditions at 37 ± 2 °C throughout the digestion period, ensuring optimal microbial activity.

These comprehensive quality control procedures ensured reproducibility of results, minimized measurement bias and systematic errors, and significantly improved the overall reliability and credibility of the experimental findings.

3.16 Summary of the chapter

This chapter provides an overview of the experimental design, materials, equipment, sample preparation procedures, analytical techniques, and data analysis methods employed throughout this study. The methodologies were carefully selected and implemented to ensure the reproducibility, accuracy, and reliability of the results. By adhering to internationally recognized protocols and maintaining rigorous quality control at every stage of the investigation, this research establishes a robust, scientifically sound foundation for evaluating the co-digestion of KW and PM as a viable strategy to enhance biogas production.

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CHAPTER 4 - EXPERIMENTAL RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results of the anaerobic co-digestion (ACoD) experiments with kitchen waste (KW) and pig manure (PM) and discusses their implications for biogas production and digestate quality. The focus is on how the KW:PM mixing ratio and the combined mechanical and enzymatic pre-treatment influenced biogas and biomethane yields, process stability, and nutrient recovery under mesophilic conditions.

The chapter begins by characterising the substrates and examining how pre-treatment affects their physical and chemical properties, before presenting biogas production results, including daily and cumulative biomethane yields for the various mixing ratios. Process stability indicators such as pH behaviour and ammonia levels are considered, followed by an assessment of digestate composition and its potential use as a fertilizer. Where appropriate, the findings are compared with published work on similar systems to provide a broader context for the study.

All substrate materials in this study underwent a combined mechanical size reduction and enzymatic pre-treatment prior to digestion. As a result, the experimental design did not include untreated (no pre-treatment) control digesters; the effect of pre-treatment is therefore inferred from biogas production and stability indicators and from comparisons with biomethane yields reported in the literature for analogous untreated systems.

4.2 Characterization of substrates

The physicochemical characterization of the substrates is a fundamental prerequisite for understanding their inherent suitability for ACoD and for accurately interpreting subsequent biogas production. Parameters including moisture content (MC), total solids (TS), volatile solids (VS), pH, and carbon-to-nitrogen (C/N) ratio influence microbial community activity, the potential for substrate biodegradability, and the operational stability of anaerobic digesters (Meegoda *et al.* 2018:6-8). These parameters collectively determine the metabolic environment within the digestion system and directly affect the efficiency of organic matter conversion.

The characterization of KW and PM was conducted prior to the initiation of the digestion process. This preliminary characterization serves multiple purposes: it establishes a baseline for evaluating process performance throughout the experimental period, enables prediction of potential synergistic interactions between co-substrates, and facilitates identification of any compositional factors that might limit digestion efficiency. Furthermore, understanding the initial physicochemical properties of these materials enables informed optimization of substrate mixing ratios and provides essential context for comparing results with previous research. The characterization data also serve as a reference point for assessing the extent of organic matter degradation and transformation during ACoD, thereby enabling a robust evaluation of pre-treatment effectiveness.

4.2.1 Physicochemical and elemental properties of KW

The physicochemical properties of the KW substrate utilized in this investigation are systematically summarized in Table 4.1.

Table 4.1: Physiochemical characteristics of KW and PM

Parameters (%)	Pig manure	Kitchen waste
Total Solids (TS)	71.47 ± 0.60	88.82 ± 0.26
Volatile Solids (VS)	38.91 ± 0.96	78.27 ± 0.13
Moisture Content	28.53 ± 0.60	11.18 ± 0.26
Concentration	27.81 ± 0.92	69.52 ± 0.32
Carbon-to-nitrogen ratio (C/N)	8.70 ± 0.12	23.91 ± 0.67

Note: Values represent mean ± standard deviation of triplicate measurements.

Table 4.2: Elemental (CHN) of KW and PM

Sample	%C	%H	%N	%R
PM	22.33 ± 0.14	2.96 ± 0.03	2.57 ± 0.03	72.14 ± 0.10
KW	40.54 ± 0.19	5.86 ± 0.05	1.70 ± 0.05	51.90 ± 0.17

(SD = standard deviation; n = 3)

The physicochemical properties of the KW used are given in Table 4.1. KW had a low moisture content (11.18%) and a high TS content (88.82%), indicating a largely solid substrate with little free water. Such TS levels are typical of food waste streams rich in pastry, bakery residues, starchy materials and fibrous vegetables, and are favourable for anaerobic digestion because they provide a high concentration of organic matter per unit mass.

The VS content of KW was 78.27% of TS, showing a large biodegradable organic fraction. This is important for methane production, as VS represents the portion of the substrate that can be converted to biogas. The high VS content suggests that the KW contained abundant carbohydrates, lipids, and proteins that can be degraded during the hydrolysis, acidogenesis, acetogenesis, and methanogenesis stages of digestion.

The elemental (carbon, hydrogen, and nitrogen, (CHN)) analysis (Table 4.2) showed that carbon was the dominant element, confirming that KW was carbon-rich. This reflects the presence of carbohydrate and lipid-rich food residues (starches, sugars, oils and fats) that are known to contribute to methane formation (Zhang *et al.* 2022:8; Ceron-Vivas *et al.* 2019:93). The C/N ratio of KW was 23.8, which falls within the broad range reported as suitable for methane production (around 20-30:1)(Energy and Sustainability Directory, 2025). However, digesting such a carbon-rich material alone can lead to nitrogen limitation over time, reduced biomass growth, and lower process performance, especially in longer-term or continuous systems (Kumar *et al.* 2024:41).

The high organic concentration in KW (69.52%) confirms its high energy content and suitability as a primary biogas substrate. At the same time, the limited nitrogen content indicates the need for co-digestion with a nitrogen-rich material to avoid pH decline, volatile fatty acid (VFA) buildup, and potential inhibition.

In summary, KW can be regarded as a highly biodegradable, energy-dense substrate with strong methane potential but an unbalanced nutrient profile when used on its own. This motivates combining it with a nitrogen-rich co-substrate such as PM to improve the C/N ratio, nutrient balance, and buffering capacity.

4.2.2 Physicochemical and elemental properties of PM

The physicochemical composition of PM is summarized in Table 4.1. Compared to KW, PM exhibited higher MC (28.53%) and lower TS content (71.47%), which can be attributed to its semi-solid consistency and the presence of residual digestive fluids along with partially digested organic matter. The lower VS content of PM (38.31%) suggests both prior digestion and a shift in composition towards more recalcitrant, lignocellulosic, and structurally complex organic matter. These compounds remain part of the volatile fraction and are biodegradable over longer time scales, but they are less readily converted to biogas than the easily fermentable carbohydrates, lipids, and proteins that KW, which explains the reduced biodegradation potential of PM in this study.

The elemental CHN analysis (Table 4.2) confirmed that PM was more nitrogen-rich than KW, with 2.57 ± 0.03% N in PM versus 1.70 ± 0.05% N in KW, and a corresponding lower C/N ratio (22.33 ± 0.14% C in PM vs 40.54 ± 0.19% C in KW). This pattern is consistent with the known composition of animal manures, in which a substantial fraction of feed nitrogen is excreted and retained as undigested or partially digested proteins, amino compounds, and urinary nitrogen - primarily urea and ammonium (Mutungwazi *et al.* 2022:4). The higher nitrogen content can support microbial growth, protein synthesis and enzyme production during digestion. However, when PM is digested alone, the low C/N ratio (8.7) is known to increase the risk of ammonia accumulation and free-ammonia toxicity, which can inhibit methanogens (Vergote *et al.* 2020:7).

The organic concentration of PM (27.38% - dry basis) was lower than that of KW, confirming its lower energy density. Despite this, PM contributes buffering capacity, alkalinity and micronutrients, thereby playing an important stabilizing role when blended with carbon-rich substrates.

In summary, PM has a smaller readily biodegradable fraction and lower methane potential than KW, but provides nitrogen, buffering and trace nutrients that are valuable in co-digestion. Its properties make it suitable as a balancing co-substrate rather than as a stand-alone feedstock.

4.2.3 Implications of substrate characteristics for co-digestion

Taken together, the characterization results highlight the complementary nature of KW and PM for ACoD. KW supplies high VS, high organic concentration and strong methane potential but limited nitrogen and buffering. PM contributes nitrogen, alkalinity and micronutrients but has a lower biodegradable fraction and methane yield. Mixing these substrates allows the combined feedstock to reach a more suitable C/N ratio, while supplying both energy and nutrients to the microbial community. This form the basis for the improved biogas yields and stable digestion performance that co-digestion demonstrates relative to mono-digestion in later sections.

The estimated C/N ratios decreased from approximately 23.9 in the 100:0 KW treatment to 8.7 in the 0:100 PM treatment. The 80:20 and 60:40 KW:PM mixtures had estimated C/N ratios of approximately 19.7 and 16.2, respectively. These intermediate ratios are closer to the range generally regarded as suitable for anaerobic digestion (AD) (Zhang *et al.* 2022:2), and likely contributed to the stable digestion performance and higher methane yields observed for the KW-rich co-digestion treatments by providing a more balanced carbon and nitrogen supply for microbial activity.

4.3 Effect of mechanical pre-treatment on substrate properties

Both KW and PM underwent mechanical pre-treatment to achieve particle size reduction and improve their physical properties before the digestion phase. Smaller particle sizes are recognized for expanding the surface area accessible to microbial activity, promoting better mixing and mass transfer dynamics, and facilitating the breakdown of complex organic materials through hydrolysis (Fernandez *et al.* 2020:3).

4.3.1 Particle size reduction and physical modification

Before pre-treatment, KW contained coarse vegetable fragments, pastry residues and irregular pieces ranging from about 5 to 40 mm, while PM contained fibrous clumps and aggregates of roughly 2 to 10 mm. After blending, both substrates appeared finer and more uniform (4-5mm), with reduced structural complexity and improved homogeneity (Figure 4.1). This change is expected to improve contact among microorganisms, extracellular enzymes, and organic material, and to support more uniform mixing within the digester (Izumi *et al.* 2010:3).

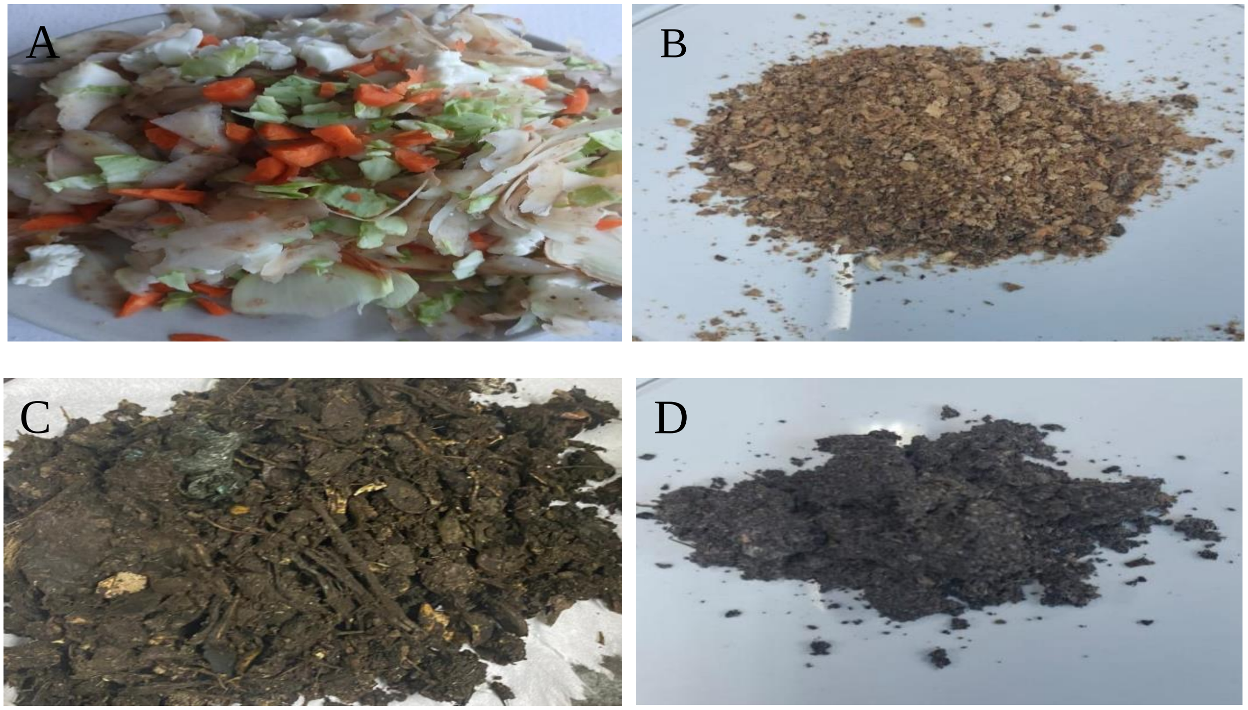

Figure 4.1: Physical appearance of substrates before and after treatment: A – KW before treatment, B – KW after treatment, C – PM before treatment, D – PM after treatment.

