



# **ANAEROBIC CO-DIGESTION OF AGRICULTURAL WASTE FOR BIOGAS PRODUCTION**

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**Submitted in fulfilment of the requirements for the degree of Master of Engineering in the Department of Chemical Engineering, Faculty of Engineering and the Built Environment at Durban University of Technology**

**By**

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## **Preface**

This research is a joint study conducted by the Department of Chemical Engineering at Durban University of Technology and REFLECT AFRICA, under the leadership of Dr. Emmanuel Kweinor Tetteh and Prof. Sudesh Rathilal, from the Green Engineering Research Group. The investigation, encompassing feed characterisation, experimentation, and analysis of results, was conducted at the Chemical Engineering Department, Faculty of Engineering and the Built Environment, DUT.

## **Declaration**

Nqobile Mkhize , the undersigned candidate declares that.

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Lastly, I would like to express my gratitude to the Mkhize family for their continued support, love, advice, and prayers.

## **Dedication**

I would like to offer this dissertation as a dedication to my parents (Mrs Clementine Mkhize and Mr. Siphon Mkhize), my brother (Siyaqala Mkhize) and my nephew (Asekhona Mkhize). I have been motivated by them to dream high and succeed.

## Abstract

South Africa today has an issue of high demand for energy, water, and waste management. The utilisation of promising alternatives can be achieved through the sustainable management of agricultural wastes (AW) and their valorisation to produce biogas. This management adds to the fight to move away from the environmentally detrimental fossil fuels. The method of waste management being used nationwide is landfilling which has been found to be a significant risk to the environment since it emits greenhouse gases (such as carbon dioxide and methane). In response to this problem, this study aimed to valorise the AW and market wastewater (MWW) obtained from a local fruit and vegetable bulk market to produce biogas using co-digestion. The study utilised the apples, bananas, oranges, spinach, tomatoes, butternuts, potatoes, and carrots as the AW. The activated sludge (AS) was obtained from a local wastewater treatment facility and was used as the inoculum.

The first objective was to characterise the AW to determine the properties that allow application in the anaerobic co-digestion (Co-AD) process. The solid AW was subjected to mechanical pre-treatment to increase the surface area and enhance a better co-digestion application. The analysis was conducted using the Fourier-Transform Infrared Spectroscopy (FTIR), Brunauer–Emmett–Teller (BET), Scanning Electron Microscopy (SEM) and oxygen bomb calorimeter. The second objective investigated the feasibility of the process of Co-AD of different AW, such as apples, bananas, carrots, butternuts, and potatoes, combined with market wastewater (MWW). The Co-AD process used activated sludge (AS) as the inoculum. A biochemical methane potential test (BMP) in 1 L capacity digesters assessed the potential for biogas production. The digesters were fed feedstock with an organic loading rate (OLR) of 2.5 kgVS/m<sup>3</sup>.day maintained at a temperature of 40°C for a hydraulic retention time (HRT) of 21 days. The BMP used a mixing ratio of 1:1 (% w/w) between MWW and AWs and a ratio of 1:2 between the co-substrates and inoculum.

The third objective was to develop a predictive model using the Box-Behnken design (BBD) model matrix on the response surface methodology (RSM). The input factors were temperature (32 - 42°C), pH (6 - 8), HRT (10 - 21 days) and OLR (1 - 4 kgVS/m<sup>3</sup>day). The final objective was to analyse the kinetics of the Co-AD system through the application of Modified Gompertz and first-order kinetic models.

Among the AW, spinach waste was found to have the highest BET surface area of 1.70 m<sup>2</sup>. The apple and banana waste developed pore volumes of 0.068 nm and 0.69 nm, respectively.

The common functional groups included the O-H, C-O and carboxylic acids with wavelengths ranging from 720 to 3280  $\text{cm}^{-1}$  for all the AW. The energy content in the agricultural waste ranges between 14 and 15 MJ/kg. These results confirmed the successful mechanical pre-treatment of agricultural waste and its viability in being a feedstock for Co-AD.

Among the different substrates, the apple and banana substrates showed the highest biogas output, with 595 mL/day with a methane composition of 68% and 585 mL/day with a methane composition of 65%, respectively. The control digester produced 450 mL/day of biogas, with 60% methane composition. The second stage evaluated the effect of substrate-to-substrate (SS) ratios and the different combinations of agricultural waste. The best-performing combination, Mix-1, produced 680 mL/day at an SS ratio of 2, and it also produced the highest biogas production with a methane composition of 75%.

At optimum conditions of OLR of 3.98  $\text{kgVS}/\text{m}^3\cdot\text{day}$ , a temperature of 40°C, HRT of 10 days, a pH of 7.2, a biogas production of 716.53 mL/day, a VS reduction of 73.37% and COD removal of 79.24%, with a desirability of 100% was obtained. The correlation between the predicted results and the experimental data was high, with a correlation coefficient ( $R^2$ ) value between 0.9 and 1. The findings from the kinetics analysis indicated that the Modified Gompertz model provided a more accurate description of the kinetics and dynamics of the system compared to the first-order kinetic model. This conclusion was supported by a correlation coefficient ( $R^2$ ) exceeding 0.99 and an error margin of less than 2%.

Finally, when co-digested with market wastewater, agricultural waste exhibited good performance in biogas production, especially apples, bananas, and Mix-1 combination. The study supports finding new ways to mitigate agricultural waste and protect the environment. Further studies may include more types of agricultural waste and evaluate other co-substrates that are easily obtainable. The study also proposes new energy sources that investigated further that will promote environmental sustainability.

## Research outputs

### Journal Articles

The following publications are numbered (1-4). The author is the corresponding (main) author, and co-authors provided valuable inputs and corrections.

1. **Mkhize, N.**, Mjoli, N.S., Khumalo, S.M., Tetteh, E.K., Mahlangu, T.P., and Rathilal, S. **2023**. Enhanced biogas production through anaerobic co-digestion of agricultural wastes and wastewater: A case study in South Africa. *International Journal of Energy Production and Management*, 8 (2): 123-131. Doi: <https://doi.org/10.18280/ijepm.080209>
2. **Mkhize, N.**, Khumalo, S.M., Tetteh, E.K., and Rathilal, S. **2024**. Fruits and vegetables waste performance in co-digestion with local market wastewater: operating conditions and kinetics (**Ref. No. AE-511 , submitted under review**)
3. **Mkhize, N.**, Tetteh, E.K., and Rathilal, S. **2025**. The influence of the substrate-to-substrate ratios: Fruit and vegetable waste and market wastewater co-digestion (**Under preparation for submission by April 2025**)
4. **Mkhize, N.**, Tetteh, E.K., and Rathilal, S. **2025**. Co-digestion of agro-waste: A critical review on the prospects of large-scale applications (**Under preparation for submission by April 2025**)

### Conference Papers

The author was the main presenter and author at the conferences and papers (5-7)

5. **Mkhize, N.**, Mjoli, N.S., Tetteh, E.K., and Rathilal., **2022**, Co-digestion of different agricultural waste from a local market for generation of biogas. 2<sup>nd</sup> Annual Sustainable Bioenergy and Processes Conference (SBP2022), Dec. 12-14, 2022, Cape Town (South Africa), Cape Town International Convention Centre, Address: Convention Square, 1 Lower Long Street, Cape Town, 8001, South Africa.
6. **Mkhize, N.**, Khumalo, S.M., Tetteh, E.K. and Rathilal, S. **2023**. Agro-Waste from A Local South African Market: Characteristics and Co-Digestion Biogas Application. 39<sup>th</sup> JOHANNESBURG International Conference on “Chemical, Biological and Environmental Engineering” (JCBEE-23) Nov. 16-17, 2023,

Johannesburg (South Africa), Birchwood Hotel & OR Tambo Conference Centre, Address: 14 Viewpoint Rd, Bardene, Boksburg, 1456, South Africa. Available: <http://iicbe.org/upload/1637C1123015.pdf>

7. **Mkhize, N.**, Tetteh, E.K. and Rathilal, S. **2024**. Fruit waste anaerobic co-digestion with market wastewater: Process kinetics. 41st CAPE TOWN International Conference on “Chemical, Biological and Environmental Engineering” (CCBEE-24) Nov. 21-22, 2024, South Africa, Cape Town Lodge Hotel & Conference Centre, Address: 101 Buitengratcht, Cape Town, 8001, South Africa. Available: <https://iicbe.org/upload/2471C1124169.pdf>
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## Nomenclature

|       |  |
|-------|--|
| AD    | Anaerobic Digestion                                |
| APHA  | American Public Health Association                 |
| APHA  | American Public Health Association                 |
| AS    | Activated Sludge                                   |
| ASBR  | Anaerobic Sequencing Batch Reactor                 |
| AW    | Agricultural Waste                                 |
| BBD   | Box-Behnken Design                                 |
| BBD   | Box-Behnken design                                 |
| BET   | Brunauer-Emmett-Teller                             |
| BET   | Brunauer-Emmett-Teller                             |
| BMP   | Biochemical Methane Potential                      |
| BMP   | Biochemical Methane Potential                      |
| BOD   | Biological Oxygen Demand                           |
| C/N   | Carbon and Nitrogen Ratio                          |
| Co-AD | Anaerobic Co-Digestion                             |
| COD   | Chemical Oxygen Demand                             |
| COD   | Chemical Oxygen Demand                             |
| CSIR  | Council for Scientific Industrial Research         |
| CSTR  | Continuous Stirred Tank Reactor                    |
| DEFF  | Department of Environment, Forestry, And Fisheries |
| DOE   | Design of Experiment                               |
| DTIC  | Department of Trade, Industry and Competition      |
| DWAF  | Department of Water Affairs and Forestry           |
| FAO   | Food and Agriculture Organisation                  |
| FTIR  | Fourier-Transform Infrared Spectroscopy            |
| FVW   | Fruit and Vegetable Waste                          |
| GC    | Gas Chromatography                                 |
| GHG   | Greenhouse Gases                                   |
| GPS   | Global Positioning System                          |
| HCL   | Hydrochloric Acid                                  |
| HRT   | Hydraulic Retention Time                           |
| HRT   | Hydraulic Retention Time                           |
| I/S   | Inoculum to Substrate Ratio                        |
| KW    | Kitchen waste                                      |
| MT    | Million Tonnes                                     |
| MWW   | Market Wastewater                                  |
| NAOH  | Sodium Hydroxide                                   |
| NDP   | National Development Plan                          |
| NERSA | National Energy Regulator of South Africa          |
| NPC   | National Planning Committee                        |
| OLR   | Organic Loading Rate                               |

|       |  |
|-------|--|
| OLR   | Organic Loading Rate                         |
| RSM   | Response Surface Methodology                 |
| RSM   | Response Surface Methodology                 |
| SEM   | Scanning Electron Microscopy                 |
| SEM   | Scanning Electron Microscopy                 |
| SSR   | Sum of Residual Squares                      |
| SS    | Substrate to Substrate Ratio                 |
| TDS   | Total Dissolved Solids                       |
| TOC   | Total Organic Carbon                         |
| TS    | Total Solid                                  |
| UNSDG | United Nations Sustainable Development Goals |
| VFA   | Volatile Fatty Acids                         |
| VS    | Volatile Solids                              |
| WD    | Width Distance                               |
| WW    | Wastewater                                   |
| WWTP  | Wastewater Treatment Plant                   |

# CHAPTER ONE: INTRODUCTION

---

## 1.1 Background

Energy is crucial for domestic, heating, transportation and as fuel in various industries. Fossil fuels have been the primary energy sources for the human population (Ritchie *et al.* 2023). Rising crude oil prices and the impending threat of climate change have led to the review of the world's economic development and energy policies, as well as investigating other energy resources (Kumar 2013; Khan *et al.* 2015). The South African energy industry has also been affected, where the energy produced majorly from coal has not been sufficient to meet the energy demands and has resulted in periodic power outages and load-shedding which is caused by outdated infrastructure, running out of coal, and insufficient generation, which also negatively affects the economy (Sadiki 2015; Longe *et al.* 2019).

According to Östergren *et al.* (2014), food waste encompasses all types of food, including all the inedible components, that are extracted from the food supply chain for the purpose of recovery or disposal by either composting, anaerobic digestion, bio-energy production, cogeneration, incineration, disposal in sewers, landfills, or disposal in the sea. Approximately 30-33% of fruits and vegetables are lost during production activities i.e. harvesting, storage, packaging, and transportation, as well as in consumption stages to produce agricultural waste (AW) (FAO 2019; Yahaya *et al.* 2019; Jun *et al.* 2022).

Food waste has accumulated in large amounts, where it was estimated that about 1.3 billion tons of waste is produced annually globally and that accounts for approximately 25% to 33% of the global food production (FAO 2016, 2019). The amount of food waste in South Africa is estimated to be 10 million tonnes annually (Nahman *et al.* 2013; DEFF *et al.* 2021). Chhandama *et al.* (2022) attributed the excessive amounts of the food waste produced to be a result of a defective waste management, urbanisation, and an increase in industrialisation. Food waste produced in large amounts results in economic losses and environmental destruction through the release of greenhouse gases (Liu 2014). The cost of the edible and inedible food waste in South Africa which are associated with opportunity and disposal costs was estimated to be R75 billion per annum from harvesting to domestic use (De Lange *et al.* 2015). Food waste has been found to have a negative effect by increasing food insecurity, whereby the food prices increase due to the high expenses which are included into the pricing of food and it also results in the wastage of water, electricity, seeds, fertiliser, and other resources required in food

production (DEFF *et al.* 2021). The carbon foot print of agricultural waste was estimated to be 3.39 kg in 2017 (Jun *et al.* 2022).

The fresh produce markets in South Africa, were first established as a venue for farmers and producers to interact with consumers (Simelane 2015). The four primary markets for fresh food in South Africa include Durban, Johannesburg, Cape Town, and Pretoria (Simelane 2015). The Competition Commission South Africa determined that, apples, bananas, pears, avocados, oranges, and grapes are the most common fruits grown and consumed in South Africa (DTIC 2022). The most common vegetables are potatoes, onions, tomatoes, carrots, and cabbage (DTIC 2022). These fresh produce markets face problems with the management of the large amounts of fruits and vegetable waste produced, (which will now be referred to as agricultural waste (AW)) (Fredes *et al.* 2023).

There are currently a few methods for managing agricultural waste (AW) are composting, incineration, landfilling, and anaerobic digestion (AD) (Chiu *et al.* 2016). Landfill disposal of the AW is the one majorly used method and is a challenge due to the biodegradation of the agricultural waste into methane and carbon dioxide (CO<sub>2</sub>) which adds to greenhouse gases release (Swati *et al.* 2018). Landfilling also produces contaminated runoff also referred to as leachate, toxic substances, and the release of odours that have a negative impact on both the environment and people (Srivastava *et al.* 2014; Swati *et al.* 2018). The valorisation of the AW is used to produce value-added products such as chemicals, materials, and fuels and it has been applied in food waste to create environmentally friendly and sustainable energy sources in the form of biogas, bioethanol, and biodiesel (Chhandama *et al.* 2022; Magama *et al.* 2022). AD is regarded as an efficient waste treatment method since it offers a way to reduce waste volume, minimise odours, and generate several revenue streams (Uddin *et al.* 2022).

South Africa is a semi-arid country that has considerable water stress, ranging from 40% to 60%, primarily because of its low annual rainfall of around 500 mm (FAO 2016). The utilisation of wastewater has gained significant significance in the management of water resources due to its environmental and economic benefits (Chaidez *et al.* 2014). Various treatment techniques can be employed to treat the wastewater for reuse. AD is a technique that can be applied in wastewater treatment, which aids in reducing the presence of harmful microorganisms, eliminating unpleasant smells, preserving the quality of the environment and promoting energy efficiency (Abdeshahian *et al.* 2016; Bella *et al.* 2023).

## 1.2 Anaerobic digestion

Anaerobic digestion (AD) is a process conducted in the absence of oxygen to produce biogas, where the bacteria degrade the organic materials referred to as substrates (Curletto *et al.* 2023). Figure 1-1 shows the schematic process of biogas production from organic waste. The other product of AD is an organic waste referred to as digestate, which can be used as a high-quality organic fertiliser (Andriamanohiarisoamanana *et al.* 2020). The utilisation of AD in the process of breaking down organic waste into biogas has been documented as a very promising environmentally friendly technology (Bong *et al.* 2018; Wainaina *et al.* 2019).

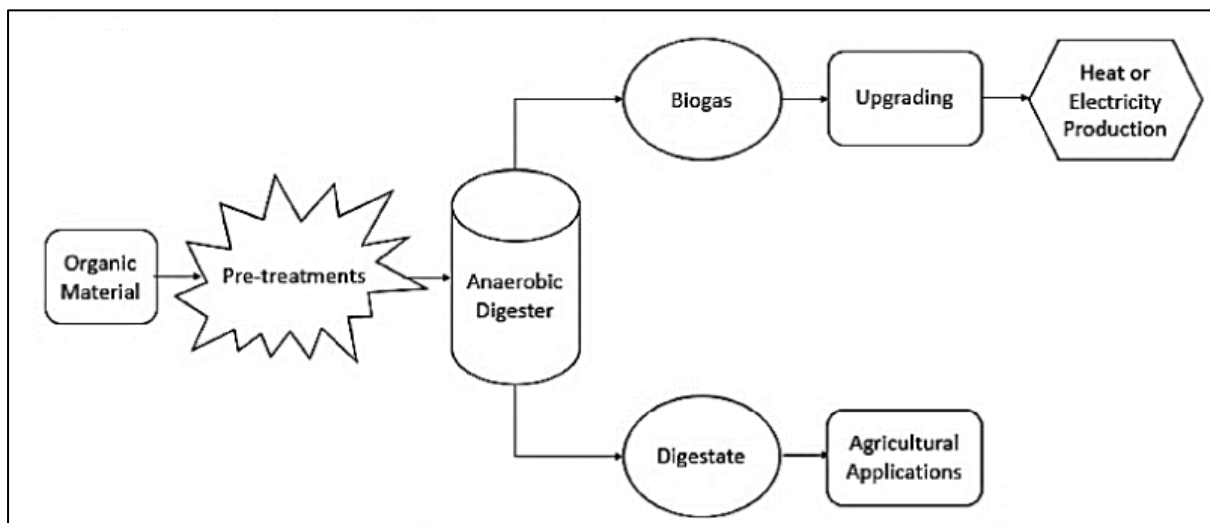


Figure 1-1: Process flow diagram of anaerobic digestion process adapted from (AL-Farajat *et al.* 2022)

### 1.2.1 Anaerobic co-digestion

AD process, which uses a combination of two or more substrates, is referred to as anaerobic co-digestion (Co-AD) (Chiu *et al.* 2016). Co-AD is a better application of the AD process as it increases the generation of biogas, improves synergy, and prevents the scarcity or abundance of certain elements caused by using a single substrate (Scano *et al.* 2014; Bong *et al.* 2018; Kuczman *et al.* 2018). The typical parameters that will affect AD include the ratio of carbon and nitrogen (C/N), volatile solids (VS), pH, particle size, toxic materials, organic loading rate (OLR) etc. (Mao *et al.* 2015; Uddin *et al.* 2022). The biogas yield in AD is also influenced by variations in the carbohydrate, protein, and lipid contents of AW (Chiu *et al.* 2016). Researchers have demonstrated that anaerobic mono-digestion (with one particular substrate) is a difficult task since it encourages rapid acidification, which inhibits the action of methanogenic bacteria and as a result, using two or more is desirable (Scano *et al.* 2014; Bong *et al.* 2018; Kuczman *et al.* 2018; Chow *et al.* 2020).

The increased generation of biogas by the co-digestion of various co-substrates is considered a beneficial use in anaerobic digestion. The study aimed to improve the production of biogas by combining anaerobic digestion of various agricultural waste from a fresh produce market with wastewater generated by the market.

### **1.3 Problem statement**

The agriculture industry faces pressure to meet the rising demand for fruits and vegetables caused by the population growth and changing diets (Sagar *et al.* 2018). The agriculture industry is huge in South Africa, where different fruit and vegetable crops are produced in different provinces. The agricultural waste (AW) is, therefore, produced in large amounts and is majorly landfilled across the country. Landfilling poses a threat to the environment as they add to the problem of greenhouse gases released through anaerobic digestion of the organic matter in the waste in landfills (Nanda *et al.* 2021).

The local market has two waste streams, which include agricultural waste (AW) and market wastewater (MWW). Waste management strategies must align with the United Nations Sustainable Development Goals (UNSDG) (UN 2015). The UNSDG Goal 6 relates to the provision of safe water and sanitation, Goal 7 addresses the availability of clean and affordable energy, and Goal 12 includes environmentally friendly consumption and production, which necessitates a decrease in the use of fossil fuels. The investigation of environmentally friendly alternatives to traditional waste management techniques is also of immense importance. Alternative energy sources should be explored and utilised in response to the existing energy crisis that can be applied to sustain the demand (Quaschnig 2014; Nematollahi *et al.* 2016; Duque-Acevedo *et al.* 2022; El-Ramady *et al.* 2022).

The application of food waste in AD to produce biogas has been investigated and is in application worldwide (Norouzi *et al.* 2022). The process of producing biogas from waste through AD is both appealing and also difficult due to many challenges that may occur during the digestion stage (Tetteh *et al.* 2019; Uddin *et al.* 2022). The application of Co-AD of AW has been investigated in the industry, where several types of AW as individual or mixed substances were co-digested with several co-substrates. The market wastewater (MWW) was used as the co-substrate to produce biogas through co-digestion (Co-AD).

### 1.3.1 Study area

The study site, as shown in Figure 1-2, represents a pictorial overview of the Durban Fresh Produce Market, in Durban, eThekweni Municipality, Kwazulu-Natal Province. The latitude and the longitude of the study site are respectively -29.9152536 and 30.9926509. It can also be found at the global positioning system (GPS) coordinates of 29°54'54.9144"S and 30°59'33.5436"E. The eThekweni Municipality produces approximately 1.4 million tonnes of waste, and the landfills are overburdened, while the fresh produce market facility in Clairwood can generate approximately 3,000 tons of AW every month (Goba 2022).



Figure 1-2: Durban Fresh Produce Market, Clairwood, Durban, Kwa-Zulu Natal (Goba 2022)

### 1.3.2 Organic waste and wastewater at the fresh produce market

There are two trade halls at the market: Sales Hall 1 features cold rooms for storing fresh goods, while Sales Hall 2 sells non-frozen vegetables. Bananas ripen in thirty (30) rooms at the market. All the AW from all the sections is dumped in a dumpsite inside the market, where non-biodegradable waste is recycled. AW is compacted for conventional waste collection trucks to take to landfills. The MWW at this plant is caused by various factors, such as ablution facilities, general waste, decomposing fruits and vegetables, dissolved salts, trace elements, and water vapour generated during the cooling tower operation.

## 1.4 Research aims and objectives

The local market has two waste streams: organic waste (agricultural waste) and market wastewater. The management of waste streams is critical, and their value must be recognised. Therefore, the aim of this research was to evaluate and optimise a bio-digestion process for biogas production from agricultural waste obtained from a local fresh produce market.

The specific objectives to achieve this aim were as follows:

1. To characterise the agricultural waste and establish their potential for biogas production.
2. To evaluate and compare the biogas production from the co-digestion of different feedstocks using the biochemical methane potential (BMP) test.
3. To evaluate and develop a predictive model for optimising biogas production as a function of temperature, hydraulic retention time, pH and organic loading rate for biogas production using different feedstocks.
4. To establish the kinetics of producing biogas from different feedstocks.

## 1.5 Approach

The approach carried out in this study involved four different phases:

**Characterisation of agricultural waste, market wastewater, and inoculum:** The first steps included collecting, preparing, and characterising agricultural waste, market wastewater, and inoculum. The agricultural waste was sent for mechanical pre-treatment, after which, it was characterised for surface shape, surface adsorption, and functional groups. The analysis included SEM, BET, FTIR, and calorific values. Wastewater analysis standards were used to characterise the inoculum and market wastewater.

**Biochemical methane potential (BMP) tests:** Eight different agricultural wastes were used to conduct a feasibility test to ascertain their potential for bioenergy production when co-digested with market wastewater in the presence of inoculum. The gas chromatography technique (GC-Solution, Shimadzu 2014, Japan) was employed to evaluate the biogas composition.

**Predictive model development:** Response surface methodology (RSM) was used for the design of the experimental runs and the development of the predictive models using the Box-Behnken design (BBD) matrix on RSM, including the input operating factors such as the OLR, pH, temperature, and HRT. The outputs included the biogas production, the percentage of

chemical oxygen demand (COD) removal, and the percentage of volatile solids (VS) removal. Established procedures were employed to investigate the pH, total solids (TS), VS and COD.

**Kinetic study:** The last phase of the project involved comparing and analysing the experimentally obtained biogas production results using Modified Gompertz and First-order kinetic models to determine the most effective model for the system.

## **1.6 Thesis outline**

This dissertation consists of five chapters structured as follows:

### **CHAPTER 1: Introduction**

This chapter will set the tone of the thesis and include background information, a problem statement, aims and objectives, an approach, and the thesis's structure.

### **CHAPTER 2: Literature Review**

This chapter presented literature on the historical background of agricultural waste, its economic value, treatment technologies, operating conditions of the AD process, advantages and applications of the Co-AD process, and the research gap.

### **CHAPTER 3: Materials and Methods**

Equipment and chemicals used in experimentation will be discussed. Experimental procedures used will also be discussed.

### **CHAPTER 4: Results and Discussions**

The experimental data will be provided and discussed using tables and graphical analysis.

### **CHAPTER 5: Conclusions and Recommendations**

The conclusions drawn from the investigation and the recommendations for further work are presented in this chapter.

In addition, works cited and used in this dissertation will be put under the **References**, while **Appendices** will include additional data to support the dissertation.

## CHAPTER TWO: LITERATURE REVIEW

---

This chapter presents a literature review on the anaerobic digestion and co-digestion process to produce biogas using agricultural waste and wastewater from markets. It also includes the performance of the anaerobic and co-digestion at different operating conditions and the comparison of different treatment technologies.

### 2.1 South African environmental concerns from agricultural waste

South Africa, as a country with a huge agricultural industry, produces large amounts of agricultural waste and wastewater. Worldwide agricultural production has tripled since the 1960s because of the broadening of the agricultural land and the technological advancements of the green revolution, which has led to enhanced crop yields to satisfy the needs of a fast-expanding global population (Pingali 2012).

#### 2.1.1 Agricultural waste

Agricultural wastes (AW) are the unconsumed and residual parts of vegetables, fruits, and other agricultural products which are generated during harvesting, post-harvesting, and consumption (Jena *et al.* 2022). Figure 2-1 shows the estimated contributions by mass of different food commodities in South Africa's food waste, where fruit and vegetables have the highest contribution. The fruit and vegetables sector was estimated to account for 44% of the total food waste produced in South Africa (Nahman *et al.* 2013).

Figure 2-2 demonstrates the different stages that occur in fruit and vegetable production that can lead to the accumulation of AW., which include cultivation, harvesting, transportation, processing etc. The accumulation of AW can be further explained by separating into different stages, where an estimated amount of 24% waste is produced in primary production, 23% during processing and manufacturing, 5% at the retail level, 39% at the household level, and 9% from the hospitality industry (FAO 2016; Caldeira *et al.* 2019). The causes of high amounts of AW include the financial, managerial and technical issues that arise during harvesting, processing, packaging and storage areas in underdeveloped countries facilities (Parfitt *et al.* 2010; FAO 2019)

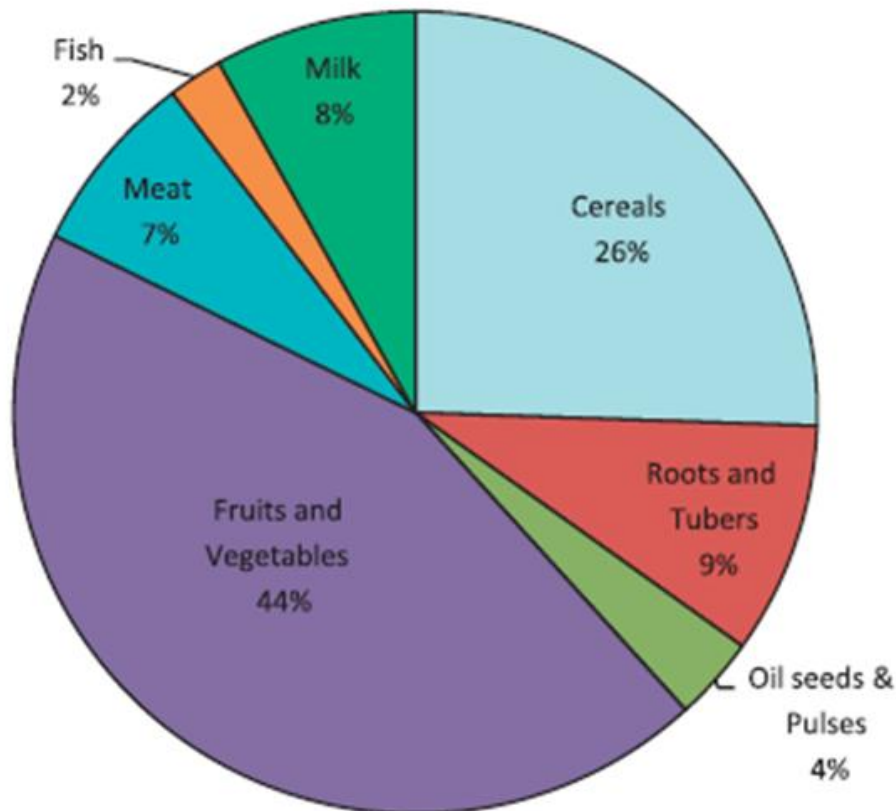


Figure 2-1: The percentage by mass contribution of each commodity group to South Africa's food waste (Nahman *et al.* 2013)

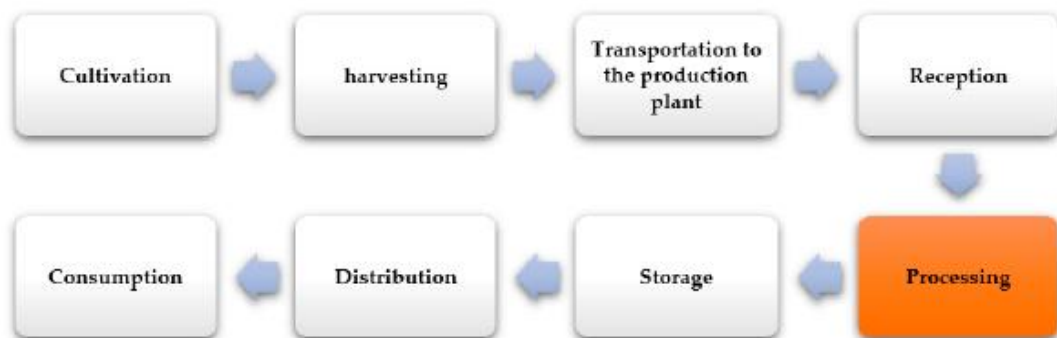


Figure 2-2: Stages in producing fruits and vegetables for consumption (Chhandama *et al.* 2022)

Large quantities of waste are produced from fruits and vegetables in the processing business, which is of major concern (Parfitt *et al.* 2010; Banerjee *et al.* 2017). Figure 2-3 shows the estimates of production of the different agricultural fresh produce. Globally, the production of citrus is estimated to be 124.73 million tonnes (MT). At the same time, bananas account for

114.08 MT, and apples contribute 84.63 MT to the total production, with tomatoes having a production volume of 171.00 MT (Mohd Basri *et al.* 2021).

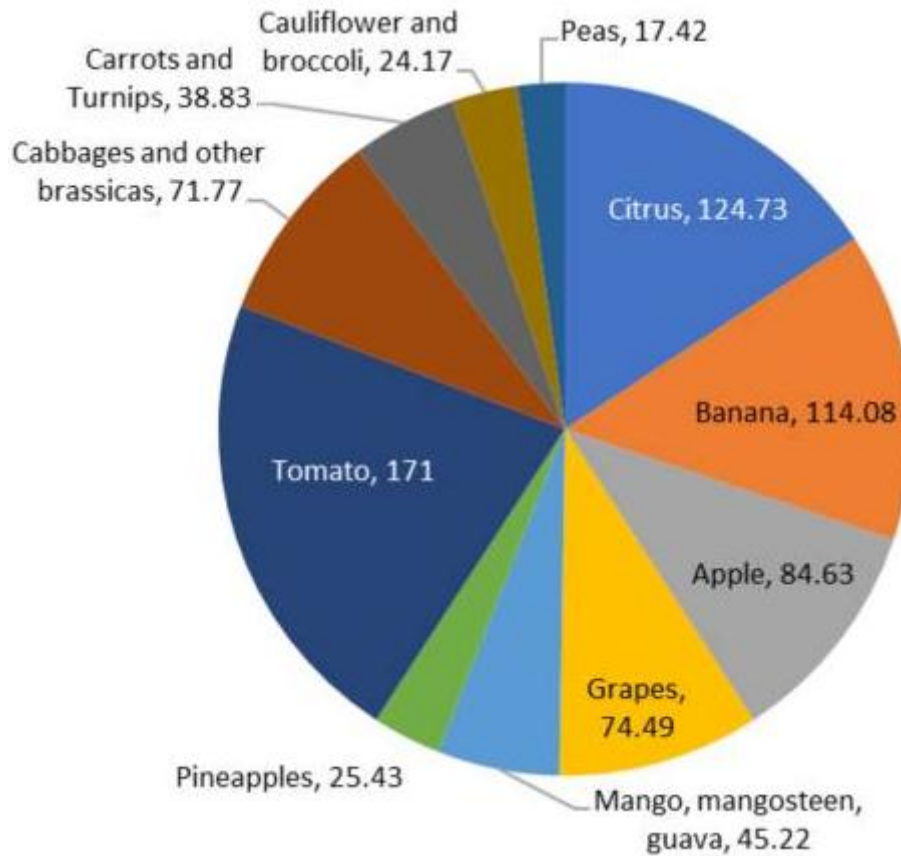


Figure 2-3: Estimate of global production of the agricultural fresh produce (Mohd Basri *et al.* 2021)

### 2.1.1.1 Environmental effects of agricultural waste

The majority of the AW is landfilled across South Africa, and this has led the country to be recognised as the 12th highest producer of greenhouse gases (GHG), with the waste industry accounting for 4.3% of the total GHG emissions in the country (Nahman *et al.* 2012; Borello *et al.* 2018). The AW has a detrimental impact on the soil, water, and air environment when they decay inside the environment. The impact results in the contamination of groundwater, leading to eutrophication and the production of foul odours and the elevated chemical oxygen demand (COD) in surface waters results in the deaths of fish (Loehr 2012; Iravanian *et al.* 2020; Alghamdi *et al.* 2021; Siddiqua *et al.* 2022). The soil environment is also heavily contaminated by AW with an increase in the amount of nutrients, including nitrogen, phosphate, potassium, and other organic substances (Maji *et al.* 2020; Koul *et al.* 2022). The

open incineration of the AW, which is a prevalent practice in numerous poor nations, results in the contamination of the air (Nagendran 2011)

### **2.1.2 Wastewater**

Wastewater has been found to include numerous harmful substances that can harm the streams it flows into, resulting in ecological imbalance and posing a possible threat to human health (Sonune *et al.* 2004; Edokpayi *et al.* 2017). The wastewater sources in South Africa have been experiencing the presence of several new pollutants, including medicines and personal care items (Archer *et al.* 2017). The global issue of sewage sludge management is on the rise, leading to a continuous increase in sludge generation due to the expansion of industrial activities. In South Africa, the agricultural sector accounts for around 61% of annual water consumption, followed by the municipality at 27%, and subsequently other industrial and domestic activities (Loki *et al.* 2021).

#### **2.1.2.1 Wastewater treatment**

Conventional methods of water and wastewater treatment typically encompass a range of physical, chemical, and biological techniques (Saravanan *et al.* 2021). Bacterial and fungal biosorption, as well as anoxic and anaerobic/aerobic processes, are employed in the biological treatment (Jagaba *et al.* 2021). Several physio-chemical treatments include membrane filtration, coagulation-flocculation, flotation, precipitation, ion exchange, adsorption, ultrasonic mineralisation, ion-pair extraction, and electrolysis (Abu Hasan *et al.* 2020; Saravanan *et al.* 2021). The concentration of several substances such as nutrients, heavy metals, microbes, and selected pollutants primarily determines the water quality (Ahmed *et al.* 2021a).

The treatment processes address the key water quality parameters, including turbidity, colour, pH, alkalinity, biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS), total organic carbon (TOC), and total faecal coliforms. (Hussain *et al.* 2020). These processes are guided by the regulations set by the local or regional government. Anaerobic digestion has been researched and applied as a low-temperature technology for the treatment of wastewater and energy recovery in the form of biogas (McKeown *et al.* 2012; Silvestre *et al.* 2015). The General Authorisation, Section 39 of the National Water Act No 36 of 1998, recorded all the discharge limits of the wastewater in South Africa (DWA 1998). Table 2-1 shows South Africa's regulated discharge limits to be met by treated effluent to the streams.

Table 2-1: Discharge limits of treated wastewater as per South African regulations (DWAF 1999).

| <b>Contaminant</b>      | <b>Discharge limit</b> |
|-------------------------|------------------------|
| Colour, odour and taste | None                   |
| pH                      | 5,5 – 9,5              |
| Suspended solids        | 25 mg/L                |
| COD                     | 75 mg/L                |
| Total faecal coliforms  | 1000 (cfu/per 100 ml)  |

## **2.2 Agricultural waste management practices**

### **2.2.1 Waste management policies in South Africa**

The South African government has put forward legislations to manage the AW. All stakeholders who handle agricultural food and waste must adhere to these legislations. One of the specific goals of South Africa's National Development Plan (NDP) is to achieve Sustainable Development Goal Number 12, which involves reducing the amount of waste sent to landfills and increasing the reuse, recycling, recovery, and production of environmentally friendly products (NPC 2011). The National Environmental Management Act, 1998 (Act No. 107 of 1998), The Waste Act, and the National Waste Management Strategy 2020 are also some of the major regulations that the government has set up to regulate waste management practices across South Africa (DEFF *et al.* 2021).

### **2.2.2 Traditional agricultural waste management technologies**

The traditional AW management practices include but are not limited to incineration, composting, anaerobic digestion, landfilling, gasification, pyrolysis and hydrothermal processes (Kassim *et al.* 2022). Thermal conversion techniques that are frequently used include burning of the waste (combustion), a gasifier is used to produce gas, which may then be utilised to heat a building or power a generator gasifier (gasification), and the thermal conversion of degraded AW into products like biomethane and bio-oil when there is no oxygen present (pyrolysis) (De Medina-Salas *et al.* 2019; Chhandama *et al.* 2022). Biochemical conversion is the microbial breakdown of biomass by the process of fermentation, composting or anaerobic digestion (Chen *et al.* 2016; Gouveia *et al.* 2017). Figure 2-4 shows the waste management hierarchy based on the most preferable solution to the least preferable solution. Waste prevention has been chosen as the best solution, followed by recovery and re-utilisation

(anaerobic digestion), while elimination (landfilling) is the least favourable (Chereji et al., 2023).

