



INVESTIGATING THE UPSCALING OF AN ANAEROBIC DIGESTER FOR BIOGAS PRODUCTION FROM INDUSTRIAL WASTEWATER

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PREFACE

This research was carried out under the collaboration of the Durban University of Technology, Green Engineering Research Group at the Department of Chemical Engineering and Water Research Commission (WRC) South Africa under the supervision of Dr EK Tetteh and Prof S Rathilal. Activated sludge and wastewater samples were collected from the local industrial and municipal wastewater treatment plants located in Durban, Kwa-Zulu Natal. Analysis, including wastewater characterisation, and experimental runs were conducted at the research lab in the Department of Chemical Engineering, Faculty of Engineering and the Built Environment, Durban University of Technology.

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DEDICATION

I dedicate this dissertation to my beloved grandmother (Mrs N Ngema) for your unwavering love, prayers, and support. To my three daughters (Siphesihle, Lisakhanya and Lwandile), my late mother, my sisters, and brothers, you have inspired me to get this far.

ABSTRACT

South Africa faces challenges of water scarcity and carbon-based energy pollution, and is currently under pressure to explore and capitalize on alternative methods to produce green energy. In addressing these challenges, the application and optimization of anaerobic digestion (AD) technology presents a robust solution for the treatment of wastewater and bioenergy production if operated efficiently. Under the South African National Development Roadmap and the United Nations Sustainability Development Goals on clean water and sanitation (#6), climate action (#13), and clean and affordable energy (#7), this research addressed the water scarcity, energy, and environmental concerns in South Africa. The study was aimed at up-scaling and optimizing an anaerobic digester for biogas production from industrial wastewater. Sequentially, characterisation of the wastewater streams, evaluating and optimising the operational factors of the AD process as well as cost-benefit analysis were among the specific objectives carried out to achieve the overall aim of the study.

The characterization results show that the sugar refinery and industrial sewage have the highest organic content with COD of 18770 mg/L and 4320 mg/L respectively. The sugar refinery stream had the highest concentration of volatile solids at 0.026 g/mL, as well as a high concentration of phosphates and nitrates, followed by industrial sewage, and the least concentration of volatile solids, phosphates and nitrates was observed from the oil refinery stream. The results of biochemical methane potential tests show the highest biogas production from the sugar refinery stream (148 mL/gTDS), followed by the industrial sewage (76 mL/gTDS), and the least production from the oil refinery (64 mL/gTDS) and municipal wastewater (45 mL/gTDS). Although the sugar refinery stream produces the highest biogas, it also shows difficulty in the removal of organic content with COD removal of 62.8%, indicating that a major fraction of organic content is not degraded and converted into biogas.

Using the OFAT (one-factor-at-time) approach, the hydraulic retention time (HRT) and magnetite load were among the key operational factors evaluated. It was observed that, at the initial stage there was a poor response in biogas production and COD removal, followed by an exponential increase in biogas production between 9-18 days, a decline in biogas production between 19-22 days, and retardants or no production between 22-30 days. This suggested that there was a long lag phase due to poor microbial activity and an optimum HRT can be obtained between 18 – 22 days. The addition of magnetite reduced the lag phase significantly from 9 days to 3 days, indicating that the addition of magnetite improves the interspecies electron transfer and ultimately enhances biogas production. The addition of 0.4 – 0.6 g/L magnetite achieved high biogas production rates of 23 mL/d and 20 mL/d

respectively between 9-12 days while the addition of 0.8 g/L resulted in catalyst overloading which inhibited the microbial activity and a rapid decline in biogas production to 2 mL/d after 9 days was observed. An increase in organic content from 4320 mg/L to 18770 mg/L resulted in a 30% increase in biogas production, however, a decline of over 40% in the removal of contaminants was noted due to a decrease in pH leading to the accumulation of long-chain fatty acids which are inhibitory to groups of microorganisms.

At the upscale of 50 L AD, the operating conditions were optimised using the response surface methodology (RSM). The statistical analysis revealed that the quadratic models for the three responses studied (biogas yield, COD removal and colour removal) were significant with p-values below 0.05. The R^2 values for the three responses were 0.99 with differences between predicted and adjusted R^2 below 0.2, suggesting reasonable agreement with observational data points. The optimum operating conditions obtained were an HRT of 21 days, pH of 7.01 and magnetite load of 0.42 g/L with a desirability of 0.99. The optimum conditions were validated against two streams, industrial sewage (low organic content) and sugar refinery (high organic content) and achieved over 85% and over 60% in the removal of the contaminants, respectively. The addition of sensors to the up-scaled 50 L AD ensured accurate control and monitoring of operating conditions, thus enhancing methane content to 90%.

Furthermore, a cost-benefit analysis of the optimized AD system with biogas production and its applicability as an alternative source of energy was conducted. The annual operating costs for the up-scaled AD system exceeded the annual revenue, resulting in a net cash flow of -R8 506. The 50 L AD has a net present value of -R121 016, indicating that the system is not economically viable due to the high capital cost required for the design and commissioning of the system. Based on the cumulative cash flow, the 1 L, 5 L, 10 L, and 50 L systems will take 24.8, 21.9, 25.3, and 19.03 years to pay back the initial investment. Also, the benefit-cost ratio increases with an increase in AD size: 1 L (0.05), 5 L (0.12), 10 L (0.13), and 50 L (0.4), indicating that up-scaling has the potential to generate more revenue with a decrease in operating cost for the optimized and stabilized system. Therefore, up-scaling of an anaerobic digester (AD) with the optimum conditions for treating industrial wastewater and biogas production was feasible. However, up-scaling beyond 50 L capacity is essential to increase biogas production, enhance revenue generation and improve the benefit-cost ratio to 1 and above. This would make the AD system economically viable for large-scale operations.

RESEARCH OUTPUTS

The candidate (L Ngema) under supervision was responsible for conceptualization, experimental design and execution, data collection, writing and discussion of the results; has authored and presented the following manuscripts (1-3) and conference proceedings (I-III) listed below.

Journal Publications

1. **Ngema L**, Sathiyah D, Tetteh EK, Rathilal S. Enhancing Biodegradation of Industrial Wastewater into Methane-Rich Biogas Using an Up-Flow Anaerobic Sludge Blanket Reactor. *Applied Sciences*. 2023; 13(7):4181. <https://doi.org/10.3390/app13074181>
2. **Ngema L**, Sathiyah D, Tetteh EK, Rathilal S. Optimization and Up-scaling of an Anaerobic Digester for the recovery of high-quality biogas from the sugar refinery effluent (**Under preparation**)
3. Sathiyah D, **Ngema L**, Tetteh EK, Chollom MN, Rathilal S. Effect of Operational Parameters on Anaerobic Digestion of Municipal and Sugar industry wastewater. *International Journal of Energy Production and Management*. 2023, Vol 8 Issue 3. DOI: <http://dx.doi.org/10.18280/ijepm.080304>

Conference presentations and proceedings

- I. **L Ngema**, Sathiyah D, EK Tetteh and S Rathilal. Response surface optimization of up-flow anaerobic sludge blanket reactor for biodegradation of wastewater into bioenergy. 2nd Sustainable Bioenergy and Processes Conference. 12-14 Dec 2022, Cape Town (South Africa) [**Presenter**]
- II. **Ngema L**, Madondo IN, Mguni LL, Sathiyah D, Tetteh EK, Rathilal S. Optimizing Anaerobic Digestion of a Local South African Municipal wastewater using Box-Behnken Design of Response Surface Methodology. 39th JICBEE-23, Nov 16-17, 2023 Johannesburg (South Africa) [**Presenter**] <https://doi.org/10.17758/IICBE5.C1123020>
- III. **Ngema L**, Khanyile SM, Sathiyah D, Tetteh EK, Rathilal S. Economic Benefits of Scaling up a Smart Anaerobic Digester for Biogas recovery from industrial wastewater. 41st CCBEE-24, Nov 21-22, 2024 Cape Town, South Africa [**Presenter**] <https://doi.org/10.17758/IICBE6.C1124233>

Awards

- ✓ Oral Best Paper award at the 39th JCBEE-23
- ✓ Best Conference Paper award at the 41st CCBEE-24

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NOMENCLATURE

AD	Anaerobic Digestion
BBD	Box – Behnken Design
BET	Brunauer -Emmett-Teller
BCR	Benefit to Cost Ratio
BMP	Biochemical Methane Potential
CF	Cash Flow
CHP	Combined Heat and Power
COD	Chemical Oxygen Demand
DNA	Deoxyribonucleic Acid
GC	Gas Chromatography
HRT	Hydraulic Retention Time
kWh	Kilowatt-Hour
LCFA	Long Chain Fatty Acids
MNP	Magnetic Nanoparticles
NP	Nanoparticles
NPV	Net Positive Value
OFAT	One Factor at a Time
OLR	Organic Loading Rate
PSA	Pressure Swing Adsorption
RSM	Response Surface Methodology

TDS	Total Dissolved Solids
TOC	Total Organic Carbon
TS	Total Solids
UASB	Up-flow Anaerobic Sludge Blanket Reactor
VFA	Volatile Fatty Acids
VS	Volatile Solids
WWTPs	Wastewater treatment plants

CHAPTER 1: INTRODUCTION

1.1 Background

The global demand on carbon-based fuels (fossil fuels), makes environmental threats by high carbon footprint inevitable. This issue is directly linked to the rising industrialised growth and demand on fossil fuels contributing to greenhouse gas emissions and climate change (Achinas *et al.* 2020). The world currently focuses on decreasing global energy demand to minimize the emissions of CO₂ and minimize greenhouse effects. Hence, mitigating climate change has become a pressing issue globally, with significant measures and strategies adapted to safeguard the ecosystem (Yoro and Daramola 2020). Some of these strategies include the South African National Development Roadmap, the United Nations 2015 Paris Agreement and the United Nations Sustainability Development Goals on clean water and sanitation (#6), climate action (#13), and clean and affordable energy (#7) (Arora and Mishra 2019).

To mitigate the environmental effects of using fossil fuels, renewable energy is gaining significant interest in electricity generation. The major groups of renewable energy sources that are gaining significant interest are solar energy, wind energy, geothermal energy and biomass because they are sustainable, eco-friendly and cost-effective (Ang *et al.* 2022). Among these groups, bioenergy production from organic waste is classified as the most fashionable method because of the abundance of raw materials. This approach also addresses the issue of waste management while generating biogas. The most common technologies for producing biogas are anaerobic digestion, dry fermentation, landfill gas recovery and microbial electrolysis cells. One of the most researched technologies is the utilization of wastewater and sewage sludge to produce biogas by anaerobic digestion (AD). Anaerobic digestion (AD) technology is a promising approach for the treatment of wet organic waste due to its energy and nutrients benefits, where microorganisms decompose and transform organic matter into biogas in an oxygen-free environment under strict anaerobic conditions (Bowman *et al.* 2022). The produced biogas can be utilized for power generation or be converted into bio-methane for use as fuel or as feed into gas network (Calise *et al.* 2022). Thus, biodegradation of wastewater through anaerobic digestion has received global attention for biogas production.

Biogas produced through AD is the low-carbon green energy carrier resulting from the degradation of organic matter in an oxygen-free environment. Even though AD offers many advantages, it also has setbacks that prevent it from being applied in a variety of industries. Low biogas yield has been discovered to be caused by a lack of process control and optimization (Panigrahi and Dubey 2019).

Another problem is the formation of toxic intermediate composites during digestion. Other challenges involves the accumulation of volatile fatty acids (VFAs), increased financial expenditures for operation and transportation, inadequate buffering capacity and process instability (Siegert and Banks 2005; Chen *et al.* 2014). These could result from a decrease in pH during the initial phase of digestion and the inhibition of ammonia, long-chain fatty acids and hydrogen sulphide (Elsamadony *et al.* 2021). A notable difficulty is the absence of suitable reactor design, as reactor design predominantly based on the rule of thumb. Wastewater characterization, biodegradability, bacterial activity development, accessibility, CH₄ production enhancement and nutritional balance are considered significant challenges for the efficient production of biogas (Pramanik *et al.* 2019).

The efficiency of an anaerobic digestion system can either be enhanced by modifying the digester design or implementing suitable advanced operational protocols. The up-flow anaerobic sludge blanket (UASB) reactor is deemed better to conventional methods due to its exceptional sludge retention (Rajeshwari *et al.* 2000). In the UASB design, the influent enters the tank from the bottom and ascends through the sludge blanket layer before being discharged from the top of the tank. This set up aims to enhance the interaction between wastewater and mixed culture sludge by reducing the up-flow velocity (Adhikari and Lohani 2019). In a similar manner, solids retention is enhanced when the denser solid components descend to the bottom of the tank, away from the effluent discharge at the top. This therefore facilitates the development of granular solids, which is often linked to enhanced treatment efficiency (Adhikari and Lohani 2019; Cecconet *et al.* 2022). In contrast, the UASB system is favoured due to its superior settleability, capacity for high biomass concentrations, effective solid/liquid separation and the ability to function at increased organic loading rates (Poh *et al.* 2015).

Many researchers suggest introducing trace elements as nanoparticles to the AD feedstock to improve AD performance and enhance biogas production. In anaerobic digesters, nanoparticles (NPs) promote direct interspecies electron transfer and acts as good immobilization matrices. This discourages the selection of resistant bacteria and genes and dominant metabolic pathways of microbial communities in anaerobic digesters (Zhang *et al.* 2019). The metallic nanoparticles most suitable for application in anaerobic digestion, are iron-based NPs, nickel NPs and titanium dioxide NPs due to their ability to enhance microbial activity (Yang *et al.* 2013; Wang and Chen 2016). Among other metallic nanoparticles, the iron nanoparticles serves as superior additions to the anaerobic digestion process owing to their capacity to undergo electron transfer, resulting in the formation of oxides or hydroxides on the metal surface (Amo-Duodu *et al.* 2021c). The iron oxide NPs with unique properties, slowly dissolve to supply the living microorganisms with the required iron ions without causing toxicity to

the bacteria. Ganzoury and Allam (2015) reported that the application of iron nanoparticles leads to 180% enhancement in biogas production and a 234% enhancement in methane production under optimal anaerobic conditions. However, excess iron from magnetite nanoparticles produces toxic free radicals, influencing the impact of Fe^{2+} ions on the anaerobic digestion of organic matter, hence altering the resilience and growth of the microbial community (Gualdani *et al.* 2019).

The response surface methodology (RSM) is a popular mathematical and statistical method for modelling and analysing a process in which the response of interest is influenced by a variety of factors, to optimize the response (Adedeji *et al.* 2022). RSM explores a suitable approximation relation between input and output variables to determine the best operating conditions for a system under investigation or an area of the factor field that meets the operational criteria (Aydar 2018; Ghaleb *et al.* 2020). The advantages of RSM include determining the interaction between the independent variables, mathematically modelling the system, and saving time and money by lowering the number of trials (Deepanraj *et al.* 2021a).

1.2 Anaerobic digestion process in wastewater treatment

The application of AD technology in wastewater treatment provides several inherent advantages, apart from biogas production. This ranges from cleaning of wastewater to reusable water, lower greenhouse gas emissions, and enhancing the general economic status of rural populations in developing nations as well as their general health and hygiene (Khanal *et al.* 2021). During the digestion of wastewater, the reaction rate and the reactions' complexity will vary for different substrates. The breakdown of organic material is an exothermic reaction, generating energy. This energy is given to the microorganism in the form of a reduced substrate and the cell will use this energy to create more cells (Nabaterega *et al.* 2021; Rahmati *et al.* 2024). In this process, the microorganisms break down biodegradable material in the absence of oxygen. The carefully controlled conditions enable the bacteria to carry out complex biochemical reactions, which result in the production of biogas and treated water that is safe to reuse or discharge to the environment.

1.3 Biogas as a source of renewable energy

In South Africa, various industrial operations produce large quantities of organic waste that is usually left to decompose into the environment. During decomposition, it produces significant amounts of methane gas, a potent greenhouse gas, and hence biogas production is defined as an important waste management strategy (Gaur *et al.* 2020; Hajam *et al.* 2023). The produced biogas can be upgraded and used for electricity and thermal energy production. If the biogas is used for electricity production,

it can be utilized close to the point of production and there is very little energy loss during the transmission process. This makes biogas electricity production especially useful for remote or rural communities far from the electric grid, such as in many third-world countries (Nindhia *et al.* 2021). In addition, biogas is a renewable energy source that can produce electricity anytime during production and therefore possesses more advantages over other renewable energy sources like solar or wind energy (Czekala 2022).

1.4 Problem statement

South Africa faces the challenge of meeting household and industrial electricity demands due to an increasing population. The conventional ways of producing electricity do not meet the current energy demand resulting in intensive power cuts (load-shedding) witnessed over the years. Moreover, South Africa is among the largest coal consumers in Africa for electricity generation, and this coal usage contributes to CO₂ emissions in the atmosphere, worsening climate change. This crisis warrants the shift to renewable energy resources to mitigate the negative impact of coal processing in the environment. As an alternative, biogas can be used to produce clean energy and eliminate emissions of greenhouse gases.

Currently, most AD systems for biogas production operate at small-scale capacity, therefore hindering their potential impact and efficiency to global energy benefits. Industrialization of biogas production from organic fraction of wastewater by AD technology has been limited by many factors such as variations in feedstock (wastewater) properties, capital and operational costs as well as lack of control and understanding of operating conditions (Madondo *et al.* 2022). Up-scaling and optimization of AD systems for biogas production hold the key to maximizing the environmental, economic and energy benefits of AD technology. Therefore, there is an urgent need to develop AD systems that can effectively treat all wastewater streams and produce quality biogas.

1.5 Aims and Objectives

This study aimed to develop and evaluate an upscaled 50 L anaerobic digester (AD) for biogas production from industrial wastewater. The specific objectives include the following:

To conduct a feasibility study of producing biogas from four different wastewater streams with high organic content using a biochemical methane potential test.

To evaluate biogas production from a characterized wastewater stream with high organic content using 5 L and 10 L lab scale AD systems

To design the upscaled 50 L AD and optimize operating conditions (operating temperature, hydraulic retention time, pH and catalyst loading rate) using response surface methodology
To conduct a cost-benefit analysis of the optimised AD system with biogas production and its applicability as an alternative energy source.

1.6 Approach

To evaluate the suitability of local wastewater streams for biogas production using anaerobic digestion technology, a thorough characterisation of the physicochemical properties of the four local wastewater streams was conducted. Further studies involving biochemical methane potential tests were conducted to determine biogas potential for various wastewater streams.

The second phase evaluated biogas production using small-scale laboratory AD systems. It entailed evaluating the effect of operating parameters using a one-factor-at-a-time system. With the Design Expert Software, the response surface methodology (RSM) was adapted and used for optimizing the operating parameters for the digester and generating model equations for estimating theoretical biogas production and the removal of contaminants.

The final phase examined the dynamics of upscaling an anaerobic digester and optimization for biogas production. It also included collection, quantification, and characterization of biogas to estimate its energy value and suitability to be used and/or converted into electricity. This phase also examined the cost-benefit analysis for scaling up a biogas recovery AD system. This evaluated and assessed the feasibility of up-scaling and industrialization of biogas recovery AD systems.

1.7 Organization of Dissertation

The dissertation consists of five chapters which are organized as follows;

Chapter One

This chapter introduces and provides background to the entire study, highlighting its need and expected outcome. It also presents the objectives of this research and the approach to achieving these objectives.

Chapter Two

This chapter reviews the literature related to the production of biogas from wastewater, challenges encountered by anaerobic digesters, advantages, and disadvantages of using the technology, and the future of biogas.

Chapter Three

This chapter presents the experimental work's research methodology, materials, methods, equipment description, and procedures.

Chapter Four

This chapter presents the results and discussions of the study. First, the characterisation of various wastewater streams and the evaluation of producing biogas from these streams is provided. Secondly, the optimization of operating conditions for maximum biogas production and the removal of wastewater contaminants is evaluated. It also summarizes the dynamics of upscaling an anaerobic digester with changes in biogas production and contaminants removal. Lastly, it evaluates the cost-benefit analysis for up scaling an anaerobic digestion system for biogas production.

Chapter Five

This chapter concludes the entire study, declaring new findings, making recommendations for further studies in reactor design and optimization for biogas production.

CHAPTER 2: LITERATURE REVIEW

This chapter provides an overview of South Africa's energy crisis and the possible alternative energy sources. It provides an in-depth explanation of anaerobic digestion (AD) technology as a waste management technique with the benefit of producing a renewable energy source (biogas). It also highlights the operating conditions of the AD parameters and their effects on the biodegradation of organic content in wastewater for biogas production. Furthermore, it explores the addition of magnetic nanoparticles to enhance biogas production and optimisation techniques.

2.1 Energy crisis in South Africa

South Africa's power crisis characterized by continuous load shedding has adversely affected the nation's economy, society, and energy policy. Electricity consumption in the early 2000s witnessed a major jump owing to the growing economy but infrastructure investments did not keep up with the demands (Berahab 2022). The capability of the production facilities to meet the peak demand is also reduced due to poor maintenance practices and unpredicted outages. Adding to many of the plants being outdated experience regular breakdowns, requiring national concern to look for alternative energy resources and facilities. Notwithstanding coal contribute to over 80% of South Africa's electricity production as shown in Figure 2-1, which results in the production of greenhouse gases that have a negative impact on the environment.

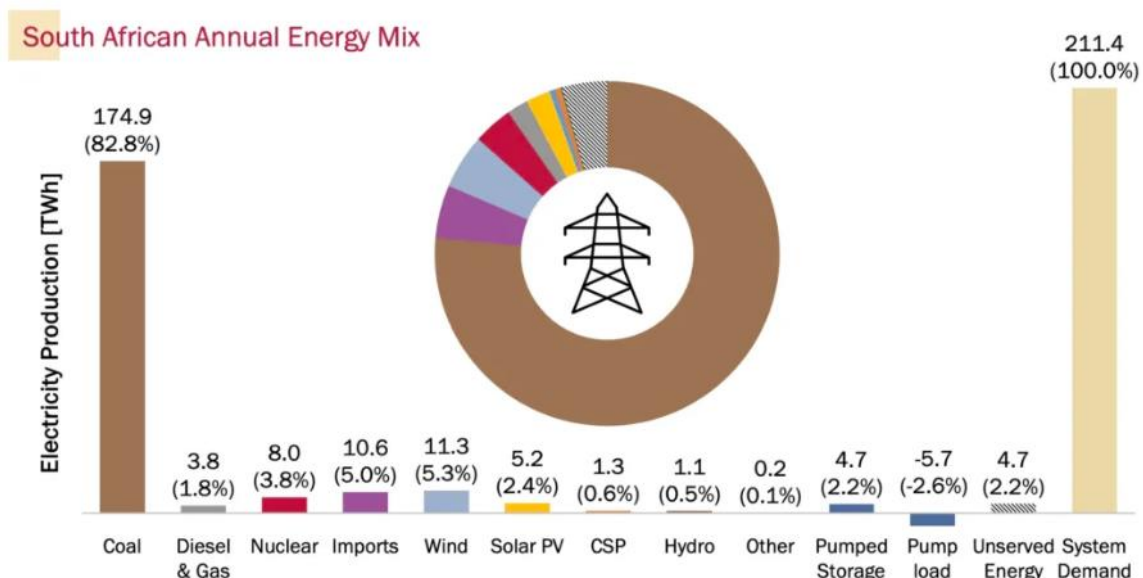


Figure 2-1: South Africa's energy production sources (www.crses.sun.ac.za/sa-energy/ 10 October 2024)

The manufacturing, mining, and agriculture sectors suffered significant economic losses due to recurring power outages. Load shedding impacts the nation's GDP growth rate, with most common small businesses experiencing losses significantly, as many cannot acquire backup energy resources like generators or solar panels. South Africa is among the largest coal consumers in Africa, and its coal usage contributes to CO₂ emissions in the atmosphere, worsening climate change. This crisis warrants the shift to renewable energy resources to mitigate the negative impact of coal processing on the environment. As the utilisation of renewable sources escalates, demand patterns may shift due to distributed generation (such as rooftop solar) reducing dependence on the grid. The current penetration of electric vehicles is minimal; however, future advancements may need a rise in electricity usage, particularly with integrating renewable energy sources. Energy-intensive industries, such as mining in South Africa, may become less dependent on coal-fired electricity as the nation progresses towards a more sustainable economy. The current crisis indicates a supply-side problem; future investments in infrastructure, renewable energy, and energy efficiency could transform demand and achieve energy sustainability. South Africa is already experiencing the effects of climate change, manifested as drought, harsh weather, and water scarcity, which are intensified by global emissions. (Berahab 2022; Behrens *et al.* 2024).

2.2 Transition from coal to green energy resources

Coal has been a fundamental source of power generation for over a hundred years, facilitating industrial growth and economic progress in numerous regions globally. Nevertheless, the ongoing dependence on coal presents considerable environmental, financial, and social challenges. Transitioning to alternative energy sources is essential for addressing climate change and promoting sustainable development. Coal combustion stands out as the predominant contributor to carbon dioxide (CO₂) emissions on a global scale. This sector accounts for more than 40% of energy-related CO₂ emissions and plays a substantial role in global warming. Most coal plants' economic viability is diminishing compared to the more cost-effective options of solar and wind energy. (Pretorius *et al.* 2015). With the current need to shift from conventional energy production methods due to climate change and CO₂ emission restrictions, there is an increase in demand for exploring alternative approaches to produce green energy. There are currently three promising sources of clean energy that warrant effort and investments for maximising energy production globally, namely wind energy resources, solar energy and biomass and wastewater. Table 2-1 summarises the benefits and limitations of the different renewable energy sources for prospects.