4.3.2 Influence of mechanical pre-treatment on TS and VS

Mechanical pre-treatment did not change the overall TS content of KW or PM, consistent with reports that size reduction primarily alters structure rather than bulk composition. However, by exposing internal surfaces and breaking up fibrous matrices, it increases the effective accessibility of VS to microbial enzymes (Kang *et al.* 2019:472; Rusanowska *et al.* 2018:221). This effect is especially important for PM, where much of the organic matter is embedded in lignocellulosic fibres that limit direct microbial contact.

Although the absolute VS values are not increased by mechanical pre-treatment, more of the existing VS becomes available for degradation as seen in a previous study (Khan *et al.* 2022:2; Orlando and Borja, 2020:4). This is expected to support higher biogas production rates, better VS conversion and more stable performance during digestion. The practical impact of this pre-treatment is evident in the biogas production results discussed in Section 4.5.

4.3.3 Implications for hydrolysis and substrate biodegradability

The observed reduction in particle size and improved substrate homogeneity resulting from mechanical pre-treatment have important implications for hydrolysis efficiency and overall substrate biodegradability in ACoD systems. Studies have established hydrolysis as the rate-limiting step in AD (Gyadi *et al.* 2024:5), particularly for substrates with complex structural compositions such as food waste and animal manure, which contain resistant polymeric substances. By expanding the surface area available for enzymatic reactions and minimizing mass-transfer and diffusion barriers at the interface between substrate and microorganisms, mechanical pre-treatment is recognized as accelerating and improving the hydrolysis of complex organic polymers into soluble intermediate compounds that acidogenic and methanogenic bacteria can easily metabolize during subsequent stages of digestion.

Improved hydrolysis supports balanced and controlled acidogenesis and methanogenesis, reducing the risk of excessive intermediate accumulation, such as VFAs, that may trigger pH depression and process inhibition. In co-digestion systems, mechanical pre-treatment improves synchronization and alignment of degradation rates between the rapidly degradable KW and the more slowly degradable PM, thereby enhancing overall process stability, preventing substrate overloading, and promoting more consistent biogas production throughout the digestion period (Zhang *et al.* 2023:547).

It can be concluded that mechanical pre-treatment is an effective, economically feasible, and environmentally sustainable physical enhancement strategy that improves substrate biodegradability without requiring chemical additives or energy-intensive thermal processes. This approach creates favourable conditions for increased biogas production, accelerated digestion kinetics, and enhanced process stability. The practical implications and benefits of these improvements are further examined and validated through the biogas production performance metrics, process stability analyses, and comparative evaluations presented in subsequent sections of this chapter.

4.4 Effect of enzymatic pre-treatment on substrate characteristics

Enzymatic pre-treatment was carried out using a laboratory-fermented cow dung slurry that served as a natural, low-cost source of hydrolytic enzymes. Prior to digestion, the slurry was combined with the mechanically shredded substrates to facilitate the breakdown of complex carbohydrates, proteins, and lipids into simpler, more easily degradable compounds. This step was intended to accelerate the hydrolysis stage of AD and improve conversion rates (Dos Santos Ferreira *et* *al.* 2020:4239). The effect of this pre-treatment was assessed through changes in TS and VS and through the resulting biomethane yields.

4.4.1 Enzyme source and pre-treatment conditions

Cow dung contains a diverse group of hydrolytic microorganisms that produce enzymes such as cellulases, hemicellulases, amylases, proteases and lipases (Behera & Ray, 2020:2). Fermenting the cow dung for 21 days under laboratory conditions encouraged enzyme production and created an enzyme-rich slurry. This slurry was then incorporated to the substrate mixtures at a fixed ratio for a defined contact time before digestion.

Using fermented cow dung as a natural enzyme source offers a practical and sustainable alternative to commercial enzymes, especially where cost and availability are limited. This approach also supports circular economy principles by transforming agricultural residue into a valuable processing aid (HomeBiogas, 2024).

4.4.2 Changes in TS and VS following enzymatic pre-treatment

Gravimetric measurements showed that enzymatic pre-treatment did not cause large changes in TS; values remained close to those of the untreated mixtures. This is expected because the enzymes mainly change the form and solubility of organic matter rather than the total mass of solids. Hydrolytic enzymes break down complex polymers into soluble sugars, peptides, amino acids and fatty acids (Saratale *et al.* 2023:6). As a result, the proportion of VS that is readily accessible to microorganisms increases, even though the measured VS percentage may remain similar.

The effects of the enzymatic pre-treatment are more evident in the digestion results than in the TS/VS values. As shown in Section 4.5, mixtures that received enzymatic pre-treatment produced higher cumulative biomethane yields than the corresponding mechanically pre-treated-only or untreated mixtures. The improved methane production profiles reported in Section 4.5 provide indirect evidence that the substrates' biodegradability increased after enzymatic treatment.

4.4.3 Comparative effect of enzymatic pre-treatment on substrate biodegradability

Compared with mechanical pre-treatment, which mainly improves substrate accessibility by reducing particle size, enzymatic pre-treatment acts at the molecular level and targets the hydrolysis of cellulose, hemicellulose, and proteins (Bonilla *et al.* 2018:81; Anacleto *et al.* 2024:4; Dincă *et al.* 2024:909). Fermented cow dung served as a low-cost, locally available enzyme source following size reduction, and the higher cumulative biomethane yields and smoother production profiles (Section 4.5.2) indirectly indicate enhanced substrate biodegradability via increased soluble, readily degradable intermediates.

4.5 Biogas production performance

Biogas production performance was evaluated using daily and cumulative biomethane yields and related methane indicators. These metrics describe how the different KW:PM mixing ratios behaved over time under the combined mechanical-enzymatic pre-treatment and reveal how this integrated strategy influenced gas production.

Table 4.3 and Figure 4.2 present the daily biomethane yield variations for all treatments (A - F).

Table 4.3: Daily biomethane yield of mono- and co-digestion of KW and PM (mL CH_4 g^{-1} VS d^{-1}; mean ± standard deviation, n = 2)

Day	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	32.77 ± 0.56	30.11 ± 0.33	27.60 ± 0.54	19.06 ± 5.44	20.19 ± 0.61	14.12 ± 0.40
2	10.33 ± 3.68	7.34 ± 0.09	7.06 ± 0.79	6.37 ± 0.04	6.22 ± 0.27	3.17 ± 0.18
3	13.61 ± 2.29	12.06 ± 2.14	11.28 ± 0.17	9.36 ± 0.39	8.32 ± 0.13	5.59 ± 0.30
4	12.12 ± 2.63	11.71 ± 2.34	8.73 ± 1.20	7.11 ± 0.18	6.14 ± 0.25	4.01 ± 0.21
5	11.10 ± 0.13	9.93 ± 1.79	6.50 ± 0.31	6.33 ± 0.50	7.15 ± 0.09	4.40 ± 0.13
6	9.20 ± 0.04	6.32 ± 1.14	6.12 ± 0.15	6.92 ± 0.09	6.51 ± 0.01	4.18 ± 0.05
7	10.01 ± 0.01	7.82 ± 0.10	7.06 ± 0.19	7.74 ± 0.09	6.87 ± 0.36	5.55 ± 1.38
8	10.63 ± 0.20	7.95 ± 0.51	7.76 ± 0.95	7.92 ± 0.57	6.40 ± 0.01	4.20 ± 0.03
9	12.64 ± 0.10	9.98 ± 0.64	10.15 ± 0.89	8.88 ± 0.94	7.03 ± 0.05	4.92 ± 0.01
10	14.65 ± 0.05	11.81 ± 0.58	11.83 ± 0.79	9.25 ± 0.80	7.62 ± 0.12	5.33 ± 0.04
11	18.39 ± 0.10	15.85 ± 0.89	14.65 ± 0.14	10.83 ± 0.53	10.10 ± 0.04	8.05 ± 0.83
12	17.45 ± 0.26	15.69 ± 0.27	14.89 ± 0.79	10.58 ± 0.81	9.48 ± 0.14	6.58 ± 0.12
13	18.37 ± 0.36	18.08 ± 0.03	15.09 ± 1.09	11.33 ± 0.58	10.24 ± 0.16	7.07 ± 0.11
14	19.88 ± 0.45	20.85 ± 0.26	15.58 ± 0.94	12.47 ± 0.54	11.33 ± 0.42	7.70 ± 0.20
15	21.87 ± 1.39	23.04 ± 0.71	16.60 ± 1.04	14.13 ± 0.03	12.78 ± 0.16	9.12 ± 0.37
16	22.20 ± 1.62	21.83 ± 0.67	16.50 ± 0.49	12.69 ± 0.98	12.51 ± 0.51	8.38 ± 0.18
17	23.17 ± 1.09	21.63 ± 0.72	16.82 ± 0.27	12.91 ± 0.65	12.15 ± 0.08	8.11 ± 0.17

18	23.88 ± 0.06	21.20 ± 0.41	16.73 ± 0.32	13.41 ± 0.25	12.82 ± 0.36	8.03 ± 0.22
19	22.58 ± 1.75	20.28 ± 0.12	16.15 ± 0.04	13.01 ± 0.32	12.55 ± 0.17	7.24 ± 0.24
20	19.71 ± 1.77	18.90 ± 0.22	15.28 ± 0.28	12.25 ± 0.47	12.14 ± 0.31	6.41 ± 0.30
21	16.26 ± 2.48	16.81 ± 0.63	14.07 ± 0.45	12.10 ± 0.72	11.53 ± 0.49	5.69 ± 0.32
22	9.79 ± 4.08	10.79 ± 1.58	9.94 ± 1.02	10.51 ± 0.18	7.30 ± 1.00	3.51 ± 0.21
23	3.50 ± 2.49	4.02 ± 1.55	5.49 ± 1.10	9.33 ± 0.25	3.42 ± 0.72	1.82 ± 0.51
24	1.84 ± 0.65	1.81 ± 0.30	3.28 ± 0.15	8.87 ± 1.53	2.01 ± 0.03	1.24 ± 0.01
25	1.48 ± 0.66	1.62 ± 0.12	4.05 ± 0.01	5.64 ± 4.59	1.71 ± 0.29	1.22 ± 0.04
26	1.60 ± 0.58	1.86 ± 0.47	6.90 ± 0.05	4.18 ± 4.82	1.44 ± 0.05	1.11 ± 0.04
27	1.94 ± 0.75	4.01 ± 1.23	9.04 ± 0.19	1.86 ± 1.46	1.23 ± 0.26	1.19 ± 0.05
28	2.80 ± 0.59	6.62 ± 0.54	8.07 ± 0.44	2.13 ± 0.06	2.22 ± 0.07	1.82 ± 0.83

Note: Data represent mean ± SD from duplicate measurements (n = 2) for each sampling day.

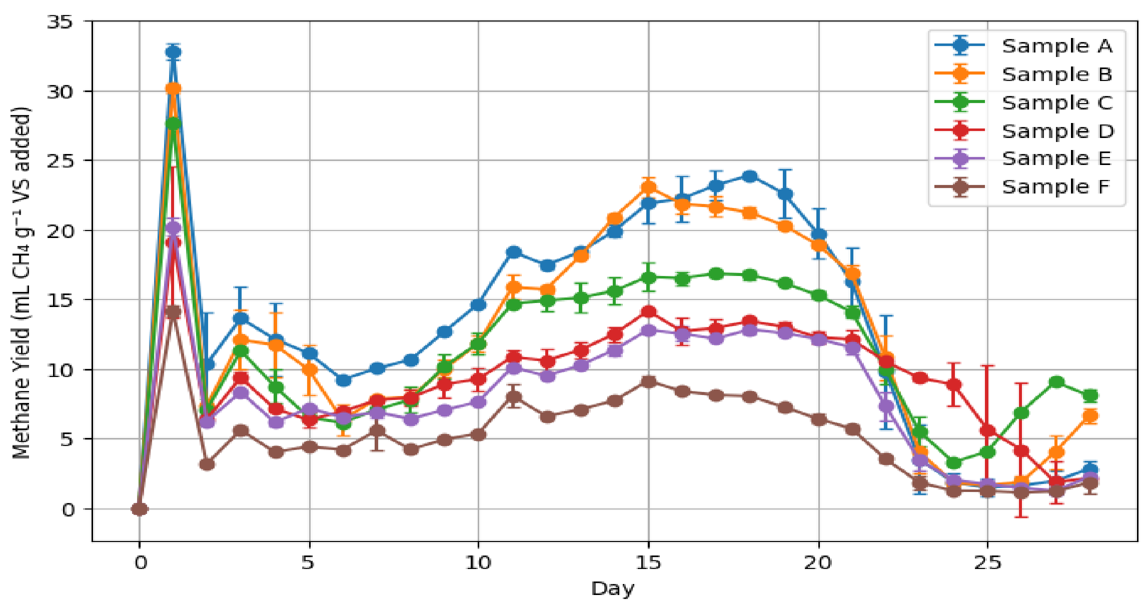

Figure 4.2: Daily biomethane production of mono- and co-digestion of KW and PM (Samples A–F). Error bars indicate 1 standard deviation of duplicate measurements (n = 2) for each day, corresponding to the data shown in Table 4.2.