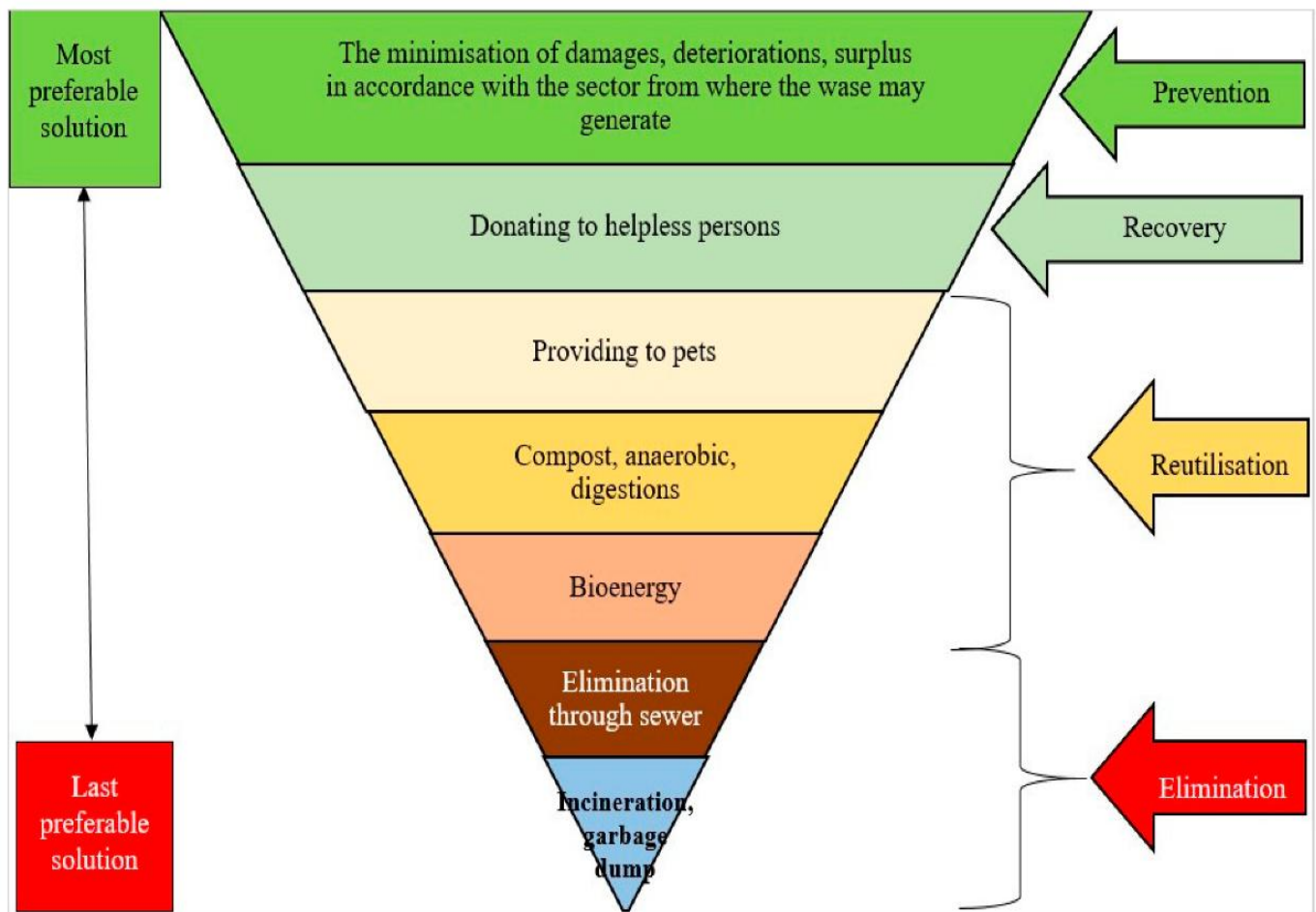


Figure 2-4: Waste management hierarchy (Chereji et al. 2023)

The process of converting waste into value-added products such as chemicals, renewable material and fuels is known as valorisation and it has garnered a lot of interest and a lot of investigations have been conducted (Magama et al. 2022). Circular economy has an avenue to incorporate the agriculture into the framework (Geissdoerfer et al. 2017; Heshmati 2017). Biomasses are one of the well-established sources of renewable energy and this also incorporates the conversion of the food waste to fuels (Negri et al. 2020). The waste-to-energy disposal methods are being investigated to find solutions that allow for partial replacement of fossil fuels and reduce the impact of the waste on the environment (Neto et al. 2021). These studies are also interested in turning food waste management processes into cheap, effective, and environmentally friendly processes to overcome the current disadvantages (Khan et al.

2016; Gumisiriza *et al.* 2017; Santolini *et al.* 2021) The AW can also be used as raw material to produce animal feed (Sabiiti 2011).

### 2.2.3 Comparison of the advantages and disadvantages of the different agricultural waste management technologies

The waste management strategies that are currently practised to handle and dispose of this kind of waste have been found to result in higher greenhouse gas emissions, such as incineration and landfilling (Azarmanesh *et al.* 2023). These waste management practices also pose a threat to the environment and human health by releasing hazardous gases, odours and leachates which results in climate change, eutrophication and water pollution (Panda *et al.* 2018; Shanthi *et al.* 2018). Anaerobic digestion has been reported to be a very promising technology for the biodegradation of organic waste into biogas (Mao *et al.* 2015; Náthia-Neves *et al.* 2018; Atelge *et al.* 2020b). AD may be converted into several forms of energy that can replace the need for traditional fossil fuels and the biogas generated from anaerobic digestion can be utilised in several types of sustainable energy (Chiu *et al.* 2016). Table 2-2 shows the advantages and disadvantages of the AW management technologies.

Figure 2-5 illustrates the different products that can also be produced using AW. The applications include the industrial production of bio-oil, promoting plant growth, producing animal feed, enhancing soil quality, and developing biosorbents such as biochar (Peng *et al.* 2023).

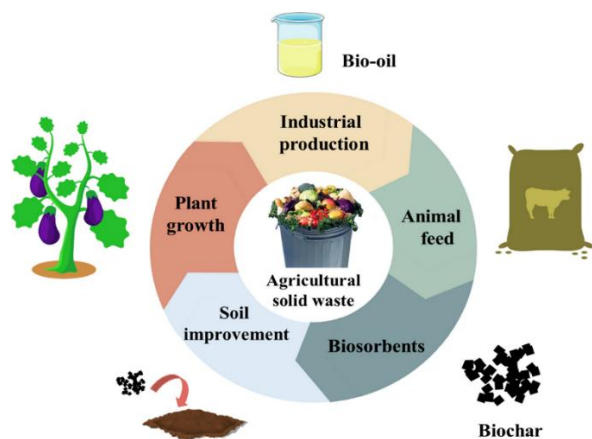


Figure 2-5: Different applications of agricultural waste for the development of useful products (Peng *et al.* 2023)

Table 2-2: Advantages and disadvantages of different waste (AW) management technologies

| <b>Process and product</b>        | <b>Advantages</b>  | <b>Disadvantages</b>  |
|-----------------------------------|--|---|
| Incineration –<br>(Heat energy)   | Incinerators have the capability to decrease the volume of (AW) by approximately 80-85%, resulting in a substantial reduction in the required volume for disposal (Pham <i>et al.</i> 2015).   | Incineration is an energy intensive process which becomes inefficient and leads to air pollution due to the high moisture content of the AW (Ahamed <i>et al.</i> 2016). The incineration technology is also characterised by a significant financial burden, which is influenced by several factors such as the costs associated with operating and maintaining the pollution control system, the acquisition of an incinerator, and the construction of a dedicated landfill for the disposal of highly hazardous fly ash (Zou <i>et al.</i> 2020; Zamli <i>et al.</i> 2021). |
| Anaerobic Digestion –<br>(Biogas) | The environmental viability and cost-effectiveness of anaerobic digestion (AD) as a treatment method for AW have been documented in the literature (De Meester <i>et al.</i> 2012; Slorach <i>et al.</i> 2019).  | The significant issue arises from the extended duration of the microbial reaction, which can last 20 to 40 days (Pham <i>et al.</i> 2015).  |
| Gasification<br>(Syngas)          | These technologies have been found to be cost-effective and capable of customising operating conditions, such as temperature and equivalence ratio, as well as specific reactor capabilities, including fixed bed, fluidised bed, entrained bed, vertical shaft, etc., which leads to the production | The release of carbon dioxide (CO <sub>2</sub> ) as a greenhouse gas, ammonia (NH <sub>3</sub> ) which may cause eutrophication, hydrogen cyanide (an explosive and poisonous gas) methane (greenhouse gas), hydrogen and carbon monoxide (combustible) (Arafat <i>et al.</i> 2015)   |

|                         |   |  |
|-------------------------|---|--|
|                         | of syngas suitable for various applications (Murugesan <i>et al.</i> 2022).   |  |
| Landfilling             | The technology is low-cost in capital and operational terms, easy to use, and has huge capacities (Trabold and Nair 2018; Ma and Liu 2019). It is one of the most commonly used techniques for managing municipal solid waste and agricultural waste. | Landfilling gives rise to several environmental concerns, such as leaching, greenhouse gas emissions (namely methane), and the generation of unpleasant odours (Srivastava <i>et al.</i> 2014; Swati <i>et al.</i> 2018; Ma <i>et al.</i> 2019). Furthermore, this technique has resulted in escalated operational expenses and concerns about landfill space availability (Badgett <i>et al.</i> 2021). |
| Composting<br>(Compost) | Composting is a cost-effective alternative to typical soil, water, and air remediation methods. It reduces methane emissions, increases crop output, and retains water (Jeevahan <i>et al.</i> 2021).   | Composting disadvantages include the cost of separating food waste from packaging, transportation limits, the release of inorganic gases, and odour issues (Sundberg <i>et al.</i> 2013).  |

## **2.3 Valorisation of agricultural waste into bioenergy**

There is a need for new and sustainable energy sources as the energy demand is increasing daily due to the economic growth, resulting from industrialisation and population increases (Omer 2008; Stern 2011; Qazi *et al.* 2019). Fossil fuels contribute approximately 80% of the overall worldwide energy generation and this not only leads to an increase in the rate of diminishing fossil fuel reserves, but also has a significant adverse impact on the environment, resulting in increased health risks and the threat of global climate change (Mohr *et al.* 2015; Ağbulut *et al.* 2021). At present, the energy sector in South Africa also has a significant dependence on coal, which it accounts for almost 80-90% of the country's energy generation, positioning it as one of the leading contributors to global carbon emissions (Pierce *et al.* 2023; Goga 2024). The increasing need for energy, along with the large quantity of untreated AW that is disposed of in landfills, has created a need to find sustainable energy solutions from waste resources. The untapped potential of waste in South Africa has not been effectively utilised due to various factors, including insufficient knowledge about the energy potential of AW, inadequate technologies for converting AW into energy, chemicals, fuels, and other valuable resources, as well as weak government policies in this area (Mohlala *et al.* 2016).

### **2.3.1 Circular economy and renewable energy**

There is a universal shift towards a circular economy at different levels to enhance the efficiency of operations and minimise the production of AW that is left with no alternative but to be disposed of in landfills (Srivastava *et al.* 2020). The circular economy offers a potential answer to the prevailing economic model that relies heavily on raw resources such as the converting of AW into useful products (Haque *et al.* 2023). These products are obtained from organic materials that possess high quality characteristics, such as their high biodegradability and convenient collecting methods (González *et al.* 2020). The processing of organic waste materials into products that have increased value is a fundamental aspect of the bioeconomy, which aligns with the principles of the circular economy and is crucial for its implementation and success (Antar *et al.* 2021). Another essential component of the bioeconomy is the idea of ramping the use of different types of waste streams and biomass (Popp *et al.* 2021). The circular bioeconomy is highly suited to contribute significantly to the sustainability of the agriculture sector and this is due to the abundant opportunities it offers for converting AW into valuable resources, hence promoting the recycling of this important organic material (Abbas *et al.* 2021).

Renewable energy refers to energy obtained from renewable resources that naturally replenish themselves within a period relevant to human activities (Owusu and Asumadu-Sarkodie 2016).

Renewable energy can be produced from biomass, the sun, water, and wind, among other sources (McGlade *et al.* 2015; Owusu *et al.* 2016; Spiru 2023).

### **2.3.1 Fertiliser or compost**

There is need to find ways to decrease the utilisation of chemical fertilisers. Fertiliser that is produced from organic AW is one of the ways that have been practised to valorise the agricultural waste into products and/or energy. Waste products, containing both organic and elemental components, can improve soil characteristics, hence enhancing crop productivity (Al-Barakah *et al.* ; Song *et al.* 2015). Granular compost is a versatile and comprehensive organic fertiliser that contains all essential nutrients and is produced through pelleting (Chew *et al.* 2019; Devianti *et al.* 2021; Eidarous *et al.* 2022).

### **2.3.2 Animal Feed**

The AW are also frequently utilised as animal feed, either in their raw form or enhanced with additional substances such as, wheat straw, which is commonly utilised after mechanical devices fragment it into minuscule pieces (Koul *et al.* 2022). The vegetable by-products were determined to be nutritionally and sanitarily suitable for inclusion in animal feed after the removal of high moisture through pulse combustion drying, oven drying, and microwave drying (San Martin *et al.* 2016).

### **2.3.3 Biogas**

The organic wastes, such as domestic wastes (e.g., food, fruits, and vegetables) and public moist wastes (e.g., restaurants, daily markets, and biological wastes from companies), are widely utilised as feedstocks to produce biogas through AD (Curletto *et al.* 2023). This preference is primarily attributed to the substantial moisture content and notable degradability of the AW (Abanades *et al.* 2022). The Biogas produced is a gas mixture containing methane (35-55%), carbon dioxide (40-60%) and other gases (<1-10%),, with methane having a high energy content (50–55 MJ/kg) that can be utilised for heat, energy, heat, and steam in homes and businesses, be injected into the natural gas system, and car fuels (Wellinger *et al.* 2013; Mortier *et al.* 2016; Khan *et al.* 2017; Rajendran *et al.* 2020). Methane burns with a blue flame and has no colour or smell (McGee 2020; Chhandama *et al.* 2022). The trace amounts of other gases may include hydrogen sulphide and ammonia (Kiyasudeen S *et al.* 2016; Pramanik *et al.* 2019).

### **2.3.3.1 Uses of biogas**

Biogas is widely recognised as a conventional form of off-grid energy on a global scale. The utilisation of biogas for power generation in the form of electricity is facilitated by advancements in technology, a decrease in dependence on fossil fuels, and a reduction in greenhouse gas (GHG) emissions (Abanades *et al.* 2022). Boilers can directly combust biogas solely for the purpose of generating heat and this is possible to make minor adjustments to natural gas boilers in order to function with biogas, this can be utilised in the small or large scale agricultural activities to heat digesters, structures such as pig housing units, greenhouses, aquafarming facilities, cool and refrigerate farm products, and facilitate drying processes (Abanades *et al.* 2022). The creation of combined heat and power (CHP) can also be accomplished through the use of biogas (Thorin *et al.* 2015).

Biogas is found to contain species such as dust particles, water saturation, siloxanes, aromatic, and halogenated chemicals in trace amounts which may increase emissions, increase corrosion, or other health risks, (Rasi *et al.* 2011; Neto *et al.* 2021). The conversion of biogas into biomethane, achieved through the processes of upgrading and cleaning, is a viable alternative to fossil natural gas for utilisation in natural gas powered vehicles and can also be delivered to the national gas network (Molino *et al.* 2013; Ardolino *et al.* 2021; Pavičić *et al.* 2022). The process of biogas upgrading involves the use of physical and chemical techniques, including adsorption, absorption, cryogenic and membrane separations, gas separation membranes, and biological technologies (Kapoor *et al.* 2019; Ahmed *et al.* 2021b). One notable advantage of biomethane is its ability to be stored for optimal use during periods of high demand (Herbes *et al.* 2018). The other applications of biogas include the generation of hydrogen and the utilisation of fuel cells (Alves *et al.* 2013; Sharma *et al.* 2024).

### **2.3.3.2 Biogas application in South Africa and challenges**

There is still a huge gap to meet in transforming the South Africa's energy sector into renewable energy such as biogas. The practical applications of biogas in South Africa, like other African countries, have been limited due to insufficient study and the estimate number of 300 installations are mostly small-scale domestic AD installations of less than 10 m<sup>3</sup> (Bond *et al.* 2011; Mugodo *et al.* 2017). The biogas industry in South Africa as an emerging sector, in 2023, had an operation of approximately 30 biogas projects across the commercial and industrial domains within the nation which are registered with the National Energy Regulator of South Africa (NERSA) (Mrwebi 2023). The current financial and economic viability of biogas generation and advancement of biogas technology for commercial purposes in South Africa is

constrained, as a result of insufficient governmental investment, support of AD research outputs, restricted domestic financial resources, insufficient feedstocks, and a scarcity of locally accessible and appropriate technologies (Parawira 2009; Smith *et al.* 2014; Mutungwazi *et al.* 2018).

## **2.4 Anaerobic digestion**

Anaerobic digestion (AD) is a process of biochemical degradation in the absence of oxygen which is commonly used to treat organic waste and produce biogas (Sawyerr *et al.* 2019). A sludge residue (digestate or bio-digest) is a by-product of the process that can be used as a soil amendment or as starting material for high-quality compost or can be used as fuel for energy generation (Scano *et al.* 2014; Klitkou *et al.* 2019). The biogas production in AD is influenced by variations in the carbohydrate, protein, and lipid contents of organic waste (Chiu *et al.* 2016). The use of AW to produce biogas is attributed to the significant amounts of hemicellulose and cellulosic materials (Kassim *et al.* 2022).

The adoption of AD for the AW waste management must overcome technical, economic, and social challenges even if it is already a mature technology for the treatment of high-strength wastewater, sewage sludge, and animal manure (Neto *et al.* 2021). The AD method is perfect for the development in the rural areas because the AD plants may be scaled down and the ability to manufacture biogas on-site at the biomass production site saves money on transportation, which is a crucial economic factor (Molino *et al.* 2013).

### **2.4.1 Anaerobic digestion mechanisms**

AD stands for anaerobic digestion, which is a coordinated procedure involving a community of microorganisms, where anaerobic bacteria break down and transforms food waste into biogas, which allows for a continual production of energy during anaerobic conditions (Morales-Polo *et al.* 2018). The process consists of four distinct mechanisms: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Sawyerr *et al.* 2019). Figure 2-6 depicts the four mechanisms and the process flow showing the reactions as well as the different bacterial populations involved during the AD process. The different bacterial populations catalyse a variety of exergonic chemical processes that release some small energy during bacterial digestion, to gain the energy required for their reproduction (Leng *et al.* 2018; Curletto *et al.* 2023). These bacterial populations that are used during these mechanisms include, acidogenic bacteria, acid-forming bacteria, acetogenic bacteria, and methanogenic bacteria (Yadav *et al.* 2022).

The process is defined by a chemical reaction (Equation 2.1) in which anaerobic bacteria decompose organic material, such as glucose, to produce carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) (Paranjpe *et al.* 2023).

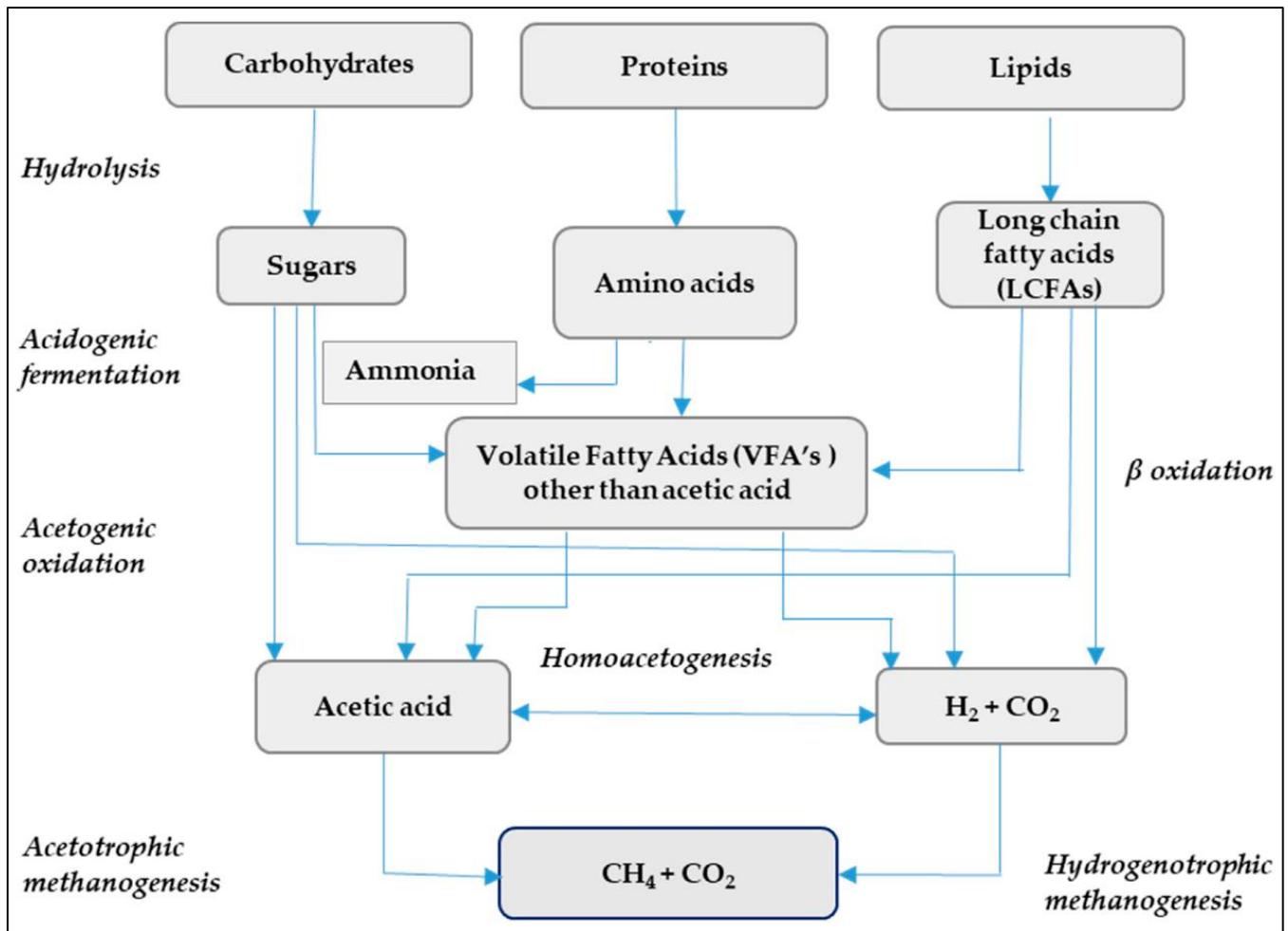


Figure 2-6: Process flow diagram of anaerobic digestion mechanism phases (Rabii *et al.* 2019)

#### 2.4.1.1 Hydrolysis:

The Complex organic matter such as carbohydrates, proteins, and lipids is broken down by acidogenic bacteria into their corresponding simple monomers, such as glucose, fatty acids, alcohols and proteins to peptides and amino acids (Molino *et al.* 2013; Paritosh *et al.* 2017; Rabii *et al.* 2019; Uddin *et al.* 2022). Some compounds in this stage are ready to be transformed into biogas, while most compounds in this step need additional breakdown through later stages

(Molino *et al.* 2013; Uddin *et al.* 2022). If the waste has a high organic content, the hydrolytic activity can become rate limiting and therefore some industrial processes use chemical reagents to improve the efficiency of the hydrolysis process to get around this restriction (Molino *et al.* 2013).

#### **2.4.1.2 Acidogenesis:**

The primary products of hydrolysis are alcohol (ethanol, methanol), short-chain volatile fatty acids (VFAs) (acetic acid, propionic acid, formic acid, and lactic acid), and ketones (glycerol and acetone) (Rabii *et al.* 2019). The acidogenic bacteria further degrades the hydrolysis by-products into volatile fatty acids (VFAs) and produce by-products such as carbon dioxide (CO<sub>2</sub>), hydrogen sulphide (H<sub>2</sub>S), and ammonia (NH<sub>3</sub>) during the acidogenesis stage (Paritosh *et al.* 2017; Cord-Ruwisch 2019; Rabii *et al.* 2019). Acidogenesis is often a relatively quick process, and if it is not adequately controlled, there is a possibility of VFA accumulation in the digester, which could lead to digester toxicity (Paritosh *et al.* 2017; Uddin *et al.* 2022; Yadav *et al.* 2022).

#### **2.4.1.3 Acetogenesis:**

The acetogenic bacteria digests the VFAs and part of the long-chain fatty acids from the hydrolysis stage to generate acetate, hydrogen gas (H<sub>2</sub>) and CO<sub>2</sub> in the acetogenesis stage (Damayanti *et al.* 2019; Uddin *et al.* 2022; Yadav *et al.* 2022). There are numerous distinct bacteria are responsible for the compounds produced during acetogenesis (Uddin and Wright 2022).

#### **2.4.1.4 Methanogenesis:**

The last AD mechanism is methanogenesis, where the methanogenic bacteria converts acetate, H<sub>2</sub>, and CO<sub>2</sub> into methane gas (Yadav *et al.* 2022). The methanogenic bacteria cannot survive in the presence of oxygen, therefore, this stage is strictly anaerobic, where, acetophilic and hydrogenophilic bacteria, metabolize into acetate and H<sub>2</sub> into CO<sub>2</sub> and CH<sub>4</sub> while hydrogenophilic bacteria turn H<sub>2</sub> and CO<sub>2</sub> into CH<sub>4</sub>, and lastly acetophilic bacteria turn acetate into CH<sub>4</sub> and CO<sub>2</sub> (Molino *et al.* 2013; Uddin *et al.* 2022; Paranjpe *et al.* 2023). The by-product of AD, the digestate, has non-digestible material that the microbes are unable to break down, as well as the remains of dead bacteria (Lv *et al.* 2020).

## **2.4.2 Challenges with the AD process**

### **2.4.2.1 Feedstock availability and collection methods**

Generally, biomass and organic material can be degraded naturally to produce energy. It is necessary for physical properties like feedstock size and moisture content to be appropriate to overcome the broad variation in the physical and chemical characteristics of the feedstocks for the digester technology; too much or too little moisture content influences bacterial development in the system and makes it difficult to feed the digester (Atelge *et al.* 2021; Pan *et al.* 2021).. The substances, including animal dung and AW, have been the primary possible feedstock, due to the carbohydrates, proteins, cellulose, hemicellulose, and lignin that they contain (Esposito *et al.* 2012; Uddin *et al.* 2022; Xu *et al.* 2023). The refractory nature and delayed hydrolysis stage, high lignin content lowers the biodegradability in the substrate (Buffière *et al.* 2006; Sawatdeenarunat *et al.* 2015; Dos Santos *et al.* 2019). It can be difficult to achieve the ideal carbon to nitrogen (C/N) ratio for anaerobic digestion, given the variety of available feedstock types (Mao *et al.* 2015; Chow *et al.* 2020). The process performance variation is frequently caused by inhomogeneity within the same feedstock, such as wastewater sludge or AW and thus, one of the biggest obstacles to process performance is maintaining ideal process conditions (Moody *et al.* 2011; Chow *et al.* 2020).

The absence of source separation for organic wastes is a prevalent issue in numerous communities, necessitating treatment facilities to engage in pre-processing of the feedstock before its integration into the waste management process as it presents significant issues in terms of operation, reduced methane production, compromised biogas quality, and, in certain instances, process failures in both single- and co-digestion systems (Forster-Carneiro *et al.* 2007; Sun *et al.* 2015; Neto *et al.* 2021; Chhandama *et al.* 2022).

The primary methods employed for the removal of inert materials include screening, sedimentation, and flotation and; Co-AD processing may necessitate the use of multiple units, such as feedstock storage and pre-treatment to eliminate inert substances and enhance digestibility, along with biogas purification (Xie *et al.* 2017).

### **2.4.2.2 Inhibition and Low Process Efficiency**

There are several factors which impede the growth of microbial populations during AW digestion, which then leads to inhibition., which is the decrease in the production of biogas and, in certain instances, results in the failure of the biological process (Mansour *et al.* 2024). Ammonia, which can exist as either free ammonia or ammonium (NH<sub>4</sub><sup>+</sup>), is critical as it

enhances the ability of a reactor to resist changes in pH by neutralising organic acids, hence preventing the adverse effects caused by the accumulation of volatile fatty acids (VFAs) from easily digestible organic waste (Han *et al.* 2020; Odejobi *et al.* 2023). Inhibition is attributed to the breakdown of proteins leading to excessive levels of free ammonia that can readily permeate the cell membrane and disrupt the equilibrium of protons (H<sup>+</sup>) and potassium, leading to a deficit, whereas the hydrolysis of fats generates significant quantities of hydrogen (Chen *et al.* 2008; Hajjaji *et al.* 2016). The amounts of ammonia should remain below 200 mg/L for the AD process to be successful, and this is due to that nitrogen is necessary for anaerobic micro-organisms (Sheng *et al.* 2013). There is a need to optimise the C/N ratio to minimise or prevent ammonia inhibition in the AD process, as an over-the-limit C/N ratio indicates a lack of nitrogen (or inefficient use of carbon), resulting in rapid consumption by methanogens and ultimately leading to reduced biogas generation (Hajjaji *et al.* 2016; Jiang *et al.* 2019).

#### **2.4.2.3 Low product quality**

The primary output of the AD process is biogas, which has a lower calorific value than natural gas and more than 40% CO<sub>2</sub>, which can render the energy content and flame speed of biogas to be low (Uddin and Wright 2022). The content of biogas can vary among different applications and even within a particular application, mostly due to variations in feed composition and operating conditions of its anaerobic digesters (Kuo *et al.* 2017).

#### **2.4.3 Characteristics of agricultural waste as substrates for anaerobic digestion**

The characterisation of waste to analyse for moisture, ash, total solids, and volatile solids are among the physical qualities that are most frequently studied and is essential since it establishes whether the waste may be used in various processes (Singh *et al.* 2012; Magama *et al.* 2022). The degradability and biogas production potential of waste in an anaerobic digester depend on the number of important constituents, such as lipids, proteins, carbohydrates (including cellulose and hemicelluloses), and lignin (Belitz *et al.* 2009; Gao *et al.* 2015). Lipids are crucial in AD, as they generate more methane than other organic compounds (Khan *et al.* 2019). Carbohydrates are the most abundant component of fruits and vegetables, accounting for 70–90% of their dry weight and leading to the production of the VFAs, resulting in acidity, low pH, and process inhibition (Díaz *et al.* 2017). The AW has been found to exhibit notable attributes such as a substantial moisture content exceeding 80-90%, a significant organic composition comprising volatile solids accounting for over 95% of the total solids, approximately 75% sugars and hemicelluloses, 9% cellulose, and 5% lignin, fats, proteins, and other nutrients like fibre, vitamins, and minerals as well as their elevated carbon content renders

them appropriate as feedstocks to produce biogas (Sharma *et al.* 2024). The co-digestion of carbohydrate-rich substrates with protein-rich substrates can enhance the efficiency of waste treatment and the stability of the process by generating significant amounts of volatile fatty acids (VFAs) while also offering a strong buffering capacity (Elbeshbishy *et al.* 2012; Ohemeng-Ntiamoah *et al.* 2018). The AW present favourable conditions for energy recovery by anaerobic digestion.

Some properties of the AW such as that of potatoes and banana peels have demonstrated potential as of the AW feedstocks to produce biogas, with carbon-to-nitrogen (C/N) ratios ranging from 35-60 and 20-30, respectively, while the calorific values of apples AW, varies between 17.15 MJ/kg and 19.85 MJ/kg, as well as their moisture content, which is around 80% (Bouallagui *et al.* 2003; Asquer *et al.* 2013; Özyuğuran *et al.* 2017). These qualities contribute to the facilitation of the biodegradation process for biogas formation. The size of particles has a critical role in the process of anaerobic digestion of solid waste, particularly during hydrolysis, and this is because smaller particles give a bigger surface area for enzyme action, leading to a more effective breakdown of the waste (Raposo *et al.* 2012; Zia *et al.* 2022).

Furthermore, due to the diversity of sources, handling and processing techniques, eating patterns, local weather patterns, and seasonal variations, even the same type of AW might exhibit significantly different features (Okewale *et al.* 2019). The total solids (TS) concentration of food waste ranges from less than 2% to more than 90% and since the VS/TS ratio that can be converted into gaseous products is typically around 90%, this makes the biomass an excellent candidate for AD (Neto *et al.* 2021). It is important to maintain a carbon-to-nitrogen (C/N) ratio of at least 20-30 in the feedstock to attain maximum biogas generation (Al-Rubaye *et al.* 2019; Pan *et al.* 2021; Magama *et al.* 2022).

#### **2.4.4 Comparison between batch and continuous AD systems**

The AD process can be operated in a single-stage batch system and a multi-stage system for different feedstocks.

##### **2.4.4.1 Single-stage anaerobic system - batch**

A single-stage anaerobic system with a simple design and little maintenance produces hydrolysis, acidogenesis, acetogenesis, and methanogenesis in the same reactor (Chhandama *et al.* 2022). The substrate is introduced into the digester, and then the digester is sealed for the full duration without any more addition of substrate until the decomposition process is practically completed, where most of the processed material is subsequently removed, and the

digester is fed with fresh substrates, initiating the digestion process again (Negri *et al.* 2020; Van *et al.* 2020). The problem of acidification of AW in the single-stage system causes acidogens to reduce the pH of the system, which disrupts the methanogens (Chhandama *et al.* 2022). The methanogenic microbial community in the reactor may be maintained by changing the feeding rate and adding a buffer to the system (Negri *et al.* 2020). The design has low input requirements, simple process control, and mechanical needs and also offers greater use flexibility than continuous systems and has high process efficiency and cost-effectiveness (Neto *et al.* 2021).

#### **2.4.4.2 Two-stage or multiple anaerobic system- continuous**

Separate reactors are used in two-stage or multiple anaerobic systems for hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which results in a high methane generation, loading rate, improved process stability, pathogen management, and increased rate of volatile material removal efficiency are some of the benefits of multiple anaerobic system (Chhandama *et al.* 2022). The maintenance and operating costs are higher when compared to a single-stage anaerobic system (Hagos *et al.* 2017). The substrate is continually introduced into and extracted from the digester in an ongoing process while all reactions related to biogas production will take place at a rather consistent rate (Sun *et al.* 2015; Neto *et al.* 2021; Uddin *et al.* 2022). The continuous process typically involves the utilisation of two digesters, such as the continuous stirred tank reactor (CSTR) and the anaerobic sequencing batch reactor (ASBR (Neto *et al.* 2021).

### **2.5 Anaerobic co-digestion (Co-AD)**

Anaerobic Co-digestion (Co-AD) involves the mixing of different wastes, which benefits the AD process by increasing methane yield, improving stability, and improving waste handling (Mukumba *et al.* 2015; Mukumba *et al.* 2016). This process also involves the use of an inoculum, which is an anaerobic digestate made from microorganisms that will break down a certain substrate after being exposed to it (Hinds *et al.* 2016). Anaerobic sludge that is produced from wastewater treatment plants was found to be a better inoculum than cow dung, corn silage, or swine sludge (Forster-Carneiro *et al.* 2007; Chhandama *et al.* 2022). Researchers have demonstrated that anaerobic mono-digestion, with one particular biomass, has negative results since it encourages rapid acidification, which has been found to inhibit the action of methanogenic bacteria and nutritional deficits and, as a result, using two or more substrates is desirable (Scano *et al.* 2014; Bong *et al.* 2018; Kuczman *et al.* 2018; Chow *et al.* 2020).

### 2.5.1 Synergistic effects of co-digestion

The Co-AD process promotes an increase in the production of a particular type of methane from the substrate combination by improving the feed qualities and synergistic effects from using the right co-substrate (Xie *et al.* 2017). Figure 2-7 shows the benefits of Co-AD, where using more than one substrate is shown to enhance biogas and methane production. The benefits of Co-AD over mono-digestion include improved macro and micro element supply, dilution of hazardous and inhibitory compounds, increased buffering capacity, decreased greenhouse gas emissions, decreased process costs, and the ability to use higher loading rates (Lee *et al.* 2019).

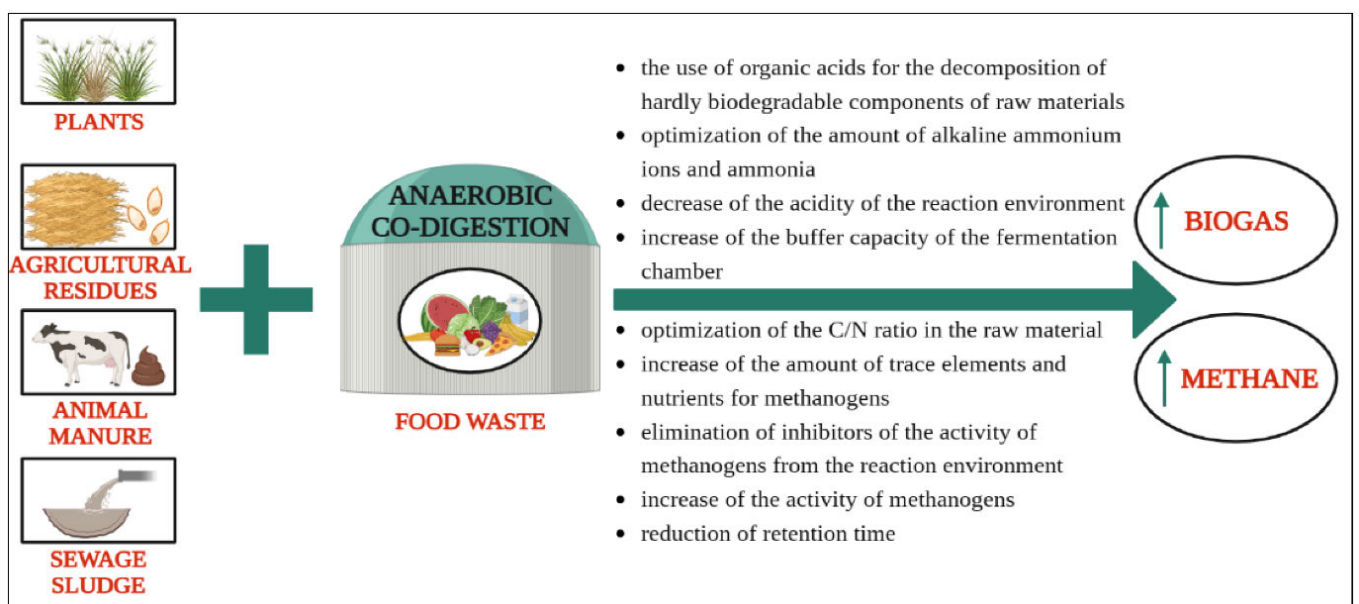


Figure 2-7: Summary of the benefits of using co-digestion process using different co-substrates (Pilarska *et al.* 2023)

#### 2.5.1.1 Prevention of inhibitory impact of anaerobic digestion on AW during Co-AD

The methanogenic activity in AD of the AW is frequently hindered by the build-up of volatile fatty acids (VFAs) caused by their high biodegradability as well as the inadequate levels of essential nutrients such as nitrogen, phosphorus, and other trace metals (Hagos *et al.* 2017). Several types of co-substrates to the AW were investigated to determine the influence of Co-AD to mitigate the inhibitory impact of AD on food waste (Chiu *et al.* 2016).

#### 2.5.1.2 Using the combinations of co-substrates that have content of organic matter

There is a lot of variety in the AW in terms of source, moisture, and calorific value, which has been proven to be the main factor in their conversion into valuable goods (Chhandama *et al.*, 2022). The volatile solid (VS) levels of the substrates determine which substrate will be a

primary substrate (substrate with a higher content) and co-substrate (the substrate with a lower content), where AW can serve as either the primary substrate or as the co-substrate (Chiu and Lo 2016). The process is hindered and rendered unbalanced by improper co-substrate selection and substrate-to-substrate (SS) mixing ratio, which lowers the process' performance in terms of methane production and volatile solids reduction (VS reduction) (Krupp *et al.* 2005; Azarmanesh *et al.* 2023). The selection of an appropriate inoculum type (such as animal dung, rumen, wastewater sludge, etc.) and the inoculum-to-substrate ratio (I/S) are crucial because they shorten start-up time, increase digestion, and strengthen the microbial community (Guo *et al.* 2013). A mixed culture inoculum is favoured over a pure culture due to tolerance to a wider selection of substrates, and technical viability for anaerobic procedures (Ghimire *et al.* 2015).

### 2.5.2 Pre-treatment of feedstock for the anaerobic digestion and co-digestion

It is necessary to choose a pre-treatment approach that is suitable for the specific properties of the substrate, such as its biodegradability and particle size, to enhance the production of biogas (Ariunbaatar *et al.* 2014; Kondusamy *et al.* 2014; Amin *et al.* 2017; Atelge *et al.* 2020a; Bandgar *et al.* 2022). Figure 2-8 shows the application of mechanical, chemical, thermal, and biological pre-treatment methods of the AW during the AD process. The pre-treatment of biomass can be applied to produce optimal microbial growth and digestion as well as the production of useful products in an economical and environmentally friendly manner (Chhandama *et al.*, 2022). In the context of Co-AD, the pre-treatment is applied to a single co-substrate, specifically the one with lower biodegradability, rather than treating the entire mixture as a whole (Tyagi *et al.* 2018).

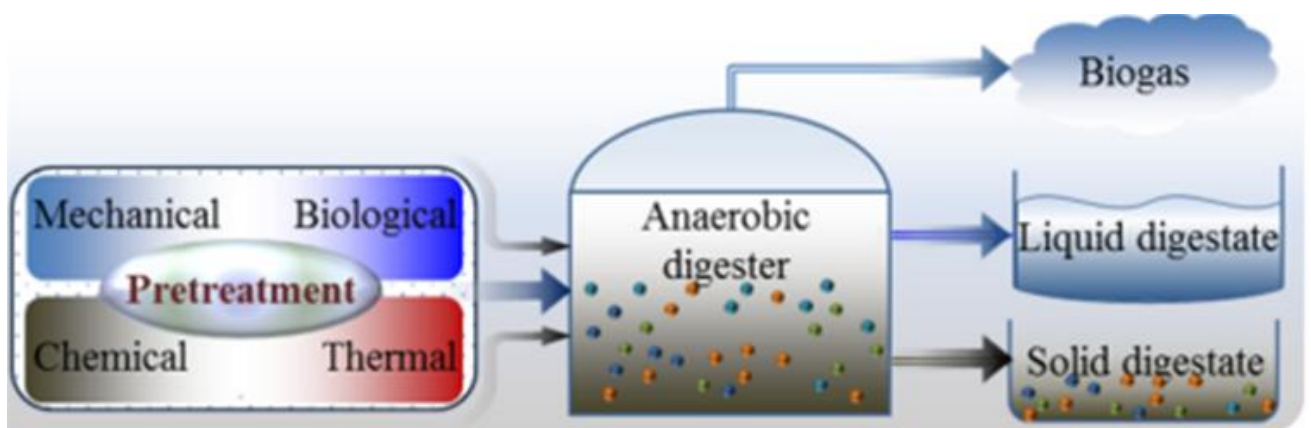


Figure 2-8: Pre-treatment methods for the feedstocks before AD and Co-AD (Nguyen *et al.* 2021)

### **2.5.2.1 Chemical Pre-treatments**

The AW is pre-treated using chemicals before AD. One type of chemical pre-treatment is acidic pre-treatment, which degrades lignocellulosic substrates into their corresponding monosaccharides and the hydrolytic bacteria can adapt to the acidity associated with this type of pre-treatment (Hu *et al.* 2012). Although using an acidic pre-treatment can help with substrate degradation and shorten the time needed for digestion, it is less cost-effective than using an alkaline pre-treatment (Nguyen *et al.* 2021).