The ongoing reliance on coal could result in economic and diplomatic seclusion for nations. Transitioning from coal to alternative energy sources is essential for tackling climate change, safeguarding public health, and promoting sustainable economic growth (Adelekan *et al.* 2024). Coal has played a significant role in historical development; however, its environmental and economic drawbacks render it unsustainable over time (Finkelman *et al.* 2021). Investment in renewables, including solar, wind, and biomass, along with supportive policies, allows nations to create a cleaner, more resilient energy future that meets the needs of both present and future generations.

For wastewater-to-energy to be effective, its energy potential must be generated through organic matter, recovered heat, and nutrient content (Hussain *et al.* 2020). This also contributes to the production of renewable energy, the decrease of accumulated waste, and the preservation of sustainable resource use. Anaerobic digestion is suggested as a method for producing bioenergy from organic waste. This process creates a form of biogas that contains CO₂ and methane. It is also utilised to generate heat and electricity in combined heat and power systems. By combining energy production with effective wastewater treatment, AD's organic waste management approach offers the added benefit of reducing methane emissions from untreated organic wastes by capturing and using biogas. This helps to prevent water contamination. Therefore, it indicates that the technology overcomes obstacles related to waste management and energy production systems and implements the circular economy concepts (Vutai *et al.* 2016; Cremiato *et al.* 2018).

Table 2-1: Summary of benefits and limitations of different energy sources

Alternative energy source	Benefits	Limitations	Reference
Solar	• Clean energy source with minimum greenhouse gas emissions and air pollution • Sustainable with an unlimited supply of energy from sunlight • Lower operating costs and minimum maintenance required	• Weather dependant and unavailable at night, requiring extra storage systems • Low efficiency with solar photovoltaics, converting less than 40% of sunlight into electricity • High installation costs required	(Kabir <i>et al.</i> 2018; Chishimba 2022)
Wind	• Conserves water as less cooling water is required and does not produce greenhouse gases • Cost effective as wind is free and unlimited, reducing reliance on imported fuels • Easy to upscale and increase electricity generation	• Dependant on weather conditions with low production in calm seasons • Excessing noise from large turbines during operation poses a health risk • High capital costs required	(Ferguson 2008; Maradin 2021)
Biomass and wastewater	• Renewable and sustainable energy • Reduces organic waste disposed to the landfills, reducing methane emission resulting from the decomposition of organic waste, thus improving waste management systems • Carbon neutral	• Lower conversion efficiency of biomass into electricity compared to fossil fuels • High capital costs required and poor infrastructure • Quality of feedstock dependent on seasons and up-stream operations	(Rulkens 2008; Maradin 2021; Tawfik <i>et al.</i> 2022)

2.3 Anaerobic Digestion Process Overview

Anaerobic digestion (AD) is one of the oldest technologies widely used to treat waste. Achinas *et al.* (2020) described anaerobic digestion as the natural process that breaks down and converts organic matter into biogas composed of carbon dioxide (CO₂) and Methane (CH₄) without oxygen. This process occurs under strict anaerobic conditions and comprises several degradation phases facilitated by the collaborated relationship of various microorganisms. Figure 2-2 summarises the process of anaerobic digestion, where four successive stages of the process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis and their intermediate products are defined

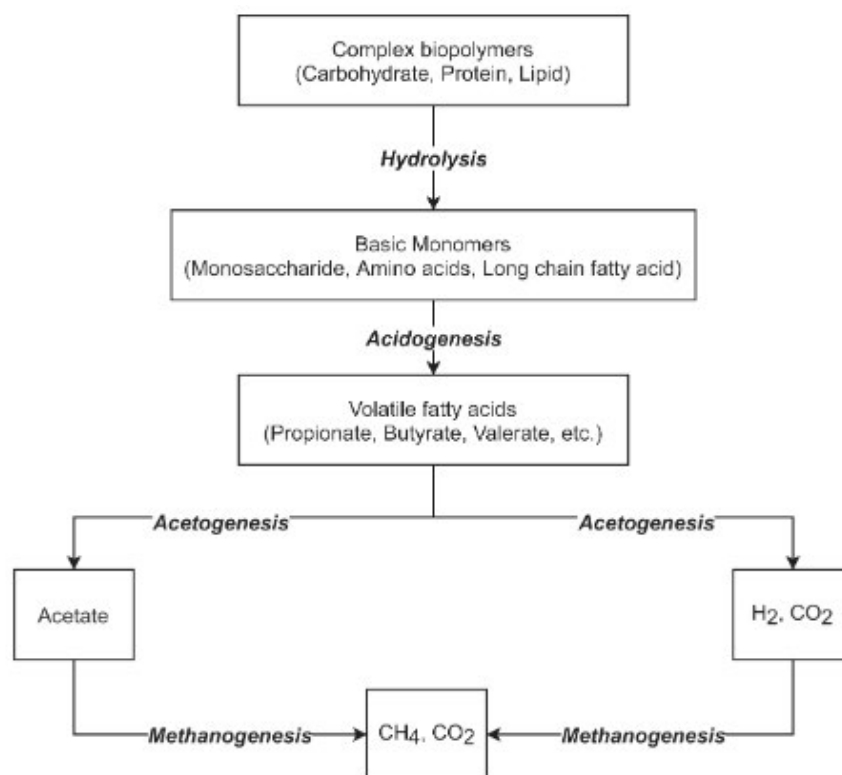


Figure 2-2: Anaerobic digestion process steps to produce biogas from organic matter (Uddin and Wright 2022)

1) Hydrolysis

Hydrolysis is the first step of the anaerobic degradation that occurs due to the encounter of complex polymers and organic waste in anaerobic digesters, which are inaccessible by microorganisms. In this step, complex organic molecules are degraded to produce soluble sugars, long chain fatty and amino acids. Hydrolysis reactions are permitted by extracellular enzymes that are made by microorganisms called hydrolytic bacteria (Kang *et al.* 2014; Kang and Yuan 2017). Previous researchers have reported hydrolysis as the rate-limiting step in anaerobic digestion due to hydrolytic bacteria' slow depolymerisation of the insoluble complex organic

matter. Meegoda *et al.* (2018b) reported that hydrolysis has an optimum temperature ranging between 30-50°C and a working pH of 5-7. However, no evidence of improved hydrolytic activity has been presented for a pH below 7.

2) Acidogenesis

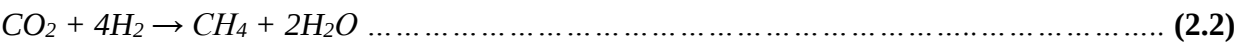
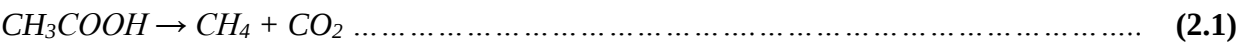
In the second stage of anaerobic digestion fermentation, acidogenic microorganisms generate intermediate products by absorbing hydrolysis products through their cell membranes. In detail, acetogenic bacteria use amino acids, long-chain fatty acids, and sugars as carbon and energy sources to produce alcohols and volatile fatty acids. Acidogenesis proceeds faster than all other stages of anaerobic digestion process (Meegoda *et al.* 2018a). This happens because acidogenic bacteria have shorter regeneration time.

3) Acetogenesis

The third step of the AD process, acetogenesis, and acetic bacteria, transforms volatile fatty acids and alcohols into acetate, CO₂, and H₂, which will be utilised in biogas production. The slow-growing acetogenic microorganisms are sensitive to high hydrogen pressure. Therefore, hydrogen is quickly consumed by existing hydrogenotrophic methanogens to maintain a favourable level of hydrogen partial pressure to acetic microorganisms (Meegoda *et al.* 2018).

4) Methanogenesis

In the final stage of anaerobic digestion, methanogenic microorganisms utilise intermediate products to produce biogas. There are two pathways for methane production in this stage: (i) the first path involves converting CO₂ into methane and water through a reaction with hydrogen, both produced in the acetogenesis step, and (ii) the conversion of acetic acid into biogas composed of methane and CO₂ (Kirk and Gould 2020). According to Achinas *et al.* (2020) About 70% of the methane produced in this process is derived from acetate consumption (2.1), while only 30% results from the reduction of CO₂ by hydrogen (2.2)



Studies by Meegoda *et al.* (2018a) suggests that methanogenic microorganisms in this step requires a higher pH than other previous stages and lower redox potential due to sensitivity of methanogenic species. The extent of methanogenesis in batch reactors can be measured when the production of biogas stops.

2.4 Advantages of AD technology in waste management and biogas production

Anaerobic digestion provides solutions for treating bio-waste to produce safe residue to dispose to the landfills. As shown in Figure 2-3, it also results in the production of biogas, which is a potential source of heat and power, and cleaner effluent suitable for disposal to the environment. Biogas can potentially be used in transportation fuel, heat, and energy generation. Most studies have shown that the conversion of biogas to generate electricity is the key element for future global projections (Calbry-Muzyka *et al.* 2022). The use of biogas in fuel cells presents a promising way to valorise biomass resources due to fuel cells' distinct efficiency.

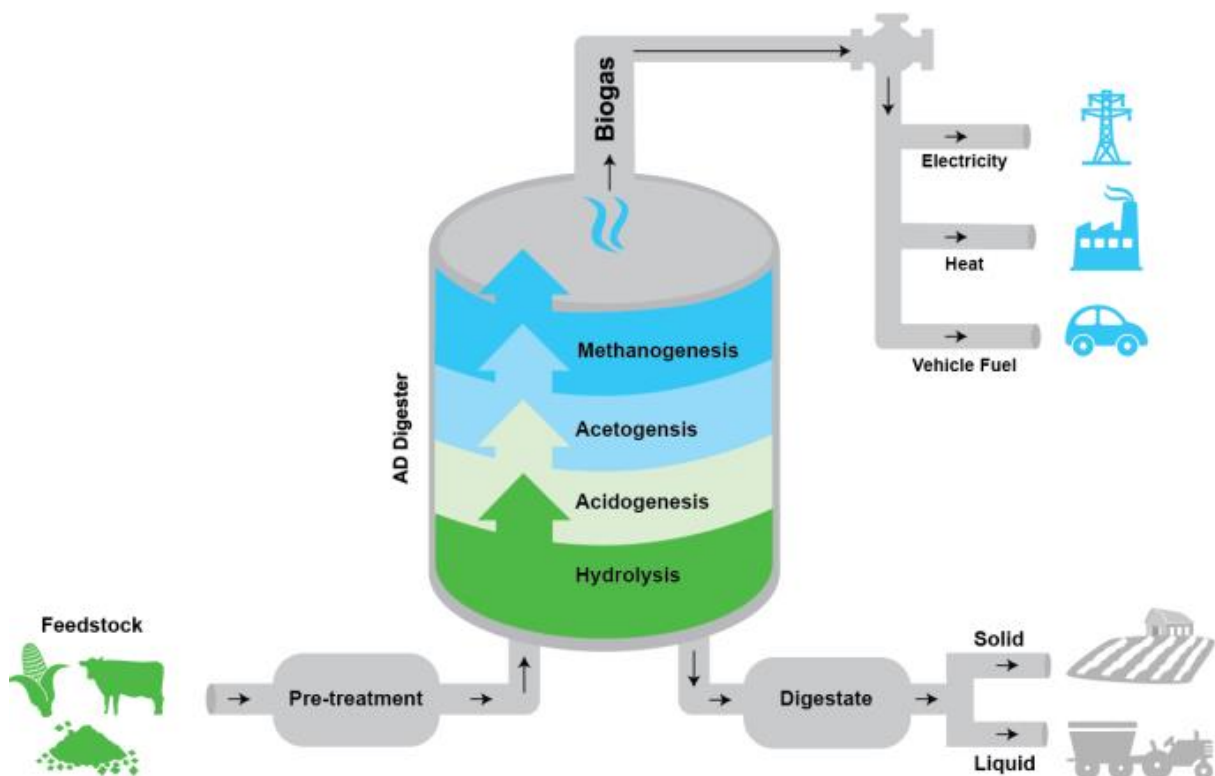


Figure 2-3: Overview of anaerobic digestion system (Global Methane Initiative 2016)

2.4.1 Waste Management Benefits

Anaerobic digestion is the perfect technology to support waste management practices in disposing of organic waste. The anaerobic process can be used to treat a sole waste stream or mixed waste. The digestion process stabilises the organic matter, reducing the putrescence of the material, which attracts vermin. The biogas produced can be used as an energy source, and the nutrient-rich digestate (comprising the solids) can be used as a fertiliser, returning nutrients and organic matter to the land. (Araya 2018). This helps sustain land productivity and can displace the production and energy-intensive manufacture of mineral fertilisers. The primary function of anaerobic digestion is to reduce the organic solid waste discarded into the environment. The treatment of waste helps to reduce water and soil pollution. The number and types of waste treated will arise because many of the wastes from the food processing and agricultural industry are suitable for digestion treatment, and the pressure to ensure more sustainable disposal of the waste will make anaerobic digestion an attractive option. The ability of digestion to reduce the mass and volume of waste or wastewater, typically by 20-40%, is an added advantage (Araya 2018).

Secondary environmental impacts of anaerobic digestion and biogas production include the generation of a renewable energy source and the potential improvement of soil quality. Previous life cycle assessment studies show that switching from electric or gas-powered heat and power systems to biogas powered CHP systems offers increased greenhouse gas savings. Organic material that has undergone anaerobic digestion is more easily integrated into the soil (Fusi *et al.* 2016; Abanades *et al.* 2022). This can lead to enrichment of the earth and decrease erosion. The overall environmental impact of treating organic waste through anaerobic digestion is a significant reduction in greenhouse gas emissions. Organic waste, when disposed in landfills, produces methane as it decomposes anaerobically. The treatment of organic waste using anaerobic digestion virtually eliminates methane emission, as the process controls the release of the gas, and the gas produced can be used as an energy source. As methane has a global warming potential 21 times greater than that of carbon dioxide, the containment of methane is of critical importance in reducing artificial climate change.

Anaerobic digestion fits within the concept of a circular economy where waste is valorised rather than disposed of in landfill sites. Using anaerobic digestion in this way helps reduce reliance on landfill as a means of waste disposal, which in turn lessens the environmental impact of harmful emissions produced from landfill sites (Tricase and Lombardi 2012). Organic waste is biodegradable, which makes it an unsuitable material for landfill because of the risk of leachate production, and subsequent pollution this causes to watercourses. Digesting organic waste can, therefore, help reduce

greenhouse gas emissions. This is because the methane produced from anaerobic digestion can be used as a replacement for natural gas. When used as a fuel, the combustion of methane produces carbon dioxide and water as opposed to methane which is 21 times more harmful to the environment as a greenhouse gas (Tricase and Lombardi 2012; Whiting and Azapagic 2014).

2.4.2 Production of electricity from renewable energy resource

The production of electricity through biogas has seen a notable increase in recent years. The process of generating electricity from biogas consists of several stages. Biogas is upgraded to remove impurities such as sulphur before combustion to prevent corrosion in the engine. Several studies have been conducted on the feasibility of AD technology to produce quality biogas that can generate electricity, and it was found viable. The uses can be categorized as either energy uses or the use of digestion effluent. Many of the energy uses for biogas, particularly electrical and heat production, are complementary. This means that the energy produced for one form of use is also useful in producing another form.

Electric production is the most efficient means of producing energy from biogas, particularly with a CHP engine. Figure 2-4 shows the flow of biogas from AD to the CHP plant for conversion into electricity. The efficiency of conversion of chemical energy to electrical energy with a CHP engine is around 35-42%. If the waste heat is captured and used to provide air conditioning or refrigeration, an overall efficiency of greater than 60% can be obtained. The efficiency of a CHP engine is greater when used for electrical production alone, which is around 30-35%. The lowest efficiency is with gas production for internal combustion engines, which is around 20-25% (Whiting and Azapagic 2014; Rios and Kaltschmitt 2016). The reason for this range in efficiencies for gas production is largely because the composition of biogas can vary according to the feedstock, and engine type/quality also varies widely. Other methods of energy production are the combustion of biogas in a burner or using biogas as a fuel for a Stirling engine. Heat production from biogas can be used in any application currently using natural gas, and the same goes for gas production for use as a vehicle fuel. This in itself represents a vast range of potential uses for biogas (Yağlı *et al.* 2016; Elsner *et al.* 2017).

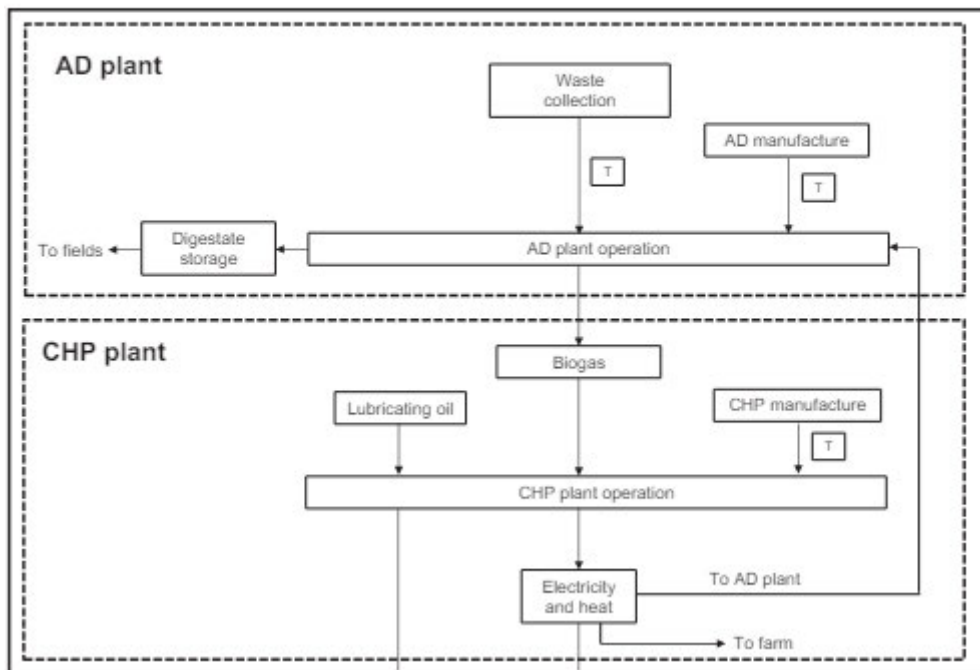


Figure 2-4: Flow diagram for conversion of biogas into electricity in a CHP plant (Whiting and Azapagic 2014)

2.4.3 Environmental impact of biogas production

Biogas utilization is an environmentally friendly, efficient, and economical way of producing electricity and heat. On a smaller scale, there are many instances in which the waste heat can be usefully employed. Greenhouses, farmhouses, and industrial processes often have a requirement for both heat and electrical energy. In the case of greenhouses, co-generation can provide a very cost-effective way of creating an ideal growing environment. The price and environmental benefits are good arguments for the use of fuel cells (Menardo *et al.* 2013). Due to the high efficiency of the fuel cell, as much as 40% of the energy can be in the form of heat. With a fuel cell running on cleaned biogas, this heat can be produced with very low emissions and the overall effect on the environment can be positive. Electricity and heat from biogas can be as good as CO₂ neutral. This is a very good argument in the case of electricity production with internal combustion engines. Combined with heat and power co-generation, the overall efficiency can be as high as 90%. The section below discusses various benefits of biogas electricity and heat, as well as challenges preventing the widespread production of biogas electricity.

The nature of biogas as a renewable source of energy provides a good opportunity for reducing environmental damage and offers several advantages in terms of mitigating global climate change.

The major advantage of biogas is that it is a clean and easily controlled source of renewable energy. It can be used as a replacement for natural gas, thus providing a means of reducing greenhouse gas emissions. In comparison to the combustion of the organic material from which the biogas was produced, using the gas in a CHP plant can yield a higher overall efficiency for energy generation. This is relevant to the issue of carbon leakage, where any use of waste materials can result in the burning or degradation of those materials being offset by increased use of fossil fuels to replace the energy that would have been produced by other means (Fusi *et al.* 2016). Another environmental benefit of biogas relates to the treatment of wastewater solids. The generation of biogas from wastewater treatment plants can be an efficient means of dealing with waste-activated sludge and can also provide a source of renewable electricity. The organic waste materials used in biogas production are often derived from a variety of agricultural, industrial, and municipal sources, which can result in widespread environmental benefits. When biogas is used to generate electricity for rural communities or businesses, it can provide an environmentally friendly and cost-effective alternative to the disposal of waste organic material. This, in turn, can help to reduce harmful emissions from decay or partial burning of waste materials and can minimize the subsequent migration of contaminants into surface and groundwater resources (Tricase and Lombardi 2012).

2.5 Quality and composition of biogas from the AD technology

Biogas is mainly composed of methane which is a useful fraction as an energy source and carbon dioxide. Additionally, biogas contains minor amounts of nitrogen, oxygen, and traces of sulphur compounds, ammonia as well as volatile organic compounds. Moreover, biogas is generally wetter than natural gas because at anaerobic temperatures it becomes saturated with moisture. The quality of biogas is affected by several impurities present which can deteriorate the downstream processes. Calbry-Muzyka *et al.* (2022) highlighted that these impurities have massive challenges to the energetic use of biogas, specifically in the use of high-temperature fuel cells or catalytic fuel upgrading units. Sulphur compounds that are generally found in agriculture biogas are of major concern whereas silicon compounds usually exist in low or undetectable levels. The biogas yield and its quality depend significantly on the anaerobic digestion of raw material. On average, biogas is composed of 50–75% methane, 25–50% carbon dioxide, and 2–8% other impurity gases (nitrogen, oxygen, ammonia, and hydrogen sulphide). Biogas quality requirements depend on its various applications. Before converting biogas into electricity by motor generators, it is necessary to employ purification steps such as desulfurization and drying processes. According to (Huerta-Reynoso *et al.* 2019) high concentrations of the colourless and flammable gas, H₂S, impose corrosion problems which can result in engine damage and production of sulphur oxides from combustion. The average

composition of biogas is shown in Table 2-2 with elevated methane content making it an attractive source of energy. Although biogas constitutes mainly methane and carbon dioxide, which are greenhouse gases that are harmful to the environment, it must undergo a burning process before releasing it to the atmosphere.

Table 2-2: Average compositions of biogas adapted from ((Bragança et al. 2020)

Component	Concentration (%)
Methane (CH ₄)	55 - 60
Carbon dioxide (CO ₂)	35 - 40
Hydrogen (H ₂)	2 - 7
Hydrogen Sulphide (H ₂ S)	0 – 0.05
Nitrogen (N ₂)	0 - 2

The presence of impurities in biogas has a detrimental effect on the calorific value of the gas, its corrosive tendencies on plants, and pollutant emissions when used in engines. Economically feasible upgrading methods exist for the removal of carbon dioxide and trace contaminants, leaving a gas consisting of over 90% methane (Bragança *et al.* 2020). The gas from these processes is of higher quality than natural gas. The energy value of the gas is calculated from its methane content and the higher heating value of methane (55.6 MJ/CH₄). High-quality gas and excess gas yield enable greater potential for electrical power production and combined heat and power (CHP) generation. The calorific value of biogas is ultimately the main factor affecting its utilisation for electricity production. It is generally considered that a gas with less than 90% methane content is not suitable for gas engine utilisation, and during a gas's transition phase to engine-grade gas, temporary cessation of production may be the most economically viable option. High-quality biogas has the potential to act as a renewable substitute for natural gas, thus opening up the possibility for injection into the national gas grid; an established method of natural gas storage and distribution (Bragança *et al.* 2020; Chaghouri *et al.* 2022).

2.5.1 Biogas utilisation for electricity generation

The breakthrough in modernizing the way of burning biogas for electricity generation is the adaptation of gas engine technology to biogas. Gas engine technology has been developed to a high level of technical sophistication and reliability in its long history in application for natural gas. Lean-burning pre-chamber engines, lean-burn Otto cycle engines (stoichiometric mixture and 3-way catalyst), and advanced design two-stroke cycle engines achieve electrical efficiencies in the range of 25-42%. (Trisakti *et al.* 2017) evaluated the performance of the Otto engine generator using biogas with a compression ratio of 10.5 and found a maximum power of 1.3 kW. All these engines are amenable to biogas fuels with varying levels of conditioning. The performance and efficiency of a biogas engine producing electricity is largely dependent on the engine's electrical generator. The engines are directly coupled to generators with the output electrical frequency and power factor depending on the type and design of the generator. Modern generators are of the brushless wound rotor design with the output frequency determined by the rotation speed of the rotor and the pole selection. Frequency control is required when paralleling with other generators and/or the grid and is usually achieved by some type of electronic or mechanical governor on the engine. High-efficiency generators, while more expensive, are often used with the rationale that the long-term economic benefits will offset the higher initial costs. For small-scale rural applications, old car alternators have been adapted to produce electricity from engines and biogas. These alternators provide a direct current electrical output which may be used for battery charging but are of low efficiency and offer no voltage or frequency regulation. Electric power from engine generators of all types may be easily connected to the local electrical grid, assuming grid access, or used in remote locations with a standalone system.