Samples A – F correspond to the mono and co-digestion configurations defined in Table 3.1 (Ratio of different digesters); where sample A = 100% KW, sample B = 80% KW and 20% PM, sample C = 60% KW and 40% PM, sample D = 40% KW and 60% PM, sample E = 20% KW and 80% PM, and sample F = 100% PM, respectively.

4.5.1 Daily biogas production profile

4.5.2 Temporal variation in gas production

The daily biomethane yield patterns for all samples (A-F) are displayed across the entire digestion period, with the day-by-day biomethane yield profiles for all treatments presented in Table 4.3 and illustrated in Figure 4.2, where error bars indicate the variability between duplicate digesters. For all the samples, the biomethane production exhibited a similar pattern, demonstrated by an initial sharp rise in methane generation during the early stages of digestion (day 2), followed by a gradual stabilization as the process matured, a subsequent increase from day 12 to day 16, and then a sharp decline at day 17 to day 23 as the substrate availability diminished.

The small standard deviations and narrow error bars observed for Samples A, B, and C indicate good reproducibility between the duplicate digesters and BMP. On the other hand, Samples E and F showed greater variation at some stages of digestion, suggesting less performance. These results indicate that the KW-rich mixtures not only produced higher biomethane yields but also performed more reliably under the experimental conditions.

A pronounced increase in daily biomethane yield was observed on day 2 for all samples, with Sample A recording the highest value (65.53 mL CH_4 g^{-1} VS), followed by Samples B and C with similar high yields. This rapid onset of methane production suggests that the microbial community quickly adapted to the substrate environment and utilized readily biodegradable organic matter such as simple sugars, soluble starches, and proteins during the early stage of digestion.

Following the initial peak, the daily biomethane yield progressively decreased from days 3-7 across all samples, reflecting the gradual depletion of readily degradable substrates and a metabolic transition toward the degradation of more structurally complex and recalcitrant organic fractions, such as cellulose, hemicellulose, and fibrous materials. This decline pattern is characteristic of batch digestion systems (Akash *et al.* 2025:10) and indicates the exhaustion of the most bioavailable organic compounds. The PM-rich co-digestion treatments (Samples D and E) and PM mono-digester (Sample F) exhibited a similar decline pattern, though with steadily reduced daily biomethane yields throughout the digestion period, indicating the lower biodegradable organic content and diminished methane potential of the manure-dominated mixtures

4.5.3 Lag, exponential, and stabilization phases

The digestion process exhibited a very short lag phase, with methane production reaching measurable levels by day 2 in all samples (Figure 4.3). This early onset of methane generation suggests that the combined mechanical and enzymatic pre-treatment likely improved substrate accessibility and bioavailability to microbial populations, thereby facilitating rapid microbial establishment and gas production. This aligns with previous reports that mechanical pre-treatment disrupts biomass structure, reduces mass-transfer limitations, and accelerates hydrolysis, thereby increasing methane yields. (Zainal *et al.* 2022:9; Dahunsi, 2019:21). In addition, the cow-dung-based enzymatic pre-treatment likely increased the proportion of soluble and readily degradable organic matter, thereby supporting the rapid transition from hydrolysis to methanogenesis (Yan *et al.* 2018:10). The integration of mechanical and enzymatic pre-treatment strategies appears to have generated a synergistic effect on both biogas yield enhancement and overall process stability maintenance.

The exponential growth phase was evident during the first few days (2 days), during which a sustained and relatively high methane production was observed for all samples. During this active degradation period, Samples A, B, and C produced higher daily biomethane yields compared to Samples D, E, and F, suggesting a stronger influence of substrate composition, particularly the KW:PM mixing ratio, on digestion kinetics and methane generation rates.

After approximately day 20, the digestion process entered a short stabilization period, followed by a decline in all samples, with daily biomethane yields progressively decreasing toward baseline levels. This decline corresponds to the near-complete exhaustion of biodegradable organic matter and indicates the approach of digestion completion. The gradual reduction in methane production reflects the diminishing availability of metabolizable substrates and the transition toward endogenous microbial metabolism, which is typical of the final stages of ACoD processes (Meegoda *et al.* 2018:11).

4.5.4 Cumulative biomethane yield

The cumulative biomethane production trends, calculated from daily yield data, are displayed in Figure 4.3. The cumulative biomethane production increased steadily throughout the digestion period across all samples, with distinct differences among substrate mixtures.

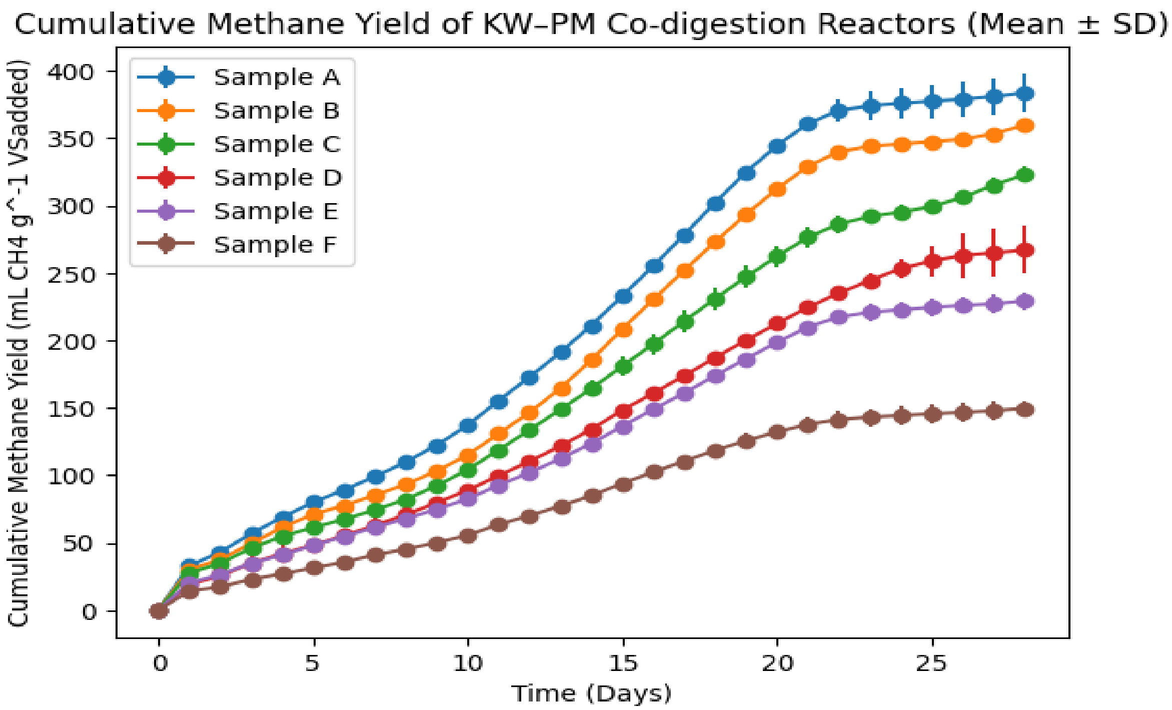

Figure 4.3: Cumulative biomethane production from mono- and co-digestion of KW and PM. Samples A, B, and C achieved higher final cumulative biomethane yields, reflecting their higher daily methane production rates during the exponential growth phase and sustained metabolic activity throughout the digestion period. In contrast, Samples D, E, and F exhibited lower cumulative yields, reflecting lower daily biomethane outputs and reduced substrate conversion efficiency.

The differences in biomethane yield are consistent with the substrate characteristics and C/N ratios presented in Tables 4.1 and 4.2 respectively. KW-rich mixtures (Samples A-C) had higher VS content and more favourable C/N ratios, whereas PM-rich mixtures (Samples E-F) contained less readily biodegradable material and lower C/N ratios. As a result, Samples A and B converted the available VS into methane more efficiently under the combined mechanical and enzymatic pre-treatment conditions.

Since all substrates underwent identical mechanical and enzymatic pre-treatment protocols, the observed differences in cumulative biomethane yield are attributed to variations in substrate mixing ratio, organic matter composition, and nutrient balance, rather than differential pre-treatment effects. The results demonstrate that an appropriate combination of substrates and optimization of the KW:PM ratio are critical to maximizing biomethane recovery, enhancing conversion efficiency, and improving overall process performance under uniform pre-treatment conditions.

Although each treatment was tested in duplicate, the small standard deviations for the KW-rich mixtures indicate that the biomethane results were uniform between reactors. This increases confidence that the observed differences reflect real treatment effects rather than random variation. However, because only two biological replicates were used, the findings should be interpreted within the scope of this experimental design, and the identified optimum ratio is specific to the specific mesophilic conditions and pre-treatments applied in this study.

The cumulative biomethane yields confirm the effectiveness of combined pre-treatment in supporting sustained methane production, efficient substrate utilization, and high biodegradability of the co-digested organic materials.

4.5.5 Methane yield and methane content

4.5.6 Methane Yield

The methane yield, expressed as daily biomethane yield per unit of VS added (mL CH_4 g^{-1} VS), is a direct indicator of substrate biodegradability and digestion efficiency under anaerobic conditions. Figure 4.3 demonstrates that samples A, B, and C attained higher methane yields across the digestion period relative to the other samples, especially during the exponential growth phase.

The higher methane yields in these samples indicate more efficient conversion of organic matter into methane, likely resulting from favourable substrate composition, an optimal C/N ratio, and balanced nutrient availability in the KW-PM mixtures. This enhanced performance reflects the synergistic effectiveness of the combined pre-treatment methods in enhancing the substrate conversion efficiency.

4.5.7 Methane content

The methane content represents the volumetric fraction of methane in the total biogas produced and reflects biogas quality and energy value rather than quantity or production rate (Cambridge Sensotec, 2024). In this study, methane composition was not measured daily; therefore, direct temporal profiles of the methane percentage are unavailable. Nevertheless, the high biomethane yields and stable biomethane production trends observed for all samples (Section 4.5), together with the absence of excessive VFA accumulation and the maintenance of a near-neutral pH throughout the digestion period (Section 4.7; Liang, 2024:128), indicate effective methanogenic activity and suggest that the biogas quality was satisfactory. These observations imply that the combined pre-treatment and co-digestion strategy used here provided conditions favourable for methane-producing microorganisms, even though the specific contribution of each pre-treatment step was not quantified separately (Bharadwaj *et al.* 2024:5).

4.5.8 Influence of substrate composition and pre-treatment

Because all samples were subjected to the same combined mechanical and enzymatic pre-treatment protocol, this study did not include a direct comparison between different pre-treatment methods or between pre-treated and untreated substrates; instead, the effect of the integrated pre-treatment strategy is reflected in the biomethane production and process stability achieved across all experimental conditions. Within this common pre-treatment framework, samples A - C, which correspond to (sample A = 100% KW, sample B = 80% KW and 20% PM, sample C = 60% KW and 40% PM, respectively), produced the highest cumulative biomethane yields and exhibited the most favourable methane production profiles. The short lag phase and rapid onset of methane production observed across all samples suggest that the integrated pre-treatment improved substrate accessibility and bioavailability, while the differences in final yields and kinetics among Samples A-F are primarily attributable to differences in substrate composition rather than to pre-treatment.