### **2.5.2.2 Thermal Pre-treatments**

The thermal pre-treatment involves subjecting the AW to high temperatures and pressure to induce hydrolysis and prevent evaporation (Arshadi *et al.* 2016). Furthermore, the high temperatures reduce the HRT for solubilisation and also decrease the overall volume of the reactors (Cesaro *et al.* 2014).

### **2.5.2.3 Biological Pre-treatments**

The processing of lignocellulosic AW can also be accelerated by using enzymes, fungus, and bacteria generated by industrial fermentation operation biological techniques to break down hemicellulose and lignin (Kucharska *et al.* 2018).

### **2.5.2.4 Mechanical Pre-treatments**

The mechanical pre-treatment method is used to reduce the particle size and increase the surface area for substrate adsorption, and this leads to an improvement of the subsequent biodegradation process and ultimately leads to increased biogas generation (Abbass Jafari *et al.* 2017; Oyaró *et al.* 2021; Zamri *et al.* 2021). The solid particles in the AW substrates undergo mechanical pre-treatment, such as cutting, grinding, compressing, or pulverising, to break them down into smaller particles that microbial enzymes can easily access for breakdown (Promon *et al.* 2018; Sindhu *et al.* 2019; Pilli *et al.* 2020). An increase in the surface area promotes digestion by strengthening the contact between the substrate and anaerobic microorganisms, hence improving the hydrolytic process and increasing the efficiency of anaerobic digestion (Elliott *et al.* 2012; Zhang *et al.* 2014). Other mechanical pre-treatment methods that are in application include collision-plate, ultrasound, and milling (Deepanraj *et al.* 2013).

Previous studies looked into the surface, physical, and chemical characteristics of AW in order to suggest specific ways to use it as biosorbents and as a source material for biofuels and biochemicals (Memon *et al.* 2008; Memon *et al.* 2009; Pathak *et al.* 2015, 2016, 2017). Studies in literature demonstrate that AW typically have a surface area that falls between the range of

0.6 m<sup>2</sup>/g to 1.7 m<sup>2</sup>/g. (Pathak *et al.* 2017); and particle size of greater than 0.7 mm and not more than 10 mm (Raposo *et al.* 2012; Zia, Ahmed and Kumar 2022). Research indicates that reducing the particle size to a range of 2.5-8 mm can increase methane output (Agyeman *et al.* 2014).

### **2.5.3 Agricultural waste application in co-digestion**

The co-digestion process of AW has been studied by different researchers using different feedstocks at different operating conditions. Table 2-3 shows the comparisons of the application of AW when co-digested with different feedstocks and inoculum. There is less literature from South Africa for the application of co-digestion of AW with wastewater and/or market wastewater as well as with other co-substrates.

Table 2-3: Table showing different applications of agricultural waste in co-digestion and mono digestion process in previous studies

| <b>Agricultural waste</b>   | <b>Co-substrate</b>                               | <b>Inoculum</b>   | <b>Application</b>  | <b>References</b>                  |
|---|---|---|---|------------------------------------|
| Fruits and vegetable waste  | None  | Butchery digestate  | An OLR of 2.68-2.97 kgVS/m <sup>3</sup> .day produced 0.87 Nm <sup>3</sup> /kgVS biogas and a 57.58% methane composition.   | (Masebinu <i>et al.</i> 2018)      |
| Fruits and vegetable waste  | Sewage sludge                                     | Anaerobically broken-down sludge  | Automatic Methane Potential Test System (AMPTS II) was used for the Co-AD of fruit and vegetable waste with sewage sludge. 1149.50 mLCH <sub>4</sub> /gVS was the final methane yield.  | (Seswoya 2019)                     |
| Fruit / vegetable waste (FVW)   | Kitchen waste (KW)                                | Activated sludge  | 0.725 LCH <sub>4</sub> /gVS biogas was produced, an optimum OLR of 3 g/Ld was found to be the best, with an FVW:KW ratio of 5:8.  | (Wang <i>et al.</i> 2014)          |
| Mixed supermarket waste (fruit and vegetable waste, bakery waste, meat waste etc.)  | None  | To prepare the batch testing, anaerobic sludge was produced in a 20 L lab-scale thermophilic reactor by digesting cattle manure and raw glycerine. (Marañón <i>et al.</i> 2021) | CSTRs, 20, 25, 30 days HRT, thermophilic conditions ± 55 °C. The best OLR was determined to be 3.6 kg VS/m <sup>3</sup> .day, which produced up to 48.1% more methane per kg of treated waste than the other OLRs that were examined. Additionally, the greatest VS elimination (89.0%) and 26.7 L CH <sub>4</sub> /kgVS were attained. | (Megido <i>et al.</i> 2021)        |
| Citrus effluents  | None  |   | It was discovered that, at standard temperature, one tonne of processed oranges produces 1.25 m <sup>3</sup> of citrus effluents and 0.72 m <sup>3</sup> methane  | (Rosas-Mendoza <i>et al.</i> 2020) |
| Vegetable waste (vegetables: 12% kale, 5% zucchini, 5% escarole, 9% eggplant, 10% chayote, 10% cabbage, 11% carrot and 10% beetroot.) | Food waste (11 percent rice and 14 percent beans) | Two granular sludges from Up flow Anaerobic Sludge Blanket reactors have been utilised.   | The best results (869 mL of CH <sub>4</sub> ./gTVS) were observed under mesophilic conditions. An organic load of 0.30 g TVS/Ld was the maximum.  | (Blasius <i>et al.</i> 2020)       |

|   |              |  |   |                                      |
|---|--------------|--|---|--------------------------------------|
| Apple pomace  | Swine manure | Activated waste sludge                             | Co-digestion of apple pomace with swine manure. The highest production of 421 mL/gVS.day was having an apple pomace: swine manure ratio of 15%:85%  | (González-García <i>et al.</i> 2022) |
| Bananas, cabbage  | None         | Pre-digested vegetable market wastes               | AD of vegetable waste was conducted in a laboratory-scale reactor with a Hydraulic Retention Time (HRT) of 30 days and an Organic Loading Rate (OLR) of 2.25 g/L.day. At 65% methane composition, the methane output was 0.387 L/gVS. | (Velmurugan 2011)                    |
| Fruit waste (fruit peel and orange bagasse)                 | None         | Industrial and sewage anaerobic sludge inoculation | 348 NmL/gVS in biogas was achieved using the Gompertz model for the kinetic study on the orange bagasse/industrial sludge combination.  | (Santos <i>et al.</i> 2020)          |
| Equal amounts of apple, banana, carrot, potato, and lettuce | None         |  | Methane output from single-phase digestion was 0.45 m <sup>3</sup> CH <sub>4</sub> /kgVS, with 83% VS removal. In comparison to a single-phase system, the energy yield for a two-phase system was 33% lower.                         | (Ganesh <i>et al.</i> 2014)          |

## **2.6 Factors affecting the anaerobic digestion process:**

The process variables such as temperature, pH, organic loading rate (OLR), hydraulic retention time (HRT), C/N ratio, the microbe pool, the nature (particle size, structure, composition), and concentration of the biomass, as well as the reactor features and others are some crucial process parameters (Sun *et al.* 2015; Neto *et al.* 2021; Uddin and Wright 2022). The control of these factors affects the effectiveness and speed of the digestion process depending on the characteristics of the feedstock and the surrounding environment (Boe *et al.* 2009; Scano *et al.* 2014; Mao *et al.* 2015; Uddin *et al.* 2022).

### **2.6.1 Temperature**

The operating conditions of the AD include temperature, which can be psychrophilic (10 – 30 °C), mesophilic (32 - 42 °C), and thermophilic (50 – 65 °C) (Al-Rubaye *et al.* 2019). Most of the available studies on agricultural waste digestion used mesophilic conditions (Sawatdeenarunat *et al.* 2015). Thermophilic digestion produces more biogas at a faster rate. Still, it requires more energy (heat) input while temperatures between 35°C and 40°C are ideal for mesophilic digestion as most of the commercial digesters function. in the mesophilic region (Uddin and Wright 2022). Mesophilic digestion produces less biogas when compared to thermophilic digestion, but it offers a more reliable operation at a cheaper cost of operation (Uddin and Wright 2022). Under the mesophilic conditions, the cost of operation is lower in terms of capital expenditure and more manageable to execute (Mortier, Velghe and Verstichel 2016; Hagos *et al.* 2017; Uddin and Wright 2022). Co-digestion systems that operate at mesophilic temperatures exhibit a greater variety of microorganisms, leading to improved stability and resilience of the entire process (Al-Rubaye *et al.* 2019). The process of mesophilic digestion of AW has shown the ability to produce higher organic liquid residues (OLRs) (Atelge *et al.* 2021; Pan *et al.* 2021).

The reduced process stability, dewatering properties, and high energy costs associated with heating are drawbacks of thermophilic anaerobic fermentation. In contrast, the thermal destruction of pathogenic bacteria at high temperatures is seen as a significant benefit (Chow *et al.* 2020). Mesophilic conditions result in the rapid decomposition of organic compounds, which contributes to an excessive accumulation of volatile fatty acids (Bouallagui *et al.* 2003; Chow *et al.* 2020)

### **2.6.2 Organic loading rate (OLR)**

The organic loading rate (OLR) gives information on how much organic material is added per unit volume of the digester and depends on the amount of organic matter in the substrate. The ideal rate is established through experimental analysis (Uddin and Wright 2022). Organic loading rate should be kept between 1 and 4 kgVS/m<sup>3</sup>.day (Atelge *et al.* 2021; Pan *et al.* 2021).

### **2.6.3 Hydraulic retention time (HRT)**

The hydraulic retention time (HRT) indicates how long the substrate stays in the digester on average and is used to ensure the substrates are allowed to remain in the digester for a long enough period to achieve the greatest possible conversion of organic materials into biogas (Uddin and Wright 2022). The HRT can be between (10 to 21 days and has been reported as the optimum for the AD of AW (Tufaner *et al.* 2016).

### **2.6.4 Carbon/nitrogen (C/N) ratio**

Microorganisms use carbon as their energy source but also need a specific amount of nitrogen for development or metabolism (Uddin and Wright 2022). The C/N level of the digester material can be optimised by co-digesting substrates that have a high C/N ratio together with nutrient-rich organic wastes that have a low C/N ratio, such as animal manure or food waste (Atelge *et al.* 2021; Pan *et al.* 2021). Low C/N ratio co-substrate ratios indicate higher N concentrations, eventually producing too much ammonia digester alkalinity, lowering biogas output (Uddin and Wright 2022). In industries, Co-AD with AW to balance is done, to attain the ideal carbon-to-nitrogen (C/N) ratio for anaerobic digestion, which is about 20-30 (Mao *et al.* 2015; Chow *et al.* 2020). A solid waste substrate with a high carbon-to-nitrogen (C/N) ratio is inappropriate for bacterial growth because it lacks adequate nitrogen, as a result, the rate at which gas is produced and the ability of solids to break down will be reduced., while at low C/N, the breakdown process leads in the formation of ammonia, which is detrimental to the bacteria (Mao *et al.* 2015; Chow *et al.* 2020).

### **2.6.5 pH**

The pH determines the concentration of hydrogen in any solution, and most microbes favour a neutral pH range; acidogenic bacteria, hydrolytic and acidogenic bacteria may survive in a wider pH range of 5.5 and 6.5, and thus researchers determined the ideal pH level should be maintained between 7 and 8 (Mortier, Velghe and Verstichel 2016; Hagos *et al.* 2017; Uddin and Wright 2022). The process of keeping all the microorganisms in the same digester at the ideal pH might be difficult, particularly for substrates with different chemical makeup, like

sewage sludge (Uddin and Wright 2022). It is advisable to ensure the life of working bacteria; the rate of alkaline addition should keep up with the rate of volatile fatty acids by maintaining pH level (Leung *et al.* 2016; Mortier *et al.* 2016).

### **2.6.6 Inoculum type and inoculum substrate ratio (ISR)**

The inoculum to be used for the Co-AD needs to exhibit a significant level of alkalinity, which contributes to its ability to effectively buffer the pH and avoid acidification during the digestion process, even in the presence of highly biodegradable substrates (Megido *et al.* 2021). The wastewater treatment plant (WWTP) sludge has been recommended for its accessibility and durability (Elbeshbishy *et al.* 2012; Li *et al.* 2020). Furthermore, the enhancement of methane generation and reactor stability in a batch AD process from AW can be achieved through the utilisation of various inoculum-to-substrate ratios (I/S) (Xu *et al.* 2014; Meng *et al.* 2018).

### **2.6.7 Mixing ratio of substrates (SS ratio)**

It is crucial to determine the ideal mixing ratio of substrates (SS ratio) to optimise the efficacy of biogas production from Co-AD and some methods for determining the mixing ratio of substrates include the optimisation of the C/N ratio or regulating the VS or volume ratio (Mata-Alvarez *et al.* 2014).

### **2.6.8 Total Solid and Volatile solids**

According to a study conducted by Higgins *et al.* (2017), the optimal advantage of co-digestion of food waste is attained when the AW loading is around 25% of the VS (AW VS to wastewater solids VS). The anaerobic digestion process can be described as dry anaerobic digestion (i.e., 20% TS (total solids) or wet anaerobic digestion 10% TS (total solids) depending on the total solids content (Karthikeyan *et al.* 2013).

The factors affecting the anaerobic or co-digestion process are important in optimising the process for maximising biogas yield and production. The next section will discuss applying the factors and operating conditions using design of experiment software.

## **2.7 Design of experiment (DOE)**

The design of experiments is a systematic and methodical approach to performing and analysing controlled investigations to assess the factors that influence a response variable by determining the specific levels at which the combinations of elements will be set, and where the individual runs of the experiment will take place (Telford 2007; Antony 2023). Research surface methodology (RSM) uses statistical and mathematical methods for predictive model

development, process optimisation and experimental design (Tetteh *et al.* 2017). Box-Behnken Design (BBD) is a symmetrical experimental design used to evaluate the relative significance of various experimental conditions and their interactions and this approach is more cost-effective as it minimises the number of experiments and provides a valuable opportunity for optimisation (Ranade *et al.* 2017; Otieno *et al.* 2023).

## 2.8 Kinetic study / mathematical modelling

The utilisation of mathematical modelling in AD serves to enhance comprehension and enhancement of the intricate process of converting organic molecules into biogas and other gases using various bacterial groups (Oyaro, Oonge, & Meshack, 2021). The results of kinetic modelling of biogas and methane production are utilised to establish and refine the reactor parameters by the experimental yields achieved, during the design of full-scale systems (Mozhiarasi *et al.* 2020). Various models can be utilised for anaerobic digestion, including the first-order, Monod, Contois, Gompertz, Modified Gompertz, Chen and Hashimoto, ADM1, Cone, and Grau second-order models (Córdoba *et al.* 2018; Momodu *et al.* 2021).

The kinetics models to be used are the first-order kinetics (Equation 2.2) and Modified Gompertz models (Equation 2.3) as applied by Oyaro, Oonge and Meshack (2021).

$$Y(t) = Y_m [1 - \exp(-kt)] \quad (2.2)$$

Where: Y(t) - cumulative biogas production (mL/gVS)

$Y_m$ - ultimate biogas production potential (mL/gVS)

t – time in day

k - first-order model constant (1/day)

$$Y(t) = Y_m \exp \left\{ -\exp \left( \frac{R_m - e}{Y_m} (\lambda - t) + 1 \right) \right\} \quad (2.3)$$

Y(t) - cumulative biogas production (mL/gVS)

$Y_m$ - ultimate biogas production potential (mL/gVS)

t - time in day

$R_m$  - maximum biogas production rate (mL/gVS.day)

e = 2.7183

$\lambda$ - lag phase time (day)

## **2.9 Summary section of literature review**

The use of co-digestion has demonstrated the highest level of effectiveness in enhancing the production of biogas. In South Africa, there is a knowledge gap in the application of co-digestion of agricultural waste and different substrates i.e. market wastewater. This study investigates this process to better understand the co-digestion process and its application using various substrates. The use of agricultural waste in co-digestion requires that the solid organic waste undergoes mechanical pre-treatment to increase the surface area and decrease particle size. For this investigation, the solid agricultural waste must be crushed and grinded into smaller particles.

The literature also determines operating conditions for the design of the experiment and to maintain an efficient system. Literature compared the application of temperatures in three ranges: mesophilic, thermophilic and psychrophilic. This study will be conducted between 32°C and 42°C at mesophilic conditions to offer a more efficient operation and to use less energy requirements. According to literature, the HRT can be maintained between 10 and 21 days, the organic loading rate for agricultural waste materials must be kept between 1 and 4 kgVS/m<sup>3</sup>day and the pH can be maintained between 6 and 8. This study aims to explore the co-digestion of several types of agricultural waste with market wastewater to enhance biogas production and optimise the system. The kinetic study will use the Modified Gompertz and First order models, which have been developed to model the biogas production.

## CHAPTER THREE: MATERIALS AND METHODS

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This chapter discusses the methodology followed for experimental research, with the aim of evaluating and optimising the anaerobic co-digestion (Co-AD) process for biogas production from agricultural waste. Section 3.1 provides information about the materials and equipment utilised which includes sampling, preparation and characterisation of all the feedstocks. Section 3.2 discusses the experimental process design for the feasibility co-digestion study. Section 3.3 provides information on the design of experiment for the optimisation of process parameters and section 3.4 discusses kinetic study.

### 3.1 Materials and equipment

The first step in conducting the experiments involved determining, quantifying, and collecting the process materials and equipment. The materials included the feedstocks (agricultural waste (AW) and market wastewater (MWW)) for the co-digestion process. The materials were collected, prepared, and characterised for use in the co-digestion process. The equipment and chemicals used were also collected and prepared for the experimental procedure.

#### 3.1.1 Agricultural waste collection

The agricultural waste was collected from the local fruit and vegetables market in Durban, South Africa. The available agricultural waste collected were apples, bananas, oranges, spinach, tomatoes, butternut, potatoes, and carrots. Figure 3-1 shows the waste collection site, and the waste collected from the waste compaction section in the market. The samples were collected in 10L plastic containers and sent to the laboratory to be kept at a temperature of  $\pm 5^{\circ}\text{C}$ .

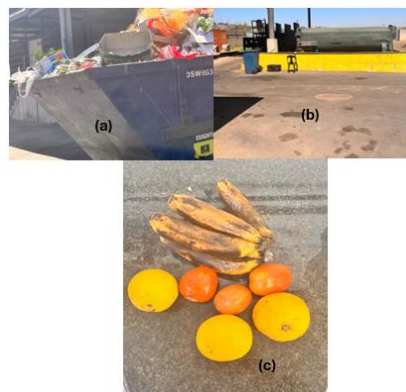


Figure 3-1: (a) Agricultural waste dumpsite (b) Market wastewater sump and agricultural waste compaction area (c) Sample agricultural waste

### 3.1.2 Market wastewater sampling

The market wastewater was obtained from the local fruit and vegetable market located in Durban, South Africa. The market has fruit and vegetable processing sections and wholesale sections for selling fruit and vegetables. Large quantity of wastewater is produced in the market, collected in different sumps around the market, and eventually collected in the final sump. All the sewerage waste is also sent into the sumps from septic tanks. The MWW samples were collected using four 25 L containers at a time, taken to the laboratory, and kept at a temperature of  $\pm 5^{\circ}\text{C}$ . Table 3-1 shows the essential chemical parameters of the MWW. The chemical parameters were determined using standard methods (APHA 2005, 2012).

### 3.1.3 Activated sludge (AS) sampling

Table 3-1 shows the characterisation results of the inoculum used for the co-digestion process activated sludge (AS) after it was characterized using standard wastewater analysis methods (APHA 2005, 2012). The activated sludge was collected from the local wastewater treatment plant in Amanzimtoti, South Africa. Figure 3-2 shows the AS sampling, which was done using 25 L containers.



Figure 3-2: Activated sludge sample collection from a local wastewater treatment plant

Table 3-1: The characterisation of MWW and AS using the APHA standard methods.

| Type                    | Volatile Solids (%) | Total Solids (%) | pH         | COD (mg/L)    |
|-------------------------|---------------------|------------------|------------|---------------|
| Market wastewater (MWW) | 47 ± 5.09           | 12.5 ± 1.98      | 5.5 ± 0.9  | 1858 ± 109.34 |
| Activated sludge (AS)   | 61 ± 6.78           | 14 ± 3.02        | 6.8 ± 1.04 | 8456 ± 278.97 |

### 3.1.4 Agricultural waste preparation and characterisation

#### 3.1.4.1 Sample preparation

The solid fruits and vegetable waste were shredded into smaller particles or pulps using the mechanical pre-treatment method. Figure 3-3 shows the process flow that was followed for the mechanical preparation of the AW. The AW samples were shredded into smaller particles to produce a substrate for digestion and in order to expand the substrate's surface area for adsorption, which would encourage more microbial activity and, as a result, produce more gas (Oyaro *et al.* 2021). The samples were grinded for duration that will allow for a production of a homogenous paste ( $\pm 15$  minutes), and this was applied for all the AW.

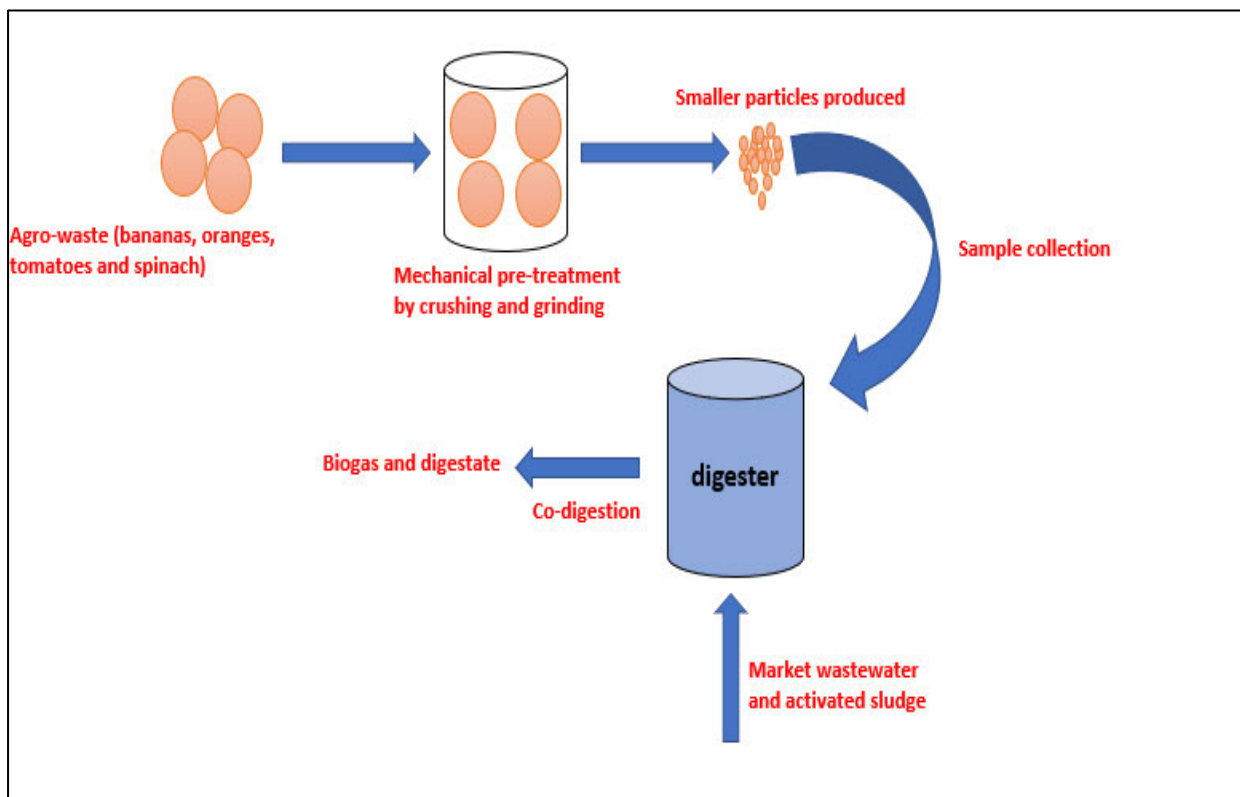


Figure 3-3: Preparation of agricultural waste for co-digestion through mechanical shredding

After being prepared through mechanical treatment to reduce the particle size and increase the surface area of the solid agricultural waste, a sample is collected and sent for analysis. Figure 3-4 shows the process flow of the method followed to send the collected samples for characterisation using the different analytical methods.

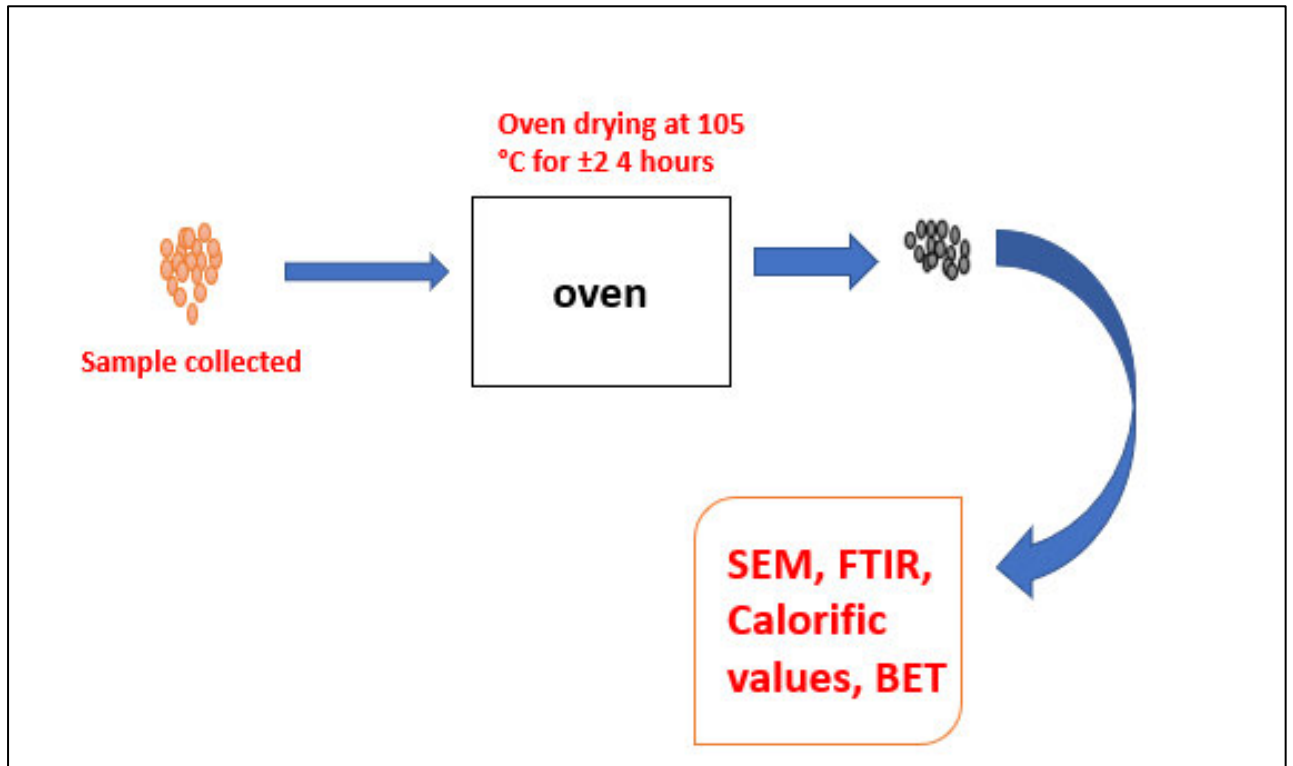


Figure 3-4: Flow diagram of characterisation process of the agricultural waste

#### 3.1.4.2 Brunauer-Emmett-Teller (BET)

The Brunauer-Emmett-Teller (BET) method was used to determine the accurate surface areas and pore size distribution of the agricultural waste. This characterisation approach is based on the physical adsorption of inert gases, such as nitrogen, onto the sample's solid surface. Figures 3-5 depict the BET analytical equipment (Micrometrics TriStar 11 PLUS, United States of America).



Figure 3-5: BET testing equipment (Micrometrics TriStar 11 PLUS)

#### **3.1.4.3 Scanning electron microscopy (SEM)**

The morphology of the waste was analysed using scanning electron microscopy techniques (JOEL JEM-2100, Japan). This analysis was conducted at the Council for Scientific and Industrial Research (CSIR) in Cape Town, South Africa. A voltage of 200 kilovolts (kV) was employed for acceleration. The specimens were dissolved in Ethanol and subjected to sonication for approximately 20 minutes. Subsequently, they were scattered onto a copper grid that had been coated with a layer of carbon, in preparation for analysis.

#### **3.1.4.4 Calorific values**

The eight collected AW samples as well as the mixture (Mix-1) were mechanically prepared and dried and sent for calorific value analysis. This process is significant for determining the energy content in the disposed AW. Figure 3-6 shows an oxygen bomb calorimeter (GLOMRO BXT-ZDHW9B, China) used to determine the calorific values of the various AW. About 0.5g of each component was used for the analysis, which quantified the energy content of the different AW.



Figure 3-6: Oxygen bomb calorimeter (GLOMRO BXT-ZDHW9B)

### 3.1.4.5 Fourier Transform Infrared analysis (FTIR)

The functional groups and molecular breakdown of the model agricultural waste were determined by employing a Fourier Transform Infrared (FTIR) spectrometer. Figures 3-7 show the analytical FTIR spectrometer (Microlab, Agilent Technologies, United States of America) used in this study. The measurements were conducted at room temperature, covering a wavenumber range of 400 – 4000 cm<sup>-1</sup>.



Figure 3-7: FTIR Spectrometer (Microlab, Agilent Technologies)

### 3.1.4.6 Total solids and volatile solids

The Total solids (TS%) were measured according to the standard analysis methods (APHA 2005, 2012). Samples with a specific weight were placed in ceramic jars and dried for 24 hours at 105°C in a convection oven to achieve constant weight to determine the total solids (TS%) (APHA 2005, 2012). For the TS measurement, the samples were weighed after cooling in the desiccators. The samples will then calcine at 550°C for two hours in a furnace (Kiln Contracts, Cape Town, South Africa) to determine the Volatile solids (VS%). Total solids and volatile solids were calculated using standard equation. The VS reduction percentage (%) was determined using Equation 3.3.

$$\% \text{ Total Solids (TS)} = \frac{W_{TOTAL} - W_{CRUCIBLE}}{W_{SAMPLE} - W_{CRUCIBLE}} \times 100 \quad (3.1)$$

$$\% \text{ Volatile Solids (VS)} = \frac{W_{VOLATILE} - W_{CRUCIBLE}}{W_{TOTAL} - W_{CRUCIBLE}} \times 100 \quad (3.2)$$

where:

$W_{TOTAL}$  - Weight of dried residue and crucible (g)

$W_{CRUCIBLE}$  - Weight of crucible (g)

$W_{\text{SAMPLE}}$  - Weight of wet sample and crucible (g)

$W_{\text{VOLATILE}}$  - Weight of residue and crucible after ignition (g)

$$\% \text{VS reduction} = \frac{VS_{\text{initial}} - VS_{\text{final}}}{VS_{\text{initial}}} \quad (3.3)$$

### 3.1.4.7 Moisture and ash percentage (%)

Other important characteristics of agricultural waste included the moisture and ash percentage (%). Standard wastewater analysis methods were applied to determine these characteristics (APHA 2005, 2012).

### 3.1.5 Market wastewater and inoculum characterisation

The collected market wastewater and inoculum were characterised before, during and after the digestion process. The standard methods for the examination of water and wastewater were used to characterise the substrate and inoculum in triplicates (APHA 2005, 2012). The different parameters, equipment and methods that have been applied in this study are shown in Table 3-2.

Table 3-2: Market wastewater and Inoculum (activated sludge) analysis

| Water characteristics | units | Instrument                       | Method          |
|-----------------------|-------|----------------------------------|-----------------|
| COD                   | mg/L  | DR3900 HACH<br>Spectrophotometer | Standard method |
| pH                    |       | pH meter                         | Standard        |
| Total solids          | %     | analytical balance and oven      | APHA            |
| Volatile solids       | %     | analytical balance and oven      | APHA            |

#### 3.1.5.1 pH

The pH of the market wastewater and the inoculum were determined before, during and after the Co-AD of the AW and market wastewater. Figure 3-8 shows the analytical instrument that was used to measured using a pH was a (Sensodirect 150, Lovibond, United States of America) model.



Figure 3-8: pH meter (Sensodirect 150)

### 3.1.5.2 COD

The COD of the market wastewater and inoculum were determined for this Co-AD process. Using the COD high-range vials high range (0-1500 mg/L) (HACH), 0.2 mL of the samples were measured and poured into the COD vials. It was then digested at 150°C for 2 hours. After the digestion, the vials were cooled at room temperature and the COD were measured using a spectrophotometer. Figure 3-9 shows the model of the spectrophotometer (DR3900, HACH, United States of America). The COD removal percentage (%) was determined using Equation 3.4.

$$\% \text{COD Removal} = \frac{\text{COD}_{\text{initial}} - \text{COD}_{\text{final}}}{\text{COD}_{\text{initial}}} \quad (3.4)$$

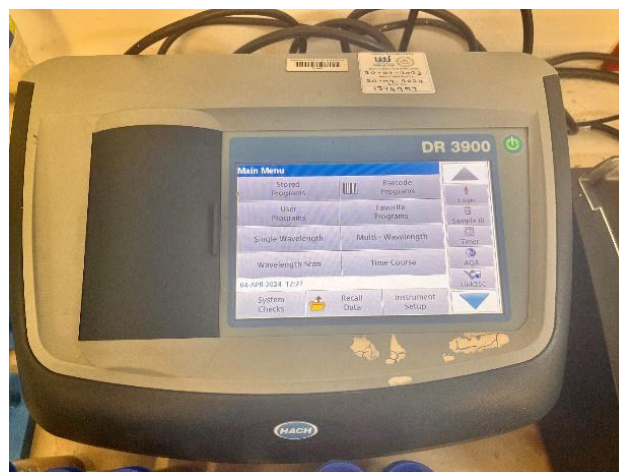


Figure 3-9: HACH DR3900 spectrophotometer.

### 3.1.6 Biogas Composition Characterisation

The AW and the market wastewater Co-AD process were used to determine the efficiency of biogas production using the two substrates. The product is biogas, and the methane composition in the produced biogas needs to be characterised. The characterisation of the biogas was conducted using gas chromatography, where the percentages of methane and carbon dioxide were determined (Okewale *et al.* 2019). Figures 3-10 show the gas chromatography equipment (GC-2014 SHIMADZU, Japan) used to determine the methane composition in the biogas.



Figure 3-10: Gas chromatography unit GC-2014 SHIMADZU

### 3.1.7 Chemicals

The experimental procedure also utilised some industry-grade chemicals. Sodium hydroxide (NaOH) (Sigma-Aldrich, United States of America) was used for the pH adjustment during the experiments, and 57% Hydrochloric acid (HCL) was also used.

## 3.2 Feasibility study of the co-digestion process of agricultural waste and market wastewater

### 3.2.1 BMP batch experiments

A feasibility investigation was carried out using five different agricultural wastes to ascertain whether the AW and MWW co-digestion process is effective in improving the performance of the anaerobic digestion system, Bananas, apples, carrots, potatoes, and butternuts were the agricultural waste used for the study. A laboratory scale batch-wise anaerobic system was set for the BMP test and operated as per standard operating conditions (Hülsemann *et al.* 2020). Figure 3-11 depicts the BMP test setup (adapted from Paper 1, on which the author designed the experiment, conducted laboratory experiments, analysed the data and authored the findings

in a manuscript). The equipment images are attached in Appendix C, Figure C-1 and C-2. The setup consists of a biodigester, a biogas collecting system, and a water bath (WBST0001) system for controlling the temperature. 1 L Schott bottles with a working volume of 800 mL were used as biodigesters, closed with Teflon caps (three ports). The outlet gas nozzle was connected to the biogas collecting unit through the downward displacement technique using a 1 L graduated cylinder placed upside down in another 10 L container filled with water.

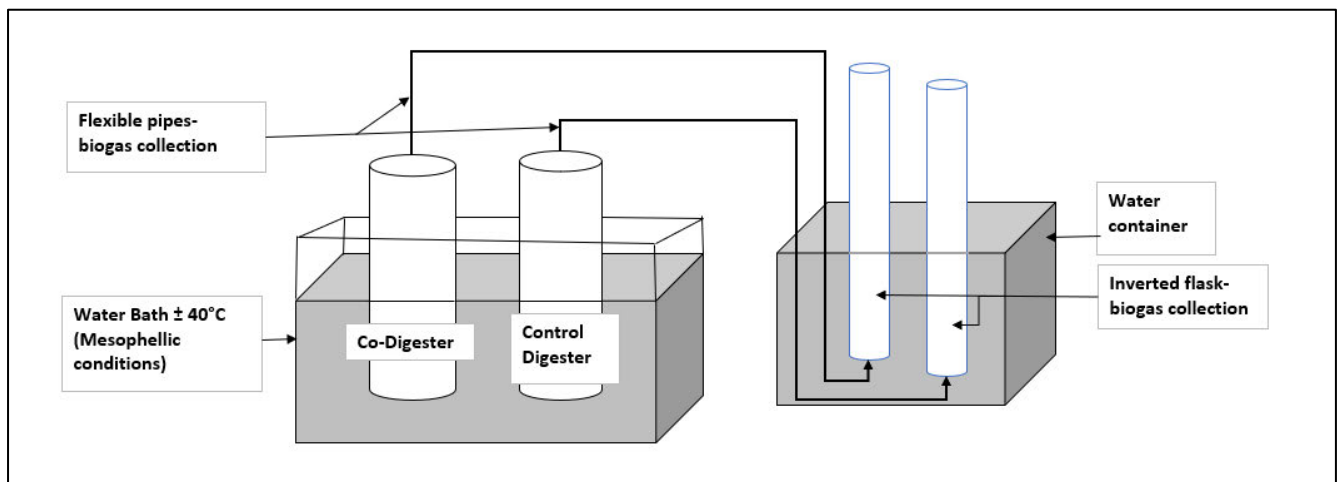


Figure 3-11: Experimental equipment for the BMP test, adapted from (Mkhize *et al.* 2023)

The initial BMP study was carried out by charging seven (7) bioreactors, with feedstocks having a weight percentage (% w/w) ratio (based on VS) of 1:1:2 (i.e., AWs: MWW: inoculum) for a hydraulic retention time of 21 days, at a fixed organic load of 2.5 kgVS/m<sup>3</sup>.day was applied to all the digesters. The design of this experiment and operating conditions were supported by previous studies that conducted co-digestion of the AWs with different co-substrates (Masebinu *et al.* 2018; Seswoya 2019). The bioreactors, after charging, were purged with nitrogen gas to establish anaerobic conditions. Daily monitoring of the biogas was done using the downward displacement method of gas collection. After the digestion period, the water quality and biogas compositions were evaluated.

### 3.2.2 BMP setups to determine the effect of substrate to substrate (SS) ratio and different mix combinations

The performance of the co-digestion process has been attributed the perfect SS mixing ratio, therefore it is important to optimise the SS ratio (Mata-Alvarez *et al.* 2014) Table 3-3 shows the set of BMP batch experiments conducted on two different combinations of mixed AW feedstocks (Mix-1 and Mix-2). The feedstocks' I/S ratio was kept constant at 2 while the SS

ratio was varied. The SS ratio (AW/MWW) was 0 (control where no MWW was added), 1, 2, and 3. The Co-AD was run for 21 days at an OLR of 2.5 kgVS/m<sup>3</sup>.day and 40°C.

Table 3-3: Two mixed AW combinations used for the BMP co-digestion experiments

| Sample | Combination of AW   |
|--------|---|
| Mix-1  | Equal amounts of apples, bananas, tomatoes, oranges and spinach   |
| Mix-2  | Equal amounts of potatoes, butternut, apples, bananas and carrots |

### 3.3 Prediction model development and process optimisation of the co-digestion process

#### 3.3.1 The design of experiment (DOE): experimental runs

The Design Expert (version 11.1.2.0) software was used for the design of the experiment (DOE), prediction model development, process optimisation, and validation of output. The Box-Behnken design (BBD) of the experiment was used from the response surface methodology (RSM) for the design of experiments (DOE) and optimisation of operating parameters. The four input variables considered included pH, hydraulic retention time, mesophilic temperature and organic loading rate, as shown in Table 3-4. The numerical values -1, 0, and +1 represented the three standardised code levels corresponding to the low, median, and maximum values. To reduce bias, the experimental runs were randomised. The validation runs were conducted under the predicted optimal conditions, consisting of three consecutive runs with identical settings.