Cost-effectiveness is particularly problematic, as the price of the electricity and the renewable energy credits generated still often cannot recover the costs of the biogas to the electricity system. Internal combustion engines are capable of handling varying compositions and gas qualities of biogas and still producing the same power. However, systems such as fuel cells and micro-turbines can have catastrophic and often irreparable damage done to the unit if the biogas quality is not of a specified level (Rafiee *et al.* 2021). This unfortunately adds greater technical risk and reduces the feasibility of electricity generation. Additional impacts to the environment can also create obstacles for biogas-to-electricity. Air and water quality impacts are regulated by environmental regulatory agencies, in many cases, the biogas electricity generator is required to meet strict emissions standards that mirror those of natural gas systems.

2.5.2 Biogas Engine Generators

The most popular and efficient system for the utilization of biogas for electricity production is a reciprocating engine generator system. The study by (Sibanda 2021) was aimed designing a small reciprocating engine for electricity generation from biogas and gasoline and revealed that the biogas outperformed gasoline with regards to fuel generation rate. Much of the infrastructure for utilizing biogas generated on wastewater treatment plants and landfills is comprised of modifications of natural gas engine-driven genets. These are internal combustion engines, operating on biogas, natural gas, or other fuels, with an electrical generator set to produce electricity. The great advantage of engine generator sets is that the electrical efficiencies are higher than with most fuel cells and turbines (Okwu *et al.* 2020). For high-temperature gas produced from gasification or pyrolysis processes the overall electrical efficiency in a combined gas engine generator set could be as high as 25%. Internal combustion engines have been used over the last 100 years and are a mature technology, with high reliability and long lifetimes for stationary applications. This makes the engine generator sets ideal for a base load operation to supply electricity to the grid. Although biogas fuel properties exhibit a wide variation depending on the feedstock and process method, gas engine generator technology is quite versatile and is capable of using low to high BTU content gas and still maintaining acceptable performance from the engine (SOUZA *et al.* 2016).

2.5.3 Biogas Turbines

Most gas turbines with biogas have been around repowering. This is the addition of a gas turbine and heat recovery boiler to an existing steam turbine plant to replace the steam turbine power generation with gas turbine power generation (Kabeyi and Olanrewaju 2022). This is especially suitable for biogas plants with combined digestion and power generation, where the increased plant efficiency can lead to increased gas production. Biogas is a versatile fuel which can be used in most internal combustion engines. However, for small-scale electricity generation, there are several types of gas turbines which are suitable for biogas. By gas turbine, the engine uses high-pressure gas to turn a turbine to produce electricity (Chang *et al.* 2019). The waste heat from the turbine is usually used to produce steam, which in turn is used in a steam turbine to produce more electricity.

2.5.4 Biogas fuel cells

Fuel cell (FC), one of the emerging technologies in distributed generation, is an electrochemical device that continuously converts chemical energy into electrical energy and heat. FCs are more efficient in comparison with internal combustion engines (Moulod *et al.* 2016). The high efficiency

and low emissions of fuel cells make them very attractive. High-temperature fuel cells may be up to 60% efficient in cogeneration mode, and with almost negligible emissions, they have the potential to be the most environmentally friendly way of producing electricity. Different fuel cells require different levels of gas cleaning and gas types. Typically, biogas needs to be reformed into hydrogen gas and cleaned of all contaminants to power commercial-grade fuel cells. This can be expensive and lower the overall efficiency of the plant (Kubota *et al.* 2002). Hydrogen gas fuel cells are the most efficient, but given the requirements, they are currently not economically viable for most applications. High-temperature fuel cells can utilize biogas with little or no reforming and are more thermally efficient than low-temperature fuel cells. As a result, they are the most suitable type for current biogas applications (Kubota *et al.* 2002). Biogas fuel cells are like battery systems because they both are electrochemical devices but differ in that fuel cells require a constant source of fuel and oxygen to run. The fuel cells use the chemical energy of biogas to cleanly and efficiently produce electricity. A fuel cell consists of an electrolyte and two catalyst-coated electrodes. The gases are electrochemically oxidized at the anode, releasing electrons and producing thermal energy. The thermal energy is utilized to electrochemically charge the electrons and provide electricity (Moulod *et al.* 2016; Beigzadeh *et al.* 2020).

2.5.5 Biogas upgrading technologies

The presence of contaminants in biogas, such as CO₂ and N₂, affects the quality of biogas as well as the energy efficiency for other applications. As a result, numerous biogases upgrading technologies have been developed to boost the heat value of the fuel while removing contaminants. Chemical scrubbing, membrane separation, pressure swing adsorption (PSA), and pressure water scrubbing (PWS) are some of the technologies. Figure 2-5 summarises the efficiencies of the commonly used biogas upgrading technologies (Abanades *et al.* 2022). The water scrubbing method has been shown to be one of the simplest and most cost-effective due to its high efficiency and low CH₄ loss, as well as the ability to remove hydrogen sulphide (H₂S). Chemical and organic scrubbers are the least used methods to alter the composition of biogas. However chemical scrubber remains the best-performing method for removal of these impurities.

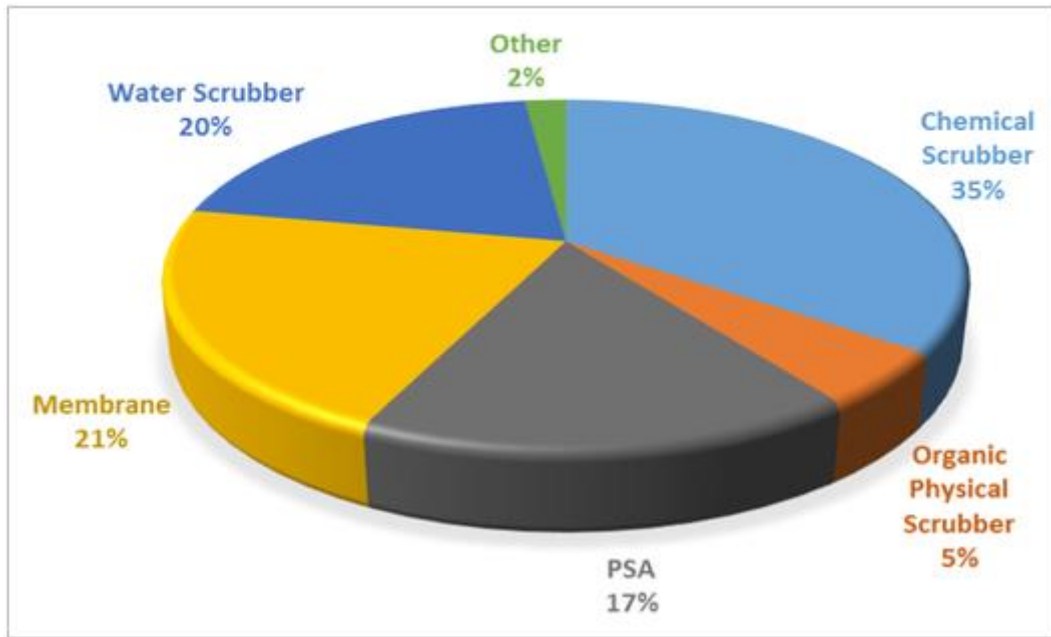


Figure 2-5: Common technologies for upgrading biogas (Abanades *et al.* 2022)

Dry reforming is another method used for upgrading biogas by adding CO₂ and H₂ to biogas. It is a high-temperature reaction utilizing a nickel catalyst. It has been found that 10% methane, 60% CO₂, and 30% H₂ mixtures have resulted in high methane conversion and good yields of syngas with some catalysts (Jung *et al.* 2021). The reaction is highly endothermic and involves high temperatures (800–1000°C). High temperature is a disadvantage to the energy efficiency; however, the heat required may be gained from the combustion of a part of the syngas stream. The carbon-forming potential in the presence of methane is significantly higher than with many other hydrocarbons reacting with CO₂ to produce CO and a mixture of free carbon and methane. The thermodynamics for the conversion of CO₂-CH₄ mixtures over nickel to produce CO is very favourable; however, the rate of reaction decreases with an increase in temperature. The overall reaction process is complex, and a problem has been that there are many undesired side reactions which lead to deactivation of the catalyst, a decrease in CH₄ conversion, and an increase in carbon formation. The addition of CO₂-H₂ mixtures was found to increase the catalyst activity enhance the production of hydrogen and increase selectivity towards the desired reactions due to the minimization of free carbon and methane production in comparison to the use of CH₄-CO₂ mixtures (Le Saché *et al.* 2019).

2.6. Challenges and limitations of anaerobic digestion for wastewater treatment

The application of AD technology for biogas production faces many challenges that hinder its industrial application and contribution to energy production globally. The integration of the AD process into the wastewater treatment settings aligns with the circular economy principles, whereby the wastewater is transformed into valuable products such as biogas and fertilizers (Chew *et al.* 2019). However, the variation in composition of the type of organic waste and other operational conditions (pH, substrate loading rate, temperature etc) are challenging in maximising the AD efficiency. Notwithstanding the difficulties of maintaining optimal conditions for microbial activity, which can inhibit the methanogenic activity and cause the acidogenic to oxidize fatty acids incompletely which can lead to an increase of volatile fatty acids in the effluent (Zacharof and Lovitt 2013). So lower organic loading rates can restrict the growth of methanogens and also the energy source (Liu *et al.* 2017).

Anaerobic digestion is a promising technology in both waste management and biogas production. It contributes to reducing landfill use, mitigating greenhouse gas emissions, producing renewable energy, and creating valuable by-products like digestate that can enhance soil health (Hussain *et al.* 2020). Despite challenges related to operational costs, feedstock variability, and infrastructure, AD remains a key player in sustainable waste management strategies and the transition toward a circular economy. With technological advances and supportive policies, AD has the potential to become an even more integral part of waste management systems globally. This section discusses the limitations of anaerobic digestion in the treatment of wastewater for biogas production.

2.6.1 Process stability and control

Process stability is an essential requirement for efficient anaerobic digestion. A stable process is defined as one that is resistant to process disturbances, where the system quickly returns to equilibrium following a disturbance. The time scale of the return to equilibrium is directly related to the rate of methane production. If the methane production rate is slow (e.g. at low organic loading rates), then a stable process cannot be distinguished from a system where the microorganisms are all inactive (Li *et al.* 2019; Ossa-Arias and González-Martínez 2021). At the other extreme, the rate of methane production can become so rapid that dangerous reactor over-pressurisation occurs, potentially leading to explosive gas release. The products of an unstable digestion process are reduced treatment efficiency, increased effluent soluble COD (which should be mineralised to methane and CO₂), and eventually a disruptive failure of the treatment system (Nguyen *et al.* 2021).

Upsetting the system can occur by making any change to the process environment, for example, loading with toxic materials, temperature change, pH changes or hydraulic shock loading. The duration and severity of the upset are largely dependent on the buffer capacity and alkalinity of the feedstock and reactor contents. The main reactions occurring during degradation of organic acids to methane and CO₂ are acetogenic rather than methanogenic (Meegoda *et al.* 2018a) . This means that consuming organic acids to methane and CO₂ is not the rate-limiting step for their formation and accumulation in the environment (Wainaina *et al.* 2019). Therefore, cessation of methanogenesis in response to organic acid buildup is an inefficient way of removing organic acids from the environment, as it simply halts their further accumulation. In contrast, organic acids may be utilized to form methane and CO₂ later, and hence accumulation of organic acids in an inhibited system can be a reversible process. High levels of accumulated organic acids lower the system pH, which may drop below the inhibition threshold for certain groups of methanogens. Failure to degrade acetolactic-specific methanogenic substrates will deplete hydrogen and acetate ions which prevents the formation of methane from CO₂ and H₂. If the environment becomes entirely void of acetate and hydrogen ions, methanogens will cease.

2.6.2 Technological and economic constraints

Technological and economic constraints have frequently been recognized as the most important barriers to the increased development and use of anaerobic processes for waste treatment and biogas production. Although the potential substrates for AD are abundant (residues from agriculture, forestry, food and paper industries, municipal solid wastes) and it is a process that can be relatively easily controlled and adapted to different scales (Varjani *et al.* 2021; Ashokkumar *et al.* 2022). It often requires relatively sophisticated and capital-intensive equipment and must compete with well-established technologies for the same end-product (e.g. wastewater treatment, incineration). The small-scale incineration of agricultural and forestry waste may be economically unattractive or technically banned, and the AD process has a clear advantage over incineration for wet wastes (Lohri *et al.* 2017). However, to compete with existing incineration facilities, the AD plants have to produce not only energy but also a waste residue that can be easily and cheaply disposed to the landfills (Makarichi *et al.* 2018). This residue (digestate) may not be produced from some wastes in sufficient quantity and quality to compete on cost with the incineration of waste with the residue being cheaper disposal of sludge from wastewater treatment.

Economic factors often constrain the use of AD for the treatment of wastes for which it is otherwise an ideal process. Waste-activated sludge from municipal and industrial wastewater treatment is an abundant source of biomass energy and bio-fertilizer. In many cases, treatment is already paid for by subsidies on agriculture and forestry and the practice of landfilling the sludge (Francini *et al.* 2019). Therefore waste disposal costs which are the main driving force behind increased AD of other wastes, are not incurred (Xu *et al.* 2014). An analogous constraint exists in developing countries, where a major hindrance to the implementation of waste treatment by AD is the relatively low cost of labour for land cultivation. This makes the compost and fertilizer products from waste treatment less economically attractive compared with the saved cost of chemical fertilizers (Romero *et al.* 2013).

2.7 Factors affecting the anaerobic digestion process

Operational parameters need to be understood and optimized to enhance the performance of anaerobic digesters for wastewater treatment and biogas production. This section discusses some of the factors affecting the AD process.

2.7.1 AD operating temperature

The temperature has a significant impact on the anaerobic digestion process, kinetically its microbial activities and biogas production efficiency. Both mesophilic (30-40°C) and thermophilic (40-50°C) conditions have been widely used in the anaerobic digestion of different types of waste, temperatures above 60°C result in the denaturalization of enzymes. Research conducted by (Kang and Yuan 2017) shows that thermophilic conditions result in high biogas generation and removal of chemical oxygen demand. However, thermophilic conditions affect the stability of the process and have high maintenance costs and high energy requirements. Khalid *et al.* (2011) suggest that temperature ranges of 35-37°C are suitable for biogas production and for sensitive microorganisms. Moreover, temperature increases from mesophilic to thermophilic will result in a sharp decline in biogas production until necessary populations of microorganisms suitable for thermophilic conditions have multiplied.

2.7.2 Alkalinity or pH

The pH of the solution is crucial in the generation of biogas by anaerobic digestion treatment. Every phase of the anaerobic digestion process possesses an ideal pH, and deviations from this range may be harmful to anaerobic bacteria (Issah *et al.*). The hydrolysis and acidogenesis processes primarily occur within an acid pH range of 5.5 – 6.5, whereas acetogenesis and methanogenesis are optimal at a neutral pH of 7.0 or in many instances, slightly alkaline conditions of 8.5. While non-optimal pH levels may directly impact microorganisms, they can also significantly influence the mitigation of the inhibitory effects of Volatile Fatty Acids (VFAs) and ammonia (NH₃). Therefore, pH stability in anaerobic digesters is the primary determinant in mitigating the inhibitory impacts of VFAs and ammonia to achieve the desired optimal level. This can be regulated by the introduction of sodium carbonate, ammonium, phosphates and sulphides to create buffering effect within the system (Dobre *et al.* 2014; Issah *et al.* 2019). In many instances, the pH in the AD system is controlled by the bicarbonate buffer system, which is dependent on the partial pressure of carbon dioxide within the system. At mesophilic temperatures, Cioabla *et al.* (2012) discovered that the pH of 6.9 gives the maximum biogas production during anaerobic treatment of wheat bran at mesophilic temperatures.

2.7.3 Hydraulic retention time and Loading rate

The hydraulic retention time (HRT) is the average amount of time that the substrate for anaerobic digestion treatment stays in the digester and comes into contact with biomass (bacterial mass) Dobre *et al.* (2014). The shorter HRT results in the accumulation of VFAs during the digestion of complex organics that require a longer acidification phase, thus reducing biogas production. Alepu *et al.* (2016) suggests that longer HRT in a period of 20 – 30 days allows for the degradation of complex organic matter allows for sufficient conversion of VFAs into biogas and enhances methane yield. Another study shows mesophilic digestion can be accomplished within 15–30 days. Shi *et al.* (2017) also suggest that longer HRT promotes AD process stability and increases the degradation of cellulose and hemicellulose, thus increasing biogas production. In a continuous AD process, HRT can be estimated using equation (2.3);

$$HRT = \frac{V_{rxn}}{Q} \dots \dots \dots (2.3)$$

where V_{rxn} represents the volume of the reactor (m³) and Q represents the inlet waste volumetric flowrate (m³/day) (Maldonado-Saeteros *et al.* 2023). The cost-benefit analysis study on municipal

wastes conducted by Meegoda *et al.* (2018b) suggests that the highest benefit is found for digesters operating on low loading rate and higher hydraulic retention time.

In AD, the loading rate for a digester is defined as the amount of the substrate introduced into the anaerobic reactor per given period. The loading rate of a digester depends on the substrate quality (amount of organic content), reactor size, HRT, and operating temperature. To ensure the stability of the AD process, the amount of organic matter loaded into the reactor must be chosen and monitored carefully. A swift increase in the loading rate usually results in the build-up of volatile fatty acids, which may result in process failure or instability. Overloading anaerobic digesters shows that the microbial community suffers a significant impact, with methane generation levels returning to normal after microbial community has developed a tolerance to higher loading rates (Regueiro *et al.* 2015; Meegoda *et al.* 2018a). Studies by Kainthola *et al.* (2019) confirms that higher loading rates results in alteration in the reactor environment and inhibit the activity of microorganisms at the beginning of fermentation. Inhibition of microbial activity occurs due to the accumulation of acids resulting from greater hydrolysis bacterial activity than methanogen activity. Furthermore, the biogas production rate was proportional to the organic loading rate. Biogas yield increased with a lowering organic loading rate. Therefore, the optimum feed rate for maximum biogas generation depends on bioreactor design and process operating parameters.

2.7.4 Availability of feedstock and preparation for anaerobic digestion

Raw material fed into the digester is another important factor affecting the production of biogas during AD. Feedstock serves as an energy source for micro-organisms to survive thus producing methane. Energy crops, plant biomass, agricultural residues like crop and animal manure, industrial residues from the food and beverage industries, sewage sludge from wastewater treatment, municipal organic waste, bio-waste from paper processing, and other organic waste sources are common feedstocks for AD (Dölle *et al.* 2020). Pre-treatment of feedstock materials by particle size reduction and co-digestion can increase the biogas output and decrease the time needed for mechanisation. Subsequently, it can reduce the size of the digester and increase the organic loading rate. Table 2-3 summarises different biogas production potentials from different feedstocks. Feed composed of fat produced more biogas than all other feeds while sewage sludge produced the least. The properties of the sewage sludge usually depend on its structure and composition, therefore affecting its performance during anaerobic digestion.

Table 2-3 Comparison of various AD feedstocks and their corresponding biogas production potentials (Bharathiraja et al. 2018)

Type	Biogas production (m ³ /ton fresh matter)	Produced (kWh) per ton of fresh matter
Cattle dung	55–68	122.5
Chicken litter/dung	120.0	257.3
Fat	826–1200	1687.4
Food waste	110	224.6
Maize silage	200/220	409.6
Municipal solid waste	101.5	207.2
Sewage sludge	47.0	96.0

Anaerobic digestion is highly dependent on composition and quantity of the feedstock. The commonly used feedstocks for biogas production are agricultural residues, energy crops, organic fractions of municipal solid waste, and animal wastes. High-energy crops like maize can supply a large amount of digestible organic matter. These crops are grown specifically for use in the biogas plant and receive undivided attention in terms of cultivation, harvesting, and storage. Maize is a renewable and fast-growing energy crop that provides a key substrate for anaerobic digestion (Zhang et al. 2007). Substrate storage is key for energy crops and must be allowed to undergo natural fermentation to increase the soluble sugars and decrease the hemicellulose content before digestion.

Crops like grass have a low dry matter production and thus could compact during storage, hence it requires airtight storage using a polyethene sheet and additives. The lower end of the energy crop spectrum is the processing of residues such as straw. Straw has a high carbon: nitrogen ratio, so it is a good complement to nitrogen-rich substrates. It must be treated with a hemicellulose and cellulose dissolving solution to increase its digestibility before it can undergo the same process as maize. High-energy crop substrates are capable of supplying a large amount of methane (Bacenetti *et al.* 2013; Sebola *et al.* 2015).

2.7.5 Design and type of the reactor

Another difficulty is the inadequacy of suitable reactor design which is to date primarily based on the rule of thumb. Overall, wastewater characterization, biodegradability, bacterial activity development, accessibility, CH₄ production enhancement, and nutritional balance are considered as significant obstacles to the efficient production of biogas (Pramanik *et al.* 2019). Anaerobic digestion reactor design is an important aspect of the efficiency, stability, and overall performance of anaerobic reactors. The reactor design dictates the process of digestion: microbial activity, substrate degradation, gas production, and overall system stability. The Stirred Tank Reactor plays an important role in solid digester treatment; it ensures the retention of enough microorganisms needed for digestion. Up-flow Anaerobic Sludge Blanket (UASB) reactors enhance solids retention time (SRT) through sludge retention. Effective reactor designs incorporate mechanisms for the collection, storage, and utilisation of biogas. Inadequate designs can facilitate gas escape, thereby diminishing yield and heightening environmental risks, such as methane leakage. Well-designed reactors optimise biogas production, reduce operational challenges, and promote long-term sustainability of the process. During anaerobic treatment in the UASB system, agitation enhances digestion efficiency by facilitating increased interaction between substrates and bacteria, while simultaneously diminishing scum formation that might obstruct gas flow at the surface. Implementing effective agitation in the anaerobic digester can prevent the localised accumulation of thermal intermediates, including acids and bases from the pH regulating system. Mechanical mixing, biogas recirculation and slurry recirculation are all viable options. Research indicates that mixing by slurry recirculation enhances the overall efficiency of anaerobic digesters more effectively than mechanical impeller and biogas recirculation methods. Incorporating all three types of mixing into an AD system significantly improves treatment efficiency relative to non-agitated digesters. Moreover, reduced mixing rates have been shown to enhance methanogenic activities in the functioning of anaerobic digesters (Poh *et al.* 2015).

The effectiveness of anaerobic digestion treatment can be enhanced by modifying the current digester design or implementing suitable advanced operational protocols. The up-flow anaerobic sludge blanket (UASB) reactor is deemed better to conventional methods due to low VFA contents in the influent, exceptional sludge retention and stable reactor performance (Rajeshwari *et al.* 2000). In the UASB, the influent enters the reactor from the bottom of the tank and ascends through the sludge blanket layer before being discharged from the top of the tank. This set-up aims to enhance the interaction between the wastewater and the mixed culture sludge by reducing the up-flow velocity. Similarly, the solids retention is enhanced when the denser solid component descends to the bottom, distanced from the effluent discharge at the top. This thus facilitates the development of granular solids which is often linked to enhanced treatment efficiency. The UASB system is favoured due to its superior settleability, capacity for high biomass concentrations, effective solids and liquid separation, and the ability to operate at increased loading rates (Poh *et al.* 2015)

2.8 Enhancing AD process with magnetic nanoparticles

Nanotechnology involves the application of scientific and engineering principles to manipulate matter at the nanoscale (1–100 nm). The incorporation of trace elements as nanoparticles diminishes the lag phase and reduces the time to reach the biogas peak as well as stimulating methanogenic archaea (Kumar *et al.* 2021). The excellent physicochemical properties of nanoparticles such as surface area, solubility, catalytic properties, etc, make them useful in an AD process. Nanomaterials possess significant characteristics in their interaction with biological systems since they can transverse cell membranes, hence enhancing their contact with the immune system (Baniamerian *et al.* 2019). Many researchers have proved that supplementation of micro-nutrients in the form of nanoparticles improves AD performance. The slow dissolution of NPs facilitates the bioavailability in the AD media and enhances the enzymatic activity. Additionally, nanoparticles can also promote the quantity and quality of biogas by the conversion of the non-volatile complex thus lowering H₂S content. (Ajay *et al.* 2020; Amo-Duodu *et al.* 2021b) found that the addition of 1g of nanoparticles was able to balance the nutritional supply of the microbes, resulting in maximum biogas yield. Moreover, the addition of nanoparticles improves the efficacy of AD with respect to the degradation of toxic contaminants in wastewater and contributes towards water sustainability.