Differences in methane yield among the samples are therefore explained by substrate composition and mixing ratio. Samples with higher shares of readily biodegradable material and a more balanced nutrient profile produced more methane, while PM-rich mixtures performed worse. Samples A (100% KW) and B (80% KW, 20% PM) gave the highest cumulative biomethane yields of 767.32 and 719.42 mL CH_4 g^{-1} VS, respectively. Although untreated substrate controls were not evaluated experimentally in this investigation, the biomethane yields across all digesters exceeded those reported for untreated-substrate digestion systems in previous peer-reviewed studies (Antognoni *et al.* 2013:24; Bernat *et al.* 2020:4; Mata-Alvarez *et al.* 2015:164). Samples E (20% KW, 80% PM) and F (100% PM), which contained less readily degradable organic matter, produced lower yields of 458.69 and 299.38 mL CH_4 g^{-1} VS (Figure 4.3). All treatments received the same mechanical and enzymatic pre-treatment; on that basis, the differences can be attributed to substrate composition rather than to pre-treatment conditions. The high biomethane yields, short lag phases, and stable production profiles observed across treatments indicate that the combined pre-treatments improved substrate biodegradability and overall process performance, consistent with a previous study (Liang, 2024:128).

Compared with recent international studies on the ACoD of KW and PM, the methane yields obtained in this study, reaching 767 mL CH_4 g^{-1} VS added for the 100:0 KW treatment and 719 mL CH_4 g^{-1} VS for the 80:20 KW:PM mixture, are within the upper range of values reported for mesophilic batch digestion systems. Previous studies have reported methane yields ranging from approximately 300 to 700 mL CH_4 g^{-1} VS for food waste and manure co-digestion under similar operating conditions (Li *et al.* 2013:2088; Meng *et al.* 2022:1752), with reviews showing that food-waste-rich substrates or balanced co-digestion mixtures can improve methane production by providing an appropriate nutrient balance and favourable digestion conditions. The present findings similarly demonstrate the benefits of KW-rich mixtures, particularly the 80:20 and 60:40 KW:PM ratios, which achieved high methane yields while maintaining stable digestion performance. Differences in reported optimum mixing ratios among studies are likely attributable to variations in substrate composition, inoculum characteristics, reactor configuration, operating conditions, and pre-treatment methods.

The maximum methane yield observed (~767 mL CH_4 g^{-1} VS added for 100:0 KW) tilts towards the upper theoretical methane potential reported for highly degradable food waste mixtures, which lies in the range 400-800 mL CH_4 g^{-1} VS depending on composition (Li *et al*. 2013:2088; Donatelli and Chang, 2024:8). This high value is plausible given the predominance of lipids and readily degradable carbohydrates in the KW used, combined with intensive pre-treatment and a well-adapted inoculum, but it also underscores the need to interpret the results cautiously and to validate them in continuous reactors and under varying operational conditions.

4.6 Effect of substrate mixing ratio on biogas production

The substrate mixing ratio is a key influence on ACoD performance, as it directly influences organic matter availability, nutrient balance, C/N ratio, and microbial metabolic activity (Kumari & Chandel, 2023:6). The effect of different KW-PM mixing ratios on biomethane production was evaluated using six digesters (A-F), configured as follows: digester A = 100% KW, digester B = 80% KW and 20% PM, digester C = 60% KW and 40% PM, digester D = 40% KW and 60% PM, digester E = 20% KW and 80% PM, and digester F = 100% PM, respectively as presented in Table

3.1. The corresponding biomethane yields obtained from these digesters are reported as Samples A-F in Table 4.3 and displayed in Figures 4.2 and 4.3.

All substrates were subjected to identical mechanical and enzymatic pre-treatment protocols prior to digestion under standardized conditions; therefore, variations in biomethane production observed among the samples are attributed solely to differences in substrate mixing ratio rather than differential pre-treatment effects. This experimental design allows for a clear assessment of the influence of substrate composition on digestion performance while maintaining consistent pre-treatment conditions across all digesters.

4.6.1 Biomethane yield at different KW:PM mixing ratios

The daily biomethane yield profile presented in Figure 4.2 reveals significant differences in biogas production performance among the various KW:PM mixing ratios tested. Digester A (100% KW), corresponding to sample A, recorded the highest daily biomethane yields throughout the digestion period, with a peak value of 32.77 mL CH_4 g^{-1} VS on day 2, reflecting the high biodegradability and readily accessible organic content of KW, which is rich in simple carbohydrates, proteins, and lipids. This was followed closely by digester B (80% KW:20% PM), which also exhibited high biomethane yields during the initial growth phase, suggesting that a small proportion of PM does not compromise methane production while providing some buffering capacity.

Digesters with intermediate mixing ratios, particularly digester C (60% KW:40% PM) and digester D (40% KW:60% PM), showed moderate biomethane yields, indicating balanced digestion performance but lower methane productivity compared to KW-rich mixtures. These intermediate ratios likely reflect a trade-off between the high biodegradability of KW and the stabilizing but less degradable characteristics of PM. Digester F (100% PM) produced the lowest biomethane yields, with a maximum daily yield of only 14.12 mL CH_4 g^{-1} VS, reflecting the lower biodegradability and higher recalcitrance of PM relative to KW, due to its fibrous lignocellulosic content and previously digested nature.

The cumulative biomethane yield patterns displayed in Figure 4.3 confirm these findings. Digesters A and B attained the highest cumulative biomethane production throughout the 28-day digestion period, demonstrating sustained high-level performance, followed by digesters C and D with intermediate cumulative yields, while digesters E (20% KW:80% PM) and F recorded the lowest cumulative yields. These outcomes demonstrate that substrate mixtures with higher KW content enhance biogas production, accelerate digestion kinetics, and improve overall conversion efficiency under the applied digestion conditions and pre-treatment approach.

4.6.2 Determination of optimal KW:PM ratio

4.6.3 Justification based on biogas and methane yield

The optimal KW:PM mixing ratio was determined by evaluating both daily biomethane yield patterns and cumulative biomethane production over the entire digestion period. Although digester A (100% KW) achieved the highest absolute biomethane yields, digester B (80% KW:20% PM) exhibited a comparable performance while offering improved substrate balance, enhanced process stability, and reduced risk of process inhibition.

Digester B demonstrated several key performance indicators:

- High peak daily biomethane yield (60.22 mL CH_4 g^{-1} VS on Day 2), which was only marginally lower than that of digester A.
- Sustained and consistent methane production throughout the exponential digestion phase without significant fluctuations.
- High cumulative biomethane yield over the entire digestion period, approaching that of digester A.

The inclusion of 20% PM likely enhanced a buffering capacity through alkalinity provision, improved microbial stability by supplying essential trace nutrients and nitrogen and reduced the potential risk of rapid acidification and VFA accumulation commonly associated with mono-digestion of rapidly degradable KW. This modest addition of PM appears to provide stabilizing benefits without substantially compromising methane yield. Conversely, digesters with higher PM proportions (E and F) produced significantly lower methane yields, indicating sub-optimal carbon availability and excessive dilution of readily biodegradable organic matter.

Based on these results, the 80% KW:20% PM mixing ratio (digester B) is considered the optimal substrate combination, as it provided a favourable balance among high biomethane yield, digestion stability, and process reliability under uniform pre-treatment conditions. This ratio maximizes methane production while maintaining stable anaerobic conditions conducive to sustained microbial activity.

The variation in biomethane yield observed across different KW:PM mixing ratios can be directly attributed to changes in the C/N ratio of the feedstock blend, with intermediate ratios offering a more balanced nutrient environment for microbial activity.

4.6.4 Comparison with reported optimal ratios reported in literature

4.6.5 Alignment or deviation from previous studies

The optimal 80:20 KW:PM ratio identified in this study agrees with many reports where food or KW dominated mixtures (typically 60-80% on VS basis) achieved the highest methane yields and stable operation when co-digested with animal manure (Ali *et al.* 2025:387; Saravanan & Thiyagarajan, 2025:307; Baek *et al.* 2020:10). For example, a mixing ratio of 1:1 (50% KW, 50% PM) has been reported to maximise methane yield and ensure stable digestion performance in co-digestion of KW and PM (Tian *et al.* 2015:52), while other studies on kitchen/food-waste co-digestion with cattle manure have identified FW:CM ratios around 2:1 or 7:3 (Xing *et al.* 2020:4) as providing the best compromise between high methane production and stable operation. Slight difference in the reported optimum may result from variations in substrate composition, reactor type, operating temperature and solids content, as well as whether pre-treatment was applied. The combined mechanical and enzymatic pre-treatment likely improved hydrolysis and enabled the use of a high KW fraction without acidification problems, which helps explain why 80% KW performed well despite its high organic loading.

4.6.6 Process stability indicators

Process stability is a critical determinant in ACoD systems, as it governs the ability of the microbial consortia to sustain methane production without metabolic inhibition or system failure (de Castro *et al.* 2025:8). In the present investigation, process stability was assessed primarily by monitoring temporal pH trends and observing any signs of foam formation, scum accumulation, odour problems, or sudden drops in biogas production during the batch BMP trials. The pH remained within a near-neutral range for all digesters, likely between about 6.8 and 7.8 based on endpoint digestate measurements (Table 4.5) and the absence of any acidification symptoms, and no obvious inhibition symptoms were observed (Sections 4.5 and 4.7), suggesting that the ACoD process operated under stable conditions with adequate buffering capacity throughout the experimental period. Although continuous pH monitoring was not performed, the final digestate pH values of 8.30-8.45 (Table 4.5) and the stable biomethane production profiles support the interpretation of overall pH stability.

4.6.7 pH Variation during anaerobic digestion

4.6.8 Stability trends and buffering effects

pH serves as a fundamental and sensitive indicator of ACoD stability, primarily because methanogenic microorganisms exhibit optimal metabolic activity within a relatively narrow pH range, typically between 6.8 and 7.8 (Diniz *et al.* 2025:6; Mao *et al.* 2017:7). In this study, pH was not recorded continuously for all reactors; therefore, detailed pH profiles over the digestion period are not available. However, the biomethane production curves did not show abrupt declines or erratic fluctuations, and no visual signs of acidification or inhibition (such as strong odour, scum formation, or gas collapse) were observed during the BMP tests, suggesting that the pH remained within a favourable range for methanogenic activity. These observations are interpreted as indirect indicators of pH stability, as seen by Naghavi *et al.* (2022:550), rather than as a substitute for measured pH data.

The minimal lag phase and the rapid onset of methane production observed in all experimental digesters suggest that the digestion system had adequate buffering capacity to avoid severe acidification during start-up. This apparent buffering effect is linked to the inclusion of PM in the substrate mixtures, as manure is well documented to contribute alkalinity and help mitigate rapid pH drops typically associated with the acidogenic degradation of readily biodegradable food or kitchen waste substrates (Arriagada *et al.* 2019:6639). Although pH and alkalinity were not monitored, the stable biomethane production profiles, without signs of process failure, indicate that no major acidification or inhibition occurred under the tested conditions, as observed in a previous study (Baek *et al.* 2020:5).

4.6.9 VFAs accumulation/degradation patterns

VFAs represent critical intermediate metabolic compounds generated during the acidogenesis and acetogenesis phases of AD and serve as sensitive indicators of process imbalance when accumulated to concentrations that exceed microbial conversion capacity (Dong *et al.* 2023:6). VFAs were not measured directly; instead, pH, ammonia concentration, and biomethane production were used to assess the likelihood of acidification and process stability. The stable pH values and consistent BMP across treatments suggest that excessive VFA accumulation was unlikely under the tested KW:PM ratios. However, this conclusion is based on indirect inference and comparisons with previous studies (Dong *et al.* 2023:6; Zainal *et al.* 2022:4) rather than direct VFA measurements. The high biomethane yields observed in the early digestion phase imply that

VFAs produced through rapid decomposition of readily biodegradable substrate fractions underwent efficient conversion to methane via methanogenic pathways. This process follows established stoichiometric mechanisms: acetate transformation to methane and carbon dioxide ($CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$), and propionate or butyrate oxidation to acetate and hydrogen, which subsequently undergo conversion to methane. The absence of abrupt drops in methane production or signs of process failure further indicates that VFA accumulation did not reach inhibitory levels during the BMP tests. Although VFA concentrations were not measured directly in this study, the observed pH behaviour, methane production trends, and ammonium-N results were used as indirect indicators of process stability.

The daily biomethane yield pattern observed in this study was characterised by a sharp initial peak within the first 2 days, a rapid decline over the next few days, a more gradual increase leading to a secondary production maximum around the mid-digestion period, and a final decrease as the readily biodegradable substrate became depleted (Figure 4.2). Such multi-phase methane production profiles, with an early peak associated with rapid conversion of easily degradable compounds followed by a slower phase linked to the hydrolysis and utilisation of more recalcitrant fractions, have been reported for batch AD of food and manure-based substrates (Mao *et al.* 2017:2). The absence of any abrupt collapse in gas production during these phases suggests that VFAs generated during acidogenesis were continuously converted to methane rather than accumulating to inhibitory levels, although VFA concentrations were not measured directly in this study.