Table 3-4: Input factors for the RSM design matrix

| Input variables                                 | Variable Code | Code levels |      |    |
|---|---------------|-------------|------|----|
|   |               | -1          | 0    | 1  |
| Temperature (°C)                                | A             | 32          | 37   | 42 |
| pH  | B             | 6           | 7    | 8  |
| Hydraulic Retention Time (days)                 | C             | 10          | 15.5 | 21 |
| Organic loading rate (kgVS/m <sup>3</sup> .day) | D             | 1           | 2.5  | 4  |

### 3.3.2 Process prediction of optimum conditions: Box Behnken design (BBD)

The BMP process was numerically optimised using the BBD of the Design-expert. This was done by fitting the variables obtained for the dependent responses while maximising the response values. The independent factors were all kept within a specified range. Table 3-5 shows each input factor ranges and desired goal. Table 3-6 shows the goals for the three chosen responses were determined for each response. The three responses (biogas production (mL/day), volatile solids reduction (%), and COD removal (%)) were investigated with RSM using the optimised conditions attained experimentally. The response variables received statistical analysis using RSM to determine the impact of interactions between the process components on the BMP process. The three responses were optimised, and the optimal solutions were created. The optimisation process produced solutions with a confidence level of 95%.

Table 3-5: BBD design constraints for the input factors co-digestion process

| Factor | Name                     | Units                    | Minimum | Maximum | Goal        |
|--------|--------------------------|--------------------------|---------|---------|-------------|
| A      | Temperature              | °C                       | 32      | 42      | Is in range |
| B      | pH                       |                          | 6       | 8       | Is in range |
| C      | Hydraulic Retention Time | days                     | 10      | 21      | minimise    |
| D      | Organic loading rate     | kgVS/m <sup>3</sup> .day | 1       | 4       | Is in range |

Table 3-6: BBD design goals for the responses in the co-digestion process

| Response       | Name              | Units  | Observations | Goal     |
|----------------|-------------------|--------|--------------|----------|
| R <sub>1</sub> | Biogas production | mL/day | 26.00        | maximise |
| R <sub>2</sub> | VS reduction      | %      | 26.00        | maximise |
| R <sub>3</sub> | COD removal       | %      | 26.00        | maximise |

### **3.3.3 Process validation of the RSM predicted optimum conditions**

The numerical optimisation of the BBD matrix was utilised to generate optimum conditions and predicted responses with a confidence level of 95%. The response models were validated by comparing the predicted responses to the experimentally generated responses. The experiments were conducted using triplicates, to ensure process repeatability.

### **3.4 Kinetic study**

The optimum operating conditions for biogas enhanced production were determined and used for the kinetic study. The kinetics models that were used were first-order kinetics (Equation 2.2) and the Modified Gompertz (Equation 2.3) models as applied by Oyaro, Oonge and Meshack (2021). This was done using the resultant experimental data from the compatible waste from the market. The kinetic constants were established by modelling the data acquired using non-linear regression analysis with Origin Lab software (2019 version).

### **3.5 Summary**

The agricultural waste was collected from the local fresh produce market in plastic containers and cleaned to remove any foreign matter and therefore stored in a low temperature of  $\pm 5^{\circ}\text{C}$  to avoid further deterioration. The market wastewater and activated sludge were also collected and kept at low temperatures of  $\pm 5^{\circ}\text{C}$ . The agricultural waste was mechanically crushed, and samples collected for characterisation. The characterisation included moisture, ash, total solids, volatile solids, SEM, FITR, BET and calorific values. The market wastewater and activated sludge were analysed using standard methods for COD, pH, total solids and volatile solids. The feasibility BMP study was conducted for HRT of 21 days, OLR of  $2.5 \text{ kgVS/m}^3 \cdot \text{day}$  and temperature of  $\pm 40^{\circ}\text{C}$  to validate the performance of AW in producing biogas. The design expert software was utilised to optimise the operating conditions such as temperature, OLR, HRT and pH. The design produced 26 experimental runs to check the responses such as biogas production, volatile solids reduction and COD removal. The kinetic study was done using Origin software with the two model equations chosen being the first order and the Modified Gompertz equations.

## CHAPTER FOUR: RESULTS AND DISCUSSION

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This chapter presents the findings derived from the research investigation. The results encompass the properties of the utilised agricultural waste, which was conducted prior to the Co-digestion (Co-AD) study and is reported in section 4.1. Section 4.2 provides information about the feasibility study, where a BMP test was conducted on different agricultural waste for the co-digestion process. In section 4.3, the study discusses a comprehensive analysis of the predictive model research carried out for the BMP system for the co-digestion process. Section 4.4 provides findings from the kinetic investigations.

### 4.1 Characterisation of agricultural waste

This section presents findings from pre-treated agricultural waste (AW) characterisation. Some of the results presented in this section are published in the candidate's Paper 6, in which the candidate was the main author, designed and conducted the laboratory experiments, analysed the data, wrote the manuscript and presented it at the conference.

#### 4.1.1 Characterisation of agricultural waste

Agricultural waste (AW) was collected from the site and sent for characterisation. Characterisation is an important step in identifying the important characteristics in the feedstocks that motivate their application in biogas production.

##### 4.1.1.1 Chemical properties of the agricultural waste (AW)

The chemical characteristics of the AW were determined for the single feedstocks (bananas, apples, tomatoes, spinach, potatoes, carrots, butternuts and oranges), and this was also determined in the mixed sample (with equal weight (wt%) contributions of the single feedstocks). The Mix-1 sample has also been characterised, and it has a combination of five agricultural wastes in equal amounts in weight (wt%): apples, bananas, tomatoes, oranges and spinach. Table 4-1 shows the parameters of the AW that were characterised before the co-digestion process was conducted. The moisture content (%), ash content (%), volatile solids (VS) (%) and total solids (TS) (%) were measured using standard procedures as discussed in section 3.1. The high ranges of moisture above 70% and above 80% for the volatile solids (as a percentage of the total solids) for the agricultural waste can be good for the co-digestion process as per literature (Sharma *et al.* 2024).

Table 4-1: Characterisation of chemical properties of the agricultural waste

| <b>Agricultural waste</b> | <b>Moisture content (%)</b> | <b>Ash Content (%)</b> | <b>Volatile Solids (%)</b> | <b>Total Solids (%)</b> |
|---------------------------|-----------------------------|------------------------|----------------------------|-------------------------|
| Bananas                   | 73 ± 7.02                   | 2 ± 0.5                | 91 ± 10.34                 | 19 ± 3.45               |
| Apples                    | 78 ± 9.08                   | 0.5 ± 0.06             | 88 ± 6.74                  | 19 ± 2.32               |
| Tomatoes                  | 90 ± 10.98                  | 0.28 ± 0.034           | 87 ± 9.45                  | 8 ± 1.00                |
| Spinach                   | 85 ± 8.08                   | 1.35 ± 0.26            | 85 ± 6.39                  | 13 ± 1.37               |
| Oranges                   | 82 ± 6.45                   | 3 ± 0.76               | 79 ± 10.2                  | 12 ± 2.00               |
| carrots                   | 84 ± 8.34                   | 3 ± 0.65               | 85 ± 8.05                  | 8 ± 1.02                |
| Potatoes                  | 85 ± 4.76                   | 3 ± 0.83               | 82 ± 7.48                  | 9 ± 1.07                |
| Butternut                 | 75 ± 6.47                   | 2.8 ± 0.4              | 87 ± 7.88                  | 15 ± 1.29               |
| Mix-1                     | 87 ± 10.76                  | 0.6 ± 0.05             | 90 ± 6.30                  | 15 ± 0.87               |

#### 4.1.1.2 Surface area, pore size, and pore volume of the agricultural waste

The process of Co-AD required that the solid AW be pre-treated using mechanical methods by crushing into smaller particles and therefore increasing the surface area. The BET surface area was measured using standard procedures as detailed in section 3.1. Table 4-2 shows the results of the BET surface area, pores diameter, and pore volumes. The surface areas of these AWs were small, and it is well-known that carbonaceous materials tend to have low surface areas (Nascimento *et al.* 2014). Spinach had the highest surface area of 1.70 m<sup>2</sup>/g. The surface area plays a significant role in enhancing the process of biogas production. The surface area of the bananas, measured at 0.92 m<sup>2</sup>/g, surpasses the surface area reported by Pathak *et al.* (2017), which was 0.65 m<sup>2</sup>/g. The discrepancies in these surface areas may be attributed to the operational intricacies involved in drying lignocellulosic samples (Pathak *et al.* 2017). The increase in surface area and reduction of particle size in pre-treatments resulted in enhanced water retention capacity of agricultural waste, which led to the change in the biochemical features, composition and behaviour of the agricultural waste resulting in better performance in biogas production (Coarita Fernandez *et al.* 2020).

The pores are very important in the biogas production process as it offers space for the microorganisms to attach and biodegrade to form biogas (Coarita Fernandez *et al.* 2020;

Fernandez *et al.* 2022). The pore volumes for the bananas were the highest at 0.19 nm, the apples had the pore volume of 0.068 nm. The lowest pore volume was from the oranges at a low value of 0.096 nm.

Table 4-2: BET surface area, pore volume and pore diameter of pre-treated agricultural waste

| <b>Agricultural waste</b> | <b>Surface Area (m<sup>2</sup>/g)</b> | <b>Pore volume (cm<sup>3</sup>/g)</b> | <b>Pore diameter (nm)</b> |
|---------------------------|---------------------------------------|---------------------------------------|---------------------------|
| Bananas AW                | 0.92                                  | 0.000079                              | 0.19                      |
| Apples AW                 | 1.07                                  | 0.000053                              | 0.068                     |
| Spinach AW                | 1.70                                  | 0.000035                              | 0.034                     |
| Oranges AW                | 0.87                                  | 0.000007                              | 0.0096                    |
| Tomatoes AW               | 1.10                                  | 0.000041                              | 0.065                     |

#### 4.1.1.3 Functional group characteristics of various types of agricultural waste

The FTIR spectra graphs were obtained to determine the different functional groups in the different AW samples, shown in Figure 4-1. Table 4-3 shows the functional groups results, and the wavelengths obtained from the FTIR spectra graphs of the different AW. The presence of aromatics, C-O stretching, C=C stretching C-H, CH<sub>2</sub> bending, and O-H stretching was determined in the analysis of the wavelengths (772 - 3280 cm<sup>-1</sup>). These functional groups can either enhance or limit the digestion process, which depends on their vibration level (Kayembe *et al.* 2012; Yanti *et al.* 2014). It was noted that aromatic structures have a relationship with their inhibitory impacts on methanogenic bacteria, which may explain the underperformance of some of these AWs (Kayembe *et al.* 2012).

Table 4-3: Table showing observed wavelength peaks of FTIR charts of the agricultural waste

| <b>Wavelength (cm<sup>-1</sup>)</b> | <b>Functional group</b>      |
|-------------------------------------|------------------------------|
| 3280, 2922                          | O-H stretching               |
| 1640                                | C=C stretching               |
| 1320                                | C-H, CH <sub>2</sub> bending |
| 1020                                | C-O stretching               |
| 772                                 | Aromatics                    |

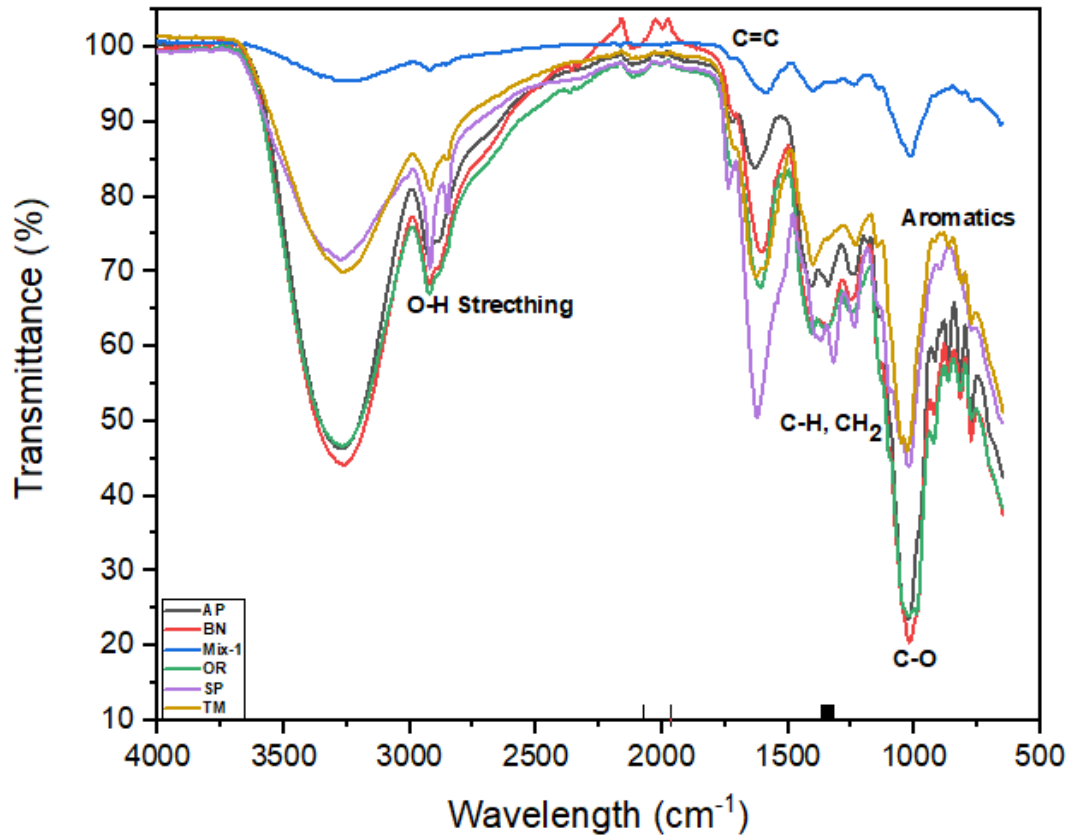


Figure 4-1: FTIR Charts of different agricultural waste

#### 4.1.1.4 Calorific values of agricultural waste

The calorific values of the feedstocks under analysis were determined using a bomb calorimeter. From Table 4-4, the bananas and apples had the highest calorific values of 14.54 and 15.00 MJ/kg, respectively. The carrots AW had the lowest calorific value of 11.15 MJ/kg. The Mix-1 sample had a calorific value of 13.33 MJ/kg. The observed calorific values were within the ranges reported for vegetables as 20.58 MJ/kg (Jiang *et al.* 2012). Scano *et al.* (2014) utilised AWs with calorific values ranging between 13.3-17.6 MJ/kg to produce biogas, which affirms calorific values obtained in this study and the resultant biogas production. Furthermore, a calorific value of 16.5 MJ/kg was obtained after pre-treatment of AWs to enhance the anaerobic co-digestion process (Edwiges *et al.* 2018). The calorific values of these AWs motivated this application of co-digestion with municipal waste (MWW) for biogas production.

Table 4-4: Calorific values of agricultural waste

| Agricultural waste | Calorific values (MJ/kg) |
|--------------------|--------------------------|
| Bananas            | 14.54                    |
| Spinach            | 14.54                    |
| Oranges            | 14.60                    |
| Tomatoes           | 14.10                    |
| Apples             | 15.00                    |
| potatoes           | 13.46                    |
| carrots            | 11.15                    |
| Butternuts         | 12.43                    |
| Mix-1              | 13.33                    |

#### 4.1.1.5 Agricultural waste surface morphology

The SEM micrographs were acquired to provide analytic characterisation of each AW. The SEM micrographs provide information about the particle geometry and the structures after mechanically pre-treatment. Pre-treatment facilitates the conversion of the complex structure of the solid food waste into a more simplified form (Deepanraj *et al.* 2017).

Figure 4-2 (a) to (d) shows the images taken for the SEM analysis. Figure 4-2(a) represents the micrograph for banana agricultural waste taken at 500kx magnification, 50µm microscale and a width distance (WD) of 8.3 mm. AW from tomatoes, spinach and oranges is depicted in Figure 4-2 (b) to (d); all micrographs have a 260kx magnification, 100µm microscale. The WD for the tomatoes, spinach and oranges is of 8.7 mm, 8.4 mm, and 7.8 mm, respectively. For bananas as seen in Figure 4-2 (a), it can be observed that the particles are rough and clumped together into larger separate clumps, also having holes and that there is a bigger surface area available for the activity of adsorption of microorganisms, and hence microorganisms becomes more accessible (Morales-Polo *et al.* 2021). A study by, which similarly employed mechanical shredding of agricultural waste feedstock, resulted in a roughened and punctured fibrous structure that is more susceptible to microbial anaerobic breakdown (Garuti *et al.* 2022).

Figure 4-2 (b) shows an image of tomatoes with many pores and an irregular shape. Spinach has a large flat structure, as can be observed in Figure 4-2 (c). Oranges, as seen in Figure 4-2

(d), have particles of the same shape and size. All these AW have surface areas that offer a good area for substrate adsorption.

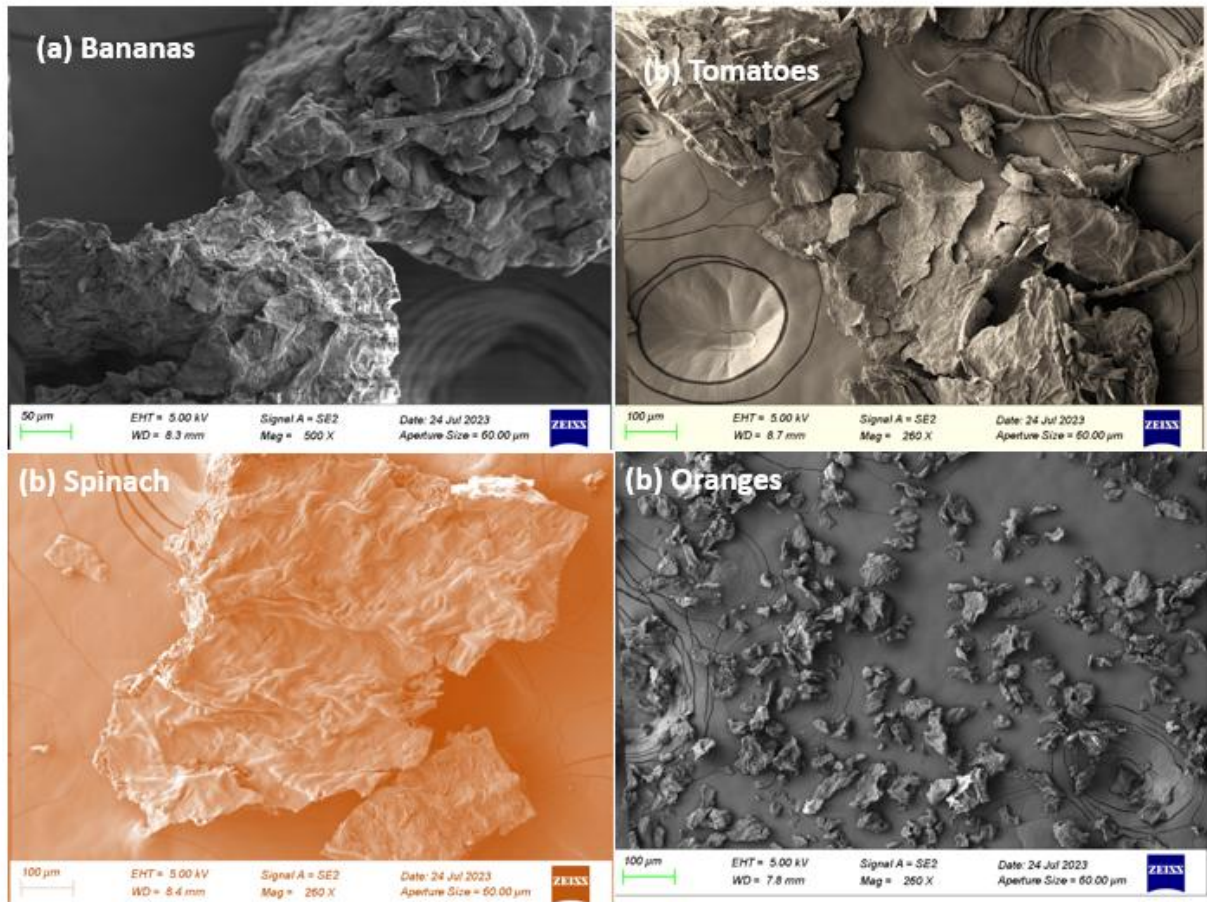


Figure 4-2: SEM micrographs for the pre-treated agricultural waste (a) Bananas AW (b) Tomatoes AW (c) Spinach AW (d) Oranges AW

The pictures captured for the scanning electron microscope (SEM) investigation for the apples, butternut, carrots, potatoes, Mix-1 are depicted in Figures 4-3 (a)- (e). The SEM micrograph of apples AW was captured at a magnification of 500x, with a microscale of the image of 500µm and a width distance (WD) of 5.95 mm. The Mix-1 SEM micrograph is depicted in Figure 4-3 (e) at a magnification of 10kx, a WD of 5.22 mm and a microscale of 5 µm. The SEM micrographs displayed the surface morphology of the AW, where the apple AW has a structure where the particles are conglomerated and have enough pores to enhance the Co-AD process. The Mix-1 AW, with a combination of different AW, provided a surface that is smooth and rough in some areas and possesses pores.

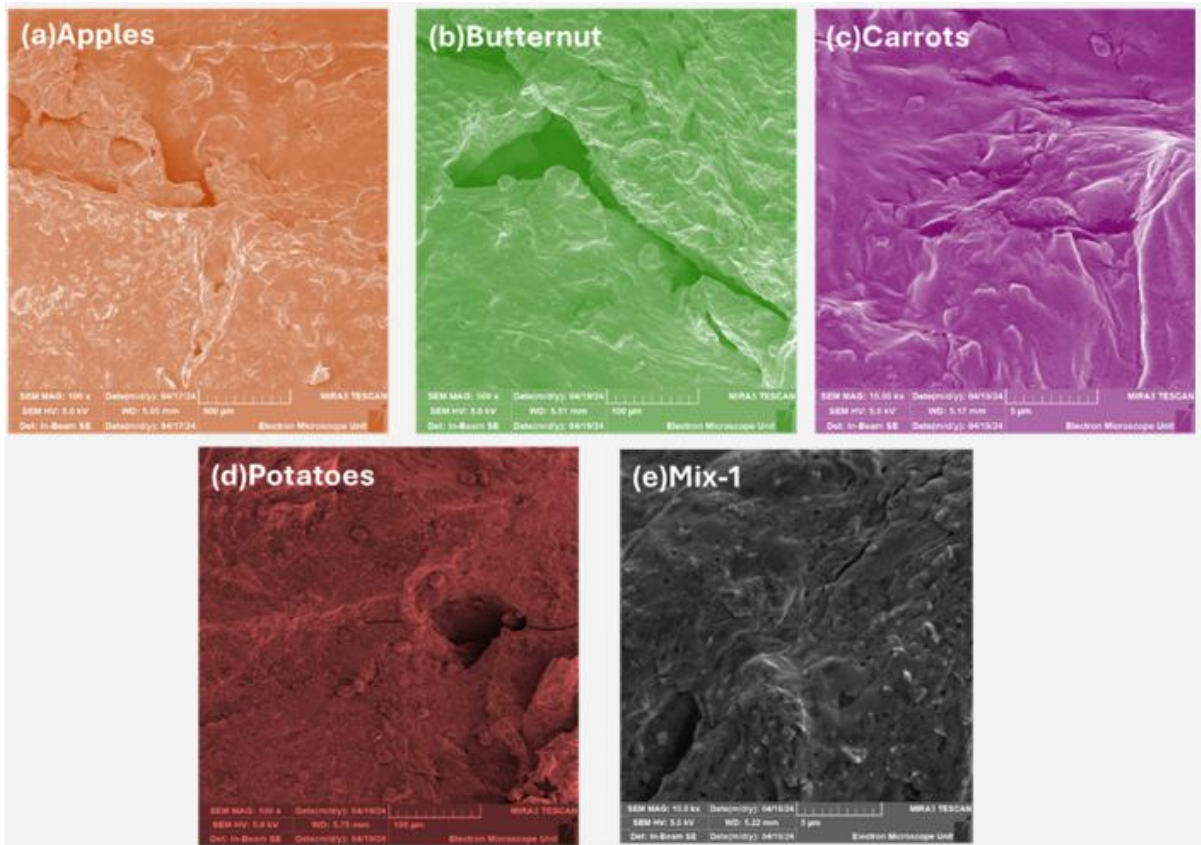


Figure 4-3: SEM micrographs for the pre-treated agricultural waste (a) Apples AW (b) Butternut AW (c) carrots AW (d) potatoes AW (e) Mix-1

#### 4.1.2 Summary

Many aspects of the mechanically pre-treated AW were evaluated for the characterisation process, including their surface shape, functional groups, surface area, pore size, volume, and energy content. The results indicate that the mechanical pre-treatment technique was effective. The energy content demonstrates the applicability of the AW in energy recovery, with the average high energy content of 13.68 MJ/kg. The surface area of AW falls between 0.92 to 1.70 m<sup>2</sup>/g, with the spinach having the highest followed by tomatoes and apples with 1.10 and 1.07 m<sup>2</sup>/g, respectively. The objective of the mechanical treatment was achieved by effectively reducing the size of the solid AW and increasing the surface areas.

## 4.2 BMP Feasibility of the co-digestion process of agricultural waste and market wastewater (Paper 1)

### 4.2.1 BMP study for the co-digestion process of the AW and MWW

This section presents some of the results published in Paper 1, where the author developed the experimental procedure, conducted experiments, collected data, analysed the data and developed a manuscript with findings as the main author. The initial stage of experiments was centred on the simultaneous digestion of AW produced from remnants of fruits and vegetables (namely potatoes, spinach, butternut, carrots, apples, oranges, tomatoes, and bananas) with wastewater (MWW), utilising activated sludge as an inoculum to enhance biogas production.

The initial investigation utilised the five agricultural waste, (potatoes, butternut, apples, bananas and carrots) and Mix-2 (which had equal parts of the individual AW), and was carried out at a temperature of around 40°C, known as mesophilic conditions, with a hydraulic retention time (HRT) of 21 days, an OLR of 2.5 kgVS/m<sup>3</sup>.day and a weight percentage (% w/w) ratio (based on VS) of 1:1:2 (i.e., AWs: MWW: inoculum). The control system digester did not contain the AW. Table 4-5 shows the seven digesters and the feedstocks that were fed into each digester.

Table 4-5: BMP digester setups for the Co-AD process

| Digester name | Feedstock                  |
|---------------|----------------------------|
| Apple-WW      | Apples AW and MWW in AS    |
| Banana-WW     | Bananas AW and MWW in AS   |
| Butternut-WW  | Butternut AW and MWW in AS |
| Potato-WW     | Potato AW and MWW in AS    |
| Mix-WW        | Mix-2 and MWW in AS        |
| Carrot-WW     | Carrot AW and MWW in AS    |
| Control       | MWW in AS                  |

MWW (market wastewater), AW (agricultural waste), AS (activated sludge), Mix-2 (potatoes, butternut, apples, bananas and carrots)

The results shown in Figures 4-4 and 4-5 clearly demonstrate a synergistic impact in the co-anaerobic digestion (Co-AD) of the model anaerobic wastewaters (AWs) with wastewater (WW). The biogas production findings clearly indicate that the co-digestion of apples-WW and banana -WW resulted in a significantly greater biogas yield of over 32% and 30%, respectively,

compared to the control. The apple-WW had the highest biogas production at 595 mL/day, and banana peels-WW with 585 mL/day and the control (450 mL/day). The Mix-2 (Mix-WW) digester produced 490 mL/day and the AW with the least amount of biogas production was the carrot-WW (475 mL/day).

This effect is achieved by combining the beneficial characteristics of both substrates, which allows for easy biodegradability and also the presence of carbohydrates, lipids, and proteins (Bouallagui *et al.* 2003; Triolo *et al.* 2012; Abbass Jafari *et al.* 2017; Rahman *et al.* 2021). The high moisture levels of apples (73%) and banana peels (78%), as shown in section 4.1 ( Table 4-1), are suitable for the anaerobic digestion (AD) process as the two substrates were shown to be among the best performing AW (Atelge *et al.* 2020a). The present investigation found that the bananas had a (VS) of 91%, which is similar to the other study that observed a VS of 87% (Tumutegyereize *et al.* 2011). The calorific values achieved in this study, as shown in Table 4-1 for the bananas and apples waste, were also high at 14.54 MJ/kg and 15.00 MJ/kg, respectively. The notable calorific values observed in these AW samples indicate that these easily accessible renewable resources possess the capacity to be converted into bioenergy in the form of biogas (Edwiges *et al.* 2018)

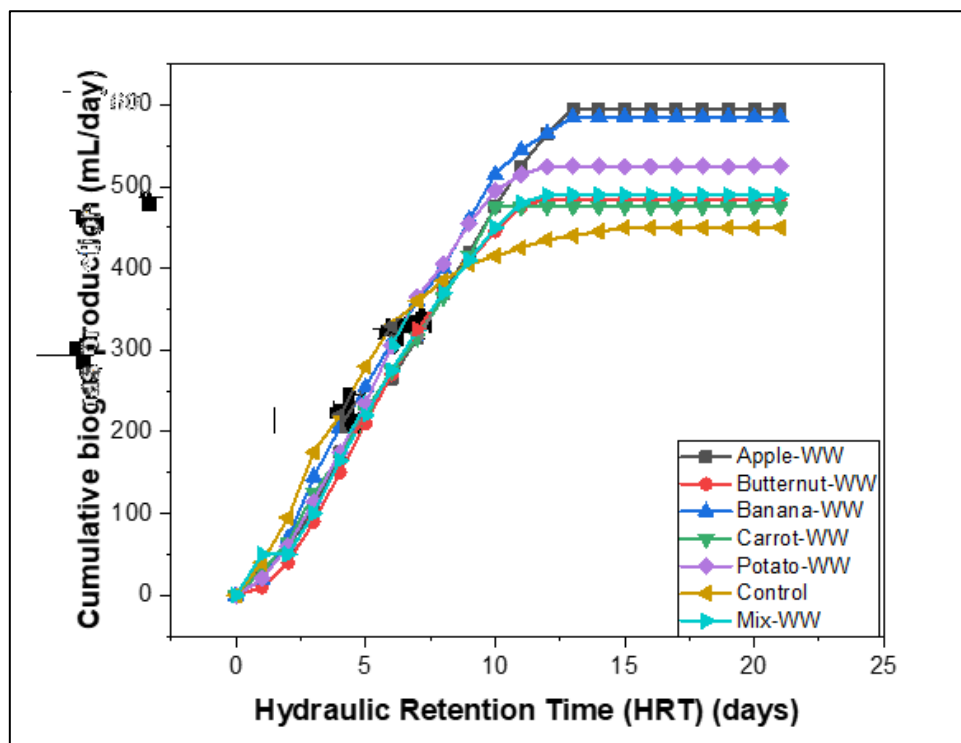


Figure 4-4: Cumulative biogas production of different agricultural waste from a BMP co-digestion, with an OLR of 2.5 kgVS/m<sup>3</sup>.day, a temperature of 40°C and an HRT of 21 days.

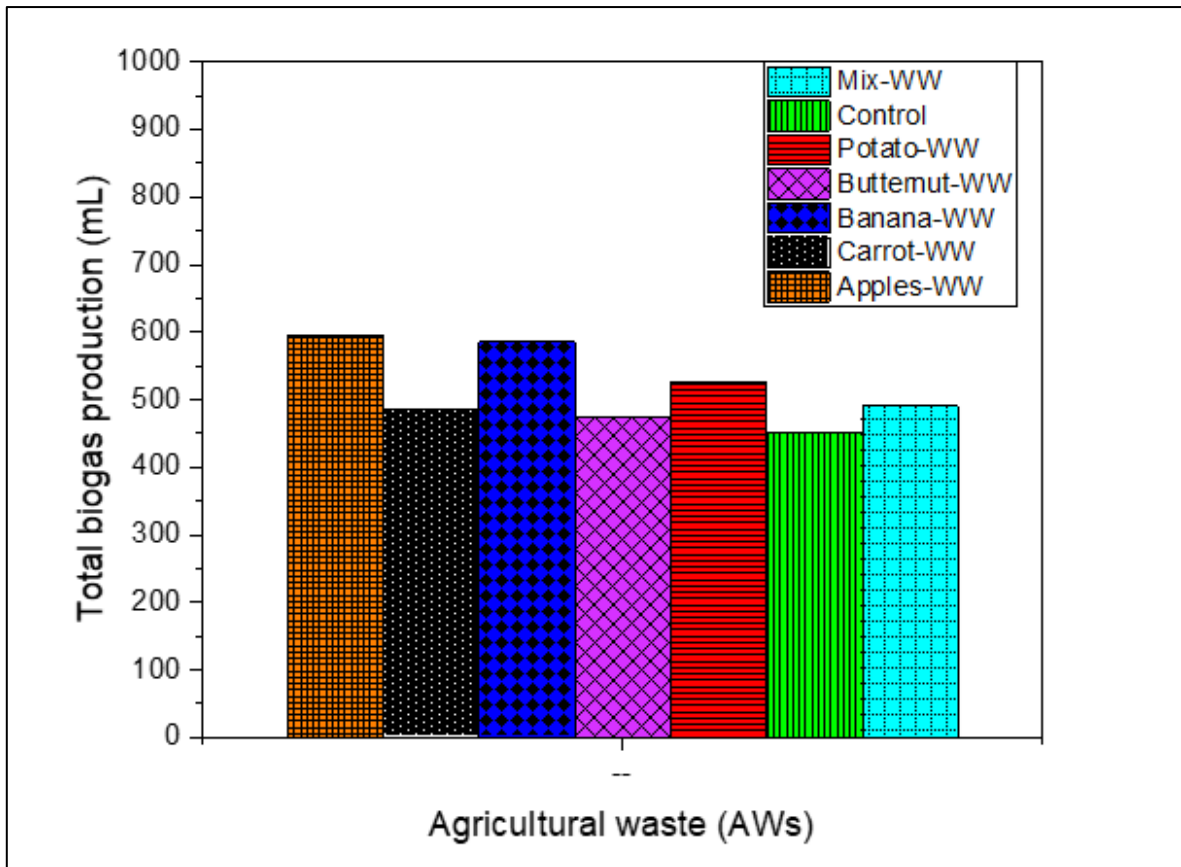


Figure 4-5: Total biogas production of different agricultural waste from a BMP co-digestion, with an OLR of 2.5 kgVS/m<sup>3</sup>.day, a temperature of 40°C and an HRT of 21 days.

Figure 4-6 shows the average daily biogas production of the digesters, where the Apple-WW had the highest daily biogas production at 28.33 mL/day, Banana-WW at 27.86 mL/day, Potato-WW at 25 mL/day, the Mix-WW 23.33 mL/day, the Butternut-WW at 23.10 mL/day, the Carrot-WW with 22.62 mL/day and lastly, Control at 21.43 mL/day. Observing Figure 4-6, it is evident that the digesters containing AWs ceased production from day 10 to day 14, which aligns with other research findings, which also found a rapid deterioration of the substrate starting from day eleven, as evidenced by the steep negative slope (Park *et al.* 2012; Seswoya 2019). The cessation of biogas generation on day between day 10 to 14 can be linked to the high volatile solids contents of the AW and the high rate of biodegradability leading to shorter running periods (Osunde *et al.* 2017). The shorter periods are not a disadvantage as the biogas quantities and quality were not affected.

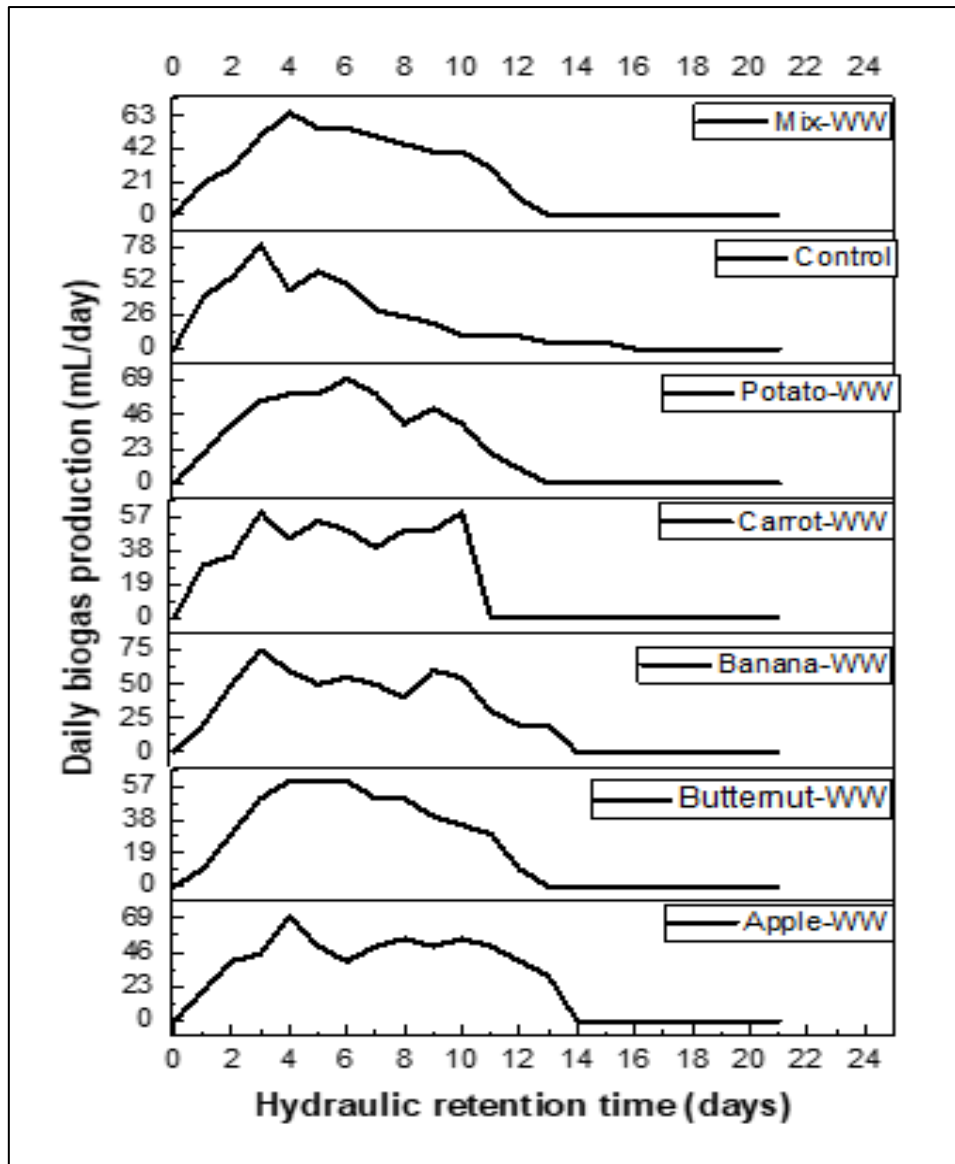


Figure 4-6: Daily production of different agricultural waste from a BMP co-digestion, with an OLR of 2.5 kgVS/m<sup>3</sup>.day, a temperature of 40°C and an HRT of 21 days.

From Figure 4-7, the impact on pH during the 21 days Co-AD period is illustrated. The pH levels within each biodigester were continuously monitored during the whole Co-AD process to guarantee that microbial activity was not hindered by acidic conditions. The optimal pH range for hydrolysis and acidogenesis is 5.5 - 6.5, which is lower than the pH range required for methanogenesis, which is 6.5 - 8.2 (Neto et al., 2021). The starting pH of the digesters was maintained at a pH level higher than 7 (Mao et al. 2015). The pH of all biodigesters with anaerobic wastewater (AWs) shows a consistent decrease during the initial stages of co-digestion (Co-AD). The pH performance in the digestion of the control exhibits a consistent

trend and this will not disturb methanogenic activity, indicating that the system has enough buffering ability to sustain a steady pH level (Widyarani *et al.* 2018).