Iron compounds may have several functions in hydrocarbon removal and degradation, helping to improve biodegradation during AD. Aside from its possible role as an electron acceptor in microbial hydrocarbon breakdown, iron is an essential component of many enzymes and cofactors (Zaidi *et al.*

2018). The iron species in nanoparticles can also act as a buffer for organic acids or as an electron donor for increased methanogenesis, facilitating the conversion of hydrocarbons into methane (Castro *et al.* 2022). Conductive iron minerals have also been shown to induce electron transfers in methanogenic processes. The study by (Zaidi *et al.* 2018) shows that Fe₃O₄ and Ni NPs have a high biogas and bio-hydrogen yield as compared to other nanoparticles. In Figure 2-6, both iron and Ni NPs produced the highest biogas during AD with better quality in terms of bio-hydrogen content, making them the most suitable NPs to add during AD. This supports the use of Fe₃O₄ NPs as a potential additive during anaerobic digestion due to its ability to gain or lose electrons. Fe²⁺ ions are crucial for energy production and DNA synthesis. The ease with which iron ions acquire or release electrons renders them an ideal and versatile cofactor for several proteins. The excess iron can readily generate highly reactive and toxic free radicals that are detrimental to several biomolecules, including nucleic acids and lipids (Aguilar-Moreno *et al.* 2020; Ziganshina *et al.* 2021).

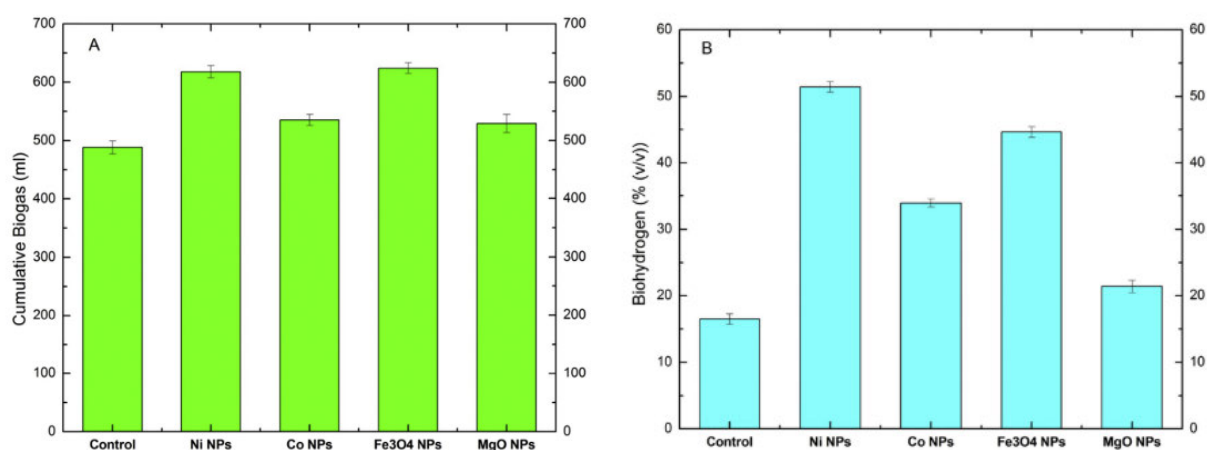


Figure 2-6; A) Cumulative Biogas Production, (B) Hydrogen content % (v/v) in biogas with the presence of NPs (Zaidi *et al.* 2018)

2.9 Application of Design Expert for the design and optimization of anaerobic digestion process

Nowadays, anaerobic digestion is one of the most important technologies in bioenergy production, especially in cases where bioconversion, organic waste, and biogas are concerned. The optimization of anaerobic digestion systems relies essentially on such parameters as temperature, pH, organic loading rate, retention time, and carbon-to-nitrogen ratio. These parameters have a very great influence on the efficiency, stability, and biogas yield in AD systems. Therefore, the challenge in knowledge and ensuring that optimal conditions are realized due to complex interactions that exist

between these parameters is crucial. It is regarding this that the software of Design Expert offers the potential to model, analyse, and optimize such multivariate conditions. Design-Expert software is a statistical tool using the full capacity of Design of Experiment, response surface methodology (RSM), and Regression Analysis in analysis and optimization of experimental conditions. It provides various techniques like Central Composite Design, Box-Behnken Design, and Factorial Designs, which are widely adopted in the optimization of biochemical processes, including AD systems. Previous researchers have adopted the use of the response surface methodology to optimize biogas production and contaminants removal during anaerobic digestion treatment of various wastes. Table 2-4 reviews and summarizes the findings of previous studies that used RSM to optimize operating conditions of an anaerobic digester.

Table 2-4: Review of previous studies on the application of RSM to optimize anaerobic digestion process

Study	Optimum condition	Reference
The production of biogas from food waste through anaerobic digestion: Optimization using the response surface methodology	• COD removal: 38% • pH: 7.0 • Temperature: 48.43°C	(Deepanraj <i>et al.</i> 2021b)
Response surface methodology as an optimization tool for anaerobic digestion of food waste	• Solid to liquid ratio: 0.19 gVS:gVS • pH: 7.5	(Parra-Orobio <i>et al.</i> 2020)
Utilization of pumice for improving biogas production from poultry manure by anaerobic digestion: Optimization using RSM	• COD removal: 68.47% • Methane content: 71.298%	(Yılmaz and Şahan 2020)
Optimization and performance evaluation of RSM in prediction of biogas production from palm oil mill effluent	• Temperature: 38.9°C • pH: 7.03 • Methane yield increase: 12.2%	(Chong <i>et al.</i> 2023)

2.9.1 Advantages of using Box-Behnken design over other designs in AD systems

BBD is a three-level design: the combination of factors uses high and low, as well as the centre point for each factor, with no extreme (corner) points, often where the maximum or minimum value of the factors can be attained. BBD requires fewer numbers of experimental runs, thus placing only points on the midpoint of each edge in the design space, along with an additional centre point, that keeps the number of experiments at a reasonable amount. BBD is ideal for studying quadratic relationships among factors, especially when extreme values (high and low) are not needed or could be harmful to the AD system. It contains factorial (corner) points, axial points, and centre points (Chollom *et al.* 2020). Factorial points allow exploration at the edges of the design space, while axial points extend beyond that range to capture more response curvature. BBD eliminates extreme values which reduce the possibility of experimental failure or system instability in AD systems where such extreme ranges-e.g., very low pH or high temperature-could be detrimental to microbial activity. BBD describes well the interactions and curvature within the studied range; hence, it is very useful when optimal performance is expected to be around mid-range conditions (Duguma *et al.* 2024; Zhan and Zhu 2024).

2.10 Summary of literature

In this chapter, the most influential factors to AD were discussed in detail. Most studies recommend mesophilic temperatures in the range of 35-40°C for a stable AD process for biogas production. The residence time (HRT) highly depends on the type of wastewater treated; however, waste-activated sludge is digested within 15-30 days depending on the operating conditions used. Another parameter, pH has an optimum value of 6.5-6.9 for methane enhancement, and up to 7.5 for the reduction of organic content. The addition of magnetic nanoparticles also proved to have significant improvement in biogas production, and the concentration of NPs should not exceed 1 g/L. Waste-activated sludge possesses many benefits with its availability, easy to handle and cheap to obtain however, its biogas production potential is low compared to other AD feedstocks. Additionally, employing BBD design is a cost-effective choice for optimizing AD systems when it is preferable to avoid extreme values, focusing on predictable responses around central conditions.

CHAPTER 3: METHODOLOGY

This chapter presents the details of the experimental work for this project. Sections 3.1 and 3.2 explain in detail the chemicals that were used, methods for wastewater collection and characterization as well as analytical equipment used. Section 3.3 summarizes the procedure for synthesizing the catalyst (magnetite). Sections 3.4 and 3.5 explain the design of the experimental matrix and optimization using response surface methodology. Section 3.6 outlines the design and optimization of the upscaled AD as well as data collection for conducting cost benefit analysis.

3.1 Materials

The chemicals that were used for the synthesis of magnetic nanoparticles included sodium hydroxide pellets (NaOH), ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), oleic acid (surfactant), and ferrous chloride hexahydrates ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), which were all analytical grade obtained from Sigma Aldrich, South Africa. Hydrochloric acid (CAS No and purity) and sodium hydroxide pellets (CAS No and purity) were used to prepare 3M solutions for HCl and NaOH which were used to control pH of the mixed liquor in the digester.

3.2 Wastewater and characterization

The four wastewater streams selected for characterization were industrial sewage wastewater, sugar refinery wastewater, oil refinery wastewater and municipal wastewater all based in Kwa-Zulu Natal.



Figure 3-1: Ariel view of the sugar refinery site supplying with the effluent



Figure 3-2: Ariel view of the municipal wastewater treatment site supplying with activated sludge

The first stream was sourced from the local sugar refinery displayed in Figure 3-1. This stream was composed of a condensed mixture of effluents from the boiler blow-down, scrubber water, contaminated cane yard run-off, condenser, and floor-washed water. The second stream shown in Figure 3-2 was the sewage wastewater from a local municipal wastewater treatment plant located in Durban, Chatsworth. This stream is composed of household wastewater and sewage from surrounding areas. Samples were taken from the two primary settling tanks in the treatment plant.



Figure 3-3: Sample collection from A) Domestic and industrial sewage and B) Oil refinery effluent

The third stream selected was from a local industrial sewage and domestic wastewater treatment plant in the KwaZulu-Natal province. It comprised of domestic, municipal and industrial sewage. This stream was sampled from four units namely screening, clarifier, digester, and sewer. Figure 3-3-A shows sampling at the clarifier for characterization. A local South African oil refinery based in KwaZulu Natal was also selected as the fourth stream. This stream consisted of waste with high water-oil emulsion strength and some oil residues. The samples were taken at the inlet of the dissolved air flotation (DAF) unit, after the oil separator and at the sewer (final effluent after the DAF process and chemical treatment).

Wastewater was collected and stored in air-tight plastic containers prior to characterization. A Hach DR 3900 spectrophotometer (Hach, Loveland, CO, USA), was used to examine the physicochemical properties of wastewater such as colour, organic content (COD, TOC) and nutrients (phosphates and nitrates). The Hach 2100N turbidimeter and pH meter were used to measure properties of water such as turbidity, pH, and total dissolved solids. Table 3-1 summarises the analytical equipment that was used for characterization. The physical properties of magnetite such as magnetic strength and surface area were examined using a portable magnet and a BET analysis. The produced biogas was characterized using Gas chromatography and an additional online gas analyser was used to measure methane content in biogas during gas production.

Table 3-1: Analytical equipment used for characterization

Substance	Equipment
Wastewater	• Hatch spectrophotometer • Turbidimeter • pH meter
Magnetite	• Scanning electron microscope coupled with an energy dispersive x-ray analyser (SEM-EDX) • X-ray powder diffraction (XRD) • Brunauer-Emmett-Teller (BET) analysis
Biogas	• Gas chromatography (GC Shimadzu 2014, Japan) • Online Gas analyser

The total and volatile solids are also a key indicator in the suitability of a stream to be treated by anaerobic digestion for biogas production. The total solids were measured by drying a 100 ml sample in an oven at 105°C for 24 hours. The difference in mass before and after drying accounts for the total solids in the 100 ml sample. The volatile solids were obtained by calcining the dried solids at 550°C

for an hour and measuring the loss in weight. The concentration of volatile solids was calculated using equation (3.1).

$$VSS \left(\frac{mg}{ml} \right) = \frac{\text{total mass after drying} - \text{total mass after calcination}}{100 \text{ ml}} \dots \dots \dots (3.1)$$

3.3 Synthesis of magnetite nanoparticles

The magnetite NPs used were prepared using the co-precipitation technique shown in Figure 3-4 (Kweinor Tetteh and Rathilal 2020; Amo-Duodu *et al.* 2021c). During the preparation of magnetite (Fe_3O_4), the solutions for $\text{Fe}^{2+}/\text{Fe}^{3+}$ were prepared in a single-step mode. In a 2 L beaker, a precursor solution was poured containing a 1:1 volume ratio (0.5 L/0.5 L) of Fe^{3+} and Fe^{2+} solutions. The pH was adjusted by dropwise addition of 2 mL surfactant to the mixture ($\text{Fe}^{3+}/\text{Fe}^{2+}$). The pH was reduced to 2 and a micro-emulsion was formed. Thereafter, 250 mL of 3M NaOH was added dropwise to the mixture while continuously stirring (15 rpm). This resulted in the formation of a thick black precipitate within the pH range of 10–12. The precipitation formed was then heated to 70°C for 2 h (ageing) and then allowed to cool to room temperature. The resultant solids were washed three times with distilled water and ethanol, and the supernatant was discarded. Following that, the samples were oven-dried for 12 h at 80°C, followed by 1 h of calcination at 550°C.

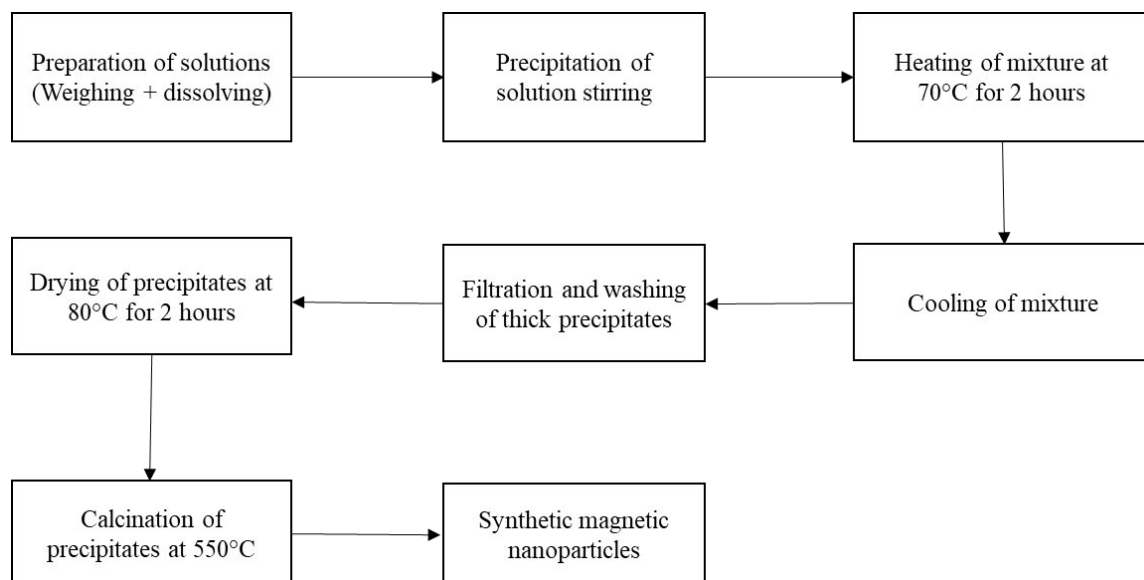


Figure 3-4: Process flow diagram for the synthesis of magnetite

3.4 Design of experiments

The Box-Behnken design (BBD) of the response surface methodology was employed for the design of the experimental matrix. The operating parameters were investigated at three levels defined as low, central point obtained from literature and high. The BBD defined 15 possible combinations for experimentation which will lead to obtaining optimum conditions for the digestion of this industrial wastewater. Table 3-2 summarises the levels of parameters used in the experimental matrix.

Table 3-2: Experimental design matrix for BBD design

Parameters	Units	Levels		
		-1	0	+1
HRT	Days	15	22.5	30
pH		5	7	9
Magnetite load	g/L	0.125	0.5	0.875

3.5 Evaluation of biogas production using 1 L and 10 L lab systems

3.5.1 Experimental procedure for 1 L reactor

The 15 experimental combinations generated by the design expert software were conducted in a 1 L reactor as shown in Figure 3-5. A magnetic hotplate was used to regulate a temperature of 37°C inside the reactor with a magnetic stirrer slowly mixing at 10 rpm for 60 seconds every 24 hours. The slow mixing was aimed at improving contact between solids and liquid and allowing for gas to escape from the mixed liquor. The characterization values were used to calculate the organic loading rate for each reactor. Each reactor was charged to a working volume of 800 mL leaving a headspace of 200 mL. This headspace was purged with nitrogen gas (N₂) to remove air from the digester for 2 minutes. These reactors were closed air-tight with rubber caps to maintain an anaerobic environment. Biogas production was monitored daily via a downward displacement technique in an inverted cylinder and characterized using gas chromatography (GC Shimadzu 2014, Japan).

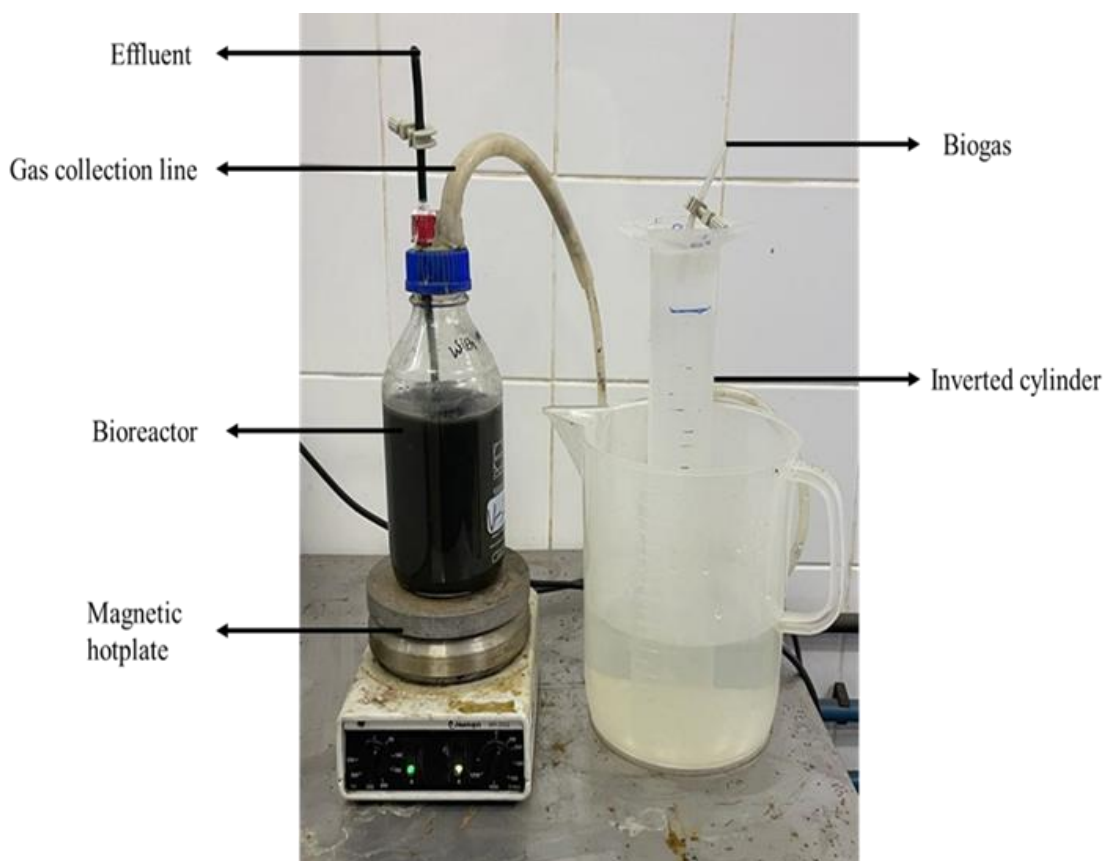


Figure 3-5: Experimental set-up for 1 L reactor used for conducting the BBD experimental matrix

3.5.2 Experimental procedure for the 10 L reactor

The up-flow anaerobic sludge blanket (UASB) reactor of 10 L capacity was employed with a working volume of 8 L and headspace of 2 L. To maintain mesophilic anaerobic conditions, water from a water bath with a constant temperature of 37.5°C was continuously circulated through the water jacket of the reactor. The reactor had several ports as shown in Figure 3-6. The two top ports were used for (1) loading and purging the system and (2) gas collection. The side ports were used as sampling points for monitoring the quality of effluent and for desludging the reactor. During operation, the system was charged with wastewater and activated sludge at a constant ratio of 5:3 with magnetite dosage varying for each experiment, purged with nitrogen to create an inert atmosphere and allowed to stabilize for two days after purging with nitrogen. Thereafter, the production of biogas was monitored using the downward displacement technique and the biogas composition was characterized at the end using the GC. The quality of effluent was examined every three days using the DR 3900 Hach spectrophotometer. The removal percentage for the contaminants was calculated using equation (3.2) where C_o and C_f represent initial concentrations and final concentrations, respectively.

$$\text{Removal efficiency (\%)} = \frac{C_o - C_f}{C_o} \times 100 \dots \dots \dots (3.2)$$

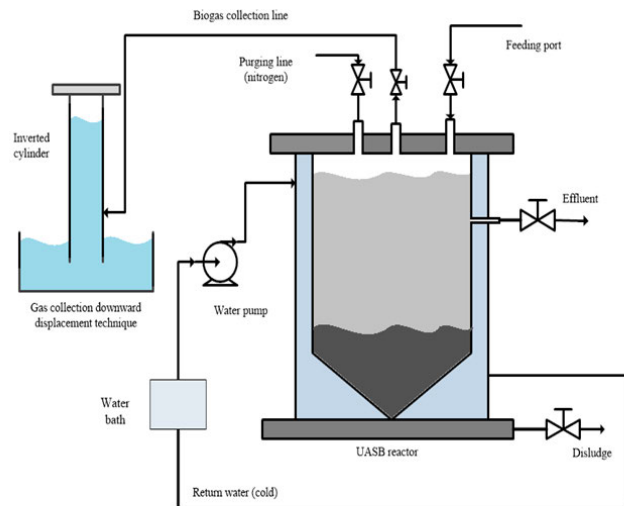


Figure 3-6: Experimental set-up for the 10 L reactor

3.6 Design of the 50 L anaerobic digester

The 50 L system was set up according to Figure 3-7A. The system feeds from the 260 L Eco branded tank that stores the effluent and gets pumped out from the bottom using the feed pump. This system comprises an anaerobic digester that has temperature and pH sensors to monitor operating conditions. The AD tank has a heating jacket that circulates hot water from the water bath. Temperature is set on the water bath and pumped to the tank's heating jacket which then transfers heat to the mixed liquor inside the tank. The temperature sensor located at the centre of the tank monitors the inside temperature and gives a signal to either increase or decrease the set temperature of the water bath. The pH being a crucial factor in AD was monitored using the sensor located at the bottom of the tank. It was connected to the automated pumps for acid and base and the control panel for setting and monitoring pH. Once the system becomes more acidic with pH dropping below the set range, the pump automatically charges the base until the pH stabilises and then switches off, a similar process is applied when the pH of the system becomes more basic, the acid pump automatically charges acid until pH comes down to the set point.

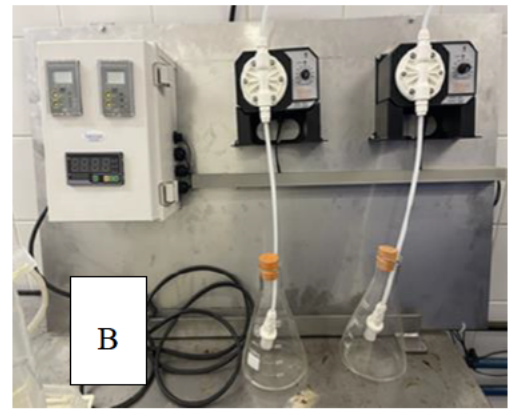
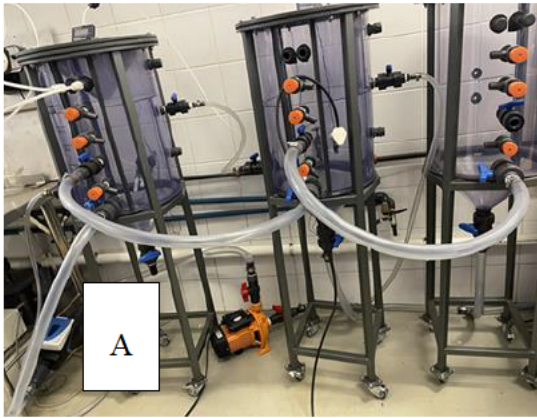


Figure 3-7: A) Set-up of the 50 L AD system, B) Acid/base storage and pumps for pH control

The main product of anaerobic digestion is biogas. In this process, the produced biogas was collected from the top of the AD tank using a rubber plastic pipe that sends the gas to the tedlar bag. The gas passes through a gas analyser (Figure 3-8A) that analyses and records methane content (percentage) in the biogas and the remaining gas is collected in the tedlar bag (Figure 3-8B). The bag has a capacity of 1.5 L for gas storage and was vacuumed before packaging to remove air and prevent contamination with biogas.