The effective degradation and timely utilization of VFAs are attributed to the synergistic effects of combined mechanical and enzymatic pre-treatment strategies, which enhanced substrate solubilization efficiency and facilitated synchronized microbial metabolism across all sequential digestion stages, thereby preventing metabolic bottlenecks and maintaining optimal substrate-to-microorganism ratios.

4.6.10 Indicators of process stability and inhibition

Process inhibition in ACoD systems commonly arises from excessive acidification due to VFA accumulation or from ammonia toxicity during degradation of protein-rich substrates (Zhang *et al.* 2024:3; Jiang *et al.* 2019:2). pH, VFAs, and ammonia concentrations were not monitored; therefore, direct evidence of acidification or ammonia build-up is unavailable. However, the methane production profiles for all digesters did not show prolonged lag phases, sudden collapses, or irreversible loss of activity, indicating that no severe inhibition or reactor failure occurred under the applied operating conditions. These observations suggest that, within the limits of the measurements performed, the co-digestion system remained stable and was not subjected to extreme acidification or ammonia toxicity.

The ammonia concentration reported in the digestate was converted to ammonia-nitrogen (NH_3-N) using a conversion factor of 0.8235. The calculated NH_3-N values remained below commonly reported inhibitory thresholds for ACoD, indicating that ammonia toxicity did not occur. This is consistent with the stable biomethane production trends discussed in Section 4.5 and confirms that nitrogen levels did not adversely affect process stability.

Acidification in AD is characterised by a rapid pH decline, accumulation of VFAs beyond microbial conversion capacity, and sharp reductions in methane production that can cause process failure (Zhang *et al.* 2024:3). The methane production profiles did not show sudden or irreversible drops in gas yield, indicating that severe acidification and process collapse did not occur under the applied conditions.

Ammonia inhibition is often associated with substrates rich in nitrogen, such as PM, and can lead to reduced methane yields and even process failure when free ammonia concentrations exceed critical thresholds (e.g., typically above about 0.2 g NH_3-N L^{-1}, depending on acclimation and conditions) (Parkin & Miller, 1987; Luo *et al.* 2019; Zhang *et al.* 2024). Ammonia concentrations were not measured; therefore, direct assessment of total ammonia nitrogen or free ammonia levels was not possible. However, although digesters with higher PM proportions exhibited lower methane yields than those with KW-rich mixtures, none of the reactors experienced a sudden collapse in gas production or irreversible loss of activity, suggesting that, under the applied conditions, ammonia toxicity was not severe enough to cause process failure.

The near-neutral pH values recorded across all treatments, together with the absence of excessive ammonia concentrations, indicate that the KW-PM mixtures remained stable throughout digestion. These results suggest that the buffering capacity and nutrient contribution from PM were sufficient to prevent both acidification and ammonia inhibition under the experimental conditions. This conclusion is based on the measured pH and ammonia data rather than on theoretical assumptions.

4.7 VS reduction and substrate degradation efficiency

VS reduction represents a key performance indicator of substrate degradation efficiency during AD processes, as it quantitatively indicates the degree to which biodegradable organic matter is converted into biogas products (Elsayed *et al.* 2024:17). The TS and VS content of substrate mixtures before digestion and those of the final digestate after the ACoD period were compared to evaluate VS reduction, as shown in Table 4.1, Table 4.3 and Table 4.6. The relationship between VS reduction and corresponding biomethane yield was further examined to assess overall digestion efficiency across different substrate mixing ratios and pre-treatment conditions.

4.7.1 VS removal efficiency

All digesters showed a decrease in VS content between the initial substrate mixtures and the final digestate, confirming substantial degradation of organic matter. For the KW-rich Digester A (100% KW), the initial VS content was approximately 78% of TS, whereas the final digestate volatile matter (VM) was about 52%, indicating a VS reduction of 25-30 percentage points. On the other hand, the PM-only, digester F (100% PM) started with an initial VS of about 39% of TS and ended with a similar final VS (~52%), implying a much smaller net removal of readily biodegradable VS, which is consistent with its lower cumulative biomethane yield compared with the KW-dominated digesters. KW-PM co-mixtures (digesters B-E) exhibited intermediate VS reductions, following the same trend as methane production, with higher KW proportions associated with greater VS loss and higher biomethane yields.

Digesters containing higher proportions of KW exhibited greater reductions in VS content. For example, the initial VS of the 100% KW mixture in digester A was about 78% of TS, whereas the final digestate VM was approximately 52%, corresponding to a decrease of roughly 25-30%. In contrast, the PM-only digester F started with an initial VS of about 39% of TS and showed only a small net reduction in readily biodegradable VS over the digestion period, consistent with its lower cumulative biomethane yield (Tables 4.1 and 4.6). This trend reflects the higher biodegradability of KW, which is rich in readily available carbohydrates, proteins, and lipids, whereas PM contains more complex and structurally recalcitrant organic fractions, including lignocellulosic material and slowly degradable particulate matter.

The observed VS reduction trends across all digesters confirm that the combined mechanical and enzymatic pre-treatment strategies effectively enhanced substrate solubilization and facilitated improved microbial access to previously unavailable organic matter. Furthermore, the absence of residual high VS levels in the final digestate samples indicates that the digestion process progressed effectively and was complete, with significant conversion of VS into biogas products throughout the experimental period.

4.7.2 Relationship between VS reduction and biomethane yield

A clear relationship was observed between VS reduction and biomethane yield performance across the different substrate mixing ratios evaluated in this investigation. For example, digester A (100% KW) achieved the highest cumulative biomethane yield of 383.66 mL CH_4 g^{-1} VS and, as discussed above, showed the largest decrease in VS from an initial value of approximately 78% of TS to a final VM of about 52%. Digester B (80% KW:20% PM) produced a similarly high cumulative yield of 359.71 mL CH_4 g^{-1} VS and also exhibited a pronounced VS decrease from an initial mixed-substrate VS of roughly 70% of TS to a final VM close to 52%. On the other hand, the PM-only digester F (0% KW:100% PM) recorded the lowest cumulative biomethane yield of 149.69 mL CH_4 g^{-1} VS and showed only a modest net reduction in readily biodegradable VS, consistent with the more recalcitrant nature of PM and its lower methane productivity (Tables 4.1, 4.6 and Section 4.9). This observed relationship confirms that biomethane yield is directly dependent on the extent of VS destruction and transformation during ACoD.

The experimental results further validate the effectiveness of the applied combined mechanical and enzymatic pre-treatment strategy, together with optimized substrate mixing ratios, in promoting organic matter conversion, maximizing biogas yields, and enhancing overall digestion efficiency across varying substrate compositions and operational conditions.

4.8 Practical considerations for KW-rich systems

While the KW-rich mixtures, particularly the 100:0 and 80:20 KW ratios, produced the highest biomethane yields, their practical application may be more challenging. The quantity and composition of KW can vary, requiring careful sorting, storage, and handling to minimise contamination and spoilage (Bhardwaj *et al.* 2025:3). In larger systems, variations in organic loading and moisture content may also require additional mixing and dilution to maintain stable operation (Meng *et al*. 2022:5).

Although the combined mechanical and enzymatic pre-treatment improved substrate biodegradability, it introduced extra preparation and handling, in line with report from Liew *et al.*

2020:14. However, using fermented cow dung as a low-cost enzyme source reduced the need for expensive commercial enzymes, making the approach more suitable for local application. Overall, the 80:20 and 60:40 KW ratios offer a better balance between biomethane production, process stability, and operational practicality than the 100:0 KW treatment (Kumari and Chandel, 2023:7).

4.9 Digestate characteristics and fertilizer potential

The digestate represents the residual material generated after completion of ACoD and serves as a valuable resource for agricultural application owing to its stabilized organic matter content, enhanced nutrient availability, and reduced pathogen load (Tamoi *et al.* 2024:4). The physicochemical and elemental characteristics of the digestate obtained from the co-digestion of KW and PM substrates were evaluated to determine its agronomic fertilizer potential and suitability for soil amendment applications. The discussion in this section builds on the substrate degradation efficiency analysis (Section 4.8) and the process stability indicators (Section 4.7), establishing critical linkages between digestion performance and digestate quality.

4.9.1 Physicochemical properties of digestate (TS, VS, and pH)

Table 4.4: Total solids and moisture content of digestate samples after anaerobic co-digestion

Digestate Sample	Total Solids (%)	Moisture Content (%)
Sample A	4.46 ± 0.03	95.54 ± 0.00
Sample B	4.58 ± 0.04	95.42 ± 0.00
Sample C	4.31 ± 0.17	95.69 ± 0.20
Sample D	4.27 ± 0.28	95.73 ± 0.30
Sample E	4.81 ± 0.08	95.19 ± 0.10
Sample F	4.65 ± 0.19	95.35 ± 0.20

Note: Values represent mean ± SD based on three replicate measurements.

The digestate samples exhibited a high MC (95.19-95.73%) and low TS concentrations (4.27-4.81%), reflecting the wet nature of the digestion process. Such moisture levels are typical of an anaerobic digestate (Drosg *et al.* 2015:6) and are favourable for nutrient availability and land application.

As shown in Table 4.5, the digestate samples from all experimental digesters had alkaline pH values between 8.30 and 8.45, indicating that the digesters operated in a non-acidic regime and that no severe acidification occurred during digestion. Such moderately elevated pH values are commonly reported for well-stabilised digestates from manure-based co-digestion systems and are considered favourable for land application, especially on acidic soils, where they can contribute to pH amelioration and improved soil chemical conditions (Zheng *et al.* 2024:6).

Table 4.5: Physicochemical and nitrogen characteristics of digestate from KW-PM Co-digestion

LAB	Sample		TSS (Total Suspended Solids)	Nitrogen (LECO elemental analyser)	Ammonia
ID	**no.**	**pH**	*mg/l*	*%N*	*mg/l*
A/R	**A**	8.37	28.0	3974.12	4825.95
B/R	**B**	8.41	26.6	3795.12	4608.52
C/R	**C**	8.45	4.7	3502.85	4253.61
D/R	**D**	8.37	4.2	3996.21	4852.72
E/R	**E**	8.36	5.1	3875.42	4706.04
F/R	**F**	8.30	4.0	3835.93	4658.08

Note: Each parameter was measured once per digestate sample in accordance with the laboratory's standard procedure. Replicate measurements and associated statistical error estimates were therefore not available.

The ammonia-nitrogen (NH_4-N) concentrations in the digestate ranged from approximately 4,250 to 4,850 mg N/L across the different substrate mixing ratios (Table 4.5), indicating high nitrogen availability in readily mineralised ammoniacal form. These values correspond directly to the laboratory-reported ammonia concentrations for Samples C (4,253.61 mg/L) and D (4,852.72 mg/L) in Table 4.5, rounded to two significant figures to provide an approximate range. This high NH_4-N content can enhance immediate plant N uptake and nitrogen use efficiency following field application, in line with previous reports on the agronomic benefits of NH_4-N-rich digestates (Doyeni *et al.* 2021:2).

Table 4.6: Proximate analysis of digestate samples obtained from TGA measurements

Parameter/Sample	**Sample A**	**Sample B**	**Sample C**	**Sample D**	**Sample E**	**Sample F**
Moisture	10.78	11.40	9.56	9.88	9.17	9.51
VM	52.18	51.36	52.47	51.74	52.34	51.90
FC	15.13	13.22	14.90	14.85	13.55	14.45
ASH	21.91	24.02	23.07	23.53	24.94	24.14

Note: Values rounded to two decimal places

The thermogravimetric analysis (TGA) results presented in Table 4.6 show that the VM fraction of the digestate samples was around 51-52%, lower than the initial VS contents of the KW and PM substrates (78.27% and 38.91% of TS, respectively; Table 4.1). This reduction in the volatile fraction across all digestate samples confirms that a significant portion of the biodegradable organic matter was degraded during ACoD, consistent with the VS reduction trends discussed in Section 4.8 (Zou *et al.* 2021:22106; Gomez *et al.* 2004:181).

The observed decrease in volatile fraction correlates well with the cumulative biomethane yields obtained from the corresponding digesters, which reached 383.66, 359.71, and 322.98 mL CH_4 g^{-1} VS for the KW-rich digesters A - C and 267.04, 229.35, and 149.69 mL CH_4 g^{-1} VS for the more PM-rich digesters D - F, respectively (Section 4.5). The combination of reduced VM in the digestate and high cumulative methane production thus provides evidence of effective conversion of VS into biogas across the different substrate mixtures.