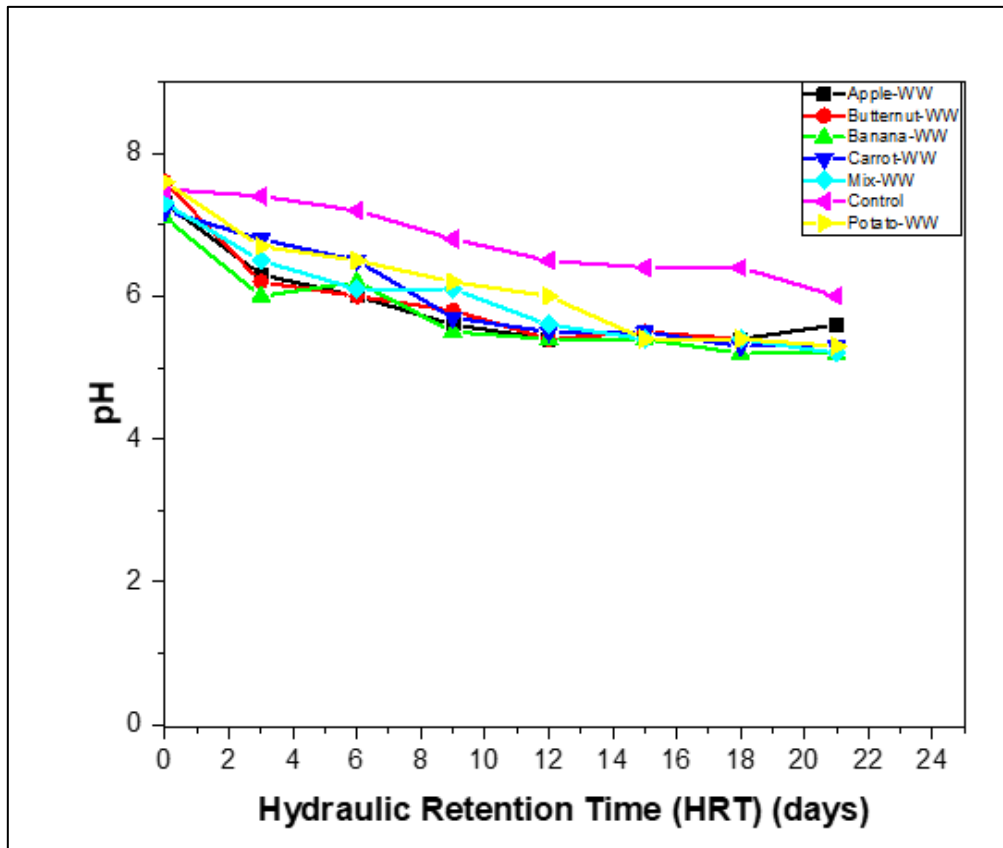


Figure 4-7: Effect of pH on the BMP co-digestion, with an OLR of 2.5 kgVS/m<sup>3</sup>.day, a temperature of 40°C and an HRT of 21 days.

The analysis of the methane composition of the systems was conducted and the results are shown in Table 4-6. The methane composition of the systems was high, with Butternut-WW system having the lowest at 55% and the Apples-WW producing the highest methane composition at 68%. The performance of the Apples-WW can be attributed to the C/N ratio, where a high ratio of 35 was able to perform better when co-digested with MWW, Apples-WW when co-digested with other co-substrates can produce a varying methane composition; when co-digested with swine manure (62%) (González-García *et al.* 2022). The solid agricultural waste substrate with a high carbon-to-nitrogen (C/N) ratio (Apples) is inappropriate for bacterial growth because it lacks adequate nitrogen, as a result, the rate at which gas is produced and the ability of solids to break down will be reduced, while at low C/N (market wastewater), the breakdown process leads in the formation of ammonia, which is detrimental to the bacteria (Atelge *et al.* 2021; Pan *et al.* 2021). Therefore, the co-digestion of the two to a C/N ratio of 35, which is still above the 20-30 ratio to mitigate the undesirable biological interactions was

good for this system and it led to the production of adequate nitrogen and no formation of ammonia (Elfeki *et al.* 2017).

The control system also produced a good methane composition at 62%. Most of the digesters had a composition above 60% which is a good performance, and it has been seen than the fruit AW usually performs better in methane production (Asquer *et al.* 2013).

Table 4-6: Methane composition produced by the BMP Co-AD systems

| System        | Methane composition (%) | Carbon to Nitrogen (C/N ratio) |
|---------------|-------------------------|--------------------------------|
| Bananas-WW    | 62                      | 25                             |
| Mix-WW        | 60                      | 21                             |
| Control       | 65                      | 12                             |
| Potatoes-WW   | 66                      | 22                             |
| Carrots-WW    | 55                      | 27                             |
| Butternuts-WW | 57                      | 20                             |
| Apples-WW     | 68                      | 35                             |

#### 4.2.2 Kinetic study of co-digestion BMP study of agricultural waste and MWW

The kinetics of the anaerobic digestion process are analysed throughout the duration of the digestion phase. The kinetics of the co-digestion BMP process in each digester were modelled by fitting their cumulative biogas production to First order and Modified Gompertz models, as shown in Equations 2.2 and 2.3. The kinetic models are known to be a valuable tool for optimising co-digestion processes. The Modified Gompertz and First order model has been employed as the kinetic model for biogas generation, under the assumption that the rate of biogas production in batch mode is directly proportional to the specific growth rate of methanogenic bacteria in the biodigester (Ghatak *et al.* 2014; Chatterjee *et al.* 2019).

Table 4-7 displays the summary of the kinetics study, demonstrating a strong correlation between the anticipated biogas production and the actual biogas production. The predicted values  $Y_m$  (model Predicted biogas production),  $\lambda$  (Lag phase),  $R_m$  (Maximum predicted biogas production),  $k$  (rate constant),  $R^2$  (Correlation coefficient) and SSR (Sum of residual squares) were determined and shown in Table 4-5. Figure 4-8 (a) displays the fitted curves of the Modified Gompertz model, first order and the corresponding experimental values for the Apples-WW system, Figure 4-8 (b) shows the fitted models and experimental data for the control system and Figure 4-8 (c) depicts the comparison of the two kinetic models and the experimental setup of the Mix-2 (Mix-WW) system. The Modified Gompertz model predicted data was observed to be closely related to the experimentally measured data and that is consistent with other studies (Chatterjee, Ghangrekar and Rao 2019; Pardilhó et al. 2022).

A strong connection between the predicted and experimental biogas production was obtained for all models. The correlation coefficient ( $R^2$ ) values for the Modified Gompertz model ( $>0.99$ ) were greater than those for the first-order kinetic model. The value of  $R^2$  represents the optimal fit of the statistical models. The experiments in the laboratory revealed a low lag phase ( $\lambda$ ) of less than 2 days, and this was also predicted by the Modified Gompertz model, with Mix-WW having a lag phase ( $\lambda$ ) of 0.12 days, Apples-WW with 0.92 days and the control with 0.22 days. A co-digestion of AW and sewage sludge study by Seswoya (2019) also had a low lag phase, as low as 0.01 days, which was consistent with the laboratory investigations.

The cumulative production acquired from the output of the accepted kinetic models are displayed against the cumulative production over the period of the experiment. The Modified Gompertz equation accurately predicted the cumulative production without significant variation from the experimental data. The maximum biogas production rate ( $k$ ) was determined to be 0.402/day for the Apples-WW Modified Gompertz model. The experimental biogas production for this condition was found to be 595 mL/gVS/day, while the predicted biogas production was 611.62 and 641.43 mL/gVS.day for the Modified Gompertz and First order models, respectively. Additionally, on the control system where only MWW was used, the biogas production rate was 0.190/day and 0.181/day for the for the Modified Gompertz and First order models, respectively. The control system had a maximum predicted biogas production of 478.58 mL/gVS.day for the First order model, while the Modified Gompertz model produced and 457.82 mL/gVS.day.

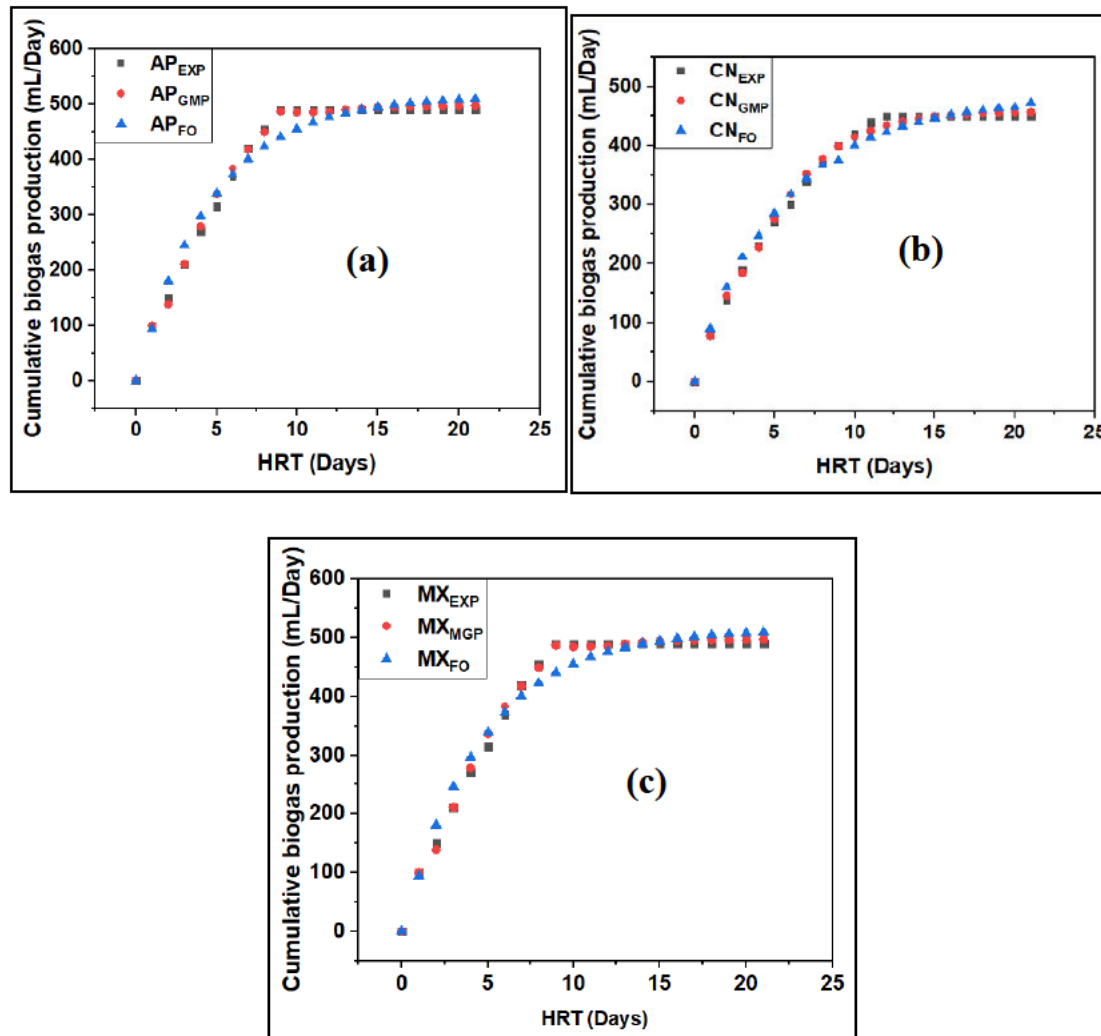


Figure 4-8: Fitting of cumulative biogas production of set-up (a) Apples-WW (b) Control (c) Mix-WW on Modified Gompert (GMP) and First-order (FO) kinetic models

Table 4-7: Summary of the kinetic study results for the BMP Co-AD Set-ups

| First Order      |                |       |                  |                |                                  |      |                | Modified Gompertz |       |      |                  |                |                                  |      |                |
|------------------|----------------|-------|------------------|----------------|----------------------------------|------|----------------|-------------------|-------|------|------------------|----------------|----------------------------------|------|----------------|
| System           | R <sup>2</sup> | k     | Y <sub>EXP</sub> | Y <sub>m</sub> | Y <sub>EXP</sub> -Y <sub>m</sub> | SSR  | R <sub>m</sub> | R <sup>2</sup>    | k     | λ    | Y <sub>EXP</sub> | Y <sub>m</sub> | Y <sub>EXP</sub> -Y <sub>m</sub> | SSR  | R <sub>m</sub> |
|                  |                | 1/day | mL/<br>gVS/day   | mL/<br>gVS/day | mL/<br>gVS/day                   |      | mL/<br>gVS/day |                   | 1/day | days | mL/<br>gVS/day   | mL/<br>gVS/day | mL/<br>gVS/day                   |      | mL/<br>gVS/day |
| Mix-WW           | 0.977          | 0.215 | 490              | 509.50         | 19.49                            | 6098 | 515.12         | 0.990             | 0.276 | 0.12 | 490              | 497.21         | 7.21                             | 4231 | 497.53         |
| Apples- WW       | 0.966          | 0.116 | 595              | 641.23         | 46.23                            | 6105 | 702.35         | 0.996             | 0.402 | 0.96 | 595              | 611.62         | 16.62                            | 6277 | 614.53         |
| Banana-WW        | 0.954          | 0.09  | 585              | 767.11         | 57.04                            | 6036 | 642.04         | 0.995             | 0.320 | 1.80 | 585              | 602.10         | 17.10                            | 6429 | 1068.05        |
| Potato-WW        | 0.956          | 0.149 | 485              | 518.23         | 33.23                            | 6471 | 541.93         | 0.994             | 0.214 | 1.13 | 485              | 494.60         | 9.60                             | 3691 | 495.29         |
| Butternut-<br>WW | 0.958          | 0.160 | 525              | 557.80         | 32.80                            | 6458 | 577.68         | 0.992             | 0.330 | 0.84 | 525              | 535.95         | 10.95                            | 5529 | 536.71         |
| Control          | 0.988          | 0.181 | 450              | 472.97         | 22.97                            | 4247 | 478.58         | 0.992             | 0.190 | 0.22 | 450              | 456.51         | 6.51                             | 3232 | 457.82         |
| Carrot-WW        | 0.947          | 0.129 | 475              | 512.28         | 37.28                            | 9687 | 549.03         | 0.991             | 0.188 | 1.34 | 475              | 486.33         | 11.33                            | 5191 | 487.50         |

Where Y<sub>m</sub> (model Predicted biogas production, Y<sub>EXP</sub> (Experimental measured biogas production, λ (Lag phase), R<sub>m</sub> (Maximum predicted biogas production), k (rate constant), R<sup>2</sup> (Correlation coefficient) and SSR (Sum of residual squares).

### 4.2.3 The effect of substrate to substrate (SS) mixing ratios and different combinations of agricultural waste in the co-digestion process

The ratio of the substrates in the feedstocks (SS) is an important parameter as well as the inoculum ratio to substrate (I/S). It is crucial to select a compatible co-substrate and determine the correct mixing ratio to optimise biogas production (Mata-Alvarez *et al.* 2014). This experiment was to determine the optimum SS ratio in the feedstock to be fed in inoculum-to-substrate ratio of 2, as was used in the initial co-digestion BMP experiments. The performance of different combinations was also conducted and compared using the different ratios. The two different combinations of the various agricultural waste were Mix-1 (apples, bananas, tomatoes, oranges and spinach) and Mix-2 ((potatoes, butternut, apples, bananas and carrots). Table 4-8 shows the different digesters, as well as the SS and I/S ratios that were applied. The Co-AD system conditions was that the OLR was kept at 2.5 kgVS/m<sup>3</sup>.day with a temperature of 40°C and an HRT of 21 days. The VS reduction and COD removal percentages (%) were also determined for the systems.

Table 4-8: Experimental setups of the co-digestion process at different substrate-substrate ratios and different mix combinations

| Co-digester setup          | CO-substrates | Substrate to substrate ratio (SS) | Inoculum to substrate ratio (I/S) |
|----------------------------|---------------|-----------------------------------|-----------------------------------|
| D <sub>X1</sub> (control)  | Mix-2 Only    | -                                 | 2                                 |
| D <sub>X2</sub>            | Mix-2 and MWW | 1                                 | 2                                 |
| D <sub>X3</sub>            | Mix-2 and MWW | 2                                 | 2                                 |
| D <sub>X4</sub>            | Mix-2 and MWW | 3                                 | 2                                 |
| D <sub>Y1</sub> ((control) | Mix-1 Only    | -                                 | 2                                 |
| D <sub>Y2</sub>            | Mix-1 and MWW | 1                                 | 2                                 |
| D <sub>Y3</sub>            | Mix-1 and MWW | 2                                 | 2                                 |
| D <sub>Y4</sub>            | Mix-1 and MWW | 3                                 | 2                                 |

MWW\*\* (market wastewater)

From Figure 4-8, the production of biogas and the methane concentrations (%) for six co-digestion setups with different substrate-substrate ratios (D<sub>X2</sub>, D<sub>X3</sub>, D<sub>X4</sub>, D<sub>Y2</sub>, D<sub>Y3</sub> and D<sub>Y4</sub>) and the two controls with mono-substrate (agricultural waste), D<sub>X1</sub> and D<sub>X5</sub> are displayed. Throughout the digestion period, the daily biogas generation from the co-substrate mixture was consistently higher than that of the control. D<sub>Y3</sub> had the highest biogas production of 680 mL/day, while the controls systems D<sub>X1</sub> and D<sub>Y1</sub> produced 250 mL/day and 290 mL/day,

respectively. These results are also consistent with literature, where the addition of 100% AW only produces less biogas and thus required the addition of co-substrates such as fish waste, slaughterhouse effluent, and waste activated sludge to the AW (Bouallagui *et al.* 2009; Scano *et al.* 2014).

The graph in Figure 4-9 illustrates the fluctuation of methane levels at various (SS) ratios for different mix combinations throughout the digesting period. The methane concentration in the reactors varied between 29% and 75%. The highest methane achieved (75%) was in the digester with the D<sub>Y3</sub> (Mix-1 and MWW, with a SS ratio of 2). The production of methane exhibited a regular pattern during the entire digestion period, whereby an initial period of slow progress was seen, succeeded by a later phase of more rapid growth, and ultimately reached a phase of stability. The controls (D<sub>X1</sub> and D<sub>Y1</sub>) had a low methane production of 29% and 37%, respectively. These results show the need for the co-digestion process and the synergetic effects of adding the market wastewater as a co-substrate. These low methane compositions from the control system with only AW are consistent with other studies, where % methane composition of 55% was achieved at an OLR of 2.5 kgVS/m<sup>3</sup>.day (Scano *et al.* 2014).

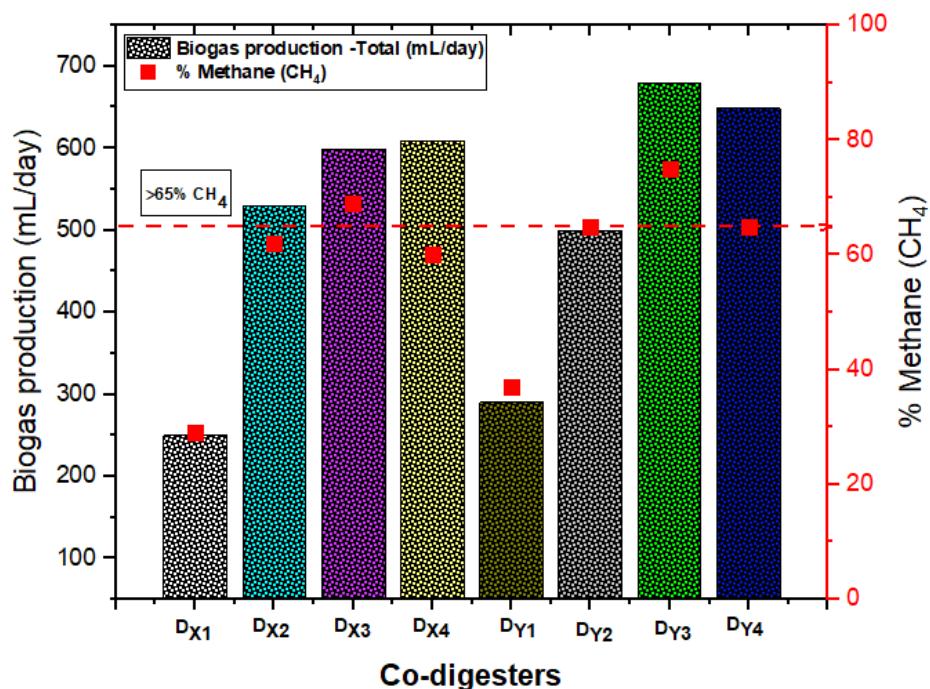


Figure 4-9: Biogas production for co-digestion BMP systems with SS ratio (1-3) and I/S ratio of 2, OLR of 2.5 kgVS/m<sup>3</sup>.day, a temperature of 40°C and an HRT of 21 days.

Figure 4-10 shows the performance and efficiency of the reactors based on COD removal and VS reduction percentages. A 75% reduction in volatile solids (VS) was achieved in reactor D<sub>Y3</sub>. The high VS removal has been attributed to high moisture contents of the AW and with moisture of the Mix-1 and Mix-2  $\geq 75\%$  (Seswoya 2019; Moretti *et al.* 2021). It was noted that when the (SS) ratio increased beyond 2, the effectiveness of VS removal in the reactor decreased. The reduction in volatile solids (VS) for D<sub>X1</sub>, D<sub>X2</sub>, D<sub>X3</sub>, D<sub>X4</sub>, D<sub>Y1</sub>, D<sub>Y2</sub>, D<sub>X4</sub> and D<sub>Y4</sub> was observed to be 53%, 65%, 72%, 68%, 60%, 69%, and 73%, respectively. The COD removal was also measured, and D<sub>Y3</sub> had a COD removal of 72%.

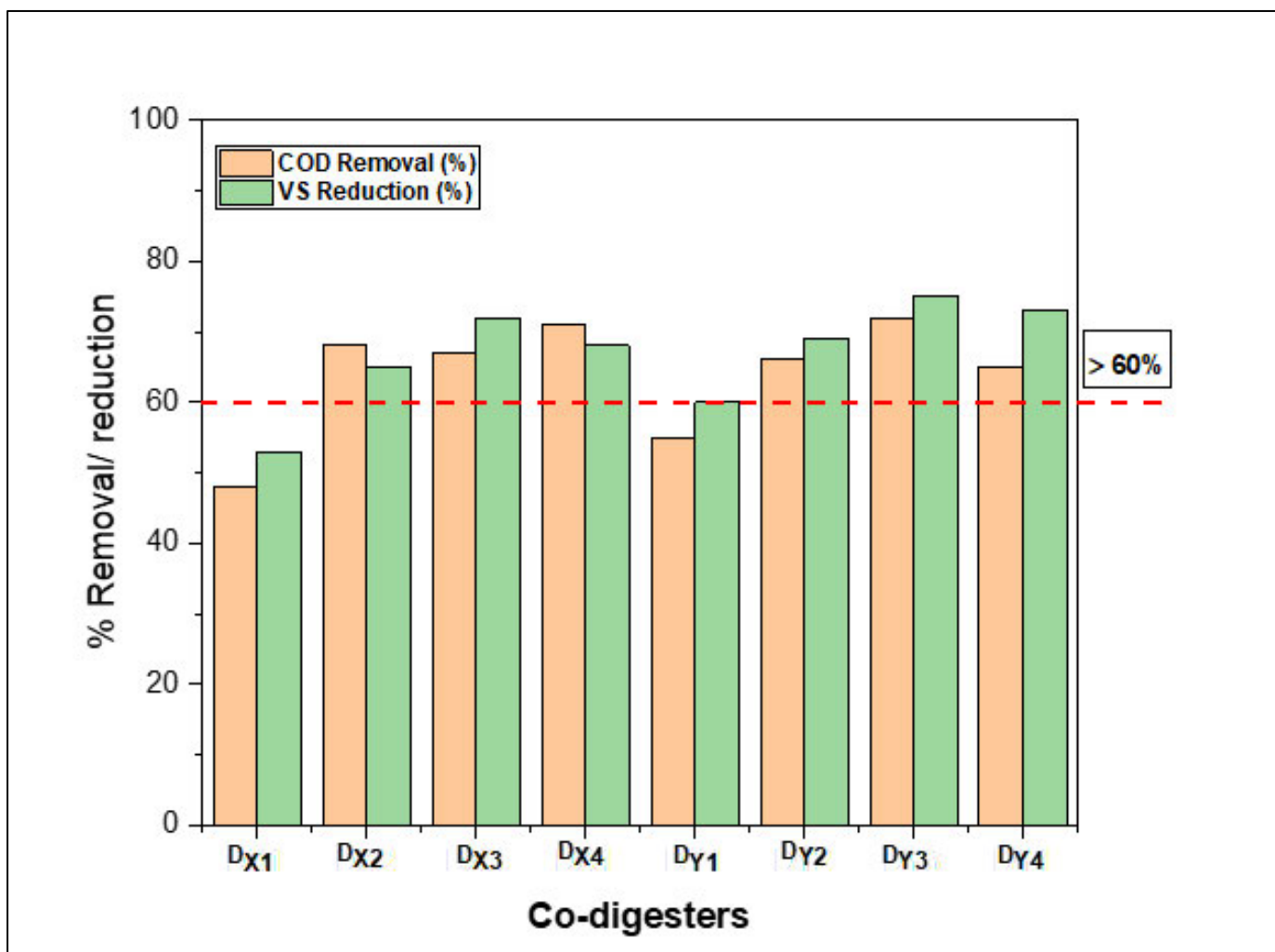


Figure 4-10: COD removal (%) and VS reduction (%) BMP systems with SS ratio (1-3) and I/S ratio of 2, OLR of 2.5 kgVS/m<sup>3</sup>.day, a temperature of 40°C and an HRT of 21 days.

#### **4.2.4 Summary**

This study examined and verified the applicability of co-digestion of agricultural wastes with market wastewater in the presence of activated sludge. The performance was measured in terms of biogas production, methane composition, VS reduction, and COD removal (%). The findings of this feasibility study indicate that the co-digestion process influences biogas production performance as it resulted in a biogas production increase of more than 5 to 30% in all co-digesters. The best performing AW was the apples at a biogas production of 595 mL/day and a methane composition of 68%. The Mix-2 AW also performed well with 490 mL/day compared to the control with an 8% increase.

A comparative study compared the performance of two combinations of single AW, Mix-1 (apples, bananas, tomatoes, oranges and spinach) and Mix-2 (potatoes, butternut, apples, bananas and carrots). This study also investigated the effect of the substrate-to-substrate mixing ratios of the two combinations of waste when co-digested with market wastewater. Mix-1 was the better-performing combination at a substrate-to-substrate ratio of 2, with a biogas production of 680 mL/day, a VS reduction of 74%, a COD removal of 72% and a methane composition of 75%. The findings demonstrated that the organic matter in the agricultural waste exhibited favourable degradability, leading to increased production of biogas and methane compared to the control system. The kinetic study determined that the data followed closely to the Modified Gompertz model. Further research is suggested to gain a deeper understanding of the microbial communities and the effects of using other co-substrates and different combinations of single AW.

### **4.3 Model development and evaluation for the co-digestion BMP study using response surface methodology (RSM)**

AD as a technology has been linked to using different organic waste matter to allow for biodegradation in an oxygen-deficient environment to produce biogas. A design matrix was developed using the Box-Behnken design (BBD) approach, employing the response surface methodology (RSM) in Design Expert software (version: 11.1.2.0). Table 4-9 depicts the results of the BBD RSM matrix developed, which produced 26 experimental runs. In developing this predictive model, for this study, hydraulic retention time, organic loading rate, pH and temperature were the chosen operational factors which were used as inputs for the BBD design ranges in RSM as depicted in Section 3.3 and included are the three levels (+1, 0, and -1) as well as two centre points. Table 4.9 also presents the data that was achieved in the laboratory investigation (Exp.) and the BBD RSM predicted data (Pred.) for the responses  $R_1$  (biogas production in mL/day),  $R_2$  (VS reduction in % ) and ( $R_3$  (COD removal in %).

In this section, the focus was the use of two substrates for the co-digestion process to produce biogas. The feedstocks that were considered for this model prediction were mixed agricultural waste (Mix-1), which was found to be the best-performing combination of AW in the BMP Co-AD. The findings show that it is advantageous to use co-digestion by proving the synergistic effect of using two or more co-substrates. Therefore, there was a need to develop a predictive model for the co-digestion process, focusing on evaluating and optimising factors such as hydraulic retention time, organic loading rate, pH and temperature.

The analysis of variance (ANOVA) was used, which compares the variation caused by changes in the combination of variable levels with the variation caused by random errors in the measurements of the generated solutions (Bezerra *et al.* 2008; Khumalo *et al.* 2023).

Table 4-9: BBD design Matrix for the co-digestion process (mixed agricultural waste (Mix-1) and market wastewater (MWW))

| Run | A: Temperature (°C) | B: pH | C: HRT (days) | D: OLR (KgVS/m <sup>3</sup> . day)) | R <sub>1</sub> = Biogas production (mL/day) |        | R <sub>2</sub> =VS reduction (%) |       | R <sub>3</sub> = COD removal (%) |       |
|-----|---------------------|-------|---------------|-------------------------------------|---|--------|----------------------------------|-------|----------------------------------|-------|
|     |                     |       |               |                                     | Exp.  | Pred.  | Exp.                             | Pred. | Exp.                             | Pred. |
| 1   | 42                  | 7     | 15.5          | 4                                   | 680   | 692.62 | 68.52                            | 67.68 | 74.56                            | 73.29 |
| 2   | 37                  | 7     | 15.5          | 2.5                                 | 460   | 464.29 | 59.25                            | 58.69 | 71.89                            | 70.74 |
| 3   | 32                  | 8     | 15.5          | 2.5                                 | 350   | 360.83 | 58.96                            | 58.06 | 69.25                            | 68.63 |
| 4   | 32                  | 7     | 10            | 2.5                                 | 440   | 428.87 | 63.78                            | 62.06 | 69.86                            | 68.33 |
| 5   | 32                  | 7     | 21            | 2.5                                 | 340   | 354.70 | 52.58                            | 53.39 | 69.45                            | 69.29 |
| 6   | 42                  | 7     | 15.5          | 1                                   | 380   | 380.95 | 53.12                            | 51.63 | 66.78                            | 65.81 |
| 7   | 37                  | 8     | 10            | 2.5                                 | 485   | 470.42 | 70.2                             | 70.52 | 71.13                            | 72.78 |
| 8   | 42                  | 8     | 15.5          | 2.5                                 | 490   | 505.83 | 65.17                            | 66.48 | 72.18                            | 72.49 |
| 9   | 42                  | 7     | 10            | 2.5                                 | 570   | 573.87 | 65.52                            | 63.99 | 77.78                            | 77.58 |
| 10  | 32                  | 7     | 15.5          | 1                                   | 255   | 235.95 | 48.87                            | 49.70 | 60.96                            | 61.95 |
| 11  | 37                  | 7     | 21            | 1                                   | 260   | 271.37 | 45.96                            | 46.33 | 60.87                            | 61.66 |
| 12  | 37                  | 7     | 10            | 1                                   | 315   | 345.54 | 55.24                            | 55.00 | 65.63                            | 66.09 |
| 13  | 37                  | 8     | 15.5          | 4                                   | 630   | 629.17 | 69.01                            | 70.30 | 79.21                            | 78.47 |
| 14  | 37                  | 6     | 15.5          | 4                                   | 500   | 505.00 | 63.63                            | 63.13 | 55.58                            | 55.90 |
| 15  | 37                  | 6     | 21            | 2.5                                 | 350   | 352.08 | 54.8                             | 54.68 | 59.89                            | 60.37 |
| 16  | 37                  | 8     | 15.5          | 1                                   | 250   | 237.50 | 52.35                            | 54.25 | 57.56                            | 56.40 |
| 17  | 32                  | 6     | 15.5          | 2.5                                 | 315   | 316.67 | 57.63                            | 57.38 | 61.78                            | 60.66 |
| 18  | 37                  | 7     | 15.5          | 2.5                                 | 480   | 464.29 | 59.12                            | 58.69 | 70.56                            | 70.74 |
| 19  | 37                  | 8     | 21            | 2.5                                 | 395   | 396.25 | 55.25                            | 54.03 | 67.78                            | 68.34 |
| 20  | 42                  | 7     | 21            | 2.5                                 | 520   | 499.70 | 57.54                            | 55.32 | 66.59                            | 67.76 |
| 21  | 32                  | 7     | 15.5          | 4                                   | 540   | 547.62 | 67.32                            | 65.74 | 68.56                            | 69.43 |
| 22  | 37                  | 6     | 10            | 2.5                                 | 420   | 426.25 | 54.1                             | 55.53 | 63.78                            | 64.80 |
| 23  | 37                  | 7     | 10            | 4                                   | 670   | 657.20 | 69.77                            | 71.05 | 72.45                            | 73.57 |
| 24  | 37                  | 6     | 15.5          | 1                                   | 280   | 273.33 | 46.89                            | 47.08 | 63.12                            | 63.02 |
| 25  | 42                  | 6     | 15.5          | 2.5                                 | 470   | 461.67 | 50.87                            | 52.83 | 65.12                            | 64.52 |
| 26  | 37                  | 7     | 21            | 4                                   | 590   | 583.04 | 60.45                            | 62.38 | 69.45                            | 69.14 |

### 4.3.1 The evaluation of the analysis of variance (ANOVA) for the response quadratic models and statistics

After conducting the experimental BMP co-digestion study based on operation factors, the next step is to develop a predictive model to evaluate and optimise the operational factors. ANOVA was conducted, and the summarised results are tabulated in Table 4-10. The coefficient of determination ( $R^2$ ) is used as a statistical measurement that determines the overall deviation of the predicted values from the mean (Khumalo *et al.* 2023). The overall significance of the model was assessed by considering both the coefficient of determination,  $R^2$ , and the modified coefficient of determination, adjusted  $R^2$ . The adjusted  $R^2$  accounts for the sample size and the number of terms in the model when correcting the  $R^2$  value, which means that when there are numerous terms in the model and the sample size is small, the adjusted  $R^2$  will not rise as the number of terms increases (Alimohammadi *et al.* 2017). For the model to be robust and have a high prediction effectiveness,  $R^2$  values should be close to 1 and the difference between the  $R^2$  and adjusted  $R^2$  values should be less than 0.2 (Montgomery 2017; Kweinor Tetteh *et al.* 2021; Madondo *et al.* 2022).

The process response variables of biogas production, VS reduction, and COD removal, obtained  $R^2$  values of 0.991, 0.978, and 0.977, respectively, as shown in Table 4-10. The fit statistics indicate that the predicted  $R^2$  values of 0.984, 0.939, and 0.936 for biogas production, VS reduction, and COD removal were reasonably close to the adjusted  $R^2$  values of 0.988, 0.966 and 0.966. The difference between these values is less than 0.2, showing the models' good predictability. The adequate precision of the model is determined by the signal to noise ratio, which was found to be 62.14 for biogas production, 31.23 for VS reduction, and 17.85 for COD removal. The values are considered acceptable as they exceed the threshold of 4.

Table 4-10: Analysis of variance results (ANOVA) for the quadratic models: responses

| Parameter                              | Biogas production | VS reduction | COD removal |
|--|-------------------|--------------|-------------|
| Standard Deviation                     | 14.16             | 1.37         | 1.09        |
| Mean                                   | 439.81            | 58.70        | 67.38       |
| Coefficient of variance (CV, %)        | 3.22              | 2.33         | 1.61        |
| Coefficient of determination ( $R^2$ ) | 0.991             | 0.978        | 0.977       |
| Adjusted $R^2$                         | 0.988             | 0.966        | 0.966       |
| Predicted $R^2$                        | 0.984             | 0.939        | 0.936       |
| Adequate Precision                     | 62.14             | 31.23        | 35.34       |

Figure 4-11 (a) - (c) shows the fit summary for the responses, where the quadratic was suggested for the biogas production and COD removal, while the 2FI was suggested for the VS reduction. The cubic was aliased for the responses. After conducting the Analysis of variance (ANOVA), the chosen models were the reduced 2FI for the VS reduction and the reduced quadratic for the biogas production and COD removal models.

| Fit Summary (a)               |                    |                     |                         |                          |                  | Fit Summary (a)          |                    |                     |                         |                          |                  |
|-------------------------------|--------------------|---------------------|-------------------------|--------------------------|------------------|--------------------------|--------------------|---------------------|-------------------------|--------------------------|------------------|
| Response 1: Biogas production |                    |                     |                         |                          |                  | Response 2: VS reduction |                    |                     |                         |                          |                  |
| Source                        | Sequential p-value | Lack of Fit p-value | Adjusted R <sup>2</sup> | Predicted R <sup>2</sup> |                  | Source                   | Sequential p-value | Lack of Fit p-value | Adjusted R <sup>2</sup> | Predicted R <sup>2</sup> |                  |
| Linear                        | < 0.0001           | 0.2896              | 0.9166                  | 0.8916                   |                  | Linear                   | < 0.0001           | 0.0278              | 0.8829                  | 0.8445                   |                  |
| 2FI                           | 0.5322             | 0.2809              | 0.9137                  | 0.8369                   |                  | <b>2FI</b>               | <b>0.0005</b>      | <b>0.0480</b>       | <b>0.9619</b>           | <b>0.9238</b>            | <b>Suggested</b> |
| <b>Quadratic</b>              | <b>&lt; 0.0001</b> | <b>0.6043</b>       | <b>0.9846</b>           | <b>0.9619</b>            | <b>Suggested</b> | Quadratic                | 0.1324             | 0.0542              | 0.9713                  | 0.9272                   |                  |
| Cubic                         | 0.3767             | 0.6234              | 0.9894                  | 0.8862                   | <b>Aliased</b>   | Cubic                    | 0.1967             | 0.0663              | 0.9884                  | 0.7999                   | <b>Aliased</b>   |

| Fit Summary (c)         |                    |                     |                         |                          |                  |
|-------------------------|--------------------|---------------------|-------------------------|--------------------------|------------------|
| Response 3: COD removal |                    |                     |                         |                          |                  |
| Source                  | Sequential p-value | Lack of Fit p-value | Adjusted R <sup>2</sup> | Predicted R <sup>2</sup> |                  |
| Linear                  | 0.0020             | 0.1651              | 0.4497                  | 0.2861                   |                  |
| 2FI                     | 0.0146             | 0.2194              | 0.7006                  | 0.4760                   |                  |
| <b>Quadratic</b>        | <b>&lt; 0.0001</b> | <b>0.5643</b>       | <b>0.9622</b>           | <b>0.9060</b>            | <b>Suggested</b> |
| Cubic                   | 0.4166             | 0.5487              | 0.9715                  | 0.6519                   | <b>Aliased</b>   |

Figure 4-11: Fit summary for the three responses (a) biogas production, (b) VS reduction and (c) COD removal

### 4.3.2 Evaluation of the effect of factors on the biogas production response using ANOVA

The effect of the operation factors in the responses is important for the process of co-digestion to determine their interactive effect in the responses. The BBD RSM was used for developing the predictive model using the factors. The statistical significance of the model and its validity were determined using the ANOVA. This involved analysing Table 4-11, Table 4-12 and Table 4-13, which provided information such as the Fisher variation ratio (F-value), adequate precision, coefficient of variance (CV), probability value (Prob > F), and lack of fit. The effect of a factor in the reduced 2FI predictive model was assessed based on its F-value, which should be greater than the other parameters, and its p-value (probability value), which should be less than 0.05.

The results from Tables 4-11 indicated that the model and their independent parameters were statistically significant, as their p-values were less than 0.05. Additionally, there was only a 0.01% chance that an F-value of this magnitude could occur due to random variation. Therefore, model terms with p-values greater than 0.05 (considered insignificant) were not

included in the model reduction process, as they were not necessary to support the model hierarchy.

The regression design model was determined to be a reduced quadratic. The model provides the p-value and F-value for the model and the individual model terms. The model was determined to be statistically significant with a p-value of less than 0.0001 and an F-value of 333.49. When evaluating the factors, HRT, OLR, temperature and pH exhibited p-values less than 0.05, indicating their substantial impact on biogas production. Therefore, it can be concluded that all four input factors have an interaction effect on biogas production. The p-value for lack of fit was beyond the threshold of 0.05 (precisely, 0.6687), indicating that it is not statistically significant. The equation for the biogas production. can be represented by Equation 4.1a for the coded equation and Equation 4.1b for the actual equation. The response predictions for each factor were determined using the three codes as shown in section 3.3.1, Table 3-4. The factors A,B,C and D were included in the polynomial and influenced the biogas production response. Furthermore, coefficients A, B and BD were positive, indicating that as temperature, pH, and OLR increased, so did biogas production. The actual equation (Equation 4.1b) utilises the actual measurements of the factors.

Table 4-11: ANOVA for reduced quadratic model – biogas production

| Source                     | Sum of Squares | Degree of freedom (df) | Mean Square | F-value | p-value  |                 |
|----------------------------|----------------|------------------------|-------------|---------|----------|-----------------|
| Model                      | 4.014E+05      | 6                      | 66902.07    | 333.49  | < 0.0001 | significant     |
| A-Temperature              | 63075.00       | 1                      | 63075.00    | 314.41  | < 0.0001 |                 |
| B-pH                       | 5852.08        | 1                      | 5852.08     | 29.17   | < 0.0001 |                 |
| C-Hydraulic Retention Time | 16502.08       | 1                      | 16502.08    | 82.26   | < 0.0001 |                 |
| D-Organic loading rate     | 2.914E+05      | 1                      | 2.914E+05   | 1452.60 | < 0.0001 |                 |
| BD                         | 6400.00        | 1                      | 6400.00     | 31.90   | < 0.0001 |                 |
| B <sup>2</sup>             | 18174.93       | 1                      | 18174.93    | 90.60   | < 0.0001 |                 |
| Residual                   | 3811.61        | 19                     | 200.61      |         |          |                 |
| Lack of Fit                | 3611.61        | 18                     | 200.64      | 1.00    | 0.6687   | not significant |
| Pure Error                 | 200.00         | 1                      | 200.00      |         |          |                 |
| Cor Total                  | 4.052E+05      | 25                     |             |         |          |                 |

$$Y_{R1} = 464.29 + 72.50A + 22.08B - 37.08C + 155.83D + 40. BD - 53.04B^2 \quad (4.1a)$$

$$Y_{R1} = -2514.10 + 14.50 \times \text{Temperature} + 697.92 \times \text{pH} - 82.78 \times \text{HRT} + 5.35 \times \text{OLR} + 22.67 \times \text{pH} \times \text{OLR} - 53.04\text{pH}^2 \quad (4.1b)$$

### 4.3.3 The analysis of variance (ANOVA) effect of the factors on the VS reduction for reduced 2FI model

The ANOVA was conducted to determine the significance of the reduced 2FI model and the interactions between the dependent variable, VS reduction, and the four independent variables A, B, C, and D. Typically, the importance of factor associated to a process can be analysed by calculating the sum of squares value. A higher total of square values indicates a substantial impact on the related variable within the context of a typical process. The ANOVA results indicate that OLR had the largest sum of squares value of 756.36, suggesting that OLR had a substantial impact on VS reduction compared to HRT (257.61), pH (211.85), and temperature (17.35) as shown in Table 4-12.