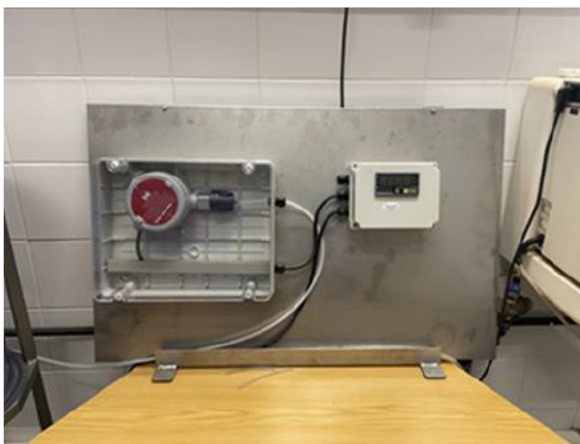


Figure 3-8: A) Gas analyser for biogas characterization and B) Tedlar bag for biogas collection

CHAPTER 4: RESULTS AND DISCUSSION

This chapter presents the results and discussion of findings obtained from this research work. It summarizes the characterization of different wastewater streams in section 4.1 and the feasibility of biogas production from the various streams using the BMP technique. Sections 4.3 and 4.4 present the effect of catalyst addition to enhance biogas production. Sections 4.5 – 4.11 present the details of investigating different operating conditions of AD and optimization using RSM. Section 4.14 summarizes the AD upscaling work and the effect of upscaling in biogas production and removal of contaminants. Lastly, section 4.15 presents the cost-benefit analysis for the optimized AD systems based on biogas production.

4.1 Wastewater characterization

The characterization of the four selected wastewater streams was conducted and the results are presented in Table 4-1. Industrial sewage and sugar refinery wastewater streams respectively had organic contents with COD of 4320 mgCOD/L and 18 770 mgCOD/L. In addition, high quantities of phosphates and nitrates makes these streams suitable for biological treatment for biogas production.

Table 4-1: Summary of characteristics for the four wastewater streams in Durban, KwaZulu Natal

Contaminants	Units	Industrial wastewater	Oil refinery wastewater	Sugar refinery wastewater	Municipal wastewater
COD	mgCOD/L	4320 ± 12	820 ± 12	18 770 ± 58	450.5 ± 15
Colour	Pt-Co	2725 ± 24	1278.5 ± 24	78 580 ± 60	413 ± 14
TOC	mg/L	4705 ± 42	730 ± 15	20 605 ± 72	696.6 ± 23
Turbidity	NTU	365 ± 2	50.75 ± 3	762 ± 1.4	61.5 ± 4
Phosphates	mgPO ₄ /L	3.62 ± 0.2	457 ± 7	52.1 ± 0.24	11.4 ± 0.8
Nitrates	mgNO ₃ /L	0.081 ± 0.001	12.37 ± 1.1	512 ± 0.18	0.0585 ± 0.001
pH		6.91 ± 0.1	5.89	4.12 ± 0.2	7.1 ± 0.1
Ammonia	mgNH ₃ /L	0.137 ± 0.1	7.24 ± 0.8	10.08 ± 0.2	24.3 ± 0.7
TDS	ppm	569 ± 2	6.29 ± 0.15	15.69 ± 0.7	618 ± 5
Volatile suspended solids	mgVSS/L	0.000785	0.0000946	0.026	0.00135

Although industrial sewage wastewater has relatively high organic content, its low colour and turbidity show that the concentration of dissolved solids is low. This stream is suitable for treatment via anaerobic digestion for clean water production with low biogas generation. On the other hand, the sugar refinery stream had a dark-yellow colour and highly viscous with high concentration of volatile solids. This is the most suitable stream for biological treatment for biogas production, however, the high strength in colour and organic content may require post treatment before discharging the effluent to the environment. As presented in Table 4-1, the effluent is highly acidic (pH of 4.12) with high organic (18770 mg COD/L) content, which is due to the presence of sugar and other organics in the wastewater. Aside the high COD concentrations, the presence of phosphorous (52.1 mgPO₄/L) and nitrate (512 mgNO₃/L) makes it nutrient rich for biological treatment. However, the high COD:NO₃ ratio can be controlled by treating the sugar refinery effluent simultaneously with domestic effluent (Yasar *et al.* 2015; Kumar *et al.* 2020). The high organic content makes it a recommendable wastewater stream for biogas production that can be converted into electricity and utilised by the WTP.

The characteristics of the oil refinery stream as presented in Table 4-1 suggests that the effluent is slightly acidic with higher organics of 730 mgTOC/L than degradable organics of 820 mg COD/L. Also, the presence of phosphorous (457 mg PO₄/L) and nitrate (12.37 mg NO₃/L) with high COD:NO₃ ratio suggests the wastewater can be subjected to biological treatment (Muenmee *et al.* 2022). In addition, the hydrocarbons in this stream can be degraded via anaerobic digestion (methanogenic bacteria) to produce methane-rich biogas. The municipal wastewater has low organic content, clear colour and less solids concentration which does not necessitate anaerobic treatment. The samples were observed to be light-yellowish in colour (413 Pt.Co) with neutral pH of 7.1-7.12. The average organic strength (450.5 mg COD/L) and organic carbon (696 mg TOC/L) were noted to be low as compared to that of the industrial sewage, oil refinery and sugar refinery effluents. Although the COD for this stream is low, the organic contents can easily be degraded by biological systems with low biogas production. This stream can be used as a medium to control the pH and promotion of stability in a biological processing of the sugar refinery stream. (Yasar *et al.* 2015; Muenmee *et al.* 2022; Patil *et al.* 2022).

In AD, COD and volatile solids are key indicators of how much biodegradable material is available for microorganisms to convert into biogas. The sugar refinery stream is selected as the most suitable feed for biogas production due to its high organic content as presented in Figure 4-1, followed by

industrial sewage. The high concentration in volatile solids for sugar, industrial sewage, and municipal wastewater in Figure 4-2 also suggests that these streams contain biodegradable material.

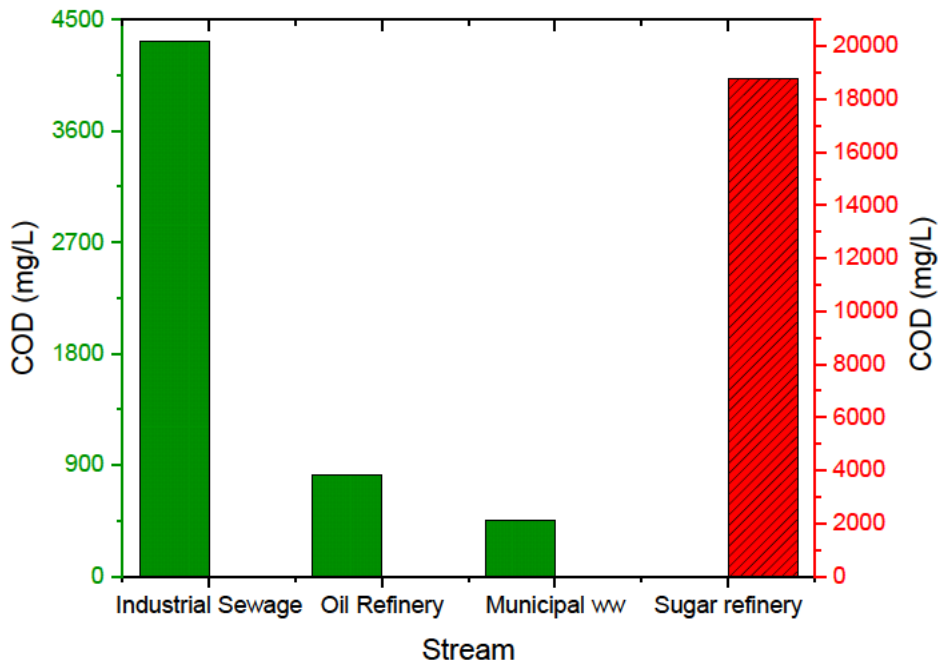


Figure 4-1: Comparison of organic content for the four wastewater streams

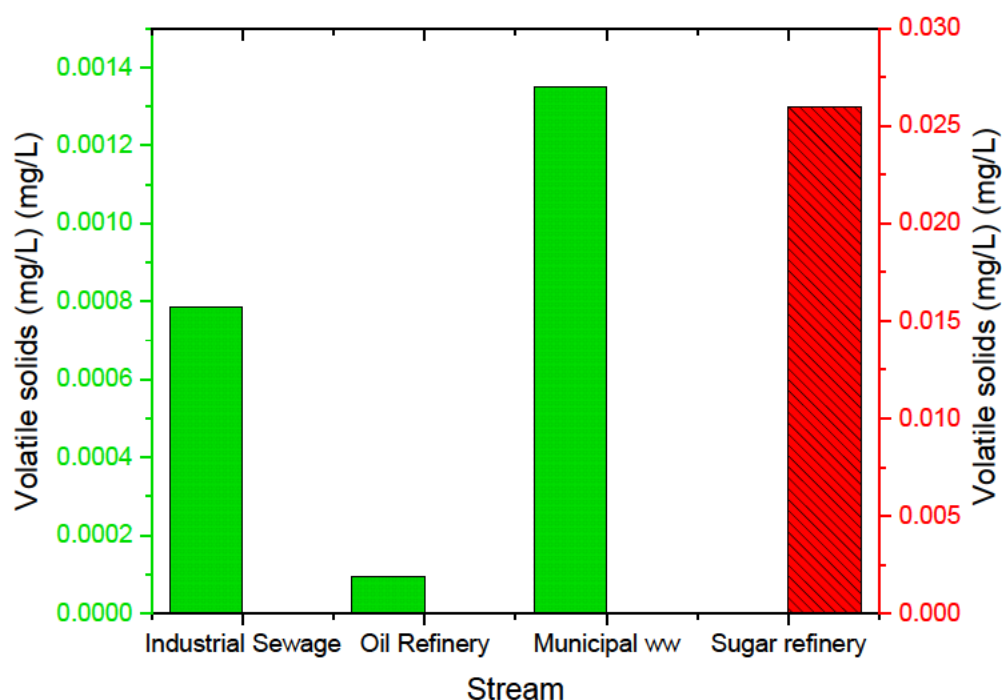


Figure 4-2: Comparison of volatile solids for the four wastewater streams

4.2 Biochemical methane potential tests

The biochemical methane potential (BMP) tests were conducted to determine methane yield from various wastewater streams at constant temperature and solid-liquid ratio. These tests rely on the amount of biogas produced to relate directly to methane potential during anaerobic digestion. Table 4-2 presents a summary of biogas production and contaminants removal during biochemical methane potential tests on the four wastewater streams.

Table 4-2: Biochemical methane potential tests for the four characterized wastewater streams

Stream	Biogas (mL)	COD removal (%)	Colour removal (%)
Industrial WW	76	79.2	81.5
Oil refinery WW	64	65.4	78.6
Sugar refinery WW	148	62.8	67.2
Municipal WW	45	74.29	89.6

Through this experiment, sugar refinery wastewater produced the maximum volume of biogas (148 mL), indicating a very high potential for methane production. This could be due to a higher organic content in this stream from sugar refineries, as it is often constituted of sugars and other easily degradable organics. The industrial sewage and oil refinery effluents indicate moderate biogas production while the oil-refinery effluent gave a slightly lower methane yield. These results indicate the presence of complex organic molecules that are non-degradable compared to other streams. Finally, the municipal wastewater stream produced the smallest amount of biogas. This is because municipal wastewater carries a smaller load of organic matter compared to the potential industrial sources.

The removal of impurities is also a key indicator of the suitability of wastewater streams to be treated by anaerobic digestion. The removal of COD reflected in the degradation of organic material in wastewater for different streams is presented in Table 4-2. Industrial sewage and municipal wastewater had a higher removal efficiency of COD, which indicated efficient degradation of organic matter by microbial community during anaerobic digestion. Also, municipal wastewaters usually contain easily degradable organics such as household wastes which makes it easy to treat. In general, sugar and oil refinery streams gave lower COD removal, suggesting that these waste streams might contain more resistant organic materials or inhibitors which reduce the efficiency in a breakdown of organic matter. Colour removal is many times associated with the removal of organic dyes, pigments, or other agents responsible for colour (Hao *et al.* 2000; Pavithra and Jaikumar 2019). Oil and sugar refinery streams showed the least colour removal, indicating thereby that colour-causing compounds in sugar refinery wastewater may be more resistant to degradation. In summary, biogas production does not correlate with COD removal for the sugar refinery stream. Although this stream produced the highest biogas, the COD removal suggests that the other fraction of COD is not readily biodegradable and may require further treatment before this stream could be discharged to the environment. Higher COD and colour removal for municipal and industrial wastewater suggests that this stream is easy to treat via AD although it may not be suitable for biogas production.

4.3 Characterization of the catalyst (magnetite)

The BET analysis results for the magnetite catalyst gives an insight into its surface properties, which are crucial for applications where surface area, pore size, and pore volume influence catalytic activity and efficiency. Table 4-3 presents a summary of physical properties of the magnetite catalyst.

Table 4-3: BET characterization of magnetite catalyst

Catalyst	Surface area (m ² /g)	Pore volume (cm ³ /g)	Pore size (nm)
Magnetite	28.468	0.0087	1.532

The characteristic of this magnetite provides a relatively high surface area for catalysts, considering the value of 28.468 m²/g for magnetite. In general, a higher surface area allows more active sites for catalytic reactions to take place. Surface areas of magnetite catalysts largely vary, depending on general synthesis methods, particle sizes, and preparation conditions. The moderate surface area could thus mean that this magnetite is either low in porosity or has a dense structure. The smaller surface area may impose limitations on the reactivity of the catalytic reactions in some cases, for instance, when such processes are gas-phase or require high accessibility to active sites (Yin *et al.* 2022). This surface area can be more appropriate for reactions in which the limitation caused by diffusion is not high, or in systems where the activity can be improved by other factors such as the nature of the active sites.

The small pore volume of 0.0087 cm³/g indicates that there is little porous structure inside the catalyst. Generally, for porous materials, the pore volume could provide storage and distribution of reactants. The higher the pore volume, the easier the diffusion and accessibility would be to the reactants. A very small pore volume would indicate that the sample of magnetite consists of compact, non-porous particles or primarily microporous structures. This is likely to be less favourable in reactions where high mass transport or retention of reactants within the pore structure is advantageous. A pore size of 1.532 nm classifies the material porosity within the microporous range since the diameter of the pores is less than 2 nm. Such conditions may provide complications in certain reactions where larger molecules are necessitated since there would be a limitation in the access to active sites in such small pores. Microporous structures support reactions involving smaller molecules which can access and interact more easily in these confined spaces. Sometimes, the micropores give, in catalytic applications, a size-selective environment in which only the reactants smaller than the pore sizes can participate in the reaction, which could be of importance for improving selectivity. This will make the catalyst suitable for reactions dealing with small molecules or gases, considering the microporous nature with pores at 1.532 nm whereby the reaction selectivity could be enhanced based on the size exclusion. Further characterization of elemental compositions of this magnetite indicates that the

concentration of iron and oxides amounts to over 60%, indicating successful synthesis of the magnetite catalyst (Amo-Duodu *et al.* 2021a; Amo-Duodu *et al.* 2024).

4.4 Evaluation of biogas production using 5 L and 10 L reactor with magnetite

This section covers the evaluation of biogas production using the 10 L and 5 L laboratory systems by investigating the effect of hydraulic retention time, catalyst load and organic content using the one-factor at a time system. This work has been published in the journal of applied science in MPDI (Paper 1).

4.4.1. Effect of hydraulic retention time on biogas production and COD removal

The effect of the retention time of HRT on natural anaerobic digestion (AD) of municipal wastewater with low organic content without additives was monitored. Microorganisms require time to adapt to a new environment before they can produce methane. In this study, the first five days had no production of biogas and COD degradation. The microorganisms responsible for the biodegradation process were probably still adapting to the environment. After five days, a gradual increase in biogas production and COD reduction was achieved as presented in Figure 4-3 and Figure 4-4, with an exponential increase in both biogas and COD reduction between 9 – 18 days. A slow performance towards the end of digestion was achieved between 19 - 21 days and thereafter, no AD activity was observed between 22 – 30 days.

These findings are in agreement with the study by Kim *et al.* (2006), which reported that the best performance in anaerobic digestion of municipal sewage waste was achieved between 10 – 20 days under mesophilic AD conditions.

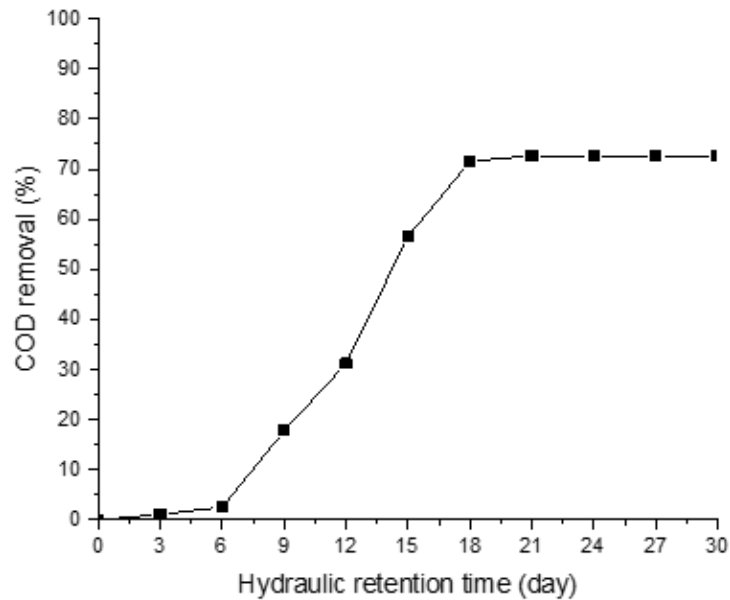


Figure 4-3: Effect of hydraulic retention time on COD removal

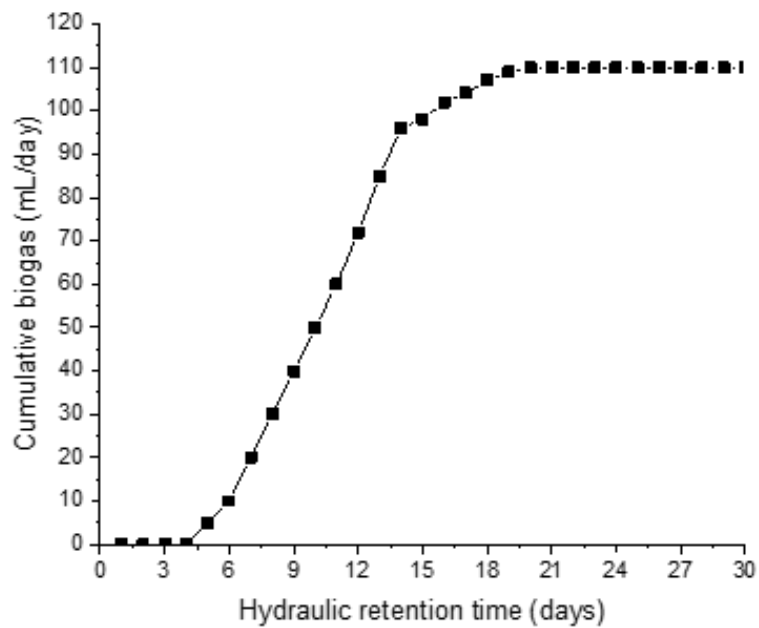


Figure 4-4: Effect of hydraulic retention time on Cumulative biogas production

4.4.2 Effect of magnetite loading on biogas production and COD removal

The AD system was operated with various magnetite doses in a range of 2 – 8 g (magnetite load: 0.2 – 0.8 g/L) with an increment of 2 g at a constant HRT of 21 days. The graph of cumulative biogas can be useful in investigating bacterial adaptation and growth during AD. Figure 4-5 shows the performance of each dose in biogas production and bacterial adaptation.

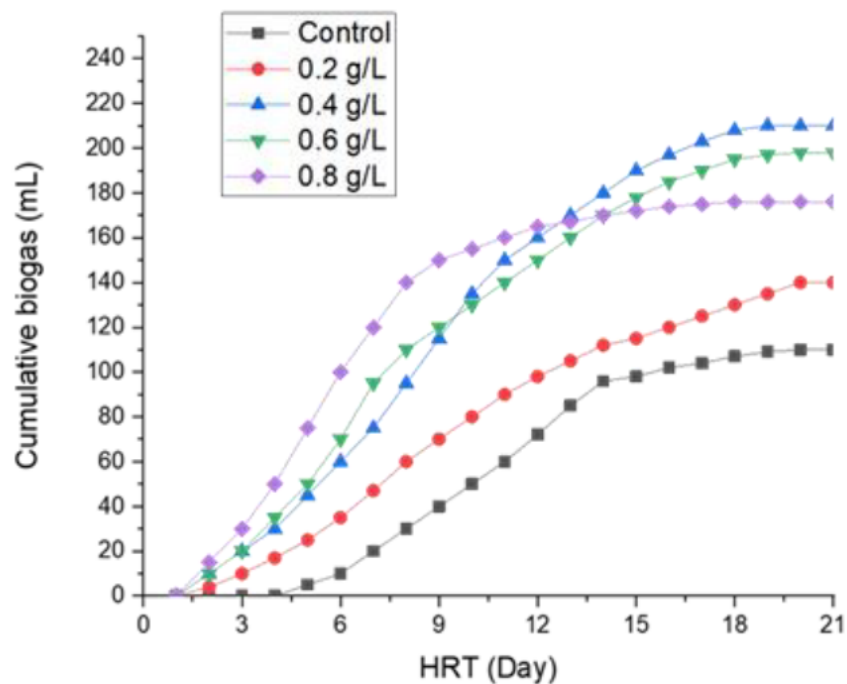


Figure 4-5 Effect of HRT on cumulative biogas produced from different AD systems with different magnetite loads

The biogas production at the beginning of the HRT for the different digesters varies with the amount of magnetite loaded. The initial gradual rise of biogas accumulation, normally called the lag phase period, indicates the amount of time it takes for the microorganisms to adapt to the new surroundings (acclimatize) (Zwietering *et al.* 1992). As evident from the graph, the control had a lag phase of 4-5 days, suggesting that it took longer for the microorganisms to fully adapt to the traditional AD system. The use of magnetite was found to reduce the lag phase of the AD process. This is because the use of magnetite in anaerobic digestion improves the interspecies electron transfer, which ultimately enhances biogas production (Yang *et al.* 2016; Amo-Duodu *et al.* 2021; Tetteh and Rathilal 2022). Because of the high surface area of magnetite at the nanoscale, the reaction is relatively fast

(Mainardis *et al.* 2020), making it interact with the sludge cell membrane, causing structural changes in the cells that eventually result in bacteria-permeable membranes. As a result, more bacteria find their way to attack sludge, increasing overall biogas production (Yang *et al.* 2016; Zhang *et al.* 2019). The best-performing digesters in terms of lag phase were the 0.4 g/L, 0.6 g/L and 0.8 g/L MNP digesters which had the lowest lag phase of fewer than 3 days. After passing the lag phase, all digesters approached the exponential phase, and the cumulative biogas generation increased substantially due to the exponential growth of methanogens. The 0.6 g/L and 0.8 g/L MNP showed the highest maximum growth rate between days 4 and 10. However, due to the very high magnetite concentration, the 8 g digester experienced overloading, which resulted in a rapid decrease in biogas generation after 9 days. The addition of excess Fe ions to the anaerobic microbial community gives rise to toxicity and excess reactivity, thus hindering the growth and activity of microorganisms (Casals *et al.* 2014).

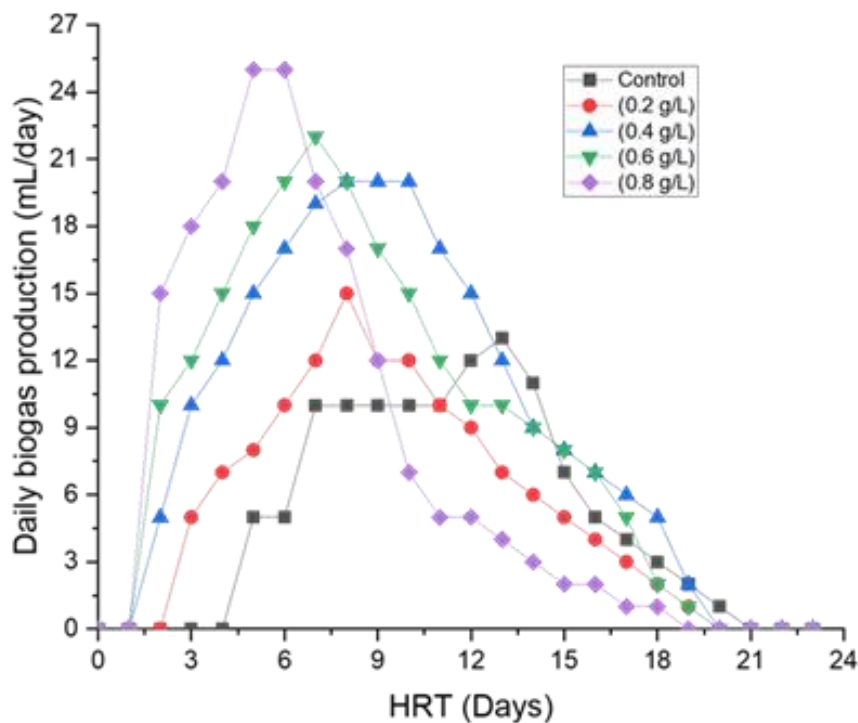


Figure 4-6: Effect of HRT on daily biogas production from different AD systems with different magnetite loads

The trend of 0.6 g/L digester in Figure 4-6 suggests that there was a disturbance in biogas generation between days 9 – 10, which sees the 4 g digester surpassing all other digesters with a steady increase in biogas generation. This could be the effect of excess magnetite which promotes the formation of toxicants and inhibits the sensitive methanogenic activities during AD, thus a decrease in biogas production up to day 18. The daily biogas production in Figure 4-6 confirms that the 0.8 g/L MNP digester had the highest biogas production reaching its peak of 25 mL/day within six days, however, after 9 days the biogas production was very low. The 0.6 g/L digester also reached its peak production of 23 mL/day on day 7 and gradually decreased after day 9 due to MNP overload. The 0.4 g/L digester was at peak production between days 9 – 12 with a production rate of 20 mL/day, followed by a gradual decrease after day 12 towards the completion of organic degradation within the digester. The 0.2 g/L and control digesters reached their peak production of 15 and 12 mL/day on days 8 and 7, respectively, because of low and no magnetite in the reactor to promote the growth and activity of microorganisms. The five digesters generated cumulative biogas in this order: 0.4 g/L > 0.6 g/L > 0.8 g/L > 0.2 g/L > control with values of 210, 190, 175, 140 and 110 mL, respectively. Therefore, the addition of 0.4 g/L to the 10 L UASB reactor results in the highest biogas production. This agrees with studies by Tetteh and Rathilal (2022) that 0.4 g/L catalyst load was found to be the optimal quantity using response surface methodology for optimization.