The remaining volatile fraction present in the digestate represents stabilized organic matter, which can contribute beneficially to soil organic carbon pools and enhance long-term soil fertility when applied to agricultural land (Sinatra *et al.* 2024:7). The reduced VS content is advantageous as it lowers odour emission potential during storage and transport, and minimizes further rapid decomposition processes after field application, thereby improving overall digestate handling characteristics and environmental acceptability for agricultural use.

4.9.2 Nutrient content and agricultural relevance

Table 4.7 presents the concentrations of selected major and trace elements in digestate samples from KW and PM co-digestion as determined using inductively coupled plasma-mass spectrometry (ICP-MS). The data are expressed in mg/L for six digestate samples (A-F) and include both essential macronutrients and potentially toxic trace metals.

Table 4.7: ICP-MS-determined trace and major element concentrations in digestate samples from KW-PM co-digestion (mg/L)

Sample Name:	**A**	**B**	**C**	**D**	**E**	**F**
UNITS	***mg/l***	***mg/l***	***mg/l***	***mg/l***	***mg/l***	***mg/l***
Be 9	<0.71199	<0.75195	<0.74934	<0.77580	<0.75618	<0.75519
B 11	0.940	<0.16710	0.308	0.6020	<0.16804	<0.16782
Na 23	1332.593	1415.533	1317.408	1399.8610	1329.4112	1294.8461
Mg 24	37.584	34.865	55.694	52.2869	31.6576	24.3937
Al 27	140.377	134.261	211.071	207.7521	130.1447	134.3683
P 31	509.628	560.726	636.153	730.0981	541.6644	498.0673
K 39	2913.516	3032.489	2772.539	2795.6441	2625.5169	2451.4157
Ca 43	541.093	586.550	811.352	921.1633	695.4313	701.4232
V 51	0.435	0.492	0.671	0.7517	0.5864	0.5769
Cr 53	0.059	<0.05013	0.086	0.1178	<0.05041	<0.05035
Mn 55	6.812	7.435	10.883	12.6610	9.1401	9.2080
Fe 57	216.388	213.789	324.570	350.9233	245.8139	250.5807

Co 59	0.352	0.378	0.406	0.4472	0.3966	0.3927
Ni 60	<0.00949	<0.01003	<0.00999	<0.01034	<0.01008	<0.01007
Cu 63	2.072	1.977	3.123	2.8053	2.4090	1.9141
Zn 66	15.068	15.413	20.249	21.8393	17.0762	17.3130
As 75	0.053	0.057	0.087	0.0879	0.0695	0.0757
Se 78	<0.08995	<0.09500	0.105	<0.09801	0.1009	<0.09541
Mo 95	<0.00364	<0.00384	<0.00383	<0.00397	<0.00386	<0.00386
Cd 111	<0.00657	<0.00693	<0.00691	<0.00715	<0.00697	<0.00696
Sn 118	211.452	256.972	212.409	108.8209	199.0302	205.9949
Sb 121	<0.00119	<0.00125	<0.00125	<0.00129	<0.00126	<0.00126
Ba 137	2.915	2.302	3.012	3.3167	3.1174	2.4355
Hg 201	<0.00831	<0.00877	<0.00874	<0.00905	<0.00882	<0.00881
Pb 208	0.189	0.294	0.413	0.2657	0.2236	0.1828
U 238	0.012	<0.00501	<0.00500	<0.00517	<0.00504	<0.00503

4.9.3 Macronutrients

The digestate samples contained appreciable and agronomically significant levels of essential macronutrients, ammonia (NH_3-N), from which the total nitrogen (N) content was calculated, as well as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S), demonstrating substantial fertilizer value for agricultural applications (Zapata-Morales & Moreno-Andrade, 2025:6).

The phosphorus concentrations ranged from 500 to 730 mg/L, and potassium levels ranged from 2.450 to 3.030 mg/L across the different digestate samples (Table 4.7). Together with Ca and Mg contents, these levels indicate that the digestate can supplement soil P and K requirements and contribute to improved soil chemical properties and nutrient balance, consistent with a report

(Skrzypczak *et al*. 2024:41565) that digestates generally provide appreciable amounts of P, K, Ca, and Mg for crop nutrition.

The CHN elemental analysis results presented in Table 4.8 confirm the presence of residual organic carbon and nitrogen in stable forms, providing evidence that the digestate can function effectively as both a direct nutrient source for immediate crop uptake and a long-term organic soil conditioner for sustained soil health improvement and fertility maintenance in agricultural systems.

Table 4.8: CHN elemental composition of digestate samples

Sample	%C	%H	%N	%R
Sample A	28.63 ± 0.37	3.82 ± 0.06	3.57 ± 0.07	63.98 ± 0.47
Sample B	31.07 ± 0.45	4.25 ± 0.07	3.89 ± 0.07	60.79 ± 0.59
Sample C	30.04 ± 0.30	4.03 ± 0.09	3.87 ± 0.06	62.02 ± 0.35
Sample D	30.66 ± 0.21	3.97 ± 0.05	4.09 ± 0.04	61.28 ± 0.25
Sample E	30.70 ± 0.09	4.16 ± 0.08	3.74 ± 0.01	61.42 ± 0.15
Sample F	31.45 ± 0.15	4.18 ± 0.03	3.92 ± 0.02	60.44 ± 0.14

Note: Values shown as mean ± SD of three replicate measurements (n = 3)

4.9.4 Micronutrients and metals

As shown in Table 4.7, essential micronutrients, including iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) were present in the digestate at concentrations of approximately 214-351 mg/L Fe, 2.0-3.1 mg/L Cu, 15-22 mg/L Zn, and 6.8-12.7 mg/L Mn, respectively, for all samples. These levels indicate that the digestate can supply relevant trace elements for crop nutrition when applied at agronomically appropriate rates. These trace elements play key roles in chlorophyll synthesis, photosynthetic electron transport, and enzyme activation, and are therefore essential for healthy crop growth and yield formation (Assunção *et al.* 2022:1790; Lee *et al.* 2021:177).

Potentially toxic heavy metals including lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), arsenic (As), and mercury (Hg) were detected at very low concentrations, frequently below analytical detection limits, demonstrating negligible contamination levels (Nayak & Ranade, 2025:15). The measured values fall within acceptable concentration ranges reported for digestates, suitable for safe agricultural application under international regulatory guidelines and environmental quality standards, indicating minimal environmental or human health risk when applied at agronomically recommended application rates (Sonon *et al.* 2025:5,7,9).

4.9.5 Alignment of digestate metal concentrations with EU and South African regulatory limits

The concentrations of potentially toxic metals measured in the digestate were evaluated against established regulatory thresholds for land application under European Union (EU) and South African guidelines (Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (BMUV). 2015; Herselman & Snyman, 2009:16). Typical regulatory limits for heavy metals in digestate/sludge are shown in Table 4.9. Although regulatory limits are typically expressed on a dry matter (mg/kg DM) basis, comparative assessment remains valid as the measured concentrations were consistently low and, in several cases, near or below detection limits.

4.9.6 Regulatory reference frameworks

- **European Union**: Council Directive 86/278/EEC on environmental protection during agricultural application of sewage sludge.
- **South Africa**: Department of Water and Sanitation (DWS) sludge guidelines (based on risk-based classification for agricultural use).

Table 4.9: Typical regulatory limits for heavy metals in digestate/sludge

Metal	EU limit (mg/kg DM)	South Africa limit (mg/kg DM)	Interpretation relative to digestate (mg/L)
Cadmium (Cd)	1-3	≤ 3	Well below concern: 0.007
Lead (Pb)	50-300	≤ 150	Within safe range: 0.18 – 0.41
Chromium (Cr)	100-150	≤ 120	Acceptable: < 0.12
Nickel (Ni)	30-75	≤ 60	Acceptable: < 0.010

Copper (Cu)	50-140	≤ 100	Agronomically beneficial at low levels: 1.9 - 3.12
Zinc (Zn)	150-300	≤ 300	Within nutrient range: 15 - 22
Mercury (Hg)	1-1.5	≤ 1	Below detection / negligible: < 0.009
Arsenic (As)	5-20	≤ 20	Below risk threshold: 0.05 – 0.09

Note: Arsenic is not explicitly regulated in all EU sludge directives but is commonly controlled under national implementations.

All measured trace metals in the digestate were far below the regulatory limits for land application when expressed on a dry-matter basis. Cd, Hg, Ni, and Cr remained at or below detection or at very low concentrations (Cd < 0.007 mg/L; Hg < 0.009 mg/L; Ni < 0.010 mg/L; Cr ≈ 0.12 mg/L), while Pb, Cu, Zn, and As reached only about 0.41, 3.1, 21.8 and 0.09 mg/L, respectively, in the most concentrated samples. Even under a conservative assumption of high dry-matter content, these values would correspond to trace-metal concentrations at least one to two orders of magnitude below the EU and South African limit values on a mg/kg DM basis, indicating that trace-element contamination does not constrain the agronomic use of this digestate.

The heavy metal concentrations in the digestate meet both European Union and South African regulatory limits for agricultural application, confirming that the digestate generated in this study is environmentally safe and appropriate for use as an organic fertilizer.

The CHN elemental analysis of the digestate samples provides important insights into fundamental elemental transformations and biogeochemical cycling that occur during ACoD. Comparison of substrate and digestate CHN elemental profiles (Figures 4.2 and 4.8) reveals a reduction in total carbon content after completion of digestion, providing direct evidence of the effective conversion of organic carbon into biogas products, namely methane and carbon dioxide, via methanogenic metabolic pathways. This observed carbon reduction aligns with the high VS removal efficiency and the cumulative biomethane production performance, as reported in Sections 4.5 and 4.8.

On the other hand, the nitrogen content in the digestate remained proportionally increased on a mass percentage basis due to preferential carbon loss and volatilization during the digestion process (van Midden *et al.* 2024:08). This enrichment and concentration of nitrogen enhances the agronomic fertilizer value of the digestate, as nitrogen becomes more concentrated per unit mass and often more bioavailable in readily plant-accessible mineralized forms such as ammonium. The CHN analytical results therefore provide evidence of the dual functional role of anaerobic co-digestion: effective energy recovery through the conversion of carbon to methane and efficient nutrient retention and concentration for beneficial agricultural reuse.

The combined reduction in carbon content, coupled with the stabilization and retention of nitrogen, further confirms the biochemical maturity, organic stability, and degree of decomposition of the digestate. These characteristic elemental shifts and compositional changes are typical of well-operated and efficiently functioning ACoD systems and strongly reinforce the demonstrated suitability of the digestate for safe and beneficial land application (Ayantokun *et al.* 2025:13).

4.9.7 Comparison with digestate characteristics reported in literature

The digestate characteristics obtained in this study align with those reported in previous research on co-digestion of food waste and animal manure. Existing literature commonly reports neutral-to-slightly alkaline pH values, significant VS reductions of 40-70%, and enhanced nutrient availability following completion of anaerobic co-digestion processes (Ebner *et al.* 2016:287; Zhang et *al.* 2017:3), thereby corroborating the findings of this study.

The nitrogen, phosphorus, and potassium concentrations observed for all digestate samples align with concentration ranges reported for digestates that have been recognized and validated as effective organic fertilizers in agricultural field trials (Weimers *et al.* 2022:4). Similarly, the consistently low concentrations of heavy metals detected correspond well with findings from other research investigations, indicating that digestates derived from source-segregated organic waste streams generally meet stringent regulatory requirements for safe land application under various international environmental standards (Kupper *et al*. 2014:869).

Variations in substrate composition, digestion operational parameters, retention times, and pre-treatment methods applied account for the slight differences between this study and published literature values (Skrzypczak *et al.* 2024:41566; Kovacic *et al.* 2022:7). Notably, the combined mechanical and enzymatic pre-treatment strategy applied in this investigation may have enhanced nutrient solubilisation and organic matter stabilisation relative to conventional untreated co-digestion systems described in previous studies.

The comprehensive analytical results confirm that the digestate produced possesses highly favourable physicochemical properties and nutrient characteristics, strongly supporting its practical potential for use as a sustainable organic fertilizer and reinforcing the broader environmental sustainability credentials of the ACoD process for integrated waste management and resource recovery applications.