The p-values and F-values were analysed for this regression model. The ANOVA results indicate that the factors of the VS reduction predictive model have F-values in the following order: OLR (404.05) > HRT (137.62) > pH (113.17) > temperature (9.27). The pH, HRT, and OLR are the factors that had a p-value that were less than 0.0001, while temperature had a p-value of 0.00073, showing that all the factors are significant ( $p < 0.05$ ). The ANOVA statistical results indicate that the reduced 2FI model for VS reduction is a better fit for the output response. The selected combinations are highly significant at a 95% confidence level. Moreover, p-value of the model is less than 0.05 and F-value of 89.94 indicate that the VS reduction model is statistically significant. In the context of ANOVA, p-values below 0.05 suggest that the model terms A, B, C, D, AB, AC, AD, and BC, are statistically significant. The probability of a lack of fit, indicated by an F-value of 0.052, with a p-value is more than 0.05, meant that the model failure is not significant, and the independent parameters have a significant impact on VS reduction. The VS reduction model equation is depicted by equation 4.2a for the coded equation and equation 4-2 (b) for the actual equation. From the coded equation, the factors with a negative sign such as C, AD and BC have a negative effect in the VS reduction. The pH has the greatest effect in the VS reduction percentage.

$$Y_{R2} = 58.70 + 1.20A + 4.20B - 4.63C + 7.94D + 3.95AB + 0.81AC - 0.76AD - 3.02BC \quad (4.2a)$$

$$Y_{R2} = 172.63 - 5.49 * \text{Temperature} - 16.52 * \text{pH} + 1.92 \times \text{HRT} + 9.05 \times \text{OLR} + 0.79 \times \text{Temperature} \times \text{pH} + 0.03 \times \text{Temperature} \times \text{HRT} - 0.10 \times \text{Temperature} \times \text{OLR} - 0.71 \times \text{pH} \times \text{HRT} \quad (4.2b)$$

Table 4-12: ANOVA for reduced 2FI model for the VS reduction response

| Source                     | Sum of Squares | Degree of freedom (df) | Mean Square | F-value | p-value  |                 |
|----------------------------|----------------|------------------------|-------------|---------|----------|-----------------|
| Model                      | 1346.99        | 8                      | 168.37      | 89.94   | < 0.0001 | significant     |
| A-Temperature              | 17.35          | 1                      | 17.35       | 9.27    | 0.0073   |                 |
| B-pH                       | 211.85         | 1                      | 211.85      | 113.17  | < 0.0001 |                 |
| C-Hydraulic Retention Time | 257.61         | 1                      | 257.61      | 137.62  | < 0.0001 |                 |
| D-Organic loading rate     | 756.36         | 1                      | 756.36      | 404.05  | < 0.0001 |                 |
| AB                         | 62.41          | 1                      | 62.41       | 33.34   | < 0.0001 |                 |
| AC                         | 2.59           | 1                      | 2.59        | 1.38    | 0.2555   |                 |
| AD                         | 2.33           | 1                      | 2.33        | 1.24    | 0.2805   |                 |
| BC                         | 36.48          | 1                      | 36.48       | 19.49   | 0.0004   |                 |
| Residual                   | 31.82          | 17                     | 1.87        |         |          |                 |
| Lack of Fit                | 31.82          | 16                     | 1.99        | 235.32  | 0.0512   | not significant |
| Pure Error                 | 0.0085         | 1                      | 0.0085      |         |          |                 |
| Cor Total                  | 1378.81        | 25                     |             |         |          |                 |

#### 4.3.4 ANOVA regression model for reduced quadratic model COD removal

The results of the ANOVA regression model for the COD removal response are tabulated in Table 4-13. The design model for the COD removal is the reduced quadratic model. The model had p-values p that are less than 0.0001, which made the model to be significant. The model had F-value of 89.08. The significance of the model term was also assessed, and this study found that HRT, temperature, OLR, pH and temperature all had significant effects on COD removal prediction. These components were found to have interacting effects, but the quadratic terms did not. All the factors interacted with the COD removal with p-values less than 0.0001.

The model equation for the COD removal is separated into equation 4-3 (a) for the coded and equation 4-3 (b) for the actual equation.

$$Y_{R_3} = 70.74 + 1.93A + 3.99B - 2.22C + 3.74D - 2.70AC + 7.30BD + 4.17B^2 - 3.13D^2 \quad (4.3a)$$

$$Y_{R_3} = -155.45 + 1.90 \times \text{Temperature} + 50.19 \times \text{pH} + 3.22 \times \text{HRT} - 24.62 \times \text{OLR} - 0.098 \times \text{Temperature} \times \text{HRT} + 4.87 \times \text{pH} \times \text{OLR} - 1.39 \times \text{OLR}^2 \quad (4.3b)$$

Table 4-13: ANOVA for the COD removal response

| Source                     | Sum of Squares | Degree of freedom (df) | Mean Square | F-value | p-value  |                 |
|----------------------------|----------------|------------------------|-------------|---------|----------|-----------------|
| Model                      | 839.65         | 8                      | 104.96      | 89.08   | < 0.0001 | significant     |
| A-Temperature              | 44.66          | 1                      | 44.66       | 37.91   | < 0.0001 |                 |
| B-pH                       | 190.72         | 1                      | 190.72      | 161.88  | < 0.0001 |                 |
| C-Hydraulic Retention Time | 58.96          | 1                      | 58.96       | 50.05   | < 0.0001 |                 |
| D-Organic loading rate     | 167.93         | 1                      | 167.93      | 142.53  | < 0.0001 |                 |
| AC                         | 29.05          | 1                      | 29.05       | 24.66   | 0.0001   |                 |
| BD                         | 213.01         | 1                      | 213.01      | 180.80  | < 0.0001 |                 |
| B <sup>2</sup>             | 105.93         | 1                      | 105.93      | 89.91   | < 0.0001 |                 |
| D <sup>2</sup>             | 59.53          | 1                      | 59.53       | 50.52   | < 0.0001 |                 |
| Residual                   | 20.03          | 17                     | 1.18        |         |          |                 |
| Lack of Fit                | 19.14          | 16                     | 1.20        | 1.35    | 0.5974   | not significant |
| Pure Error                 | 0.8844         | 1                      | 0.8844      |         |          |                 |
| Cor (Total)                | 859.68         | 25                     |             |         |          |                 |

#### 4.3.5 Validation of the ANOVA model results

The validation of all the developed models in ANOVA results is critical, done by conducting residual analysis. Figure 4-12 (a) shows the actual vs predicted plot for the biogas production response. The actual vs predicted plots for the VS reduction and COD removal responses are shown in Figures 4-12 (b) and 4-12 (c), respectively. It can be seen from Figure 4-12 (a) - (c) that the plots depicted accurate predictions as the response values of the projected and actual data were randomly distributed close to the 45° line, which means the three models exhibit negligible errors that fall within the specified operational limits (Khumalo *et al.* 2023). Therefore, in the case of the reduced quadratic model for biogas production, the reduced 2FI model for the VS reduction and the COD reduced quadratic model, no anomalies were detected, meaning that all experimentally obtained residuals were within the boundaries of the predicted. This indicates that the response variable does not need any modification. The diagnostic plots were important in assessing the model's appropriateness.

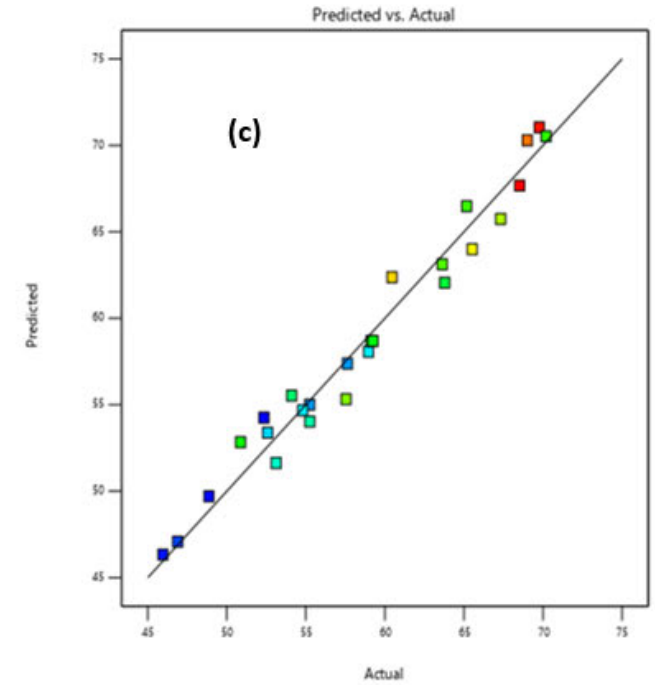
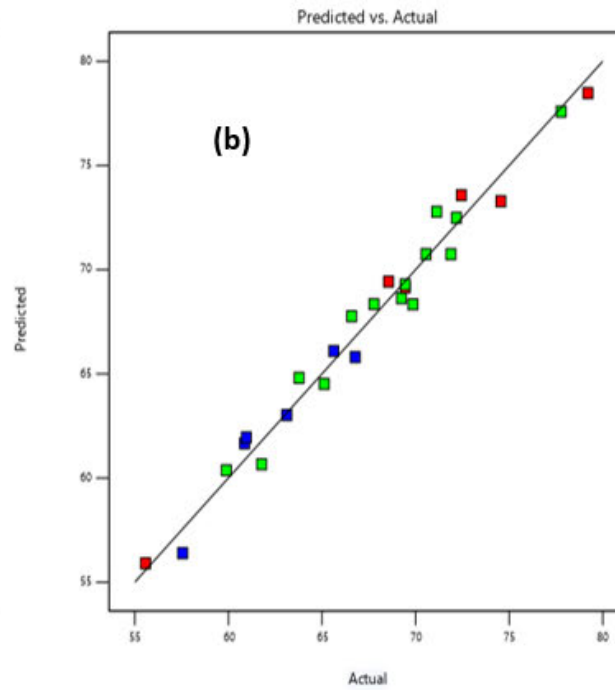
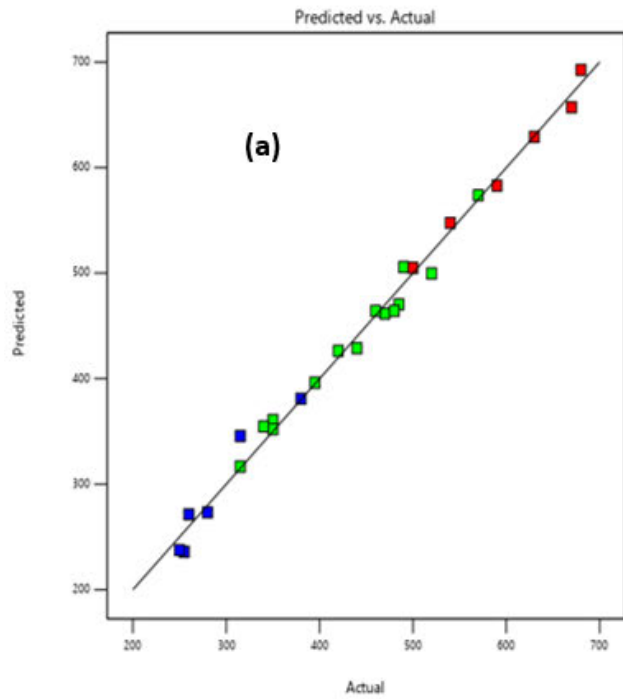


Figure 4-12: The actual vs predicted plots (a)biogas production (b)VS reduction (c) COD removal

The interaction of two factors is used to see their effect in the responses to examine the combined influence of the factors on the responses. Figure 4-13 (a) and (b) display the visual depiction of the impact of two components (OLR and pH) on the biogas production response. The 3D and contour plots depict the interactive effect of organic loading rate and pH in the predicted responses for the biogas production in mL/day. The impact of the organic loading rate (1 - 4 kgVS/m<sup>3</sup>.day) and pH (6 - 8) show that as there is an increase in the OLR and an increase in pH towards 7, there is an increase in the predicted biogas production. The biogas production rises as organic loading rate, reaching its maximum at 4 kg/m<sup>3</sup>.day. However, a rapid rise in pH towards the high range of 8 reduced the predicted biogas production.

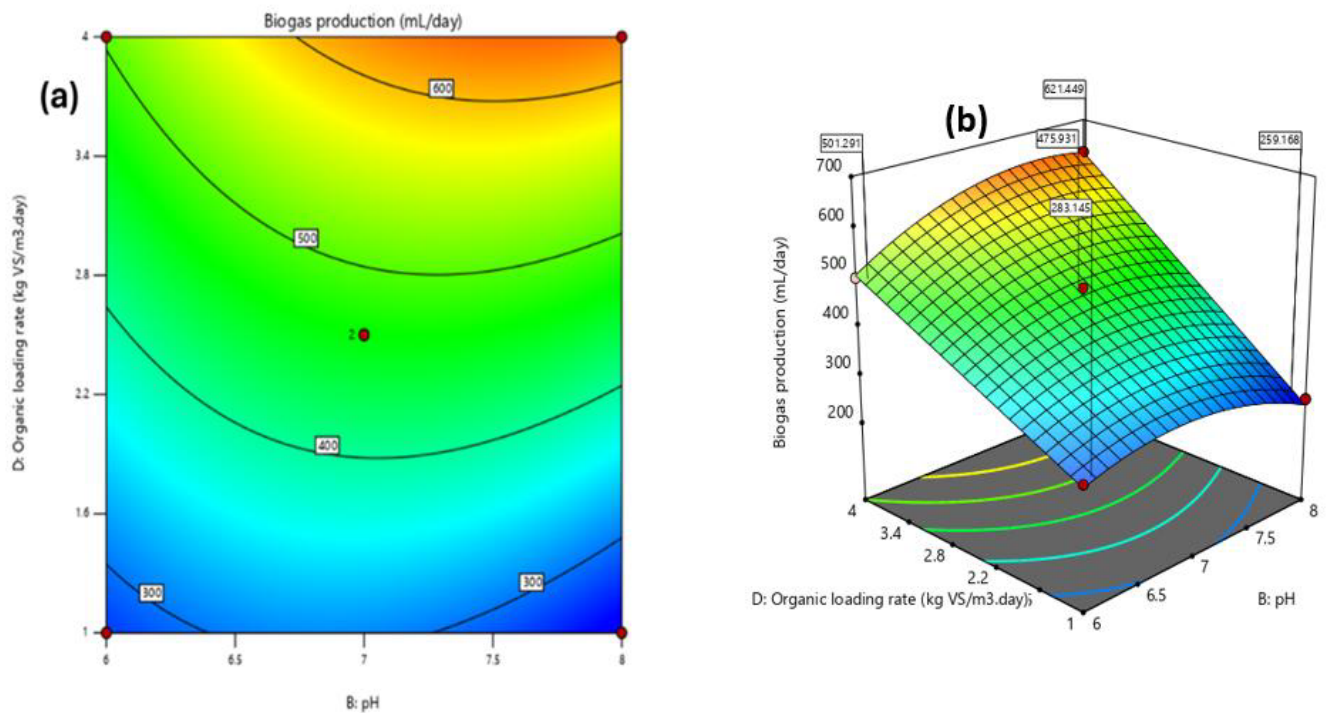


Figure 4-13: (a) Contour plots and (b) 3D plots showing interactive effect between OLR and pH in the biogas production

The second model was the reduced 2FI for the VS reduction. From Figure 4-14 (a) - (b), the relationship between pH and temperature showed that the VS reduction dropped the PH was low as 6 and with high temperatures above 40°C. As the pH is increased above 7, at temperatures around 37°C, the VS reduction also increases. after a pH of 7, as the temperature increases with an increase in pH, so does the VS reduction.

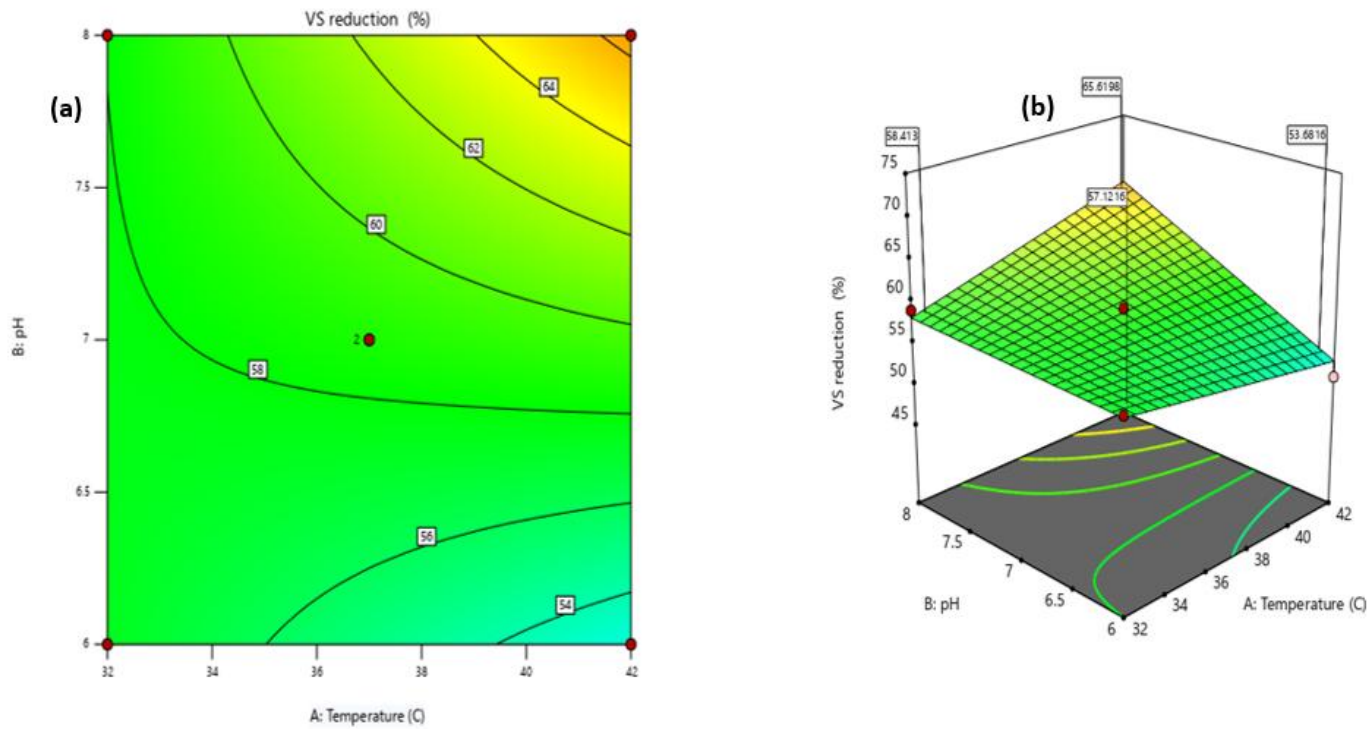


Figure 4-14: (a) Contour and (b) 3D plots showing interactive effects of temperature and pH on the VS reduction

From Figures 4-15 (a) - (d), the influential variables that affected the regression model of the COD removal responses separated into two, HRT with temperature and organic loading rate with pH. Consequently, it was shown that increasing the temperature, while decreasing the HRT has a positive impact on COD removal. The OLR when compared with the interactive effect with pH, show that a high OLR results in high COD removal at pH of above 7. All input factors have a huge effect in the COD removal and must be monitored.

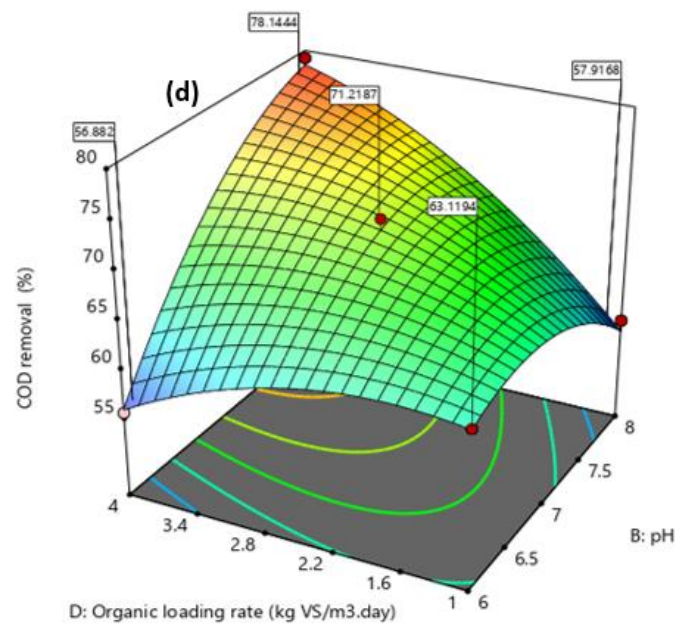
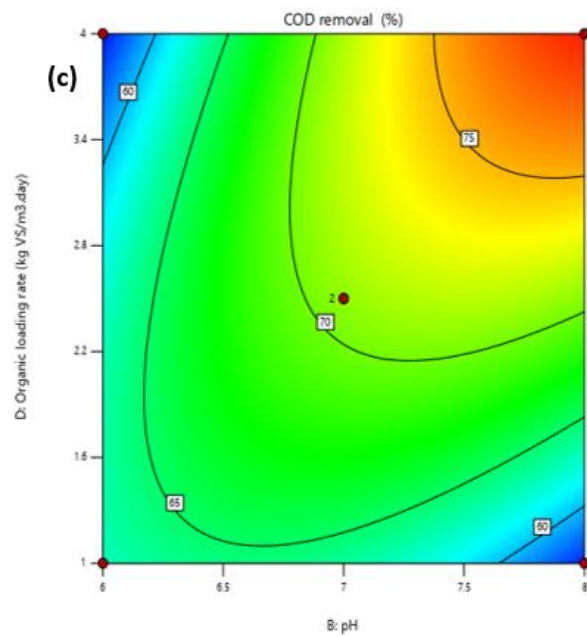
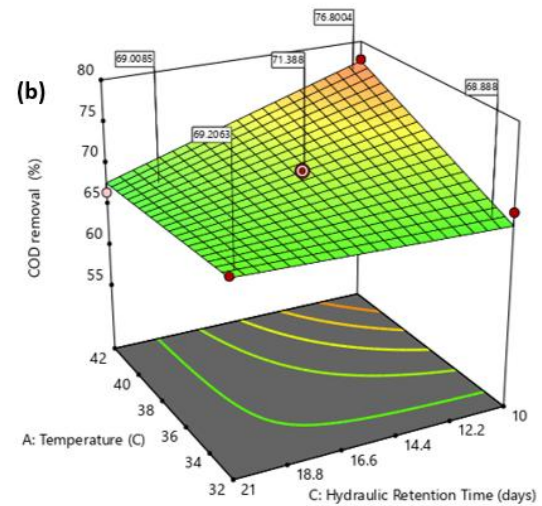
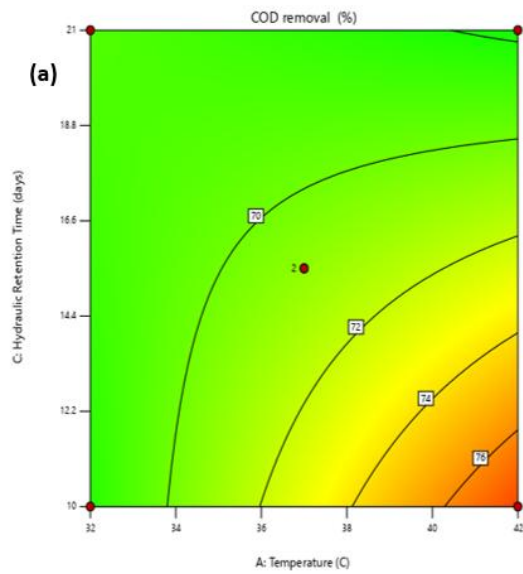


Figure 4-15: (a) Contour plots showing effects of temperature and HRT (b) 3D plots showing interactive effects of temperature and HRT (c) Contour plots and (d) 3D plots for the effect of OLR and pH in the COD removal

#### 4.3.6 The determination of process optimum through the predictive RSM matrix

The design software used for RSM has predictive models which utilizes numerical and graphical optimisation techniques to discover the conditions of the input variables that had a significant impact. The optimum conditions and responses were predicted based on the limitations and specific goals specified in Section 3.3 and Table 3-6. The selected 10 out of

100 optimal solutions are presented in Table 4-14. The optimum conditions of the input factors are shown in Figure 4-16, where the optimum organic loading rate of 3.98 kgVS/m<sup>3</sup>.day, a temperature of 40°C, a hydraulic retention time of 10 days, and a pH of 7.2 will result in a biogas production of 716.53 mL/day. Additionally, there will be a VS reduction of 73.37% and a COD removal of 79.24%. The first option from the possible solutions offered as depicted in Table 4-3 was chosen to validate the model results to demonstrate the applicability and repeatability of the design models with a 95% confidence.

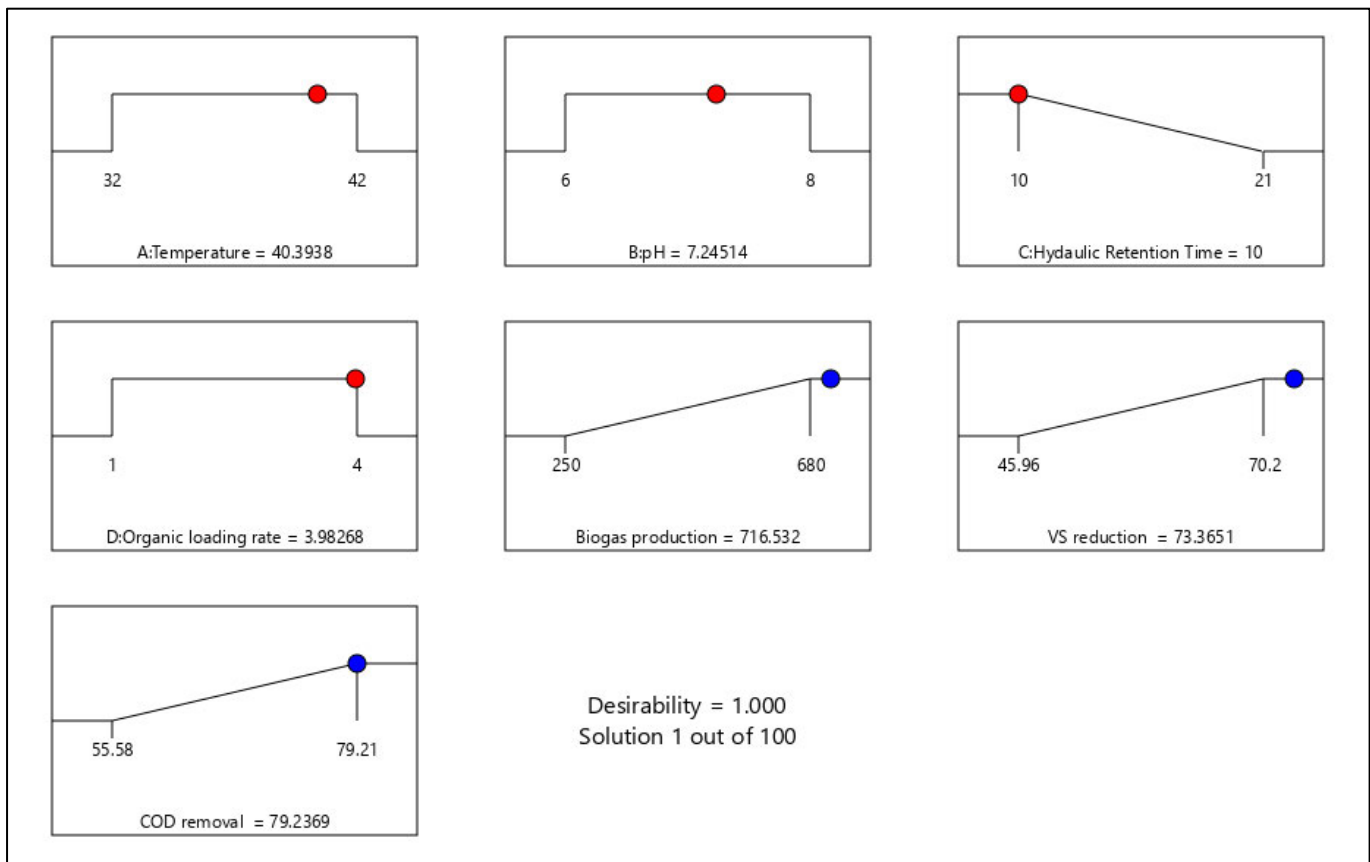


Figure 4-16: Ramp plots showing optimum conditions of the co-digestion BMP system

Table 4-14: Solutions for the predicted optimisation using BBD RSM

| Number   | Temperature   | pH           | Hydraulic Retention Time | Organic loading rate | Biogas production | VS reduction  | COD removal   | Desirability |                 |
|----------|---------------|--------------|--------------------------|----------------------|-------------------|---------------|---------------|--------------|-----------------|
| <b>1</b> | <b>40.394</b> | <b>7.245</b> | <b>10.000</b>            | <b>3.983</b>         | <b>716.532</b>    | <b>73.365</b> | <b>79.237</b> | <b>1.000</b> | <b>Selected</b> |
| 2        | 41.630        | 7.391        | 10.000                   | 3.622                | 697.285           | 73.366        | 81.348        | 1.000        |                 |
| 3        | 38.694        | 7.741        | 10.000                   | 3.954                | 692.990           | 77.254        | 81.121        | 1.000        |                 |
| 4        | 41.815        | 7.106        | 10.000                   | 3.671                | 697.891           | 70.506        | 79.405        | 1.000        |                 |
| 5        | 39.775        | 7.415        | 10.000                   | 3.808                | 691.976           | 74.009        | 79.985        | 1.000        |                 |
| 6        | 39.864        | 7.519        | 10.000                   | 3.950                | 710.803           | 75.739        | 80.913        | 1.000        |                 |
| 7        | 40.179        | 7.720        | 10.000                   | 3.903                | 708.593           | 77.566        | 82.288        | 1.000        |                 |
| 8        | 39.149        | 7.601        | 10.000                   | 3.815                | 684.308           | 75.532        | 80.557        | 1.000        |                 |
| 9        | 40.586        | 7.407        | 10.000                   | 3.942                | 719.081           | 74.816        | 80.769        | 1.000        |                 |
| 10       | 38.064        | 7.963        | 10.000                   | 3.989                | 681.778           | 78.903        | 81.527        | 1.000        |                 |

#### 4.3.7 Validation of RSM model predicted results

The BDD RSM optimisation study was used to assess the optimal conditions and determine if the experimental response results aligned with the predicted responses. Figure 4-17 displays the results obtained for biogas production, COD removal and VS reduction from an organic loading rate of 3.98 kgVS/m<sup>3</sup>.day, a temperature of 40°C, a hydraulic retention time of 10 days and a pH of 7.2. The findings demonstrated a robust association between the RSM predicted responses and experimental response outcomes, with minimal variance of less than 2%. Consequently, the design models were accurately adjusted and effectively account for the range of possibilities in the system with a 95% level of confidence and a 100% level of desirability. The comparison of the COD removal and VS reduction percentages of the BDD RSM predicted results, and the validation experimental investigations is shown in Figure 4-18. The triplicated laboratory investigations were able to produce results that are consistent with RSM, with the variance less than 2%, with the RSM results of 716.53 ± 14.14 mL/day biogas production and the laboratory validation producing 730 ± 20.56 mL/day.

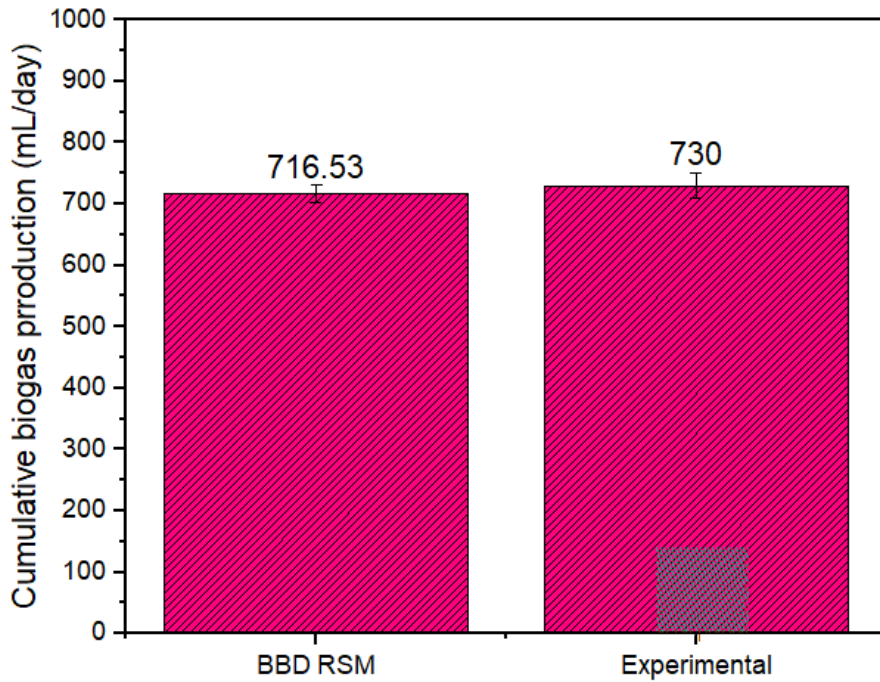


Figure 4-17: Comparing the BBD RSM predicted response value to the experimental results of biogas production at an organic loading rate of 3.98 kgVS/m<sup>3</sup>.day, a temperature of 40°C, a pH of 7.2 and a hydraulic retention time of 10 days.

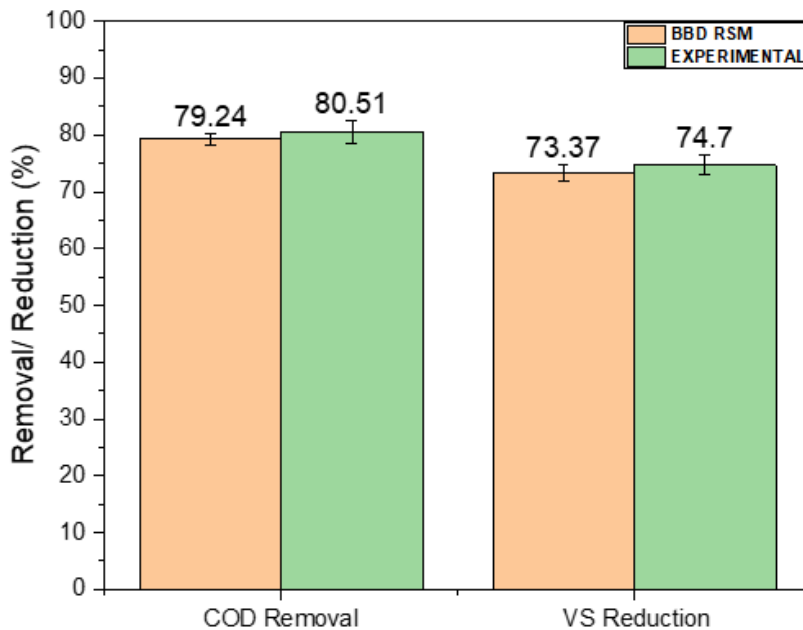


Figure 4-18: Comparing the BBD RSM predicted response value to the experimental results of COD removal and VS reduction at an organic loading rate of 3.98 kgVS/m<sup>3</sup>.day, a temperature of 40°C, a pH of 7.2 and a hydraulic retention time of 10 days

#### 4.3.8 Model development and optimisation using Apples AW and Banana AW

The performance of Apple AW and the Banana AW in producing biogas was good using the Co-AD process with MWW, as depicted in the BMP batch investigations. The operation of the Co-AD can also use a single AW as feedstock; this is the motivation for also developing predictive models for the two AWs and predicting the optimum operating conditions to achieve the predicted optimum biogas production. The BBD RSM matrix used for the bananas system with 26 experimental runs is attached in Appendix B, Table B-1. The ANOVA statistics (Table B-2 to Table B-4), model equations, actual vs predicted results and the predicted optimum results (Figure B-10) in graphs for the bananas system are attached in Appendix B. Also attached in Appendix B are the BBD RSM matrix (Table B-6) for the apple system with 26 experimental runs, as well as the ANOVA statistics (Table B-7 to Table B-9), model equations, actual vs predicted results and the predicted optimum (Figure B-19) results.

Table 4-15 compares the performance of the single agricultural waste (apples and bananas) with the mixed agricultural waste (Mix-1). The single AW predicted higher biogas production when compared to the Mix-1 system, with the Apple AW system predicting a biogas production of  $922.32 \pm 30.45$  mL/day when compared to Mix-1, which was  $716.53 \pm 14.14$  mL/day. The Mix-1 is expected to produce more due to the synergistic effect of adding more organic single substrate however the underperformance can be attributed to the Mix-1 containing different AW, which needs careful consideration of the type and amounts of the single AW to add to the mix to offer a perfect balance of nutrients that will help stabilise the system and, therefore, more biogas production (Ji *et al.* 2017). The VS removal 73.37 to 77.26%, predicted is within the performance as achieved by other studies (Zia *et al.* 2022)

Table 4-15: Comparison of predicted optimum input operational factors and responses of the Apples AW, Bananas AW and the mixed AW

|            | Input factors |             |                            |     | Responses          |                  |                  |
|------------|---------------|-------------|----------------------------|-----|--------------------|------------------|------------------|
|            | HRT           | Temperature | OLR                        | pH  | Biogas production  | COD removal      | VS reduction     |
|            | (days)        | °C          | (kgVS/m <sup>3</sup> .day) |     | (mL/day)           | (%)              | (%)              |
| Mix-1      | 10            | 40          | 3.98                       | 7.2 | $716.53 \pm 14.14$ | $79.24 \pm 1.09$ | $73.37 \pm 1.37$ |
| Apples AW  | 10            | 40          | 3.54                       | 7.7 | $922.31 \pm 30.45$ | $80.81 \pm 3.15$ | $77.26 \pm 1.84$ |
| Bananas AW | 12            | 37          | 4.00                       | 7.8 | $820.00 \pm 19.36$ | $77.26 \pm 2.44$ | $77.00 \pm 1.56$ |

#### 4.3.9 Summary

The co-digestion of the AW study investigated the use of the single AW as well as the two different combinations to determine the best performance in terms of biogas production; the results showed that Mix-1 AW performed well in the comparative study while the apples and bananas AW performed well as single AW. This study then utilised these best performing AW to develop a predictive model using RSM (response surface methodology), Box-Behnken Design (BBD), of the Design Expert's software. The BBD conducted 26 experimental runs, considering four operational factors: Organic loading rate (1 - 4 kgVS/m<sup>3</sup>.day), temperature (32 - 42°C), pH (6 - 8), and hydraulic retention time (10 - 21 days). The study measured four responses: biogas production (mL/day), COD removal (%), and VS reduction (%).

The use of Mix-1 for the Co-AD with the BBD investigation resulted in three response models, for which reduced quadratic (biogas production and COD removal) and 2FI (VS reduction) regression models were built. The analysis of variance for each response model demonstrated a substantial coefficient of determination of greater than 0.9 and closer to 1 and having low standard deviations. The p-value and F-value fell inside the permitted range, suggesting that the model is statistically significant. All the response models had an accuracy greater than 4. After optimisation at a 95% confidence level, the predicted optimum operating factors were developed, where organic loading rate of 3.98 kgVS/m<sup>3</sup>.day, temperature of 40°C, pH of 7.2, and hydraulic retention time of 10 days. These values resulted in a desirability of 100%. The subsequent validation of the findings demonstrated that the regression model exhibited satisfactory applicability, with a minor variance of less than 2%.