In addition to biogas production, the degradation of organic content in the form of chemical oxygen demand (COD) increased exponentially with the addition of magnetite. The trends in Figure 4-7 confirms that the highest COD removal of <90% was achieved in the digester dosed with 0.4 g/L of magnetite. The reduction of organic content corresponds to the generation of biogas with microorganisms degrading and converting it into biogas.

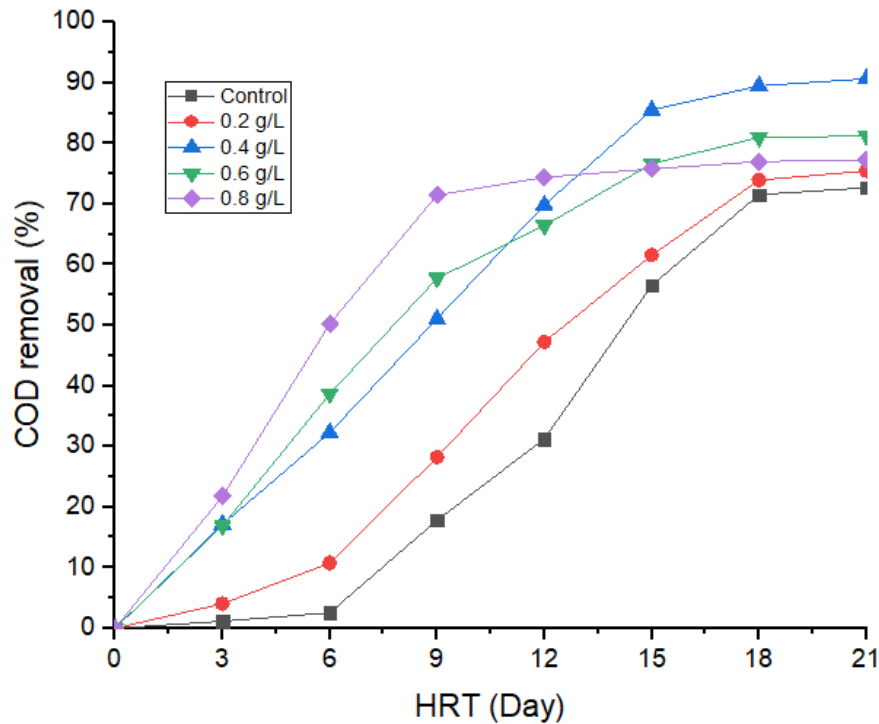


Figure 4-7: Effect of HRT in COD removal for different AD systems with different magnetite loads

4.4.3 Effect of organic content on biogas production and quality

The impact of the organic loading rate was studied on the response of the digester using the HRT for 21 days and a magnetite load of 0.4 g/L. The two streams compared were municipal wastewater (Low OLR) and sugar refinery stream (High OLR) as presented in Table 4-1. The third stream was a mixture of municipal and sugar refinery with an average organic content (Average OLR) of 9590 mg/LCOD. Figure 4-8 shows a 200% increase in cumulative biogas with the increase in organic content. This is a result of high organic content and total dissolved solids exposed to microorganisms for degradation. Compared to low OLR, the average and high OLR streams achieved a low percentage removal for COD as presented in Figure 4-9. An increase in organic content results in a decrease in pH due to the accumulation of VFAs and long-chain fatty acids (LCFA) (Wijekoon *et al.* 2011). The study by Boe *et al.* (2010) also revealed that LCFAs and the presence of organics that are difficult to degrade are inhibitory to all groups of microorganisms hence the AD of high organic content results in 70 – 80% removal for contaminants.

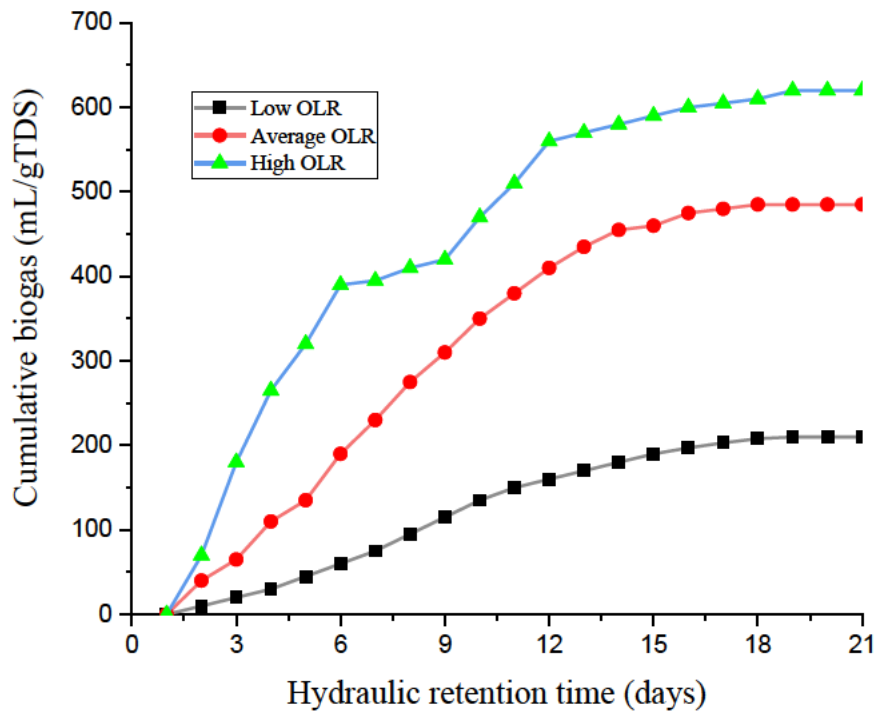


Figure 4-8: Effect of HRT on cumulative biogas produced from three AD systems with different OLR

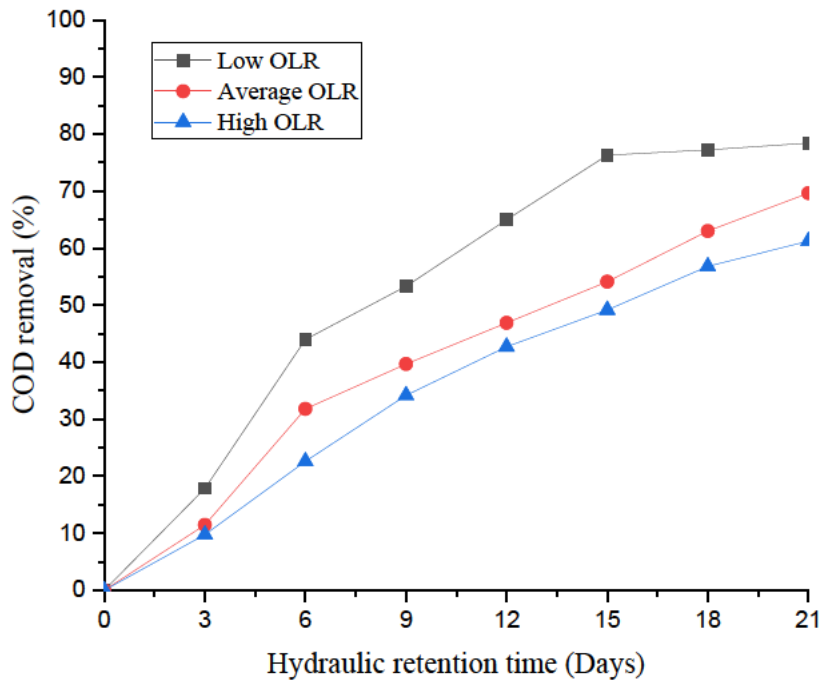


Figure 4-9: Effect of HRT on COD removal for three AD systems with different OLR

The effect of magnetite addition on the quality of biogas is presented in Figure 4-10. The use of magnetite supports the methane-producing microorganisms during anaerobic digestion. Figure 4-10 shows that the methane content in biogas generated without the addition of magnetite is low (55%), however, when magnetite was introduced, the grade of biogas improved with methane content reaching 73%. Previous studies have revealed that the methane content in biogas produced during the digestion of municipal wastewater is generally low because of relatively small concentrations of suspended and dissolved organic and inorganic solids. Zhang *et al.* (2022) revealed that the utilization of iron in magnetite by the microbial community and the potential mechanism of promoting interspecific iron uptake improves the carbon conversion efficiency of organic matter during anaerobic digestion, thus increasing the methane content.

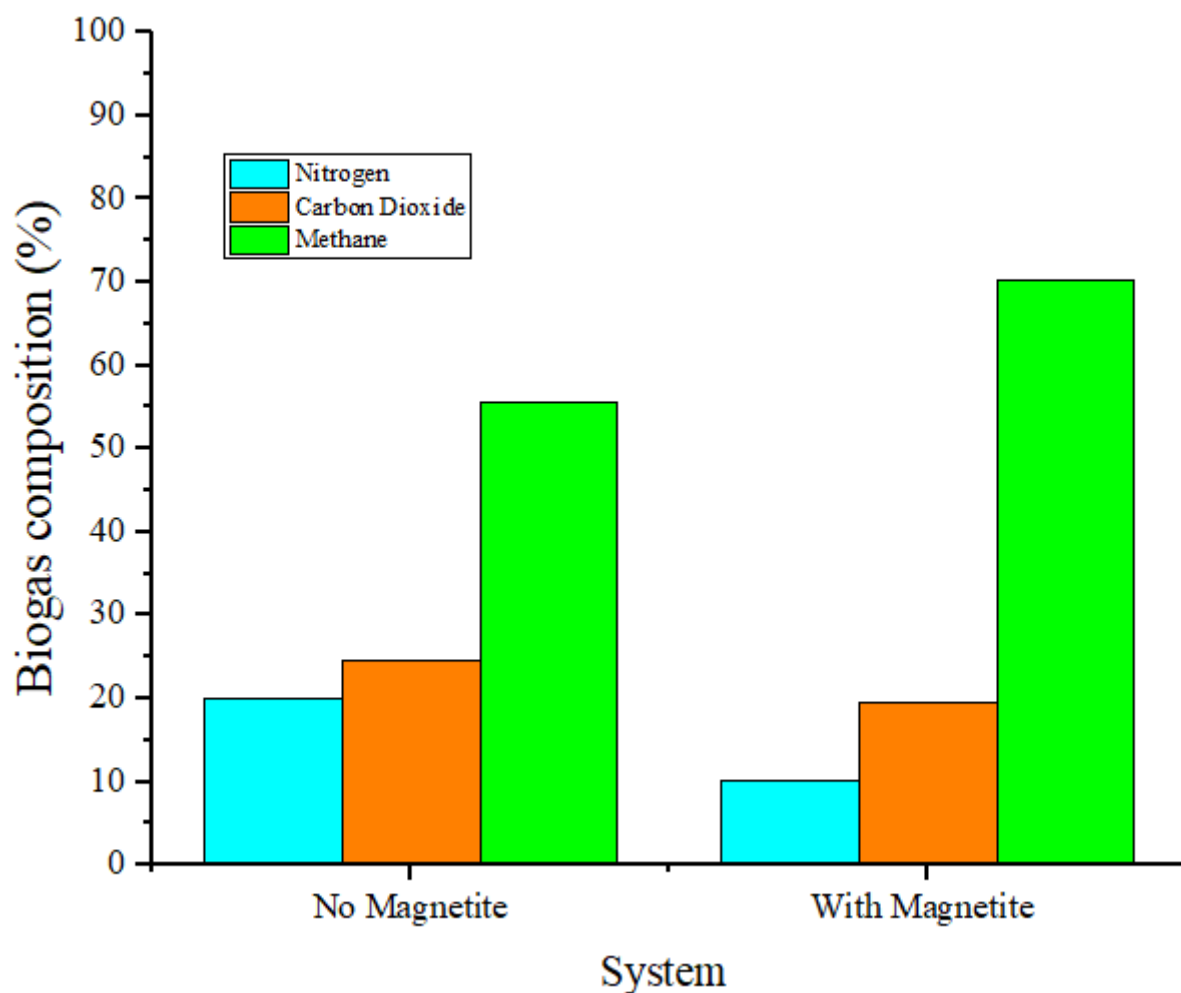


Figure 4-10: The effect of magnetite addition on the quality of biogas (%methane content)

4.4.4 Overall treatability efficiency

The overall treatability efficiency of the digester was monitored with the removal of turbidity and color in the final effluent. The control digester achieved the lowest removal percentages of 67 and 79% for the removal of color and turbidity, respectively. On the other hand, the 0.4 g/L digester outperformed all the other digesters in the removal of color (95.2 %) and turbidity (98.9 %). The removal of color and turbidity corresponds to the degradation of dissolved and suspended solids which are converted to methane-rich gas by microorganisms. The microbial community in the 0.4 g/L digester was well adapted to the environment which sees them continuously converting the organics in wastewater throughout the digestion period to produce cleaner effluent. The 0.6 g/L digester was the second best performing with color and turbidity removals of 89% and 92%. The 0.8 g/L and 0.2 g/L digesters both achieved removals of 70-80% in color and above 80% for the removal of turbidity. The digester with high organic content achieved 65% in the removal of color and 75% in the removal of turbidity. This confirms that this stream had highly dissolved and suspended solids, with dark-yellow color and was highly viscous. Although biogas production increased exponentially for this digester, a post-treatment step would be necessary for further reduction of containments to produce cleaner water. The overall treatability of the digesters with the removals for color and turbidity is presented in Figure 4-11.

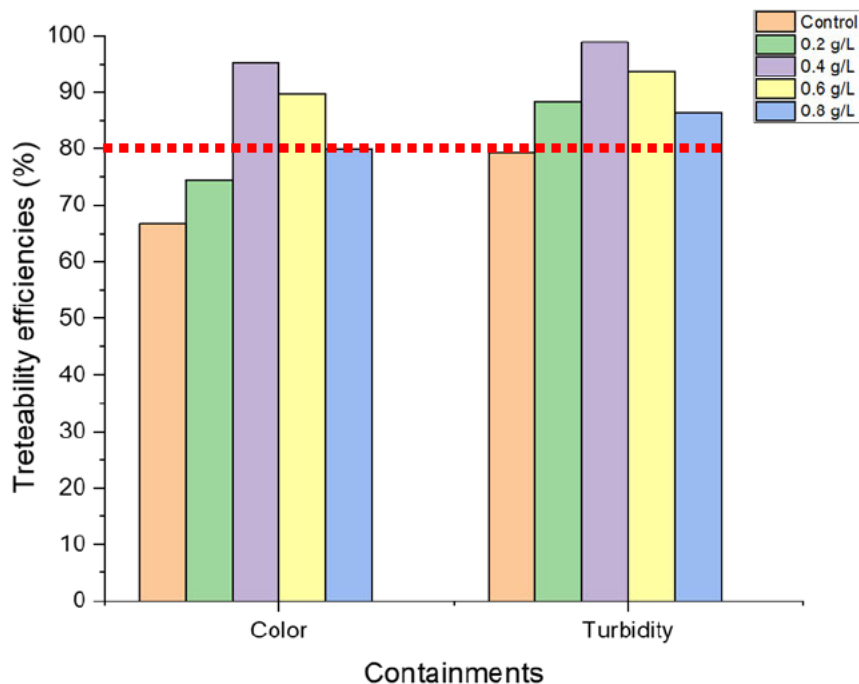


Figure 4-11: Overall treatability efficiencies for different AD systems with different magnetite loads

4.5 Optimizing anaerobic digestion using response surface methodology

This section outlines the optimization of operating conditions for the anaerobic digestion process. The selected parameters for optimization were HRT, pH, and catalyst (magnetite) load using the 1 L laboratory bio-digester. The Box Behnken design was used to design the experimental matrix, and the response surface methodology was used to optimize the operating conditions. The optimum conditions were considered in the upscaling to the 50 L AD size. This work has been published in a conference proceeding (Paper I).

In anaerobic digestion (AD), the bacteria remove contaminants via biodegradation of organic contents to produce methane-rich biogas and reduce costs for effluent and sludge disposal. At the end of AD, the effluent was characterized and the removal percentages for the contaminants of interest and biogas production is summarized in Table 4-4 and compared with the predicted values. The COD removal is a key indicator of the performance of AD, indicating how much organic content is converted to biogas by anaerobic bacteria. In this study, COD removal ranged from 65% to 90% for all experimental conditions investigated. This suggests that in experiment 15, where 90% COD removal was achieved, microorganisms were exposed to an environment that promotes growth and stimulates their activity at a faster rate to convert organic content to biogas. Volatile solids also indicate the biodegradability of the organics in wastewater as they generally consist of solids from plants and other organic matter. The reduction of up to 98% in volatile solids as summarized in **Error! Reference source not found.**, indicates the suitability of the digester to digest the solids with other contaminants in wastewater to produce reusable water and biogas. Colour removal is an important factor in measuring the quality of effluents being discharged into the environment or for reuse. It is quite evident that colour removal is affected by both the digestion and settleability of free solids in the effluent. In this study, the application of magnetic nanoparticles permitted microorganisms to degrade contaminants (dissolved and non-dissolved) and longer retention times allowed free solids to settle due to gravity while dissolved solids are being digested and hence higher removal for colour was achieved across the experiments. The comparison in Table 4-4 suggests that the actual values for the outputs agree with predicted values and the percentage difference between all values is below 2%.

Table 4-4: Experimental matrix showing investigated conditions with the predicted and actual outputs

Inputs factors			Biogas (mL/gTDS)		COD removal (%)		Colour removal (%)	
HRT (day)	MNP dose (g/L)	pH	Actual	Predicted	Actual	Predicted	Actual	Predicted
22,5	0,4	7	96	99	89,78	89,8	98,29	98,21
30	0,7	7	50	50,5	78,84	79,1	96,76	96,75
22,5	0,7	5	31	30,25	68,62	68,6	95,45	95,42
22,5	0,1	9	44	44,75	74,29	74,31	97,26	97,29
30	0,1	7	58,5	59	82,56	82,71	96,46	96,48
30	0,4	5	38	38,25	63,69	63,45	96,93	96,97
15	0,7	7	51,5	51	84,25	84,1	93	92,99
22,5	0,4	7	100	99	89,51	89,8	98,22	98,21
15	0,4	9	45	44,75	70,3	70,54	95,4	95,36
30	0,4	9	48	46,75	65,24	65,08	98,93	98,88
22,5	0,1	5	67	66,25	64,08	64,18	96,02	95,96
22,5	0,7	9	48	48,75	65,31	65,21	95,45	95,5
15	0,4	5	55	56,25	65,27	65,43	95,81	95,85
15	0,1	7	75	74,5	85,43	85,16	95,58	95,59
22,5	0,4	7	101	99	90,11	89,8	98,13	98,21

4.5.1 Influence of input parameters on output variables using correlation plots

The correlation plots indicate the strength and direction of the relationship between two variables (input and output). The correlation value below zero indicates that there is no correlation between the input and output. The correlation value between 0 – 0.3 indicates a weak positive correlation, 0.3 – 0.7 indicates a moderate positive correlation and above 0.7 indicates a strong positive correlation. In this study, the input variables comprise three observational points (i.e., -1, 0, 1), and a linear plot is drawn for all variables. Thus, the correlation can be represented as the slope of a straight-line graph. If that is the case, a correlation with a positive value would suggest that the output increases with increase in the input variable (that is, the input variable is directly proportional to the output variable), whereas a correlation with a negative value signifies that the output decreases with an increase in the input variable (that is, the input variable is inversely proportional to the output variable).

In this investigation, the correlation coefficient of HRT and biogas yield, as presented in Figure 4-12, was -0.134, the correlation coefficient of HRT and COD removal, as presented in Figure 4-13, was -0.136 and the correlation coefficient for HRT and colour removal, as presented in Figure 4-14, was 0.574. An increase in hydraulic retention time increased both biogas yield and COD removal, however, the correlations obtained were very low. This could reveal that: a) hydraulic retention time does not affect biogas yield and COD removal, or b) hydraulic retention time has maximum value in the centre point of 22.5 days, or c) hydraulic retention time has minimum value in the centre point of 22.5 days. Based on Figure 4-12 and Figure 4-13, most data points were found at retention time of 22.5 days, with the maximum responses were obtained at this HRT. Therefore, the low correlations revealed that the optimum hydraulic retention time for biogas production and COD removal is around 22.5 days. In contrast, Figure 4-14 showed a positive correlation value of 0.574, suggesting that increasing HRT increased colour removal.

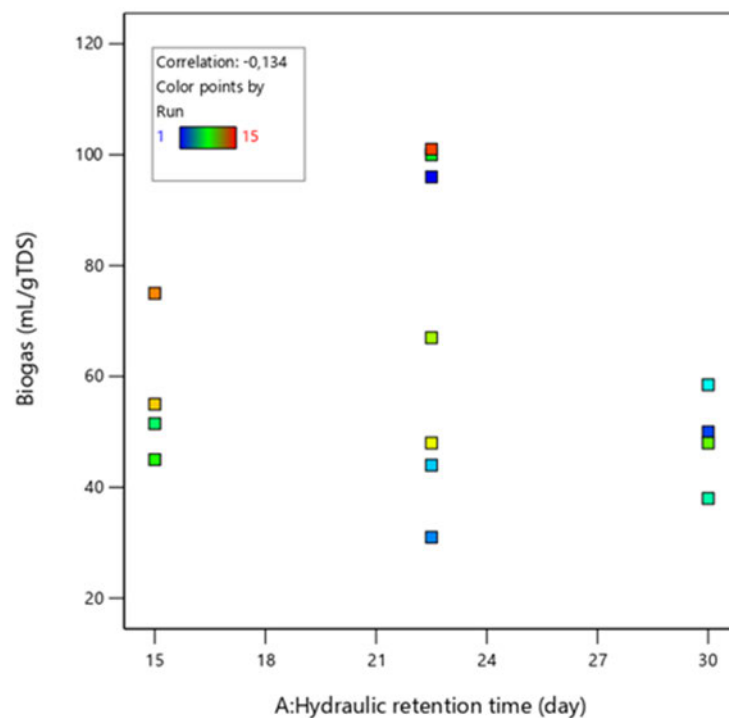


Figure 4-12: Correlation graph showing the influence of hydraulic retention time on biogas production

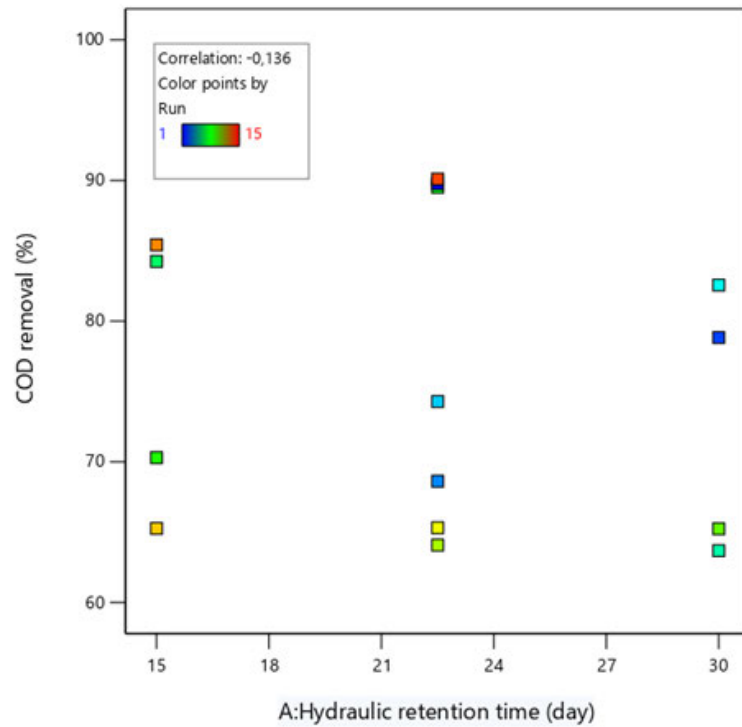


Figure 4-13: Correlation graph showing the influence of hydraulic retention time on COD removal

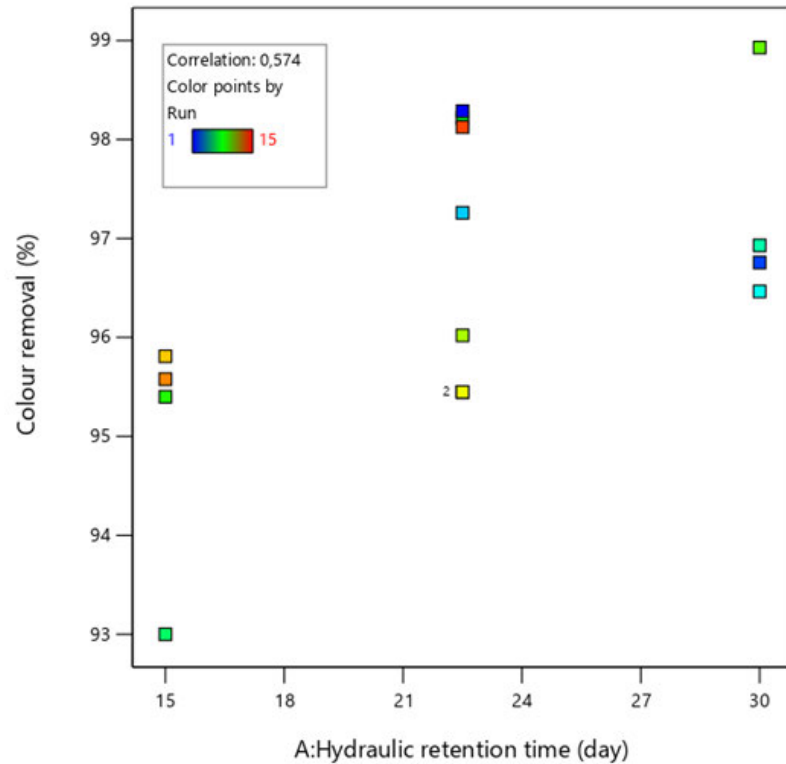


Figure 4-14: Correlation graph showing the influence of hydraulic retention time on colour removal

Based on the absolute values of the correlation values, the decreasing order of the correlations showed the following: colour removal (0.574) > COD removal (0.136) > biogas yield (0.134). These results revealed that HRT had the greatest influence on colour removal, but it had the least influence on biogas yield.