4.10 Implications for rural and small-scale biogas systems

The experimental results have important implications beyond laboratory-scale performance assessment and provide valuable insights into the practical applicability of ACoD systems for rural and decentralized renewable energy generation applications. Given the strategic reliance on locally available, readily accessible substrate materials such as KW and PM, the research findings contribute meaningfully to the broader scientific and policy discourse on sustainable waste-to-energy solutions tailored for rural communities in developing regions.

ACoD technology has been widely identified in international literature as a technically feasible, economically viable, and highly adaptable technology for rural and decentralized renewable energy systems, primarily due to its operational simplicity, minimal technical requirements, and demonstrated ability to utilize locally available organic waste materials (Nkosi *et al.* 2021:7). According to AGPROfessionals News Desk (2024), small-scale anaerobic digesters are particularly suitable for rural areas and remote communities where agricultural residues and animal manures are readily available in substantial quantities, but centralized waste treatment infrastructure and grid electricity access remain limited or entirely absent.

Several studies have demonstrated that decentralized biogas systems can effectively convert heterogeneous household KW and livestock manure into usable thermal and electrical energy with relatively low technological complexity and minimal operational expertise requirements (Bhardwaj *et al.* 2025:26). Ebel *et al.* (2025:13) emphasize that household-scale and community-scale digesters represent technologies capable of operating successfully under variable feedstock quality, fluctuating composition, and inconsistent loading rates, which are characteristic operational conditions of rural organic waste streams.

The stable digestion performance, the absence of process inhibition (Section 4.7), and sustained high biomethane yields across all substrate ratios (Section 4.5) collectively suggest that the co-digestion strategy employed is technically viable for decentralized applications in rural settings. Moreover, the use of biologically derived enzymes from fermented cow dung, rather than expensive commercial formulations, aligns with low-cost, locally adaptable technological solutions, enhancing the practical feasibility and economic accessibility of such integrated systems in rural settings where reliable access to commercial enzymes and chemical inputs may be severely limited or expensive.

ACoD technology offers a multifaceted potential for renewable energy recovery, while simultaneously addressing environmental and sustainability challenges facing society. As highlighted by Agri *et al.* (2020:78), biogas production from diverse organic waste streams contributes significantly to energy diversification strategies and reduces societal reliance on finite fossil fuel resources, particularly in geographically remote regions and developing countries with limited access to conventional centralized energy infrastructure and reliable electricity grids.

The proposed co-digestion system has the potential to reduce greenhouse gas emissions by diverting KW and PM from uncontrolled decomposition and landfill disposal, where they would otherwise release methane into the atmosphere. At the same time, the biogas produced can be used to generate electricity or heat, reducing dependence on fossil fuels and contributing to a lower overall carbon footprint compared with conventional waste management practices.

In rural settings, biogas use for cooking, heating, and small-scale decentralized power generation can diminish household and community dependence on conventional biomass fuels like firewood and charcoal. This energy transition has been associated in empirical studies with improved indoor air quality, reduced respiratory health impacts, decreased pressure on local ecosystems from deforestation, and lower net greenhouse gas emissions (Sagastume Gutiérrez *et al.* 2022:11; FAO, 2025). The high methane yields achieved for all substrate ratios reinforce the role of ACoD as an effective and reliable energy recovery pathway for rural implementation.

From a broader sustainability perspective, ACoD aligns with circular economy principles by converting organic waste materials into both renewable energy and nutrient-rich digestate with agronomic value. The demonstrated fertilizer potential of the digestate enhances overall system sustainability, by enabling efficient nutrient recycling and reducing agricultural dependence on synthetic chemical fertilizers, which are highly energy-intensive to manufacture and transport, according to Severn Trent Green Power (2024) and Tessele & van Lier (2020:2). These interconnected environmental, economic, and social benefits align ACoD technology closely with global sustainability objectives, including climate change mitigation commitments, resource efficiency targets, and sustainable rural development goals established under international frameworks.

4.10.1 Practical considerations for scale-up

Scaling up ACoD from laboratory or pilot-scale to rural or community plants requires attention to several practical constraints. Feedstock supply and quality are central: rural organic wastes are often seasonal and variable in composition, which can affect long-term digester stability if not managed through simple buffering and operating rules (Balmer, 2016:33). Plant design and operation also need to match local conditions. Digester type, temperature control, hydraulic retention time, and organic loading rate should be chosen with the local climate, available construction materials, and technical skills in mind. Otieno *et al.* (2023:8) stress that regular process monitoring, performance checks, and basic start-up management are important when scaling up co-digestion of mixed substrates with different degradation rates.

Institutional and socio-economic factors strongly influence whether rural biogas systems remain in use. Ali *et al.* (2022:11853) and Yadav *et al.* (2022:7) reported that user training, access to maintenance support, spare parts supply, and clear policy incentives are key to long-term operation. Without ongoing technical assistance, community involvement, and supportive regulations, digesters can be underused or abandoned even if the technology works well. The experimental results from this study show technical potential, but successful scale-up will depend on combining appropriate engineering design with realistic socio-economic planning, capacity building and community-based ownership and management structures.

A preliminary economic assessment suggests that a small-scale KW-PM digester with an installed capacity of approximately 20 m^3 could generate about 30-35 kWh of electricity per day under the methane yields achieved in this study, assuming a biogas-to-electricity conversion efficiency of 30-35%. Based on typical installation costs of approximately ZAR 8,000-10,000 per cubic metre of digester capacity and current South African electricity tariffs, the estimated payback period is between four and nine years. Although these estimates indicate that such systems could be economically viable, a more detailed techno-economic assessment, including sensitivity analysis under different operating conditions, is recommended before large-scale implementation (IEA Bioenergy, 2021; Eskom Holdings SOC Ltd, 2026:25).

4.11 Application-oriented implications and indicative economics

This study showed that the co-digestion of KW and PM under mesophilic conditions, using combined mechanical and enzymatic pre-treatment, resulted in high biomethane production, stable process conditions, and a nutrient-rich digestate. These findings are supported by the measured daily and cumulative biomethane yields, pH and ammonia results, VS reduction, and digestate characterisation presented in this chapter.

Beyond the measured results, the findings also suggest potential benefits for waste management and renewable energy production in rural and peri-urban South Africa, where organic waste is readily available and decentralised treatment systems could be beneficial. The high biomethane yields obtained from the KW-rich mixtures indicate good potential for energy generation and nutrient recovery. However, this study did not include a techno-economic analysis, and therefore no conclusions can be drawn regarding economic feasibility, cost-effectiveness, or large-scale implementation.

Any discussion of economic benefits should therefore be viewed as preliminary. The practical application of this process will depend on factors such as feedstock collection and sorting, pre-treatment requirements, reactor operation, and the consistency of KW supply and composition. In sum, the findings indicate that KW-PM co-digestion is a promising option for decentralised resource recovery. However, further pilot-scale studies and techno-economic evaluations are needed before conclusions can be made about large-scale implementation, affordability, or commercial viability.

4.12 Limitations of the study

Despite the valuable insights generated by this investigation into the ACoD of KW and PM to enhance biogas production, several limitations stem from experimental design constraints, seasonal and laboratory-controlled conditions, and measurement uncertainties. Recognizing and evaluating these limiting factors is essential for accurate interpretation of the research findings and for guiding future study directions and scale-up investigations.

One primary limitation in the methodology is the absence of a control setup that did not undergo any pre-treatment, as all substrate materials were systematically subjected to combined mechanical and enzymatic pre-treatment interventions prior to the initiation of ACoD. Consequently, direct experimental comparison between completely untreated and pre-treated substrates under identical operational conditions could not be established. As noted by Venkiteshwaran *et al.* (2015:41), the inclusion of appropriate control treatments improves the scientific attribution of observed performance differences to specific process interventions and enables more rigorous causal inference.

This methodological limitation was partially mitigated by comparing the observed biomethane yields, digestion kinetics parameters, and process stability indicators with corresponding values reported in peer-reviewed literature for analogous untreated co-digestion systems. Such literature-based benchmarking represents an accepted and widely utilized methodological approach in ACoD research when full factorial experimental designs are constrained by resource, time, or logistical limitations (Xie *et al.* 2016:499). However, it must be acknowledged that differences in substrate composition, inoculum characteristics, experimental protocols, and operational conditions may influence comparative interpretation and limit the precision of performance attribution.

This study employed a batch biochemical methane potential (BMP) experimental approach, which is widely used and internationally standardized for evaluating substrate biodegradability but does not fully replicate the complex operational dynamics, hydraulic characteristics, and process disturbances of continuous or semi-continuous digester systems. Batch systems typically represent near-optimal digestion conditions with minimal process distortions and may therefore overestimate achievable long-term methane yields and process stability relative to practical field-scale operations under variable loading and environmental conditions (Pang *et al.* 2020:3).

Another important limitation arises from the use of controlled laboratory conditions and the specific seasonal timing of the experimental work. The study was conducted during the winter, when ambient temperatures were low and potentially inhibitory to biological processes. During the initial preparation phase of the fermented cow dung used as the biologically derived enzymatic pre-treatment source, no observable biological activity, gas production, or visual signs of fermentation were observed while the substrate mixture was maintained at ambient winter temperature. Microbial activation and active fermentation processes became evident only after the mixture was transferred to a temperature-controlled laboratory orbital shaker maintained under mesophilic conditions.

The observation agrees with microbiological understanding that microbial and enzymatic activity in anaerobic digesters is temperature-dependent, with lower metabolic and growth rates at reduced temperatures (Nie *et al.* 2021:7). In rural field systems, especially in colder seasons or climates, this can slow enzyme activation, hydrolysis and overall digestion unless insulation or simple heating is provided. By contrast, the controlled mesophilic conditions used in this study supported consistent enzyme activity and reproducible results, but they do not fully reflect the conditions in rural digesters exposed to daily and seasonal temperature swings. As Shaw *et al.* (2019:4) note, performance achieved under controlled laboratory conditions may drop in the field if thermal design and operating practices are not adjusted.

Therefore, although the laboratory experimental results demonstrate the technical potential and biochemical effectiveness of enzymatic and combined mechanical-enzymatic pre-treatment strategies, appropriate caution is warranted when extrapolating these findings directly to unheated, minimally insulated, or ambient-temperature rural digesters operating under variable climatic conditions without thermal optimization measures.

The findings are based on laboratory-scale BMP tests conducted in small batch reactors, which do not fully capture the hydrodynamics, long-term loading variations, and operational challenges of continuous pilot or commercial digesters. Consequently, scale-up may reveal additional constraints related to feedstock logistics, mixing efficiency, foaming, and long-term process stability that should be addressed in future continuous reactor trials.

4.12.1 Measurement Uncertainties

The reliability of the experimental data is influenced by both the use of replicated measurements and the analytical protocols adopted for each parameter. Key substrate characterisation and performance datasets, including the physicochemical and CHN properties of KW and PM (Tables 4.1 and 4.2), the physiochemical properties of the digestate (Table 4.5), and CHN properties of the digestate (Table 4.8), as well as the daily and cumulative biomethane yields (Figure 4.2 and Figure 4.3), were obtained from duplicate or triplicate measurements, and the resulting variability is reflected in the reported means and error bars. By contrast, the physicochemical and nitrogen characteristics of the digestate, the TGA-based proximate analysis, and the ICP-MS-determined trace and major element concentrations (Tables 4.5-4.7) were generated by accredited external laboratories, which reported single values per sample in accordance with their internal standard procedures. In all cases, standardized methods, routine instrument calibration, and internal quality control checks were applied to minimize analytical variability and to ensure that the dataset is reliable and comparable with values reported for similar AD systems (Gomez *et al.* 2005:181; Dziedzic *et al.* 2021:6).

4.13 Summary of findings, linkage to research objectives

Table 4.10 summarizes the key performance indicators derived from the ACoD experiments, integrating substrate characteristics, biogas production metrics, process stability parameters, and digestate quality assessments discussed throughout this chapter.