## 4.4 Kinetic study of the co-digesters operated under RSM predicted operating conditions.

### 4.4.1 The comparison of the experimental data with the Modified Gompertz and First-order models

The behaviour and effectiveness of the co-digestion BMP systems was analysed using the total output of each co-digester using both the First order (Equation 2.2) and Modified Gompertz (Equation 2.3) kinetic models. Figures 4-19 (a) - (c) display the graphical representation of the findings obtained by fitting the cumulative production on two different models. The cumulative biogas production of the co-digesters, MX (Mix-2 and MWW), AP (apples AW and MWW) and BN (bananas AW-MWW), were found to be well-suited to the Modified Gompertz model, with just a minor variation compared to the First order Model. This was further iterated by Modified Gompertz model achieving higher  $R^2$  values than the First order models. The rate constants ( $k$ ) of the Modified Gompertz model were seen to demonstrate high values, which indicates a rapid rate of biodegrading (Kweiyor Tetteh *et al.* 2020).

Tables 4-16 present the summary of the kinetic analysis, which confirms that the Modified Gompertz model provides a more accurate explanation of the kinetics and dynamics of the BMP systems; this is also most accurately represented by the Gompertz model Figure 4-17 (a-c). The  $R^2$  values achieved for the Modified Gompertz model were greater than 99%, with an error margin of less than 2%. The kinetics for the Modified Gompertz model includes the correlation coefficients ( $R^2$ ) of 0.995, 0.996 and 0.992 for co-digesters MX (Mix-1 and MWW), AP (Apples AW and MWW) and BN (bananas AW-WW, respectively). The  $R^2$  values for the First order model were also high, with the lowest at 0.953 for the BN system, the AP system at 0.976 and the highest of 0.990 for the MX system. The BN co-digester had the largest lag phase ( $\lambda$ ) of 1.40 days, while the AP had a lag phase of 1.02 and MX with a lag phase of 0.90. The Smaller  $\lambda$  values in this study conforms to the study by (de Quadros *et al.* 2023), where a kinetic study obtained  $\lambda$  between 0.85 and 0.594 days using agricultural and co-digesting the grounds of coffee and leachate from landfills. The low lag phase suggests that the breakdown of organic materials started soon after the incubation period because microorganisms in the mixture quickly adjusted to their environment but higher lag phases have been said to be more efficient in biogas yield (Zhao *et al.* 2016; de Quadros *et al.* 2023). Although the First-Order model predicted greater values for the maximum biogas production compared to the Modified Gompertz model, it was noted that the predictions of the biogas

production from the Modified Gompertz model were more accurate when compared to the actual measured production.

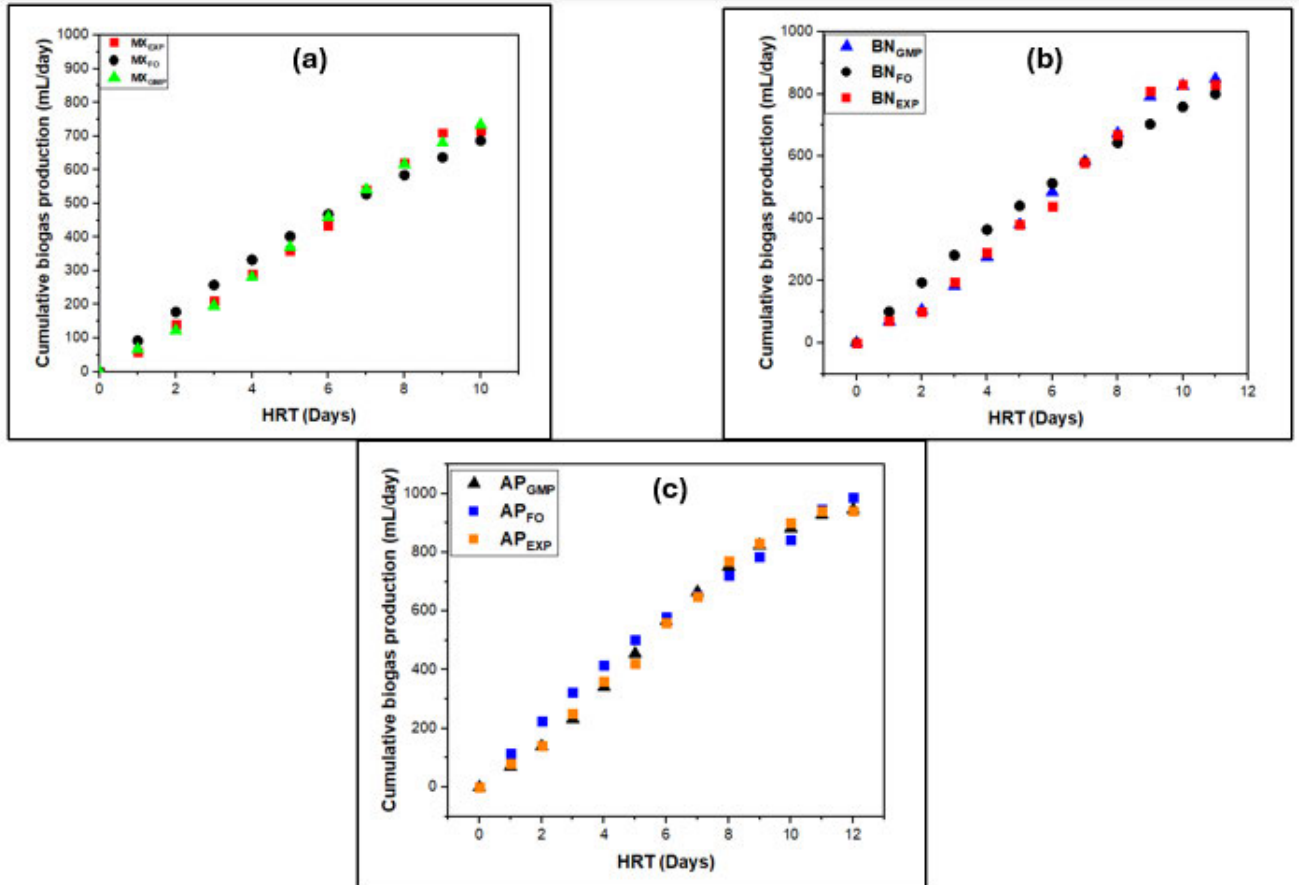


Figure 4-19: Comparison of Modified Gompertz and First order models for (a) Mix-MWW (b) Banana-MWW and (c) Apples-MWW systems

Table 4-16: Summary of the kinetic study conducted from the co-digestion process under RSM predicted optimum conditions

| System    | First order    |           |                    |                     |         |                              | Modified Gompertz |           |         |                    |                     |         |                              |
|-----------|----------------|-----------|--------------------|---------------------|---------|------------------------------|-------------------|-----------|---------|--------------------|---------------------|---------|------------------------------|
|           | R <sup>2</sup> | k (1/day) | Exp. (mL/gVS /day) | Pred. (mL/gVS /day) | SSR     | R <sub>m</sub> (mL/gV S/day) | R <sup>2</sup>    | k (1/day) | λ(days) | Exp. (mL/gVS/ day) | Pred. (mL/gVS /day) | SSR     | R <sub>m</sub> (mL/gVS /day) |
| <b>MX</b> | 0.990          | 0.02      | 715                | 686.00              | 5978.31 | 1856.98                      | 0.995             | 0.08      | 0.90    | 715                | 733.59              | 3556.18 | 949.82                       |
| <b>AP</b> | 0.976          | 0.08      | 940                | 985.97              | 6394.58 | 1574.63                      | 0.996             | 0.06      | 1.02    | 940                | 946.56              | 4547.26 | 1085.47                      |
| <b>BN</b> | 0.953          | 0.07      | 830                | 810.34              | 7900.56 | 1580.63                      | 0.992             | 0.11      | 1.40    | 830                | 848.35              | 6429.12 | 1068.05                      |

Where, Pred. (model Predicted biogas production, Exp. (Experimental measured biogas production, λ (Lag phase), R<sub>m</sub> (Maximum predicted biogas production, k (rate constant), R<sup>2</sup> (Correlation coefficient and SSR (Sum of residual squares), MX (Mix-1 and MWW), AP (Apples AW and MWW) and BN (bananas AW-MWW)

#### **4.4.2 Summary**

The investigations to validate the predicted BBD RSM results were conducted using the MX (Mix-1 and MWW), AP (Apples AW and MWW) and BN (bananas AW and MWW) systems. The data of the cumulative biogas production was used to conduct a kinetic study, where the two models were the First order and the Modified Gompertz models. The predicted data from the models using Origin software and fitting the models depicted a phenomenon where the Modified Gompertz model was the one that perfectly described the Co-AD process, with also having  $R^2$  that was higher than 0.99. The lag phase of less than 1 day was also consistent with the laboratory investigations.

## CHAPTER FIVE: CONCLUSION

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The study examined the application of the co-digestion process using the waste materials (market agricultural waste (AW)) and market wastewater (MWW) to enhance the anaerobic digestion process while removing contaminants in the wastewater. This chapter provides a concise overview of the main findings and offers suggestions for further study and decision-making.

The specific objectives of the project are as follows:

1. To characterise the agricultural waste and establish their potential for biogas production.
2. To evaluate and compare the biogas production from the co-digestion of different feedstocks using the biochemical methane potential (BMP) test.
3. To evaluate and develop a predictive model for optimising biogas production as a function of temperature, hydraulic retention time, pH and organic loading rate for biogas production using different feedstocks.
4. To establish the kinetics of producing biogas from different feedstocks.

### 5.1 Conclusion

The findings from the characterisation of the agricultural waste (apples, bananas, oranges, spinach, tomatoes, carrots, potatoes and butternuts) used in this study indicated that they had a high potential for biogas production. Among the eight agricultural wastes used, the descending order of the calorific values were apples (15.00 MJ/kg) > oranges (14.60 MJ/kg) > bananas (14.54 MJ/kg) > spinach (14.54 MJ/kg) > tomatoes (14.10 MJ/kg) > potatoes (13.46 MJ/kg) > butternuts (12.43 MJ/kg) > carrots (11.15 MJ/kg). The mixture of the agricultural waste had 13.33 MJ/kg. The energy potential (calorific values) encourages their feasibility study for biogas production. Some common functional groups obtained in the characterisation include O-H stretching and S=O stretching vibrations. The BET surface areas of agricultural waste in ascending were found to be oranges (0.87 m<sup>2</sup>/g) < bananas (0.92 m<sup>2</sup>/g) < apples (1.07 m<sup>2</sup>/g) < tomatoes (1.10 m<sup>2</sup>/g) < spinach (1.70 m<sup>2</sup>/g).

Secondly, the feasibility study of the agricultural waste and their mixture via co-digestion was found viable for biogas production. The results demonstrated an approximately 32% rise in co-digester with the apple waste, which received the highest biogas production of 595 mL/day with a methane composition of 68% in comparison to the control system (450 mL and 65%

methane composition), where the agricultural waste was not introduced to the system. All the other digesters were also performing well, and the carrots had the least amount among the five, with 475 mL/day and a methane composition of 55%. The mixture of the five agricultural wastes produced 490 mL/day and a methane composition of 60%.

Thirdly, predictive response models were developed using the BBD RSM for the three best considerable agricultural-wastes (apples, bananas and Mix-1). The optimum conditions obtain for Mix-1 system were HRT (10 days), temperature (40°C), an OLR (3.98 kgVS/m<sup>3</sup>.day), a pH (7.2) with desirability performance of 100% to produce (biogas production (716.23 mL/day), COD removal (79.24%) and VS reduction (73.37%) responses. Also, bananas system had HRT (12 days), temperature (37°C), an OLR (4.00 kgVS/m<sup>3</sup>.day), a pH (7.8) to produce (biogas production (820 mL/day), COD removal (77.26%) and VS reduction (77.00%). Finally, the apples system had had HRT (10 days), temperature (40°C), an OLR (3.54 kgVS/m<sup>3</sup>.day), a pH (7.7) to produce (biogas production (922.31 mL/day), COD removal (80.81%) and VS reduction (77.26%). The obtained results were examined and represented as a mathematical function of the input variables using ANOVA, with a statistically significant regression coefficient ( $R^2 > 0.93$  for all the responses) at a confidence level of 95%. The experimental validation of the predictive models agreed with the results predicted by the model. This suggests the models can be used for scalable predictions with the same agricultural waste and operating conditions

Lastly, the kinetic studies on the three considerable agricultural wastes were then fitted on the Modified Gompertz and First-Order model. The Modified Gompertz model is best fitted for the experimental data obtained for apples, bananas and the Mix-1 systems and can be used to better understand the co-digestion systems. These results were also supported by the better performance in the observed  $R^2 > 0.99$  for all the Modified Gompertz data. The kinetic modelling produced low lag phases for the three systems, bananas (0.90 days), apples (1.02) and bananas (1.4 days) showing that the agricultural waste had high biodegradability.

Consequently, it can be concluded that co-digestion of the agricultural waste and market wastewater to enhance the anaerobic digestion process for generating is extremely feasible and will improve the efficiency of the AD by increasing the generation of biogas and boosting the output of methane. The investigation of the co-digestion of agricultural waste is significant in the fight for environmental sustainability and zero-waste management towards a circular economy.

## 5.2 Recommendations

The findings of this research can assist with the build up from a lab scale to an upscale pilot plant. The following recommendations are suggested to handle the limitation of exploring the co-digestion of AWs into biogas.

- The results of this study could enhance the available data for both small-scale and industrial-scale applications of utilising AW as substrates and/or co-substrates in the production of biogas for commercial purposes.
- The predictive model and optimisation results can be utilised to develop a pilot scale and to upscale to larger applications.
- The study must be further investigated focusing on the cost analysis.
- The investigation of more combinations of the single AW to determine the perfect combinations with the best synergetic effect of AW.
- The investigation can be further extended to research on nutrient recovery from the digestate produced by the co-digestion process.
- The addition of nanoparticles enhances the treatment of the sludge and market wastewater.

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## A. APPENDIX A

This section will present sample raw data for the daily biogas production for the co-digestion systems with agricultural waste and market wastewater for the HRT of 21 days, OLR of 2.5 kg/m<sup>3</sup>.days and a temperature of 40 °C.

Table A-1: Sample raw data for the BMP digester setups for the Co-AD process

| Co-digester  | Days |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|--------------|------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|              | 0    | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| Apples-WW    | 0    | 20 | 40 | 45 | 70 | 50 | 40 | 50 | 55 | 50 | 55 | 50 | 40 | 30 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Butternut-WW | 0    | 10 | 30 | 50 | 60 | 60 | 60 | 50 | 50 | 40 | 35 | 30 | 10 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Banana-WW    | 0    | 20 | 50 | 75 | 60 | 50 | 55 | 50 | 40 | 60 | 55 | 30 | 20 | 20 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Carrot-WW    | 0    | 30 | 35 | 60 | 45 | 55 | 50 | 40 | 50 | 50 | 60 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Mix-WW       | 0    | 20 | 40 | 55 | 60 | 60 | 70 | 60 | 40 | 50 | 40 | 20 | 10 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Control      | 0    | 40 | 55 | 80 | 45 | 60 | 50 | 30 | 25 | 20 | 10 | 10 | 10 | 5  | 5  | 5  | 0  | 0  | 0  | 0  | 0  | 0  |
| Potato-WW    | 0    | 20 | 30 | 50 | 65 | 55 | 55 | 50 | 45 | 40 | 40 | 30 | 10 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |

Table A-2: Sample raw data for the BMP digester setups for the Co-AD process at different substrate to substrate ratio

| Co-digester                | Days |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|----------------------------|------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|                            | 0    | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| D <sub>X1</sub> (control)  | 0    | 0  | 10 | 30 | 30 | 35 | 25 | 25 | 30 | 25 | 20 | 10 | 5  | 0  | 5  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| D <sub>X2</sub>            | 0    | 20 | 35 | 45 | 70 | 55 | 70 | 55 | 55 | 45 | 25 | 20 | 10 | 10 | 5  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| D <sub>X3</sub>            | 0    | 30 | 60 | 65 | 55 | 65 | 50 | 60 | 45 | 55 | 60 | 20 | 30 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| D <sub>X4</sub>            | 0    | 25 | 40 | 65 | 65 | 60 | 65 | 60 | 55 | 55 | 50 | 50 | 10 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| D <sub>Y1</sub> ((control) | 0    | 5  | 10 | 25 | 30 | 35 | 50 | 45 | 20 | 20 | 10 | 15 | 15 | 10 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| D <sub>Y2</sub>            | 0    | 20 | 35 | 55 | 65 | 50 | 55 | 50 | 45 | 45 | 30 | 20 | 20 | 10 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| D <sub>Y3</sub>            | 0    | 35 | 35 | 65 | 80 | 80 | 75 | 65 | 65 | 40 | 55 | 55 | 30 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| D <sub>Y4</sub>            | 0    | 25 | 45 | 55 | 75 | 65 | 65 | 50 | 60 | 65 | 60 | 60 | 25 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |

## B. APPENDIX B

This section will present all the ANOVA statistics, pred vs actual and the model equations as well as the optimisation data as predicted by the BBD RSM software are attached to this appendix for the Bananas-MWW (bananas AW and market wastewater co-digestion) and Apples-MWW (apples AW and market wastewater co-digestion) systems.

### B-1 Bananas-MWW System

Table B-1: BBD-RSM Matrix for the Bananas-MWW system

| Run | A:Temperature (°C) | B:pH | C:Hydraulic Retention Time (days) | D:Organic loading rate KgVS/m <sup>3</sup> . day) | R <sub>1</sub> = Biogas production mL/day |        | R <sub>2</sub> =VS reduction (%) |       | R <sub>3</sub> = COD removal (%) |       |
|-----|--------------------|------|-----------------------------------|---|---|--------|----------------------------------|-------|----------------------------------|-------|
|     |                    |      |                                   |   | Exp.                                      | Pred.  | Exp.                             | Pred. | Exp.                             | Pred. |
| 1   | 42                 | 7    | 15.5                              | 4   | 300                                       | 357.71 | 53.23                            | 54.74 | 72.5                             | 72.22 |
| 2   | 37                 | 7    | 15.5                              | 2.5   | 605                                       | 607.50 | 70.13                            | 69.39 | 68.25                            | 67.47 |
| 3   | 32                 | 8    | 15.5                              | 2.5   | 600                                       | 586.88 | 62.34                            | 62.14 | 69.55                            | 68.51 |
| 4   | 32                 | 7    | 10                                | 2.5   | 610                                       | 607.50 | 68.65                            | 69.39 | 67.87                            | 67.47 |
| 5   | 32                 | 7    | 21                                | 2.5   | 500                                       | 485.21 | 53.86                            | 52.29 | 66.78                            | 66.08 |
| 6   | 42                 | 7    | 15.5                              | 1   | 360                                       | 408.75 | 44.44                            | 44.78 | 62.52                            | 62.76 |
| 7   | 37                 | 8    | 10                                | 2.5   | 440                                       | 395.42 | 55.85                            | 54.63 | 72.24                            | 73.15 |
| 8   | 42                 | 8    | 15.5                              | 2.5   | 200                                       | 223.54 | 39.78                            | 40.21 | 66.87                            | 66.28 |
| 9   | 42                 | 7    | 10                                | 2.5   | 280                                       | 327.71 | 40.59                            | 41.58 | 67.85                            | 67.93 |
| 10  | 32                 | 7    | 15.5                              | 1   | 660                                       | 615.42 | 62.74                            | 61.19 | 75.9                             | 76.17 |
| 11  | 37                 | 7    | 21                                | 1   | 350                                       | 398.75 | 49.52                            | 51.34 | 65.78                            | 65.79 |
| 12  | 37                 | 7    | 10                                | 1   | 700                                       | 670.21 | 64.66                            | 63.20 | 78                               | 77.53 |
| 13  | 37                 | 8    | 15.5                              | 4   | 680                                       | 646.04 | 60.02                            | 58.70 | 73.55                            | 73.44 |
| 14  | 37                 | 6    | 15.5                              | 4   | 250                                       | 222.71 | 37.15                            | 35.02 | 60.39                            | 61.02 |
| 15  | 37                 | 6    | 21                                | 2.5   | 740                                       | 761.04 | 76.99                            | 78.02 | 75.68                            | 76.41 |
| 16  | 37                 | 8    | 15.5                              | 1   | 535                                       | 575.00 | 62.51                            | 63.32 | 72.63                            | 72.76 |
| 17  | 32                 | 6    | 15.5                              | 2.5   | 280                                       | 256.04 | 46.77                            | 44.89 | 53.82                            | 53.88 |
| 18  | 37                 | 7    | 15.5                              | 2.5   | 180                                       | 127.50 | 36.75                            | 35.14 | 55.63                            | 55.98 |
| 19  | 37                 | 8    | 21                                | 2.5   | 760                                       | 775.21 | 69.3                             | 69.76 | 77.69                            | 76.67 |
| 20  | 42                 | 7    | 21                                | 2.5   | 300                                       | 311.88 | 43.18                            | 44.74 | 67.22                            | 66.17 |
| 21  | 32                 | 7    | 15.5                              | 4   | 240                                       | 211.88 | 39.64                            | 39.99 | 53.86                            | 53.40 |
| 22  | 37                 | 6    | 10                                | 2.5   | 600                                       | 569.38 | 57.58                            | 58.53 | 67.68                            | 68.53 |
| 23  | 37                 | 7    | 10                                | 4   | 320                                       | 356.67 | 40.55                            | 42.53 | 63.21                            | 64.38 |
| 24  | 37                 | 6    | 15.5                              | 1   | 820                                       | 804.17 | 60.75                            | 70.71 | 63.2                             | 72.85 |
| 25  | 42                 | 6    | 15.5                              | 2.5   | 450                                       | 416.88 | 71.74                            | 51.30 | 68.62                            | 69.20 |
| 26  | 37                 | 7    | 21                                | 4   | 500                                       | 541.04 | 80.32                            | 52.14 | 76.52                            | 70.42 |

**Response 1: Biogas production - Bananas-MWW system**

**Coded equation:**  $Y_{R_1} = 607.5 + 114.583A + 50.83B - 52.5C + 223.75D + 57.5BC + 45BD - 80.8333A^2 - 105.208B^2 - 47.7083C^2 - 60.8333D^2$  **(B-1 (a))**

**Actual equation:**  $Y_{R_1} = -11560.06 + 262.18 * \text{Temperature} + 1610.80 * \text{pH} + 112.53 * \text{Hydraulic Retention Time} + 74.35 * \text{Organic loading rate} - 10.46 * \text{pH} * \text{Hydraulic retention time} + 30 * \text{pH} * \text{Organic loading rate} - 3.23 \text{Temperature}^2 - 105.21 * \text{pH}^2 - 1.58 * \text{Hydraulic Retention Time}^2 - 27.04 * \text{Organic Loading Rate}^2$  **(B-1 (b))**

Table B-2: ANOVA for Reduced Quadratic model (Biogas Production) - Bananas-MWW system

| Source                     | Sum of Squares | df | Mean Square | F-value | p-value  |                 |
|----------------------------|----------------|----|-------------|---------|----------|-----------------|
| Model                      | 8.989E+05      | 10 | 89885.93    | 45.05   | < 0.0001 | significant     |
| A-Temperature              | 1.576E+05      | 1  | 1.576E+05   | 78.96   | < 0.0001 |                 |
| B-pH                       | 31008.33       | 1  | 31008.33    | 15.54   | 0.0013   |                 |
| C-Hydraulic Retention Time | 33075.00       | 1  | 33075.00    | 16.58   | 0.0010   |                 |
| D-Organic loading rate     | 6.008E+05      | 1  | 6.008E+05   | 301.10  | < 0.0001 |                 |
| BC                         | 13225.00       | 1  | 13225.00    | 6.63    | 0.0211   |                 |
| BD                         | 8100.00        | 1  | 8100.00     | 4.06    | 0.0622   |                 |
| A <sup>2</sup>             | 28512.12       | 1  | 28512.12    | 14.29   | 0.0018   |                 |
| B <sup>2</sup>             | 48300.19       | 1  | 48300.19    | 24.21   | 0.0002   |                 |
| C <sup>2</sup>             | 9932.01        | 1  | 9932.01     | 4.98    | 0.0414   |                 |
| D <sup>2</sup>             | 16148.48       | 1  | 16148.48    | 8.09    | 0.0123   |                 |
| Residual                   | 29929.17       | 15 | 1995.28     |         |          |                 |
| Lack of Fit                | 29916.67       | 14 | 2136.90     | 170.95  | 0.0599   | not significant |
| Pure Error                 | 12.50          | 1  | 12.50       |         |          |                 |
| Cor Total                  | 9.288E+05      | 25 |             |         |          |                 |
|                            |                |    |             |         |          |                 |
| Std. Dev.                  | 44.67          |    |             |         |          |                 |
| Mean                       | 471.54         |    |             |         |          |                 |
| C.V. %                     | 9.47           |    |             |         |          |                 |
| Std. Dev.                  | 44.67          |    |             |         |          |                 |
| R <sup>2</sup>             | 0.9678         |    |             |         |          |                 |
| Adjusted R <sup>2</sup>    | 0.9463         |    |             |         |          |                 |
| Predicted R <sup>2</sup>   | 0.8842         |    |             |         |          |                 |
| Adeq Precision             | 23.2897        |    |             |         |          |                 |

**Biogas production (mL/day)**

Design Points:

● Above Surface

○ Below Surface

180  820

X1 = B

X2 = D

**Actual Factors**

A = 37

C = 15.5

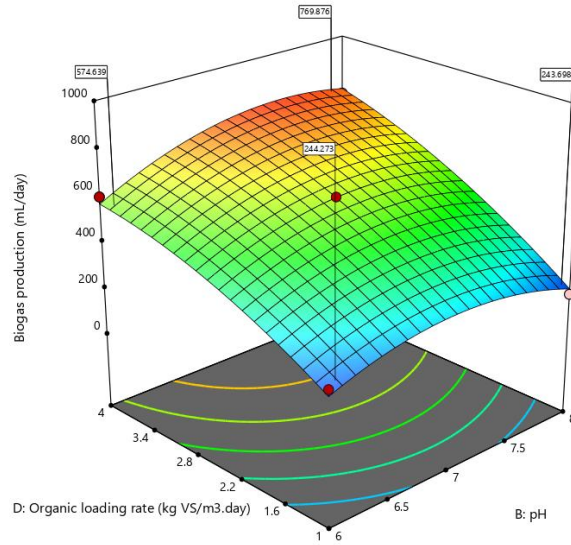


Figure B-1: 3D plot for the interaction of organic loading rate and pH in biogas production response - Bananas-MWW system

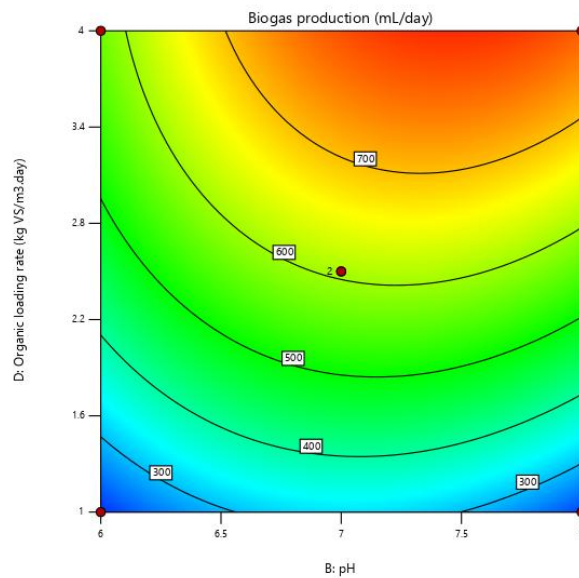


Figure B-2: Contour plot showing interactive effect between organic loading rate and pH in the biogas production - Bananas-MWW system

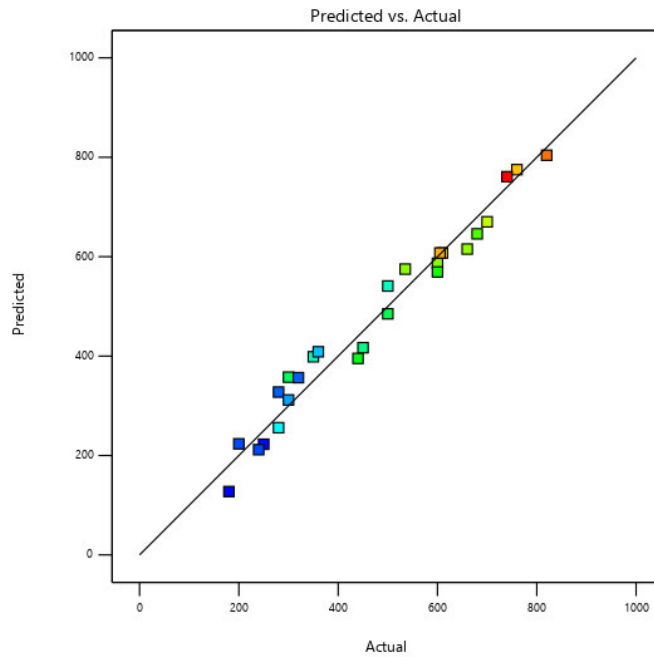


Figure B-3: The actual vs predicted plot biogas production response - Bananas-MWW system

**Response 2: VS reduction - Bananas-MWW system**

**Coded equation:**  $Y_{R_2} = 69.39 + 3.70A + 4.93B - 3.28C + 14.09D + 4.82BD - 8.57A^2 - 7.31B^2 - 9.10C^2 - 7.90C^2$  **(B-2 (a))**

**Actual equation:**  $Y_{R_2} = -871.32 + 26.10 * \text{Temperature} + 99.17 * \text{pH} + 8.73 * \text{Hydraulic Retention Time} + 4.46 * \text{Organic loading rate} + 3.21 * \text{pH} * \text{Organic loading rate} - 0.34 * \text{Temperature}^2 - 7.31 * \text{pH}^2 - 0.30 * \text{Hydraulic Retention Time}^2 - 3.51 * \text{Organic Loading Rate}^2$  **(B-2 (b))**

Table B-3: ANOVA for Reduced Quadratic model (VS reduction) - Bananas-MWW system

| Source                         | Sum of Squares | df | Mean Square | F-value | p-value  |                 |
|--------------------------------|----------------|----|-------------|---------|----------|-----------------|
| <b>Model</b>                   | 3573.03        | 9  | 397.00      | 126.94  | < 0.0001 | significant     |
| A-Temperature                  | 164.06         | 1  | 164.06      | 52.46   | < 0.0001 |                 |
| B-pH                           | 291.26         | 1  | 291.26      | 93.13   | < 0.0001 |                 |
| C-Hydraulic Retention Time     | 129.04         | 1  | 129.04      | 41.26   | < 0.0001 |                 |
| D-Organic loading rate         | 2382.34        | 1  | 2382.34     | 761.72  | < 0.0001 |                 |
| BD                             | 92.83          | 1  | 92.83       | 29.68   | < 0.0001 |                 |
| A <sup>2</sup>                 | 320.42         | 1  | 320.42      | 102.45  | < 0.0001 |                 |
| B <sup>2</sup>                 | 232.88         | 1  | 232.88      | 74.46   | < 0.0001 |                 |
| C <sup>2</sup>                 | 361.49         | 1  | 361.49      | 115.58  | < 0.0001 |                 |
| D <sup>2</sup>                 | 272.19         | 1  | 272.19      | 87.03   | < 0.0001 |                 |
| <b>Residual</b>                | 50.04          | 16 | 3.13        |         |          |                 |
| Lack of Fit                    | 48.95          | 15 | 3.26        | 2.98    | 0.4290   | not significant |
| Pure Error                     | 1.10           | 1  | 1.10        |         |          |                 |
| <b>Cor Total</b>               | 3623.07        | 25 |             |         |          |                 |
| <b>Std. Dev.</b>               | 1.77           |    |             |         |          |                 |
| <b>Mean</b>                    | 54.22          |    |             |         |          |                 |
| <b>C.V. %</b>                  | 3.26           |    |             |         |          |                 |
| <b>R<sup>2</sup></b>           | 0.9862         |    |             |         |          |                 |
| <b>Adjusted R<sup>2</sup></b>  | 0.9784         |    |             |         |          |                 |
| <b>Predicted R<sup>2</sup></b> | 0.9662         |    |             |         |          |                 |
| <b>Adeq Precision</b>          | 39.2056        |    |             |         |          |                 |

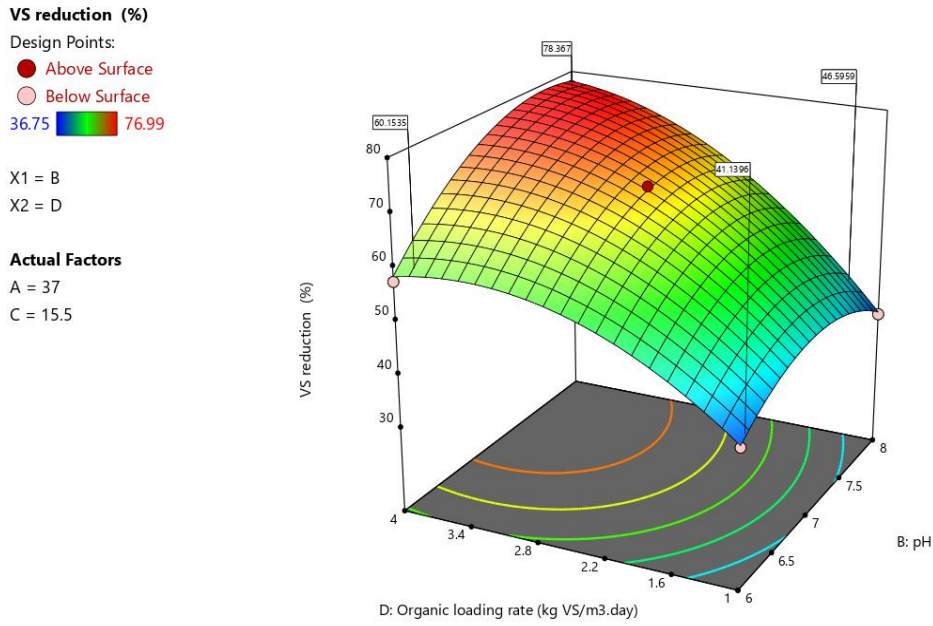


Figure B-4: 3D plot for the interaction of organic loading rate and pH in VS reduction response - Bananas-MWW system

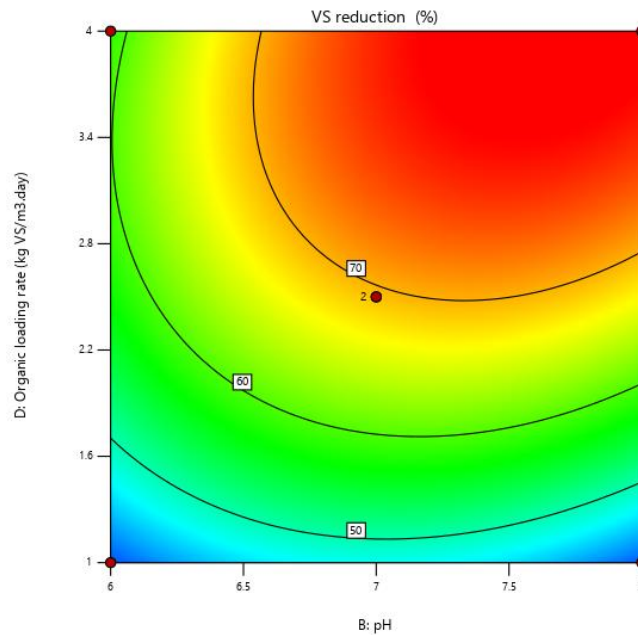


Figure B-5: Contour plot showing interactive effect between organic loading rate and pH in VS reduction response - Bananas-MWW system

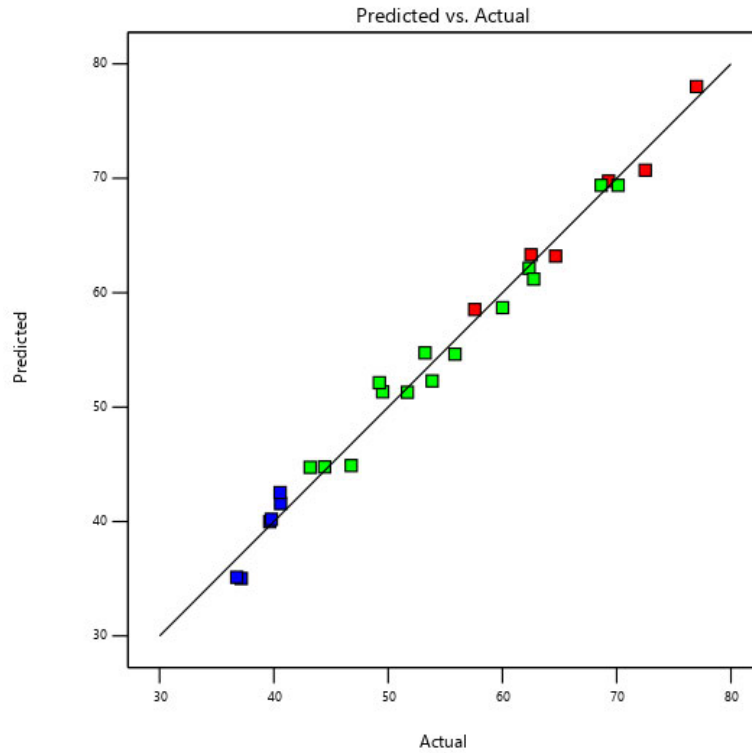


Figure B-6: The actual vs predicted plot in VS reduction response - Bananas-MWW system

**Response 3 : COD Removal - Bananas-MWW system**

**Coded equation:**  $Y_{R_3} = 67.47 + 2.12A + 5.19B - 1.51C + 6.31D - 3.98AB - 2.08AD - 1.25BD + 1.94CD - 0.98A^2 - 1.32B^2 + 3.31C^2$  **(B-3 (a))**

**Actual equation:**  $Y_{R_3} = -320.01 + 9.59 * \text{Temperature} + 55.18 * \text{pH} - 4.26 * \text{Hydraulic Retention Time} + 16.65 * \text{Organic loading rate} - 0.80 * \text{Temperature} * \text{pH} - 0.27 * \text{Temperature} * \text{Organic loading rate} - 0.84 * \text{pH} * \text{Organic loading rate} + 0.24 * \text{Hydraulic retention time} * \text{Organic loading rate} - 0.04 * \text{Temperature}^2 - 1.34 * \text{pH}^2 + 0.11 * \text{Hydraulic Retention Time}^2$  **(B-3 (b))**

Table B-4: ANOVA for Reduced Quadratic model (COD removal) - Bananas-MWW system

| Source                     | Sum of Squares | df | Mean Square | F-value | p-value  |                 |
|----------------------------|----------------|----|-------------|---------|----------|-----------------|
| Model                      | 1093.03        | 11 | 99.37       | 126.14  | < 0.0001 | significant     |
| A-Temperature              | 53.98          | 1  | 53.98       | 68.52   | < 0.0001 |                 |
| B-pH                       | 323.44         | 1  | 323.44      | 410.59  | < 0.0001 |                 |
| C-Hydraulic Retention Time | 27.42          | 1  | 27.42       | 34.81   | < 0.0001 |                 |
| D-Organic loading rate     | 478.42         | 1  | 478.42      | 607.34  | < 0.0001 |                 |
| AB                         | 63.28          | 1  | 63.28       | 80.33   | < 0.0001 |                 |
| AD                         | 17.26          | 1  | 17.26       | 21.92   | 0.0004   |                 |
| BD                         | 6.28           | 1  | 6.28        | 7.97    | 0.0136   |                 |
| CD                         | 15.09          | 1  | 15.09       | 19.16   | 0.0006   |                 |
| A <sup>2</sup>             | 5.29           | 1  | 5.29        | 6.71    | 0.0213   |                 |
| B <sup>2</sup>             | 9.57           | 1  | 9.57        | 12.15   | 0.0036   |                 |
| C <sup>2</sup>             | 60.37          | 1  | 60.37       | 76.64   | < 0.0001 |                 |
| Residual                   | 11.03          | 14 | 0.7877      |         |          |                 |
| Lack of Fit                | 10.96          | 13 | 0.8428      | 11.67   | 0.2256   | not significant |
| Pure Error                 | 0.0722         | 1  | 0.0722      |         |          |                 |
| Cor Total                  | 1104.06        | 25 |             |         |          |                 |
| Std. Dev.                  | 0.8875         |    |             |         |          |                 |
| Mean                       | 67.94          |    |             |         |          |                 |
| C.V. %                     | 1.31           |    |             |         |          |                 |
| R <sup>2</sup>             | 0.9900         |    |             |         |          |                 |
| Adjusted R <sup>2</sup>    | 0.9822         |    |             |         |          |                 |
| Predicted R <sup>2</sup>   | 0.9584         |    |             |         |          |                 |
| Adeq Precision             | 40.0282        |    |             |         |          |                 |