The correlation plots for pH and magnetite load against the three responses are presented in Appendix A. The correlation value for MNP load and biogas yield presented in Figure I-1 was -0.268, the correlation value for MNP load and COD removal presented in Figure I-2 was -0.085 and correlation value for MNP load and colour removal presented in Figure I-3 was -0.289. All correlation values were negative, and therefore this denoted that the overall increase in magnetite load resulted in a decrease in all responses, i.e., biogas yield, COD removal, and colour removal. With respect to the absolute values of correlation values, the increasing order of the correlations displayed the following: colour removal (0.289) > biogas yield (0.268) > COD removal (0.085). Therefore, these results revealed that magnetite load had the greatest effect on colour removal. On the other hand, the COD removal was less affected by magnetite load.

The correlation plots for pH and biogas, pH and COD removal and pH and colour removal are also presented in Appendix A as Figure I-4, Figure I-5 and Figure I-6 respectively. The correlations of Figure I-4 showed a negative value of -0.025, which indicated that the overall increase in pH resulted in a decrease in biogas yield. On the other hand, overall increase in pH resulted in an increase in both COD removal (Figure I-5) and colour removal (Figure I-6), with these responses indicating correlations of 0.123, and 0.175, respectively. These outcomes provide a starting point for statistical study. The hypothesis that was made was that the observational points represent a linear equation, but this must be verified in the fit summary analysis section.

4.6 Regression models and fit summary

One of the most powerful tools of statistical analyses is the response transformation. In statistics, this tool incorporates the use of numerous mathematical/statistical functions to the response variable/s. The mathematical/statistical functions that were considered for transformation were: no transformation, power, square root, inverse square root, logit, base 10 log, arcsine square root, inverse, and natural log. The transformations for colour removal, COD removal, and biogas yield regressions were 1.11, 1.55 and 2.88, respectively. These values were all below 3, which indicated that no transformation was needed in any of the investigated responses.

The next step is to find the type of regression equations for the investigated responses, namely, biogas yield, COD removal, and colour removal. There are numerous tables that may be employed in identifying the model type to choose for further investigation. The fit summary table is a statistical table that may be used for the selection of the type of regression model. This table provides a collection of substantial parameters that are employed as the starting point for the true regression equation. Figure 4-15, Figure 4-16 and Figure 4-17 for responses 1, 2 and 3 portray the fit summary tables respectively for biogas yield, COD removal, and colour removal. In Design Expert software, the types of regression equations that are considered are quadratic, cubic, two-factor interaction (2FI), and linear regression equations. The regression equation with the greatest lack-of-fit p-value predicted R^2 , adjusted R^2 , F-value, and p-value in statistical analysis is considered the best-fit model (Stat-Ease Inc., 2019). The quadratic regression equation revealed the greatest lack-of-fit p-value, adjusted R^2 , and predicted R^2 for biogas yield, COD removal, and colour removal. It can thus be said that the best-fit regression models for all responses were the quadratic equation.

Response 1: Biogas

	Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
	Linear	0,7805	0,0097	-0,1578	-0,3286	
	2FI	0,8594	0,0071	-0,4557	-0,8246	
	Quadratic	< 0.0001	0,8215	0,9920	0,9810	Suggested
	Cubic	0,8215		0,9863		Aliased

Figure 4-15: Regression model for biogas production

Response 2: COD removal

	Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
	Linear	0,9238	0,0005	-0,2209	-0,6678	
	2FI	0,9599	0,0004	-0,6202	-2,3052	
	Quadratic	< 0.0001	0,4390	0,9990	0,9958	Suggested
	Cubic	0,4390		0,9992		Aliased

Figure 4-16: Regression model for COD removal

Response 3: Colour removal

	Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
	Linear	0,0816	0,0032	0,2917	0,0711	
	2FI	0,5649	0,0027	0,2343	-0,2245	
	Quadratic	< 0.0001	0,5815	0,9975	0,9910	Suggested
	Cubic	0,5815		0,9972		Aliased

Figure 4-17: Regression model for Colour removal

4.7. Analysis of Variance (ANOVA) test and fit statistics

4.7.1 Analysis of variance

Perhaps the most important table in statistical analysis is the analysis of variance (ANOVA) table. The ANOVA tables for biogas yield, COD removal, and colour removal are respectively presented in Table 4-5, Table 4-6 and Table 4-7. The tables contain parameters that are useful in assessing the selected regression models. A statistical parameter that is useful in ascertaining the importance of the means between input variables is the F-test. From the ANOVA tables, the F-values for biogas yield, COD removal, and colour removal were 192.95, 1526.52, and 612.24, respectively. The greatest F-value of 1526.52 was obtained from the COD removal regression model, denoting that the model of this response was the most robust. Another imperative statistical term that is highly related to the F-value is the p-value. In the ANOVA test, the p-value represents the probability of the regression model (Stat-Ease Inc., 2021). The p-values of all regression models in this study were below 0.0001. Hence, for the biogas yield, COD removal, and colour removal regression equations, there is less than 0.0001% chance that the respective F-values as large as 192.95, 1526.52, and 612.25 are because of noise. If the p-value is considerably low, i.e., less than the alpha value (α), then the null hypothesis should be ignored (Stat-Ease Inc. 2019, Frost 2022). Since the confidence interval of 95% was used in this study, the value of α was therefore $\alpha = 100 - 95\% = 5\%$. As a result, p-values lower than 0.05 were considered significant, while p-values above 0.10 were insignificant and therefore were discarded. For the regression models of biogas yield and colour removal, the p-values for the terms were less than 0.05, which suggested that the existing terms have a significant impact when added to the regression equations. Conversely, the only term with a p-value greater than 0.10 for COD removal was B. This term was therefore not significant and was discarded from the regression model. The lack-of-fit test is another significant statistical parameter of the ANOVA test. The lack-of-fit of the

chosen regression model must be insignificant (Stat-Ease Inc., 2021). This will take place when the p-value is beyond 0.10. The values of lack-of-fit were greater than 0.10 for all regression models, denoting that the projected regression models were fitted well.

Table 4-5: ANOVA for biogas production

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7119,73	9	791,08	192,95	< 0.0001	significant
A-Hydraulic retention time	175,1	1	175,1	42,71	0,0013	
B-Magnetite load	30,12	1	30,12	7,35	0,0423	
C-pH	81,76	1	81,76	19,94	0,0066	
AB	56,25	1	56,25	13,72	0,0139	
AC	100	1	100	24,39	0,0043	
BC	400	1	400	97,56	0,0002	
A ²	1570,67	1	1570,67	383,09	< 0.0001	
B ²	1422,06	1	1422,06	346,84	< 0.0001	
C ²	3751,44	1	3751,44	914,99	< 0.0001	
Residual	20,5	5	4,1			
Lack of Fit	6,5	3	2,17	0,3095	0,8215	not significant
Pure Error	14	2	7			
Cor Total	7140,23	14				

Table 4-6: ANOVA for COD removal

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1506,96	9	167,44	1526,52	< 0.0001	significant
A-Hydraulic retention time	20,08	1	20,08	183,07	< 0.0001	
B-Magnetite load	0,1736	1	0,1736	1,58	0,2639	
C-pH	46,89	1	46,89	427,51	< 0.0001	
AB	1,62	1	1,62	14,8	0,012	
AC	3,03	1	3,03	27,59	0,0033	
BC	45,74	1	45,74	416,96	< 0.0001	
A ²	74,44	1	74,44	678,68	< 0.0001	
B ²	23,82	1	23,82	217,14	< 0.0001	
C ²	1358,73	1	1358,73	12387,23	< 0.0001	
Residual	0,5484	5	0,1097			
Lack of Fit	0,373	3	0,1243	1,42	0,439	not significant
Pure Error	0,1754	2	0,0877			
Cor Total	1507,51	14				

Table 4-7: ANOVA for colour removal

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	32,71	9	3,63	612,24	< 0.0001	significant
A-Hydraulic retention time	6,59	1	6,59	1110,63	< 0.0001	
B-Magnetite load	0,5894	1	0,5894	99,29	0,0002	
C-pH	1,33	1	1,33	224,32	< 0.0001	
AB	2,06	1	2,06	347,19	< 0.0001	
AC	1,45	1	1,45	244,41	< 0.0001	
BC	0,3833	1	0,3833	64,58	0,0005	
A ²	3,83	1	3,83	645,15	< 0.0001	
B ²	11,2	1	11,2	1886,11	< 0.0001	
C ²	0,6642	1	0,6642	111,89	0,0001	
Residual	0,0297	5	0,0059			
Lack of Fit	0,0166	3	0,0055	0,8469	0,5815	not significant
Pure Error	0,0131	2	0,0065			

4.7.2 Coded equations for quadratic models

The analysis of variance was used to examine the impact of input factors on the response variables. There are three regression models that were generated and can be used to estimate theoretical values for the output parameters in similar studies. The coefficient of each term shows the impact it has on the output variables. The model for biogas production in Eq. (4.1) shows that the combination of terms B and C have the highest impact in biogas production. For COD removal, term C shows the highest impact as shown in Eq. (4.2) whereas colour removal is affected by the combination of term A and C as shown in Eq. (4.3). The regression models obtained are as follows:

$$\begin{aligned} \text{Biogas} = & 99,74 - 5A + 1,97B - 3,42C + 3AB + 5AC + 8BC - 20,62A^2 - 12,56B^2 \\ & - 31,88C^2 \dots \dots \dots (4.1) \end{aligned}$$

$$\begin{aligned} \text{COD removal} = & 89,93 - 1,69A + 0,1498B + 2,59C - 0,5096AB - 0,8698AC - 2,71BC \\ & - 4,49A^2 - 1,63B^2 - 19,18C^2 \dots \dots \dots (4.2) \end{aligned}$$

$$\begin{aligned} \text{Colour removal} \\ = & 98.24 + 0.9702A + 0.2761B + 0.436C + 0.5742AB + 0.6022AC - 0.2477BC \\ & - 1.02A^2 - 1.11B^2 - 0.4241C^2 \dots \dots \dots (4.3) \end{aligned}$$

Where the terms A, B and C represents hydraulic retention time (days), magnetite load (g/L) and pH respectively.

4.8 Fit statistics summary

The fit statistics table, displayed in Table 4-8, is another important statistical table that comprises vital parameters, i.e., mean, standard deviation, coefficient of determination (R^2), predicted R^2 , adjusted R^2 , PRESS, adequate precision, and coefficient of variation. The coefficient of determination, R^2 , can be exercised to compute how well the predicted model fits the observational points. In essence, this term determines the percentage of variation of the output (described by the equation), y, in its neighbourhood. The R^2 term ranges from 0 to 1. An R^2 value close to 1 signifies that the model is a good fit, whereas lower values suggests that the model is not. The coefficient of determination revealed the following: COD removal had an R^2 of 0.9996, colour removal had an R^2 of 0.9978, while biogas yield had an R^2 of 0.9971. Although these values were high, they did not differ too much.

Even though the coefficient of determination is the mostly used parameter in mathematical/statistical analysis, the most preferred parameter is the adjusted R^2 . This is mainly because the adjusted R^2 does not increase with the addition of an input variable. In the same way as the coefficient of determination (R^2), the adjusted R^2 of the responses were high. However, they did not differ too much. In the fit statistics table, the predicted R^2 represents the proposed R^2 for the projected regression model. The increasing order of the predicted R^2 was colour removal (0.9961) > COD removal (0.9958) > biogas yield (0.9810). Therefore, the most robust regression model was the colour removal, followed by COD removal, and lastly the biogas yield. On the other hand, the difference between the predicted and adjusted R^2 values of biogas yield, COD removal, and colour removal were 0.0108, 0.041, and 0.0012, respectively. These values are below 0.2, suggesting reasonable agreement. Thus, there is no issue with both the regression models and the observational data points.

In statistical analysis, adequate precision measures the projected response limits to the projected error, thus the response-to-noise ratio (Stat-Ease Inc., 2019). In general, an adequate precision that is more than 4.0 is recommended since it denotes robust models (Stat-Ease Inc., 2019). This study revealed adequate precisions of 41.58, 97.45, and 123.85 respectively for biogas yield, COD removal, and colour removal. The values of adequate precisions were all greater than 4.0, indicating that discriminations of the regression models were adequate. Thus, the chosen regression models may be employed in navigating the response design spaces since the projected responses are less changed by noise. The colour removal model produced the highest adequate precision of 123.85, which indicates that the regression model of this response had the greatest signal-noise ratio. This further verifies that the model of colour removal was the most robust regression model.

Another significant parameter of fit statistics is the coefficient of variation, which indicates the percentage of the standard deviation and the response mean (Stat-Ease Inc., 2021). The lower the coefficient of variation, the more robust the regression model. For biogas yield, COD removal, and colour removal, the respective coefficients of variation were 3.35%, 0.4368%, and 0.1065%. As was the case with adequate precision, the model of colour removal also performed better in terms of coefficient of variation as it revealed the lowest value of 0.1065%, hence demonstrating a more authentic regression model. This means that the standard deviation of colour removal (0.0977) was very small in comparison to its related mean (96.27%), signifying that the regression model of this response was the most robust.

Predicted residual error sum of squares, i.e., PRESS, is exercised in statistical analysis to compute the variation of error (Stat-Ease Inc., 2021). Basically, the lower the PRESS value, the more

authentic/robust the regression model. The increasing order of PRESS values revealed the following: biogas yield (PRESS = 130.5) > COD removal (PRESS = 6.36) > colour removal (PRESS = 0.21). The lowest PRESS value of 0.21 was obtained on colour removal, suggesting that the regression model of this response was most satisfactory.

Table 4-8: Fit summary statistics for all responses

Statistical parameter	Biogas yield	COD removal	Colour removal
Std dev	2.02	0.3312	0.0977
Mean	60.53	75.82	96.27
Coefficient of variation (%)	3.35	0.4368	0.1065
R^2	0.9971	0.9996	0.9978
Adjusted R^2	0.992	0.999	0.9973
Predicted R^2	0.981	0.9958	0.9961
Adequate precision	41.584	97.4507	123.8489
PRESS	130.5	6.36	0.2106

4.9 Graphical analysis to evaluate validity and reliability of the fitted models

4.9.1 Diagnostic test

It is extremely essential for the regression model equation to be verified and this is attained by performing a diagnostic test (Stat-Ease Inc., 2019). The predicted versus actual graph is one of the significant graphs in model validation. Figure 4-18, Figure 4-19 and Figure 4-20 portrays the predicted versus actual graphs for biogas yield, COD removal, and colour removal, respectively. The observational points should be linearly scattered beside the 45-degree line of the predicted versus actual response figure (Stat-Ease Inc., 2019). The data points of all responses (namely, biogas yield, COD removal, and colour removal) were linearly scattered along the 45-degree straight-line, suggesting that the regression models were able to predict the true data points.

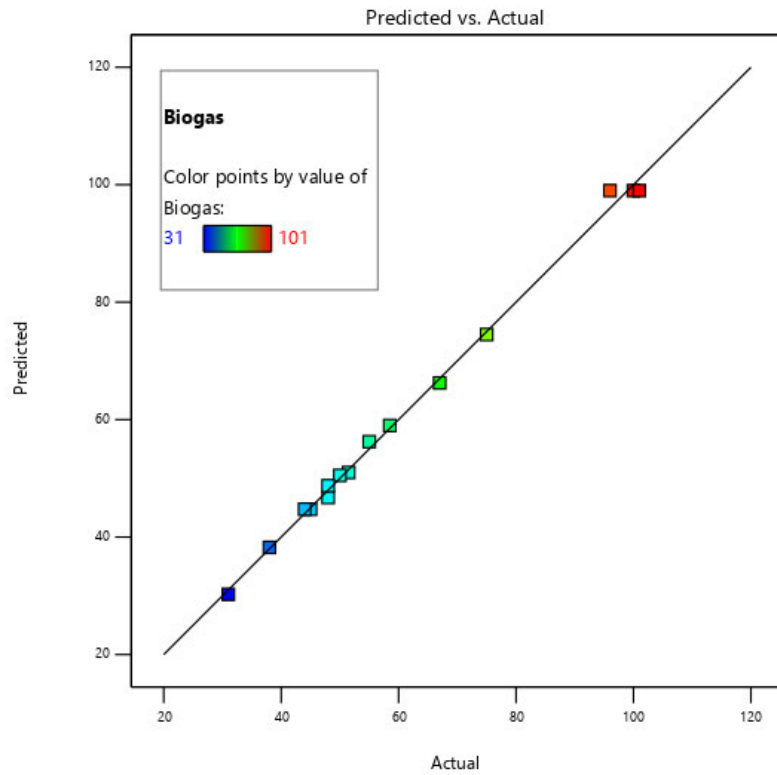


Figure 4-18: Predicted versus actual graph for biogas yield

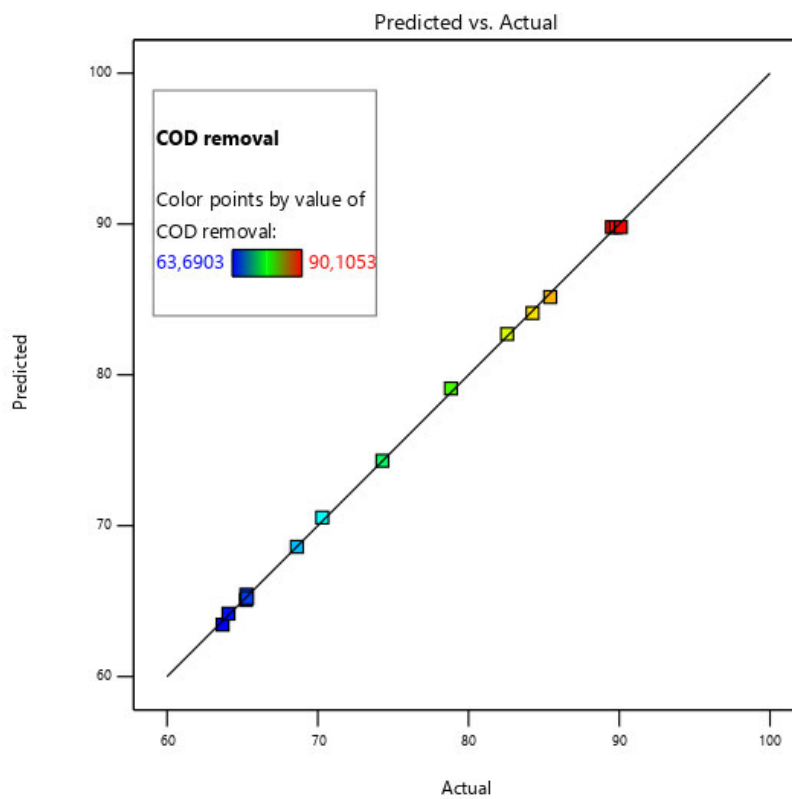


Figure 4-19: Predicted versus actual graph for COD removal

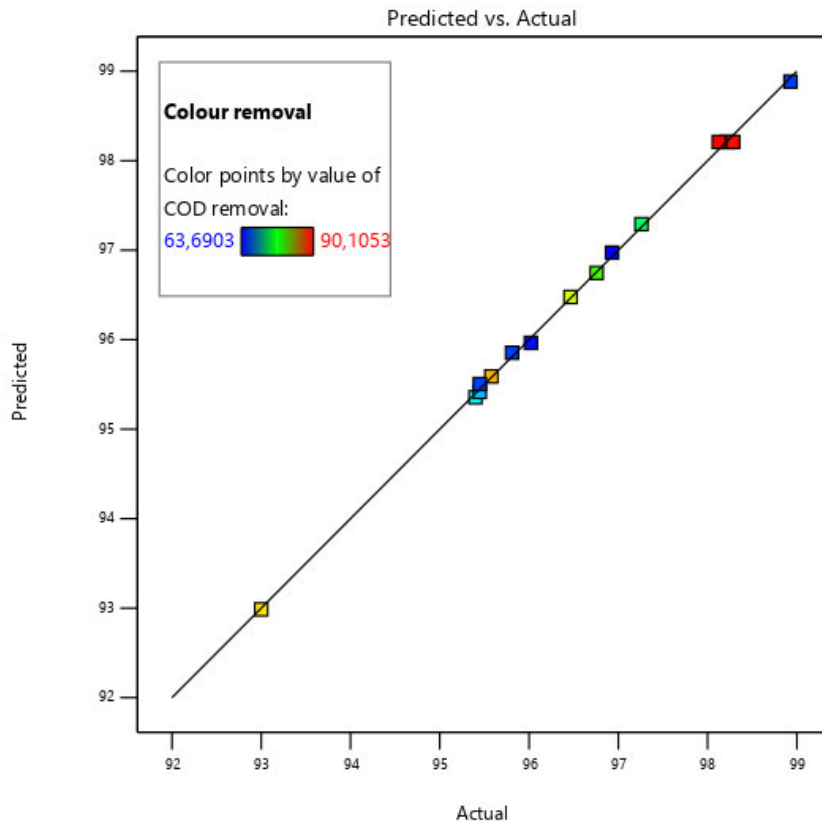


Figure 4-20: Predicted versus actual graph for colour removal.

Another statistical tool that is used to diagnose the regression model is the externally studentized residuals versus the run number shown Figure 4-212, Figure 4-223 and Figure 4-234. The graph of externally studentized residuals versus run number represents the number of t-values in an observational run that lies outside of the other experimental points; that is, “Outliers” (Stat-Ease Inc., 2021). This type of graph has limits that rely on the degree of freedom (D_f) and the tail value (t_v). Equation (4.4) can be applied to compute the term, t_v :

$$t_v = \alpha / (2N) \dots\dots\dots (4.4)$$

where the term α represents the alpha value while N denotes the total number of runs.

In this study, the term α is 0.05 whereas the N value is 15. As a result, $t_v = \alpha / (2N) = 0.05 / (2 \times 15) = 0.0017$. The term D_f can be described by Equation (4.5):

$$D_f = N - p_v - 1 \dots\dots\dots (4.5)$$

In this equation, the term p_v denotes the number of input variables.

The number of input variables in this study, p_v , is equal to 3. Thus, according to Equation (4), the value of D_f is 5. In accordance with the one-tailed/two-tailed t-table (Stat-Ease Inc., 2019), at $t_v = 0.0017$ and $D_f = 5$, the residual limit (R_L) is given by $R_L = \pm t(D_f, t_v) = \pm t(5, 0.0017) = \pm 6.25$. From the plots of biogas yield (Figure 4-21), COD removal (Figure 4-22), and colour removal (Figure 4-23), the data points were inside the residual limits (R_L), which indicated that the output variables should not undergo transformation. Thus, the regression models were able to fit-well all observational points, and therefore there was no issue with either the regression equations or the observational points.