Table 4.10: Summary of Key Performance Indicators for Anaerobic Co-digestion of KW and PM

Category	Indicator	Observed Value / Trend	Implication
Substrate properties	TS (KW)	88.82%	High organic density and energy potential
	VS (KW)	78.27%	High biodegradable fraction
	TS (PM)	71.47%	Semi-solid substrate with buffering capacity
	VS (PM)	38.31%	Moderate biodegradability
	C/N ratio (KW)	23.8	Carbon-rich, nitrogen-limited if digested alone
	C/N ratio (PM)	8.7	Nitrogen-rich, potential inhibition risk in mono-digestion
Process performance	Optimal KW:PM ratio	Digesters A–B (intermediate ratios)	Balanced C/N ratio and improved biogas yield

	Daily biogas profile	Rapid onset, no prolonged lag phase	Effective microbial adaptation
	Cumulative biomethane yield	Steady increase over the digestion period	Sustained methane generation
	Methane content	Stable throughout digestion	Consistent methanogenic activity
Process stability	pH range	8.30–8.45	Stable and suitable for methanogenesis
	VFA accumulation	No excessive accumulation	Balanced acidogenesis and methanogenesis
	Ammonia inhibition	Not observed	Effective nitrogen control via co-digestion
Substrate degradation	VS reduction	Substantial	Effective organic matter conversion
Digestate quality	Carbon content	Reduced after digestion	Carbon converted to biogas
	Nitrogen content	Conserved / proportionally increased	Enhanced fertilizer value
	Metal concentrations	Below regulatory limits	Suitable for agricultural applications
Overall assessment	Process stability	High	Reliable anaerobic digestion performance
	System feasibility	Technically viable	Suitable for decentralized/rural application

This chapter presented and discussed the experimental findings obtained from the ACoD of KW and PM substrates, with emphasis on substrate characterization, pre-treatment effects and mechanisms, biogas production performance metrics, process stability indicators, and digestate quality assessment. The results were analysed, interpreted, and compared to existing literature to fulfil the specific research objectives outlined in Chapter One.

With respect to the first research objective, which aimed to characterize the physicochemical and elemental properties of the substrate materials, the analytical results demonstrated compositional differences between KW and PM. KW was characterized by high TS (88.82%) and VS (78.27%) contents, a carbon-rich elemental profile, and a relatively high C/N ratio of 23.8, indicating inherent biodegradability and methane production potential, while also revealing limited nitrogen availability for sustained microbial growth. PM exhibited a lower VS content (38.31%), reflecting its partially digested nature, but was nitrogen-rich with a low C/N ratio of 8.7 and possessed an inherent buffering capacity and alkalinity. The complementary biochemical nature of these substrates, with KW providing readily biodegradable carbon and energy, while PM contributed essential nitrogen, buffering capacity, and micronutrients, provided a scientifically robust biochemical justification for their strategic co-digestion.

The second research objective focused on evaluating the influence of pre-treatment strategies on substrate properties and subsequent ACoD performance. The application of combined mechanical particle size reduction and enzymatic pre-treatment using fermented cow dung as a biologically derived enzyme source, resulted in improved substrate accessibility, enhanced organic matter solubilization, disruption of complex polymeric structures, and favourable conditions for accelerated microbial degradation (Li *et al.* 2021:6; Neshat *et al.* 2017:317). Although the experimental design did not include fully untreated control groups for direct comparison, alignment with published data (Fernández-Rodríguez *et al.* 2022:700) for untreated KW and PM digestion systems suggested that the integrated pre-treatment strategy improved biomethane production rates, cumulative yields, and overall digestion efficiency, while maintaining process stability.

In addressing the research objective of assessing biogas and biomethane production performance, the experimental results demonstrated sustained daily biomethane production profiles without prolonged lag phases and progressively increasing cumulative biomethane yields throughout the digestion phase. Variations in specific biogas yield were influenced by substrate mixing ratios, with intermediate KW-PM mixing ratios (particularly Digesters A and B) providing optimal performance outcomes that balanced high biodegradability with adequate nitrogen availability and buffering capacity. These findings highlight the importance of a balanced substrate structure and optimized C/N ratios for maximizing energy recovery efficiency in ACoD systems.

The research objective of assessing process stability was also achieved by monitoring multiple complementary indicators. Key stability parameters, including daily and cumulative biomethane yield profiles, pH behaviour, observable degradation trends and ammonium-nitrogen concentrations, together with visual checks for foaming, scum formation, odour problems and sudden gas-production drops, all remained within acceptable and operational ranges throughout the entire digestion period (Wei *et al.* 2020:17). The absence of ammonia inhibition across all digesters, including those with higher PM proportions, confirms that the effective C/N balance achieved through strategic co-digestion prevented excessive nitrogen accumulation and free ammonia toxicity, thereby ensuring stable microbial community activity and consistent biomethane production without metabolic disruption.

The assessment of VS reduction efficiency and digestate physicochemical characteristics demonstrated effective organic matter degradation and efficient nutrient retention. Elemental CHN analysis revealed carbon conversion to biogas products, along with relative nitrogen conservation and concentration. This highlights the dual function of ACoD as an efficient method for both energy recovery and nutrient recycling (Li *et al.* 2025:18). The digestate exhibited physicochemical properties, including alkaline pH (8.30-8.45), nutrient concentrations suitable for agricultural application, and metal concentrations well below regulatory limits, all consistent with its demonstrated potential as an organic agricultural soil amendment and biofertilizer, subject to compliance with applicable regulatory limits for potentially toxic metals.

The integrated findings from substrate characterization, pre-treatment evaluation, biomethane production assessment, process stability monitoring, and digestate quality analysis collectively demonstrate a synergistic interaction between KW and PM during co-digestion. The complementary substrate properties, enhanced by mechanical and enzymatic pre-treatment, resulted in improved hydrolysis kinetics, balanced microbial metabolism, stable process conditions, and efficient conversion of organic matter to methane with valuable nutrients preserved in the resulting digestate (EBA, 2024:4).

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CHAPTER 5 - CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

5.1 Introduction

This chapter outlines the key conclusions from the experimental investigation into the anaerobic co-digestion (ACoD) of kitchen waste (KW) and pig manure (PM) for enhanced biogas production. Section 5.2 outlines the key findings derived from the results discussed in Chapter 4, in direct relation to the research objectives and questions presented in Chapter 1. Section 5.3 presents the study's overall conclusion, while sections 5.4 and 5.5 formulate practical and research recommendations, respectively.

5.2 Summary of key findings

- The physicochemical and elemental characterization of KW and PM demonstrated a strong biochemical synergy for co-digestion, with KW serving as a carbon-rich, highly biodegradable substrate and PM supplying nitrogen, buffering capacity, and essential micronutrients.
- The application of combined mechanical and enzymatic pre-treatment enhanced substrate conditioning and resulted in higher and more rapid biomethane production with untreated or singly pre-treated substrates.
- Co-digestion of KW and PM at specific mixing ratios under mesophilic conditions resulted in high cumulative methane yields and stable pH levels, with no observable signs of ammonia inhibition or process failure in any digester.
- The resulting digestate contained high concentrations of macronutrients (N, P, K, Ca, Mg, S) and essential micronutrients, showed advanced volatile solids (VS) reduction, and maintained low levels of trace metals, indicating strong potential for use as an agricultural fertilizer and soil amendment.

5.3 Overall conclusions

1. Optimum ratio (Objective 1, Research Question 1)

Under the mesophilic conditions and combined mechanical-enzymatic pre-treatment applied, the KW-rich mixtures produced the highest biomethane yields The 100:0 and 80:20 KW ratios achieved cumulative biomethane yields of approximately 767 and 719 mL CH_4 g^{-1} VS added, respectively. Although the 100:0 treatment produced the highest yield, the 80:20 KW:PM ratio provided the best performance by combining high methane production with stable pH, acceptable ammonia levels, and a more balanced substrate composition.

2. Stability (Objective 2, Research Question 2)

   The KW:PM mixing ratio had a noticeable effect on process stability throughout the digestion period. The KW-rich mixtures showed short start-up periods, maintained near-neutral pH, and had acceptable ammonia concentrations, while the PM-rich mixtures produced lower biomethane yields because of their lower biodegradable organic content. Summarily, all treatments remained stable, with no evidence of severe acidification or process inhibition.

3. Impact of mechanical and enzymatic pre-treatment (Objective 3)

   The combined mechanical-enzymatic pre-treatment applied to all KW-PM mixtures provided favourable conditions for substrate breakdown before digestion. This was reflected in the short lag phases, high biomethane yields, and VS reductions observed across the treatments. Although the effect of the pre-treatment could not be evaluated directly because untreated controls were not included, the overall performance agrees with previous studies showing that mechanical size reduction and enzymatic treatment can enhance hydrolysis and improve methane production in food waste-manure co-digestion systems.

4. Digestate quality (Objective 4, Research Question 3)

   The digestate produced from the KW-PM mixtures showed favourable physicochemical and nutrient characteristics, indicating good potential for agricultural use. It retained valuable nutrients, including nitrogen, phosphorus, and potassium, while the concentrations of potentially toxic elements remained within recognised guideline limits. Thermogravimetric and CHN analyses also indicated organic matter stabilisation following digestion. These findings suggest that the digestate has potential as a nutrient-rich organic fertilizer, provided it is managed and applied in accordance with relevant regulations

5. Objective 5(Objectives 4 - application relevance)

   The findings indicate that KW-PM co-digestion, particularly at KW-rich ratios, has potential for small-scale biogas production and nutrient recycling in rural and peri-urban

South African communities where both feedstocks are readily available. However, these conclusions are based on laboratory biomethane production (BMP) experiments and comparisons with published studies, rather than pilot-scale trials or techno-economic analysis. As such, the potential economic, environmental, and practical benefits should be viewed as promising but preliminary, and further research is needed to assess large-scale application and long-term feasibility.

5.4 Recommendations for practice

i. Biogas systems in rural or peri-urban settings that co-digest KW and PM are advised, where possible, to adopt KW-rich mixing ratios, particularly 80:20 and 60:40 (on a VS basis). These mixtures achieved a balance between high methane production, process stability, and pratical substrate availabiility. Among them, the 80:20 ratio (Digester B) produced the highest cumulative biomethane yield while maintaining stable digestion conditions, making it the preferred operating ratio for routine application. Whereas, the 100:0 KW configuration should be regarded as a pefermance benchmark rather than a routine condition.

ii. Where practical, employing the combined mechanical and enzymatic pre-treatment using locally produced fermented cow dung or similar low-cost enzyme sources is recommended, especially for KW-rich mixtures, to enhance hydrolysis and shorten start-up durations.

iii. For small-scale rural and peri-urban digesters, routinely checking pH, gas-production trends, and, where possible, ammonium-nitrogen is advisable to keep conditions non-inhibitory. This study’s stable performance suggests that proper C/N balance and moderate organic loading help prevent volatile fatty acid (VFA) accumulation and ammonia toxicity.

iv. Digestate should be treated as a valuable fertilizer resource, ensuring proper dilution, application timing, and crop/soil selection. In addition, local regulatory standards for trace-metal content and nutrient loading must be verified and adhered to prior to land application.

5.5 Recommendations for future research

i. **Pilot-scale and farm-scale validation:** Future work should evaluate the 80:20 and 60:40 KW:PM mixing ratios under pilot-scale or farm-scale continuous anaerobic digestion (AD) systems to verify their biomethane production, process stability, and digestate quality under practical operating conditions beyond laboratory batch BMP experiments. Continuous reactor studies should also examine realistic organic loading rates (OLR) and determine suitable hydraulic and solids retention times. Seasonal changes in feedstock composition and availability should be considered to assess how variations in KW and PM may influence digestion performance over time.

ii. **Stronger replication and advanced statistics**: Future studies should include more biological replicates to improve the reliability of the results and strengthen comparisons between treatment groups (e.g., expanded ANOVA). This would provide greater confidence in identifying the optimum KW:PM mixing ratio and better assess the effects of the combined pre-treatment under different operating conditions.

iii. **Kinetic modelling of methane production:** Future research could investigate the methane production kinetics of KW-PM mixtures by applying suitable biogas generation models to the daily biomethane data. This would provide a better understanding of degradation rates, lag phases, and methane production patterns, as well as the influence of substrate ratio and pre-treatment on the digestion process.

iv. **Additional stability indicators:** Future studies should include direct measurements of additional process stability indicators, such as VFA, alkalinity, and microbial community composition. Incorporating these parameters alongside pH, ammonia, and VS data would provide a better understanding of buffering capacity, inhibition, and the wholesome stability of KW-PM ACoD systems.

v. **Techno-economic assessment under South African conditions:** Future research should include a dedicated techno-economic assessment of the preferred KW:PM mixing ratios and the combined mechanical-enzymatic pre-treatment using South African cost data and policy frameworks. Such assessment would help determine capital and operating costs, potential energy revenues, digestate value, and the financial feasibility of implementing the technology in rural and community-scale biogas systems.

vi. **Integration with policy and circular-economy initiatives:** Future research should explore how KW-PM co-digestion can be integrated into existing waste management systems, biogas programmes, and nutrient recycling initiatives in South Africa. Building on the findings of this study, such work would help identify practical pathways for implementing the technology while supporting sustainable waste management and renewable energy development.