Factor Coding: Actual

**COD removal (%)**  
53.82  78

X1 = B  
X2 = D

**Actual Factors**  
A = 37  
C = 14.4

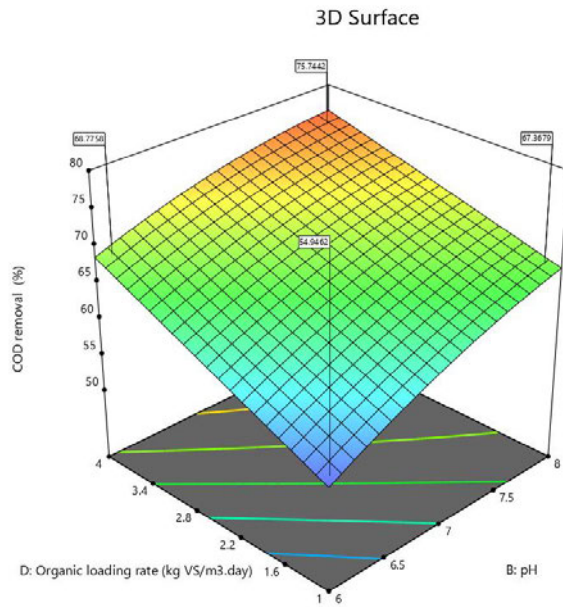


Figure B-7: 3D plot for the interaction of organic loading rate and pH in COD removal response - Bananas-MWW system

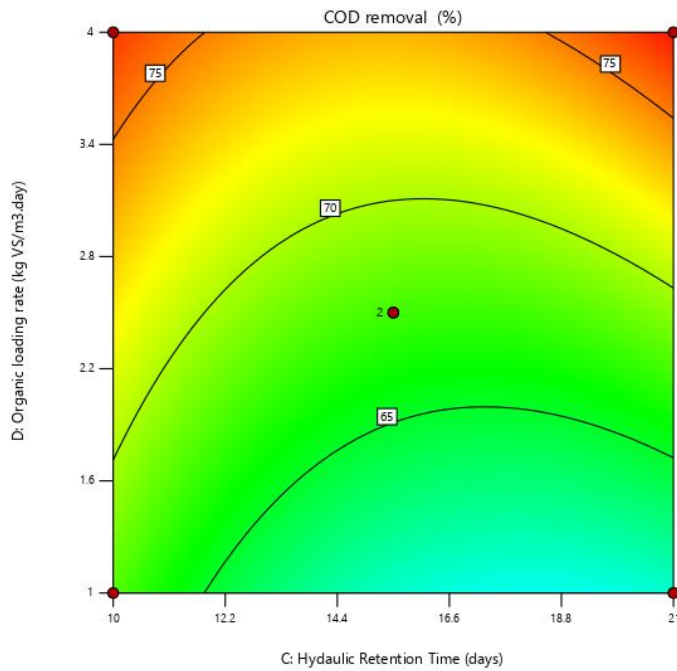


Figure B-8: Contour plot showing interactive effect between organic loading rate and Hydraulic retention time in the COD removal - Bananas-MWW system

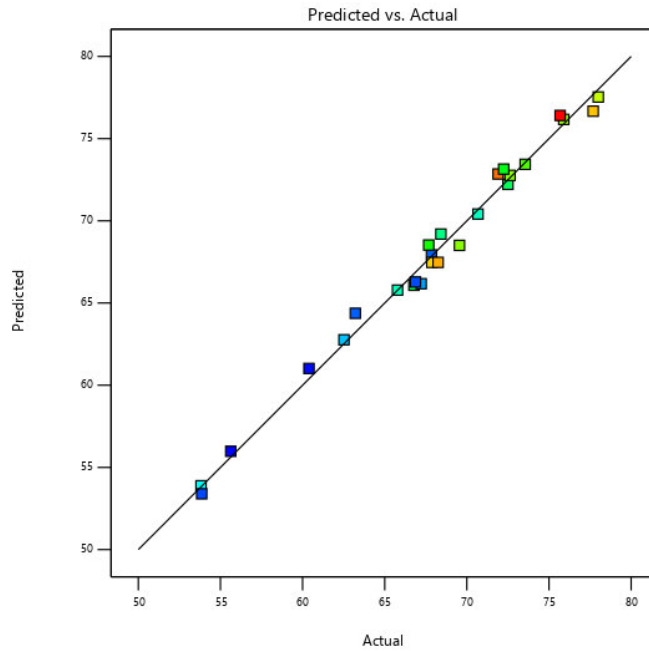


Figure B-9: The actual vs predicted plot COD removal response - Bananas-MWW system

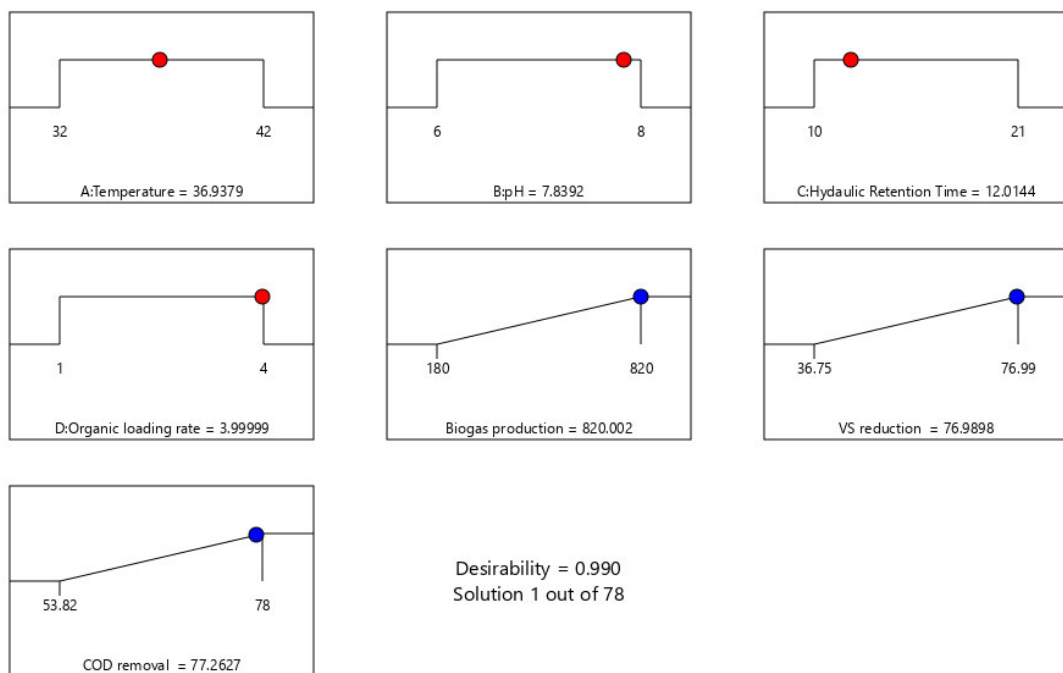


Figure B-10: Ramp plots showing optimum conditions of the co-digestion - Bananas-MWW system

Table B-5: Solutions for the predicted optimisation sing BBD RSM - Bananas-MWW system

| Number    | Temperature   | pH           | Hydraulic Retention Time | Organic loading rate | Biogas production | VS reduction  | COD removal   | Desirability |                 |
|-----------|---------------|--------------|--------------------------|----------------------|-------------------|---------------|---------------|--------------|-----------------|
| <b>1</b>  | <b>36.938</b> | <b>7.839</b> | <b>12.014</b>            | <b>4.000</b>         | <b>820.002</b>    | <b>76.990</b> | <b>77.263</b> | <b>0.990</b> | <b>Selected</b> |
| <b>2</b>  | 36.935        | 7.838        | 12.013                   | 4.000                | 820.004           | 76.990        | 77.263        | 0.990        |                 |
| <b>3</b>  | 36.958        | 7.848        | 12.020                   | 4.000                | 819.999           | 76.990        | 77.262        | 0.990        |                 |
| <b>4</b>  | 36.920        | 7.830        | 12.009                   | 4.000                | 820.000           | 76.990        | 77.262        | 0.990        |                 |
| <b>5</b>  | 36.905        | 7.823        | 12.005                   | 4.000                | 819.999           | 76.989        | 77.261        | 0.990        |                 |
| <b>6</b>  | 36.981        | 7.859        | 12.026                   | 4.000                | 819.999           | 76.989        | 77.260        | 0.990        |                 |
| <b>7</b>  | 36.891        | 7.815        | 12.000                   | 4.000                | 820.000           | 76.987        | 77.259        | 0.990        |                 |
| <b>8</b>  | 37.000        | 7.867        | 12.033                   | 4.000                | 820.002           | 76.990        | 77.256        | 0.990        |                 |
| <b>9</b>  | 37.021        | 7.875        | 12.039                   | 4.000                | 819.999           | 76.990        | 77.250        | 0.990        |                 |
| <b>10</b> | 36.949        | 7.857        | 11.940                   | 4.000                | 820.005           | 76.840        | 77.337        | 0.990        |                 |

## B-2 Apples-MWW System

Table B-6: BBD-RSM Matrix for the Apples-MWW system

| Run | A:Temperature (°C) | B:pH | C:Hydraulic Retention Time (days) | D:Organic loading rate KgVS/m <sup>3</sup> . day)) | R <sub>1</sub> = Biogas production mL/day |        | R <sub>2</sub> =VS reduction (%) |       | R <sub>3</sub> = COD removal (%) |       |
|-----|--------------------|------|-----------------------------------|--|---|--------|----------------------------------|-------|----------------------------------|-------|
|     |                    |      |                                   |  | Exp.                                      | Pred.  | Exp.                             | Pred. | Exp.                             | Pred. |
| 1   | 42                 | 7    | 15.5                              | 4  | 630                                       | 635.00 | 74.25                            | 74.54 | 66.59                            | 66.86 |
| 2   | 37                 | 7    | 15.5                              | 2.5  | 350                                       | 324.58 | 59.36                            | 59.68 | 56.63                            | 57.78 |
| 3   | 32                 | 8    | 15.5                              | 2.5  | 385                                       | 405.42 | 70.78                            | 71.11 | 76.12                            | 75.79 |
| 4   | 32                 | 7    | 10                                | 2.5  | 600                                       | 616.25 | 75.58                            | 76.72 | 74.98                            | 75.60 |
| 5   | 32                 | 7    | 21                                | 2.5  | 895                                       | 922.92 | 82.78                            | 82.08 | 75.68                            | 75.74 |
| 6   | 42                 | 7    | 15.5                              | 1  | 750                                       | 747.08 | 80.25                            | 80.72 | 80.55                            | 79.66 |
| 7   | 37                 | 8    | 10                                | 2.5  | 660                                       | 656.67 | 69.89                            | 71.01 | 63.78                            | 65.18 |
| 8   | 42                 | 8    | 15.5                              | 2.5  | 240                                       | 239.17 | 58.78                            | 59.57 | 67.11                            | 66.72 |
| 9   | 42                 | 7    | 10                                | 2.5  | 545                                       | 548.75 | 70.6                             | 69.68 | 58.74                            | 57.92 |
| 10  | 32                 | 7    | 15.5                              | 1  | 680                                       | 598.75 | 74.1                             | 72.62 | 77                               | 75.83 |
| 11  | 37                 | 7    | 21                                | 1  | 420                                       | 455.42 | 67.17                            | 68.32 | 65.01                            | 65.27 |
| 12  | 37                 | 7    | 10                                | 1  | 420                                       | 382.50 | 73.12                            | 73.49 | 67.89                            | 69.66 |
| 13  | 37                 | 8    | 15.5                              | 4  | 740                                       | 759.58 | 81.27                            | 81.51 | 79.46                            | 78.54 |
| 14  | 37                 | 6    | 15.5                              | 4  | 570                                       | 539.58 | 72.88                            | 71.43 | 70.22                            | 72.30 |
| 15  | 37                 | 6    | 21                                | 2.5  | 320                                       | 370.00 | 69.27                            | 68.14 | 72.58                            | 72.49 |
| 16  | 37                 | 8    | 15.5                              | 1  | 290                                       | 307.08 | 60.55                            | 60.22 | 54.6                             | 54.58 |
| 17  | 32                 | 6    | 15.5                              | 2.5  | 800                                       | 817.50 | 80.14                            | 79.97 | 67                               | 69.60 |
| 18  | 37                 | 7    | 15.5                              | 2.5  | 840                                       | 805.00 | 78.69                            | 79.11 | 72.52                            | 72.44 |
| 19  | 37                 | 8    | 21                                | 2.5  | 685                                       | 674.17 | 74.78                            | 75.03 | 68.52                            | 66.66 |
| 20  | 42                 | 7    | 21                                | 2.5  | 200                                       | 196.25 | 58.36                            | 58.71 | 59.67                            | 57.98 |
| 21  | 32                 | 7    | 15.5                              | 4  | 495                                       | 467.92 | 74.33                            | 73.75 | 62.25                            | 60.72 |
| 22  | 37                 | 6    | 10                                | 2.5  | 340                                       | 391.25 | 62.96                            | 63.33 | 66.62                            | 65.03 |
| 23  | 37                 | 7    | 10                                | 4  | 640                                       | 635.00 | 76.25                            | 74.54 | 65.78                            | 66.86 |
| 24  | 37                 | 6    | 15.5                              | 1  | 250                                       | 221.67 | 60.75                            | 60.04 | 63.2                             | 65.23 |
| 25  | 42                 | 6    | 15.5                              | 2.5  | 490                                       | 534.58 | 71.74                            | 72.76 | 68.62                            | 67.98 |
| 26  | 37                 | 7    | 21                                | 4  | 700                                       | 682.92 | 80.32                            | 80.86 | 76.52                            | 75.24 |

**Response 1: Biogas production - Apples-MWW system**

**Coded equation:**  $Y_{R_1} = 635.00 + 71.67A + 145.83B - 74.17C + 217.50D + 41.25BD - 41.46A^2 - 51.46B^2 - 56.46C^2 - 65.21D^2$  **(B-4 (a))**

**Actual equation:**  $Y_{R_1} = -6009.65 + 137.05 * \text{Temperature} + 797.50 * \text{pH} + 44.33 * \text{Hydraulic Retention Time} + 97.41 * \text{Organic loading rate} + 27.50 * \text{pH} * \text{Organic loading rate} - 1.66 * \text{Temperature}^2 - 51.46 * \text{pH}^2 - 1.87 * \text{Hydraulic Retention Time}^2 - 28.98 * \text{Organic Loading Rate}^2$  **(B-4 (b))**

Table B-7: ANOVA for Reduced Quadratic model : Biogas production - Apples-MWW system

| Source                     | Sum of Squares | df | Mean Square | F-value | p-value  |                 |
|----------------------------|----------------|----|-------------|---------|----------|-----------------|
| Model                      | 9.809E+05      | 9  | 1.090E+05   | 73.71   | < 0.0001 | significant     |
| A-Temperature              | 61633.33       | 1  | 61633.33    | 41.68   | < 0.0001 |                 |
| B-pH                       | 2.552E+05      | 1  | 2.552E+05   | 172.60  | < 0.0001 |                 |
| C-Hydraulic Retention Time | 66008.33       | 1  | 66008.33    | 44.64   | < 0.0001 |                 |
| D-Organic loading rate     | 5.677E+05      | 1  | 5.677E+05   | 383.92  | < 0.0001 |                 |
| BD                         | 6806.25        | 1  | 6806.25     | 4.60    | 0.0476   |                 |
| A <sup>2</sup>             | 7500.19        | 1  | 7500.19     | 5.07    | 0.0387   |                 |
| B <sup>2</sup>             | 11554.73       | 1  | 11554.73    | 7.81    | 0.0130   |                 |
| C <sup>2</sup>             | 13909.28       | 1  | 13909.28    | 9.41    | 0.0074   |                 |
| D <sup>2</sup>             | 18554.73       | 1  | 18554.73    | 12.55   | 0.0027   |                 |
| Residual                   | 23658.33       | 16 | 1478.65     |         |          |                 |
| Lack of Fit                | 23608.33       | 15 | 1573.89     | 31.48   | 0.1391   | not significant |
| Pure Error                 | 50.00          | 1  | 50.00       |         |          |                 |
| Cor Total                  | 1.005E+06      | 25 |             |         |          |                 |
| Std. Dev.                  | 38.45          |    |             |         |          |                 |
| Mean                       | 535.96         |    |             |         |          |                 |
| C.V. %                     | 7.17           |    |             |         |          |                 |
| R <sup>2</sup>             | 0.9765         |    |             |         |          |                 |
| Adjusted R <sup>2</sup>    | 0.9632         |    |             |         |          |                 |
| Predicted R <sup>2</sup>   | 0.9426         |    |             |         |          |                 |
| Adeq Precision             | 30.4712        |    |             |         |          |                 |

**Biogas production (mL/day)**

Design Points:

● Above Surface

○ Below Surface

200 895

X1 = B

X2 = D

**Actual Factors**

A = 37

C = 15.5

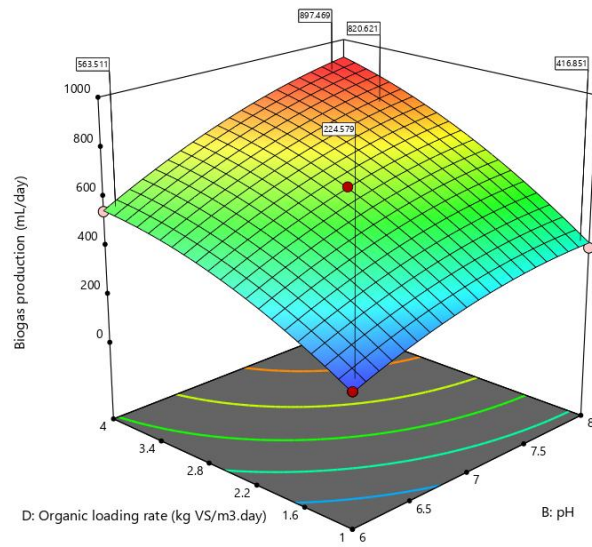


Figure B-11: 3D plot for the interaction of organic loading rate and pH in biogas production response - Apples-MWW system

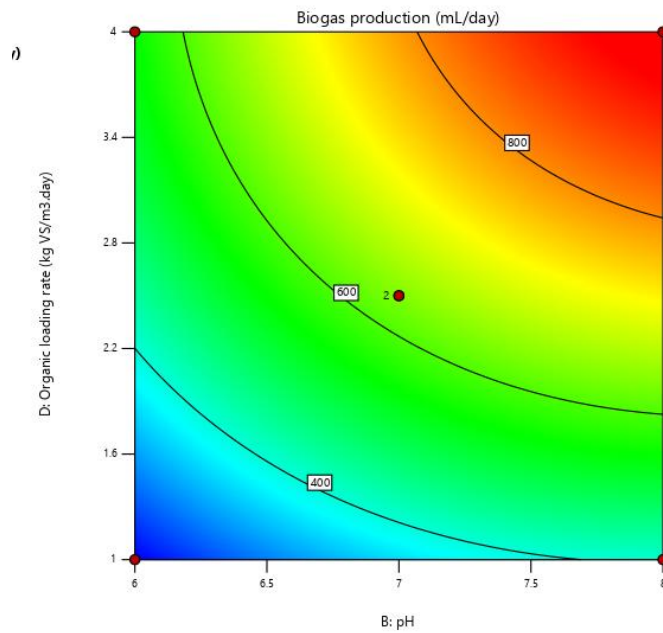


Figure B-12: Contour plot for the interaction of organic loading rate and pH in biogas production response - Apples-MWW system

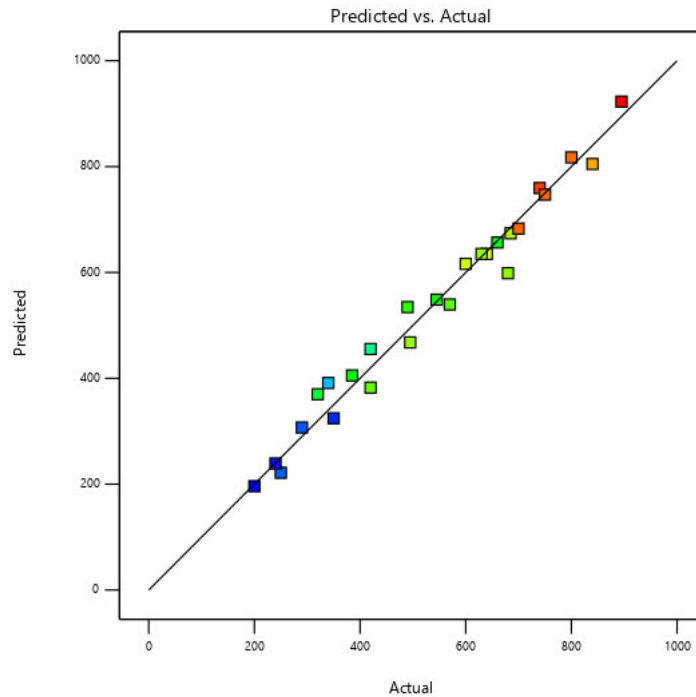


Figure B-13: The actual vs predicted plot biogas production response – Apples- MWW system


**Response 2: VS reduction - Apples-MWW system**

**Coded Equation:**  $Y_{R_2} = 74.54 + 4.71A + 6.20B - 4.05C + 5.49D - 2.32AB - 2.25AD - 1.62B^2 - 2.44C^2 - 2.52D^2$  **(B-5 (a))**

**Actual Equation:**  $Y_{R_2} = -255.24 + 4.94 * \text{Temperature} + 46.08 * \text{pH} + 1.77 * \text{Hydraulic Retention Time} + 20.33 * \text{Organic loading rate} - 0.46 * \text{Temperature} * \text{pH} - 0.30 * \text{Temperature} * \text{Organic loading rate} - 1.62\text{pH}^2 + 0.08 * \text{Hydraulic Retention Time}^2 - 1.12 * \text{Organic loading rate}^2$  **(B-5 (b))**

Table B-8: ANOVA for Reduced Quadratic model (VS reduction) - Apples-MWW system

| Source                         | Sum of Squares | df | Mean Square | F-value | p-value  |                 |
|--------------------------------|----------------|----|-------------|---------|----------|-----------------|
| <b>Model</b>                   | 1380.29        | 9  | 153.37      | 137.37  | < 0.0001 | significant     |
| A-Temperature                  | 266.77         | 1  | 266.77      | 238.96  | < 0.0001 |                 |
| B-pH                           | 461.16         | 1  | 461.16      | 413.07  | < 0.0001 |                 |
| C-Hydraulic Retention Time     | 196.75         | 1  | 196.75      | 176.23  | < 0.0001 |                 |
| D-Organic loading rate         | 361.02         | 1  | 361.02      | 323.38  | < 0.0001 |                 |
| AB                             | 21.53          | 1  | 21.53       | 19.28   | 0.0005   |                 |
| AD                             | 20.16          | 1  | 20.16       | 18.06   | 0.0006   |                 |
| B <sup>2</sup>                 | 14.48          | 1  | 14.48       | 12.97   | 0.0024   |                 |
| C <sup>2</sup>                 | 32.81          | 1  | 32.81       | 29.39   | < 0.0001 |                 |
| D <sup>2</sup>                 | 34.96          | 1  | 34.96       | 31.31   | < 0.0001 |                 |
| <b>Residual</b>                | 17.86          | 16 | 1.12        |         |          |                 |
| Lack of Fit                    | 15.86          | 15 | 1.06        | 0.5288  | 0.8107   | not significant |
| Pure Error                     | 2.00           | 1  | 2.00        |         |          |                 |
| <b>Cor Total</b>               | 1398.15        | 25 |             |         |          |                 |
| <b>Std. Dev.</b>               | 1.06           |    |             |         |          |                 |
| <b>Mean</b>                    | 71.50          |    |             |         |          |                 |
| <b>C.V. %</b>                  | 1.48           |    |             |         |          |                 |
| <b>R<sup>2</sup></b>           | 0.9872         |    |             |         |          |                 |
| <b>Adjusted R<sup>2</sup></b>  | 0.9800         |    |             |         |          |                 |
| <b>Predicted R<sup>2</sup></b> | 0.9681         |    |             |         |          |                 |
| <b>Adeq Precision</b>          | 35.6616        |    |             |         |          |                 |

**VS reduction (%)**  
 Design Points:  
 ● Above Surface  
 ○ Below Surface  
 58.36  82.78

X1 = A  
 X2 = B

**Actual Factors**  
 C = 15.5  
 D = 2.5

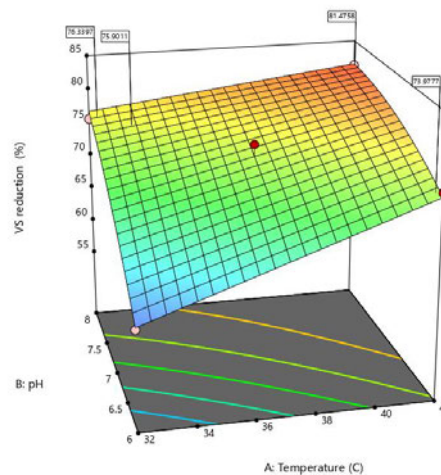


Figure B-14: 3D plot for the interaction of temperature and pH in VS reduction response - Apples-MWW system

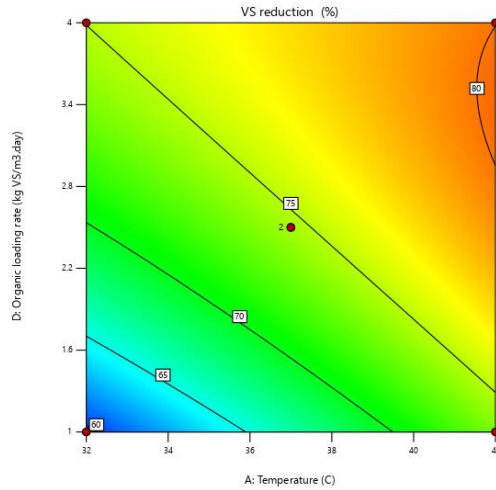


Figure B-15: Contour plots for the interaction of organic loading rate and temperature in VS reduction response - Apples-MWW system

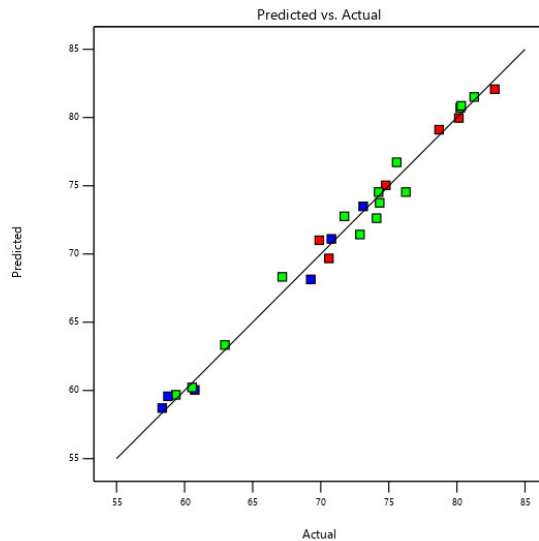


Figure B-16: The actual vs predicted plots VS reduction response – Apples- MWW system

**Response 3: COD removal - Apples-MWW system**

**Coded Equation:**  $Y_{R_3} = 66.82 + 1.47A + 8.91B - 3.63C - 0.03D + 1.722BC + 1.30A^2 - 7.31B^2 + 1.98C^2$  **(B-6 (a))**

**Actual Equation:**  $Y_{R_3} = 124.723 - 3.56 * \text{Temperature} + 4.07 * \text{pH} - 4.87 * \text{Hydraulic Retention Time} - 0.02 * \text{Organic loading rate} + 0.31 * \text{pH} * \text{Hydraulic retention time} + 0.05 * \text{Temperature}^2 + 0.07 * \text{Hydraulic Retention Time}^2$  **(B-6 (b))**

Table B-9: ANOVA for Reduced Quadratic model (COD removal) - Apples-MWW system

| Source                         | Sum of Squares | df | Mean Square | F-value | p-value  |                 |
|--------------------------------|----------------|----|-------------|---------|----------|-----------------|
| <b>Model</b>                   | 1176.44        | 7  | 168.06      | 74.72   | < 0.0001 | significant     |
| A-Temperature                  | 25.99          | 1  | 25.99       | 11.55   | 0.0032   |                 |
| B-pH                           | 952.12         | 1  | 952.12      | 423.29  | < 0.0001 |                 |
| C-Hydraulic Retention Time     | 158.27         | 1  | 158.27      | 70.36   | < 0.0001 |                 |
| D-Organic loading rate         | 0.0091         | 1  | 0.0091      | 0.0040  | 0.9501   |                 |
| BC                             | 11.76          | 1  | 11.76       | 5.23    | 0.0345   |                 |
| A <sup>2</sup>                 | 10.33          | 1  | 10.33       | 4.59    | 0.0460   |                 |
| C <sup>2</sup>                 | 23.82          | 1  | 23.82       | 10.59   | 0.0044   |                 |
| <b>Residual</b>                | 40.49          | 18 | 2.25        |         |          |                 |
| Lack of Fit                    | 40.16          | 17 | 2.36        | 7.20    | 0.2860   | not significant |
| Pure Error                     | 0.3281         | 1  | 0.3281      |         |          |                 |
| <b>Cor Total</b>               | 1216.93        | 25 |             |         |          |                 |
| <b>Std. Dev.</b>               | 1.50           |    |             |         |          |                 |
| <b>Mean</b>                    | 68.37          |    |             |         |          |                 |
| <b>C.V. %</b>                  | 2.19           |    |             |         |          |                 |
| <b>R<sup>2</sup></b>           | 0.9667         |    |             |         |          |                 |
| <b>Adjusted R<sup>2</sup></b>  | 0.9538         |    |             |         |          |                 |
| <b>Predicted R<sup>2</sup></b> | 0.9313         |    |             |         |          |                 |
| <b>Adeq Precision</b>          | 30.1448        |    |             |         |          |                 |

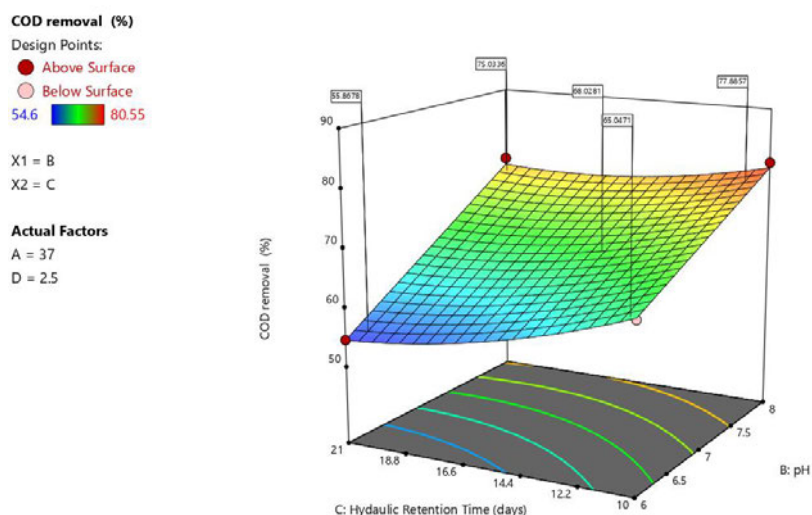


Figure B-17: 3D plot for the interaction of hydraulic retention time and pH in COD removal response - Apples-MWW system

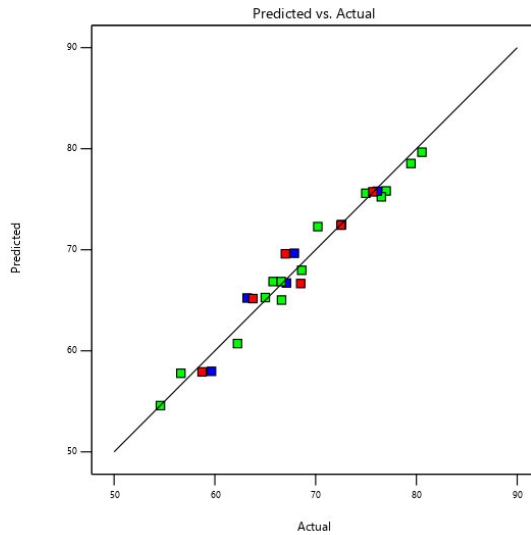


Figure B-18: The actual vs predicted plots COD removal response – Apples- MWW system

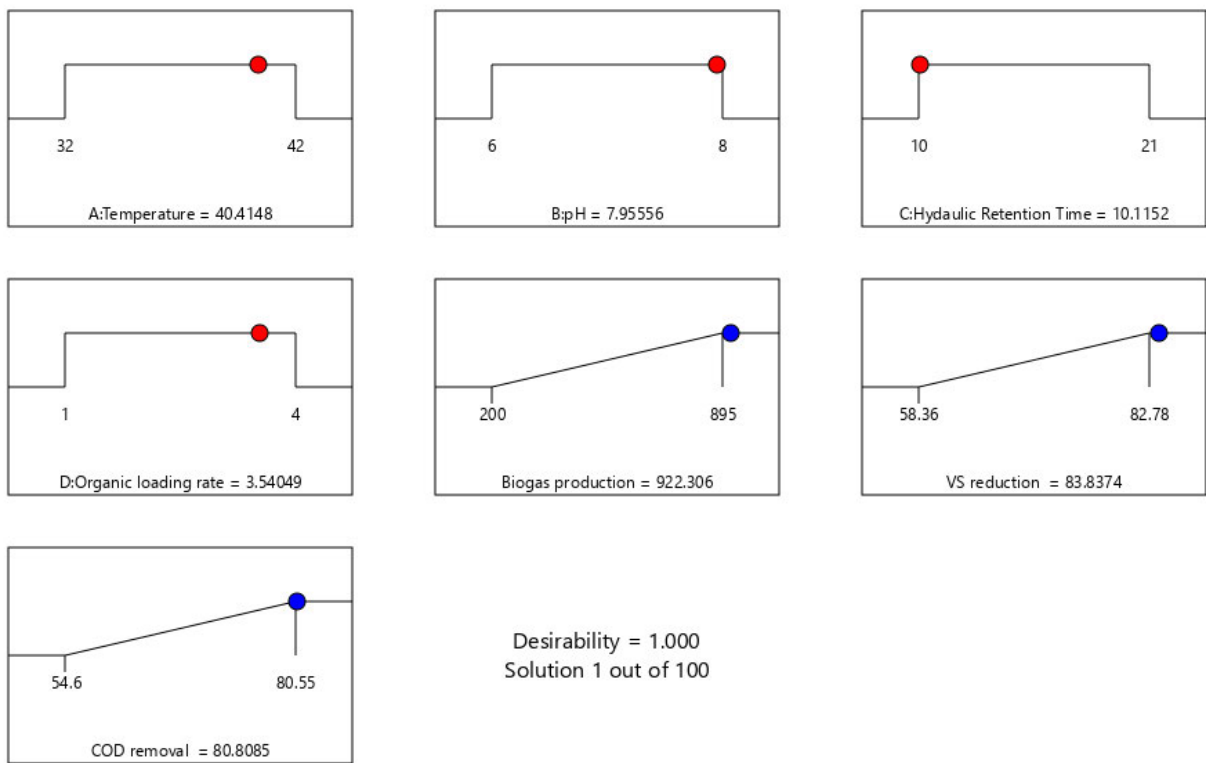


Figure B-19: Ramp plots showing optimum conditions of the co-digestion - Apples-MWW system

Table B-10: Solutions for the predicted optimisation using BBD RSM - Apples-MWW system

| Number   | Temperature   | pH           | Hydraulic Retention Time | Organic loading rate | Biogas production | VS reduction  | COD removal   | Desirability |                 |
|----------|---------------|--------------|--------------------------|----------------------|-------------------|---------------|---------------|--------------|-----------------|
| <b>1</b> | <b>40.415</b> | <b>7.956</b> | <b>10.115</b>            | <b>3.540</b>         | <b>922.306</b>    | <b>83.837</b> | <b>80.808</b> | <b>1.000</b> | <b>Selected</b> |
| 2        | 41.993        | 7.987        | 11.729                   | 3.630                | 940.876           | 84.138        | 80.655        | 1.000        |                 |
| 3        | 41.323        | 7.990        | 10.864                   | 3.614                | 938.248           | 84.095        | 80.935        | 1.000        |                 |
| 4        | 41.887        | 7.989        | 10.424                   | 3.320                | 901.380           | 84.149        | 81.801        | 1.000        |                 |
| 5        | 40.281        | 7.999        | 10.582                   | 3.281                | 896.682           | 83.758        | 80.563        | 1.000        |                 |
| 6        | 39.947        | 7.979        | 10.210                   | 3.703                | 940.345           | 83.844        | 80.585        | 1.000        |                 |
| 7        | 39.741        | 7.991        | 10.027                   | 3.771                | 945.838           | 83.817        | 80.739        | 1.000        |                 |
| 8        | 41.476        | 7.922        | 10.313                   | 3.642                | 933.161           | 84.011        | 81.103        | 1.000        |                 |
| 9        | 40.303        | 7.985        | 10.033                   | 3.643                | 934.829           | 83.873        | 81.038        | 1.000        |                 |
| 10       | 41.578        | 7.984        | 11.255                   | 3.988                | 975.056           | 83.885        | 80.716        | 1.000        |                 |

## C. APPENDIX C

### EQUIPMENT FOR THE CO-DIGESTION BMP PROCESS

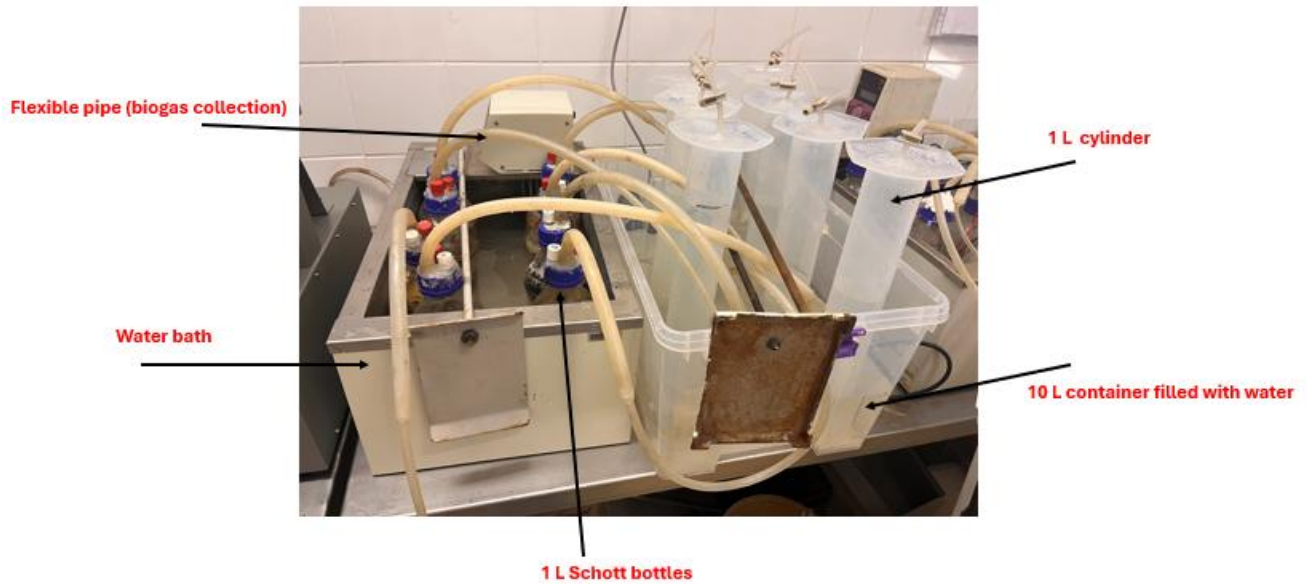


Figure C-1: Equipment for the BMP Co-digestion process setup

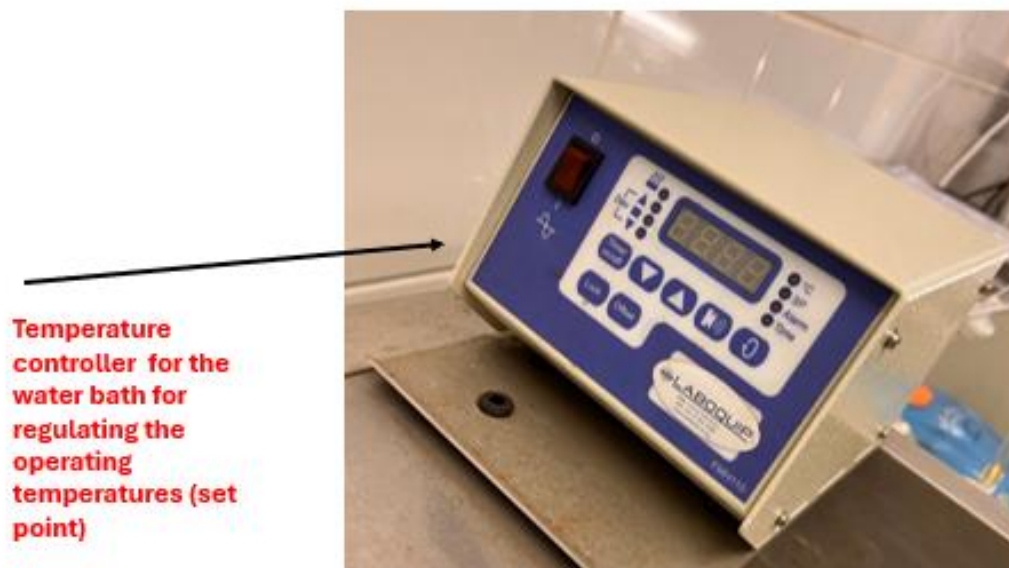


Figure C-2: Temperature control system for the water bath BMP system