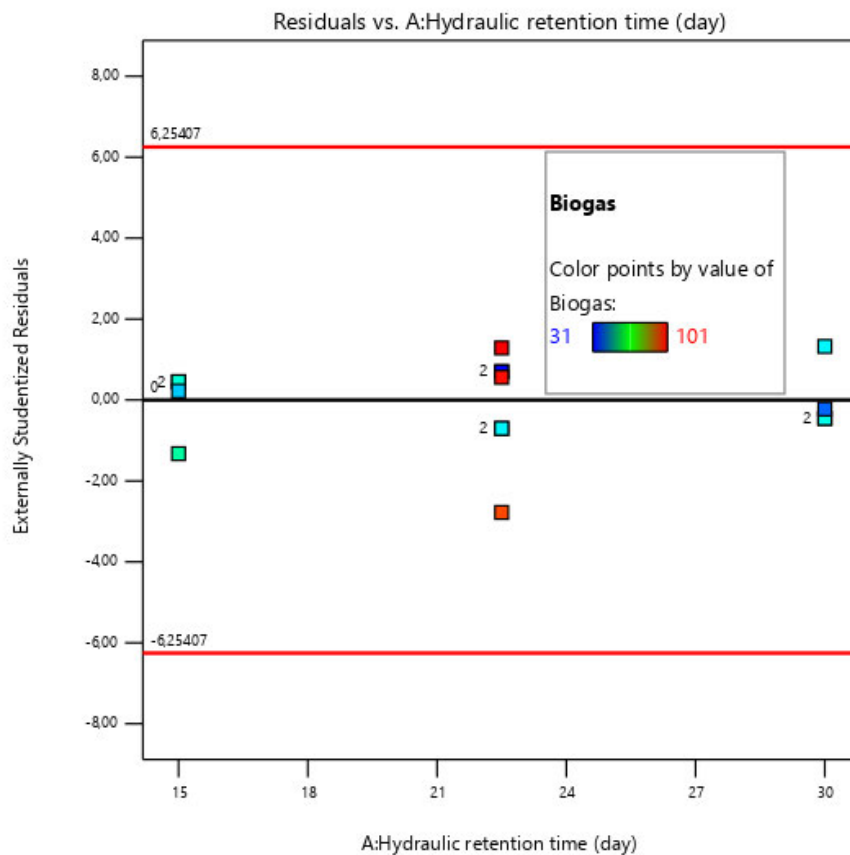


Figure 4-21: Externally studentized residuals versus run number graph for biogas yield

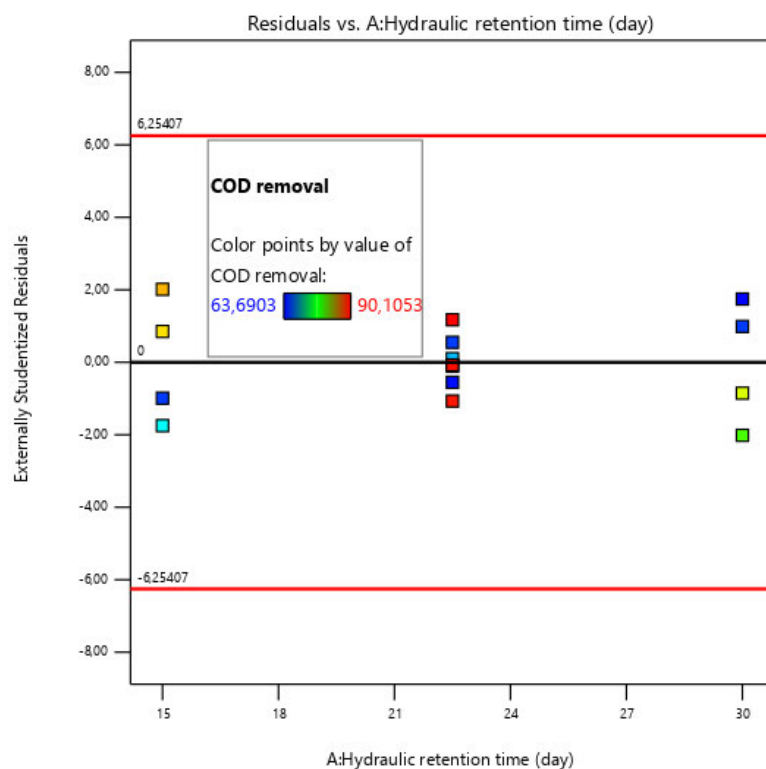


Figure 4-22: Externally studentized residuals versus run number graph for COD removal

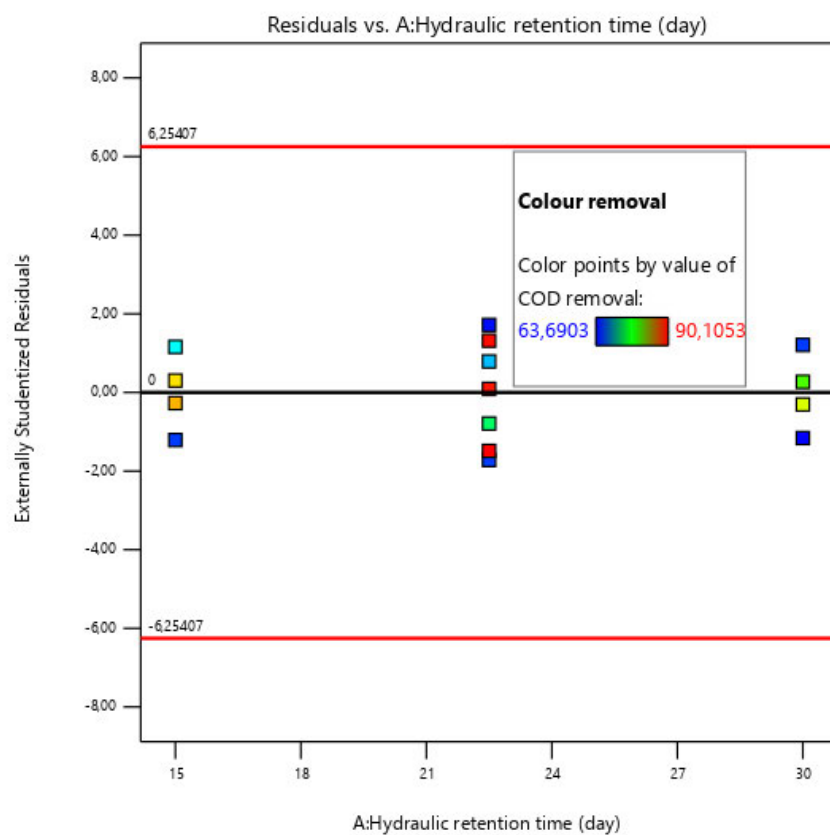


Figure 4-23: Externally studentized residuals versus run number graph for colour removal.

4.9.2 Surface plots (3-D) for biogas yield

The 3D surface plots provide a visual representation of how biogas production (measured in mL/gTDS) varies as a function of three key parameters: Hydraulic Retention Time (HRT) (in days), pH and Magnetite load (in g/L). Biogas production (mL/gTDS) is the response variable plotted on the Z-axis. The surface plot shows how biogas production changes based on different levels of HRT, pH, and magnetite load. The colour scale on the left ranges from blue (low biogas production) to red (high biogas production), making it easier to interpret regions with low vs high methane production. Contour lines on the base (X-Y plane) indicate equal biogas production levels at different combinations of HRT and magnetite load, giving a 2D view of how these variables interact. Figure 4-24, Figure 4-25 and Figure 4-26 display the surface plots for biogas production.

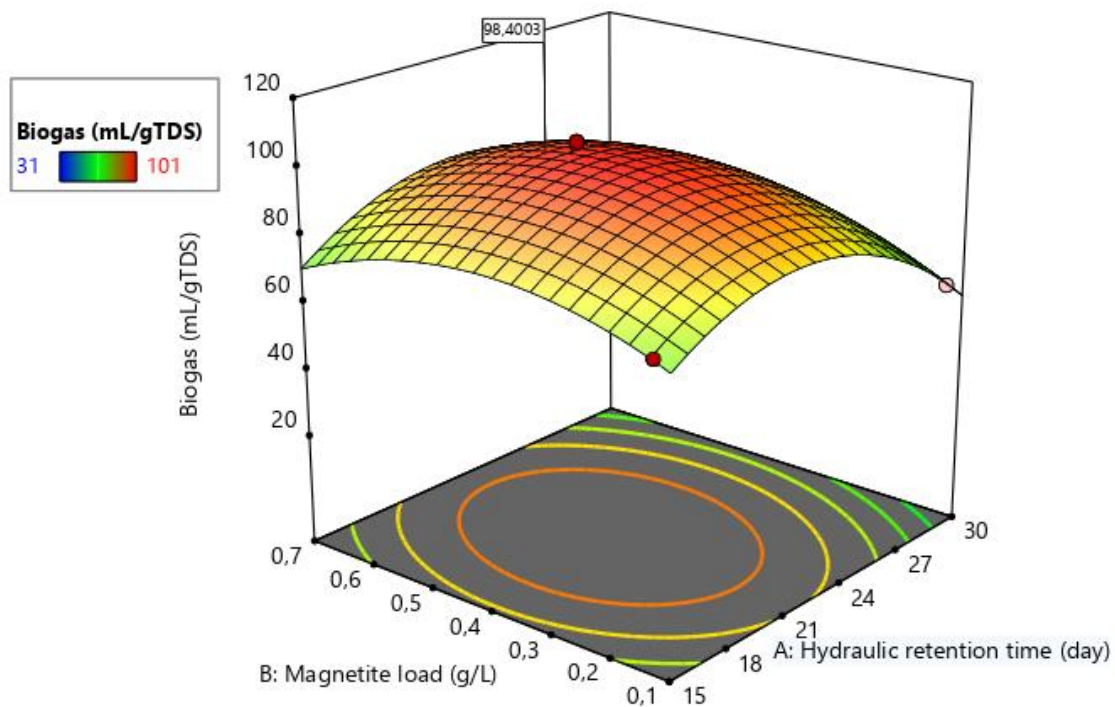


Figure 4-24: Surface plot showing the effect of the interaction of magnetite load vs HRT on biogas production

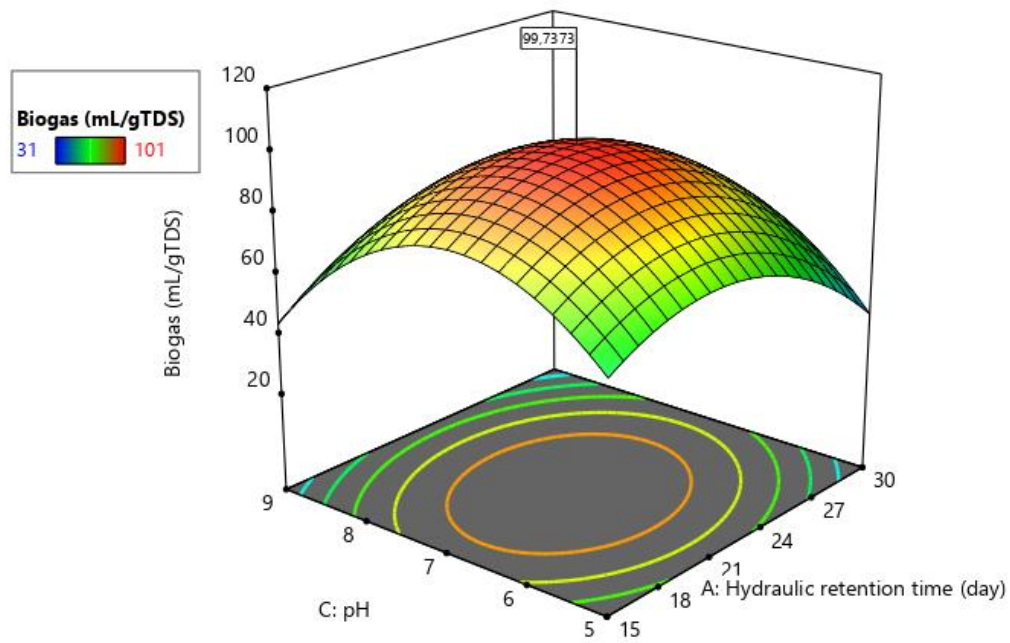


Figure 4-25: Surface plot showing the effect of the interaction of pH vs HRT on biogas production

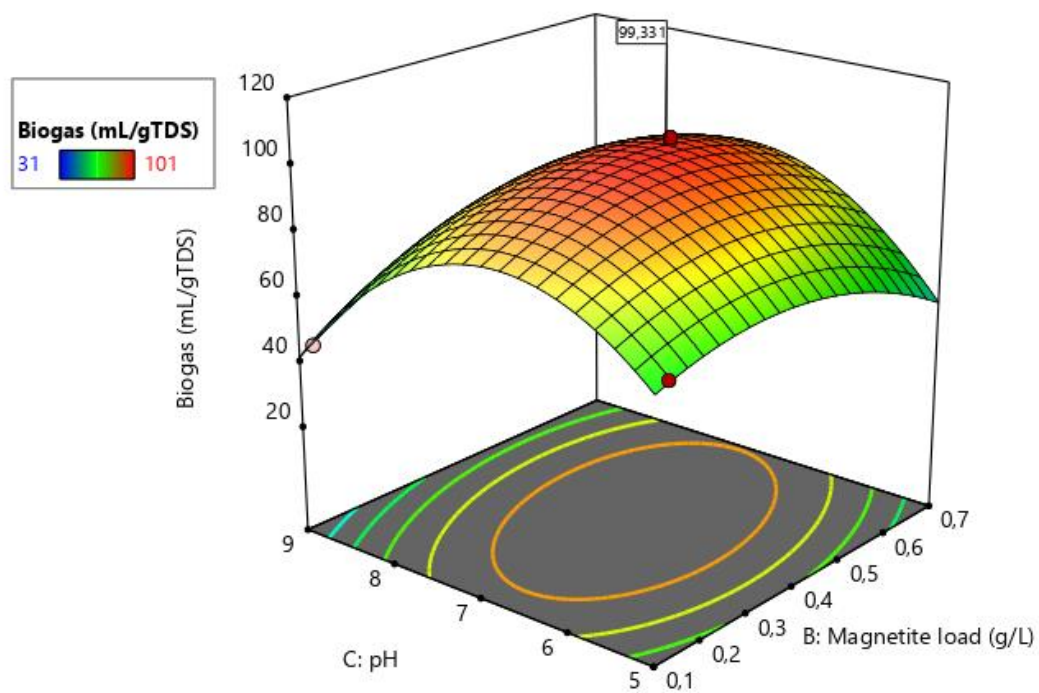


Figure 4-26: Surface plot showing the effect of the interaction of pH versus magnetite load in biogas production

The top surface (red region) represents an area where input parameters can be obtained for maximum biogas production. All three plots show that maximum biogas production can be achieved at the magnetite loading rate of around 0.4-0.5 g/L, pH of around 6.9-7.4 and hydraulic retention time of 18 – 25 days. The trend of biogas production increases with an increase in pH, HRT, and magnetite load until all the input parameters reach optimum ranges, thereafter the trend for biogas production becomes constant showing no further production of biogas. The effect of increasing HRT moving from left to right along the X-axis, increases biogas production. This is expected; with longer HRT, there is increased time for anaerobic digestion, allowing the microorganisms to break down more organic material, thus enhancing methane production. The HRT above 25 days appears to slightly lower the positive effect of HRT, suggesting that increases in HRT beyond this point will not enhance biogas production. There is a positive effect that magnetite load has on the biogas production. The biogas production increases steadily with the increase in magnetic load from 0.1 to 0.4 g/L. However, above 0.5 g/L, magnetite load tends to have a slightly lower effect, as the produced biogas graph had been showing signs of levelling off, indicating that the high loading of magnetite is poisoning the microorganisms.

The plot indicates that a synergistic effect exists; increasing the HRT and magnetite load both improve biogas production. Notice that the high values of the two variables create the highest amount of biogas. The surface has curvature in such a way that no single variable can give the maximum biogas without being in synergy with the other variable. For instance, a low magnetite load with high HRT does not yield high biogas production compared to when both are high. From the base contours, it can be observed that some combinations of HRT and magnetite load produce similar biogas production, for example, 15 d HRT and 0.6 g/L magnetite load, or 25 d HRT and 0.2 g/L magnetite produce a range of biogas production between 60-80 mL/gTDS. This allows one to visualize the trade-offs between these two variables. For example, if one intends to increase the magnetite load only up to a certain limit, then he/she may compensate by extending the HRT to achieve the yield of biogas intended. These 3D surface plots provide clear insight into how hydraulic retention time, pH and magnetite load affect biogas production. From the graphs shown, the ideal combination for maximizing methane yield is an HRT of around 21 days, a magnetite load of 0.4 – 0.5 g/L, and a pH of 6.9 – 7.1, whereby after this point the biogas production starts to decrease.

4.9.3 Surface plots (3-D) for COD removal

Three-dimensional illustrations must be used in RSM to illustrate the relationship between several input factors and one or more response variables. The response's fluctuation with changes in the input variables is well depicted by this surface plot. It helps determine the optimal operating settings, examine the response surface configuration, and analyse the interaction impacts of various components. The dependent variable, in this case, the COD removal, is plotted on the Z-axis, showing the height or depth of the surface about the inputs. A typical 3D graph in RSM would have two independent variables plotted on the X and Y axes, which in this case are (a) magnetite load versus the hydraulic retention time, (b) pH versus the hydraulic retention time, and finally (c) pH versus the magnetite load.

As seen in Figure 4-27, the removal of COD was insignificantly affected by the increase in magnetite load (0.1 to 0.7 g/L), and the hydraulic retention time also showed a slight increase between days 21 and 27 but a gradual decrease after day 27, reaching an optimum of 90% removal at 0.55 g/L of magnetite load in about 24 days. Figure 4-28 shows that the percentage of COD removal is considerably higher at pH values between 6.5 and 8 and decreases at higher pH values. In this instance, the hydraulic retention time is incredibly short and has little bearing on the elimination of COD. At a pH of 7.5 and a hydraulic retention period of 26 days, the ideal COD removal was discovered. The COD removal in Figure 4-29 likewise peaks in the neutral zone, declining after pH values of 6 and 8. The elimination of COD was not significantly impacted by the magnetite load. A pH of 7.5 produced the best COD elimination.

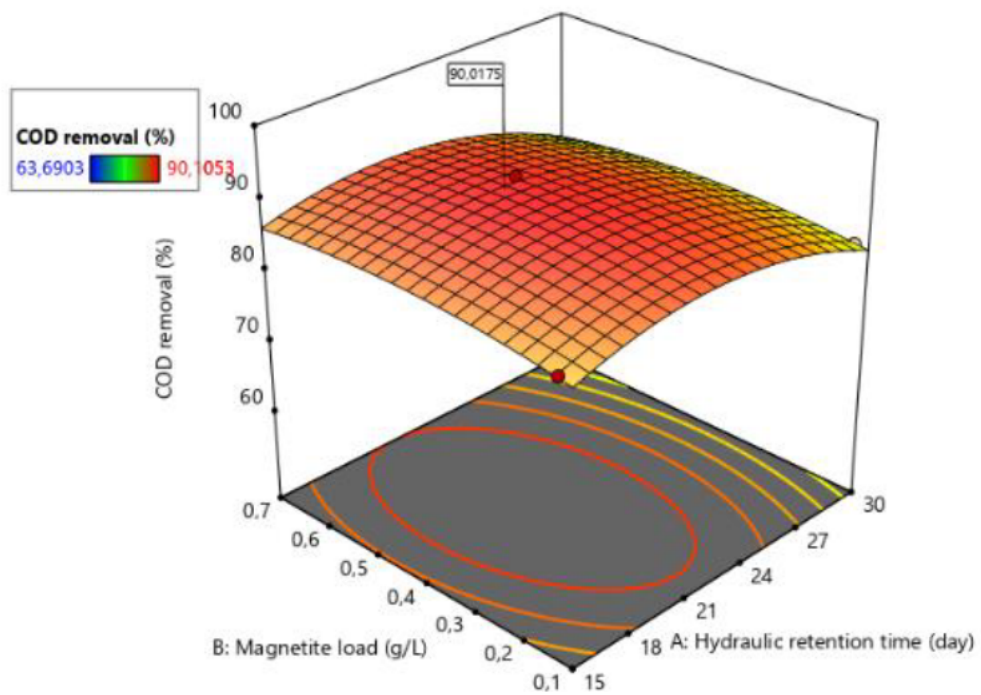


Figure 4-27: Surface plot showing the effect of the interaction of magnetite load vs HRT on COD removal

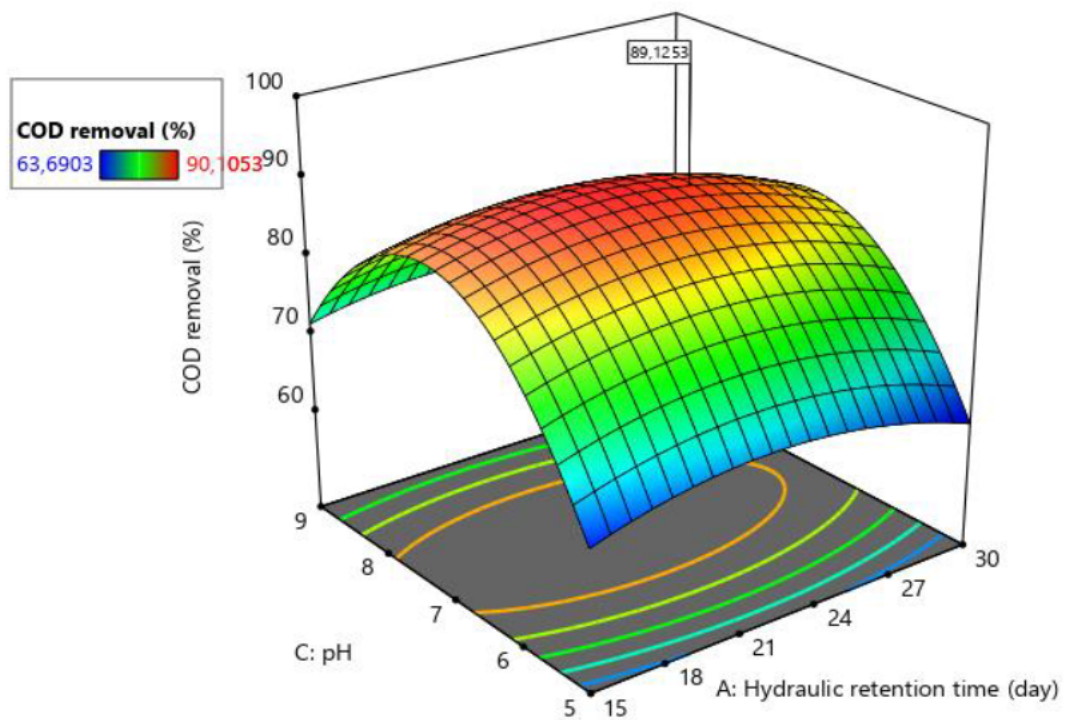


Figure 4-28: Surface plot showing the effect of the interaction of pH vs HRT on COD removal

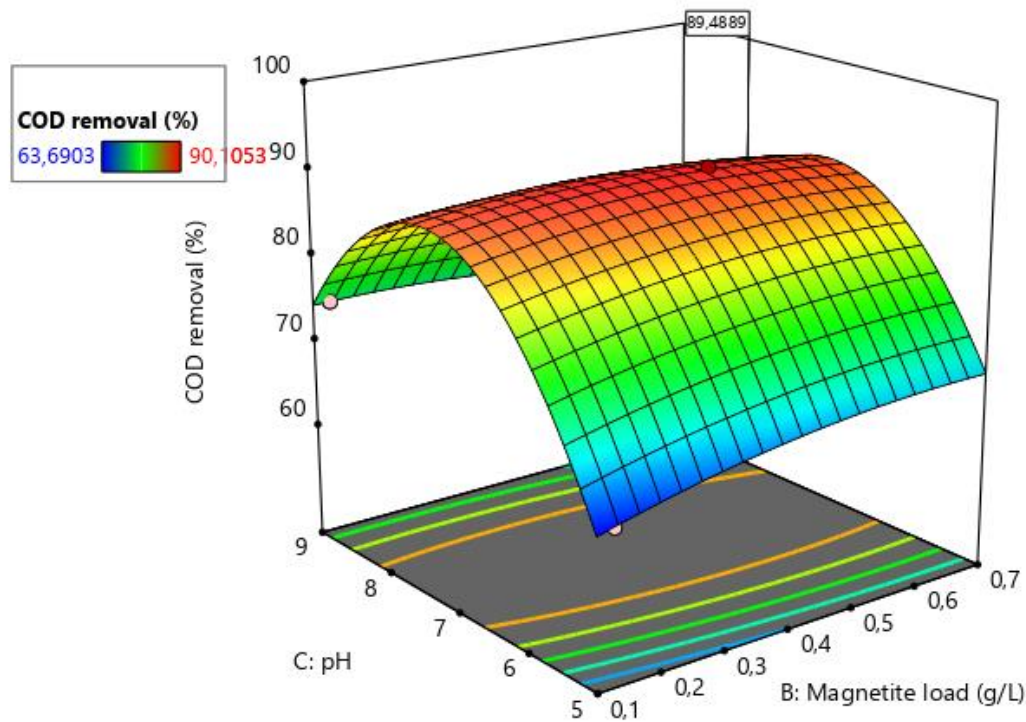


Figure 4-29: Surface plot showing the effect of the interaction of pH versus magnetite load on COD removal

4.9.4 Surface plots (3-D) for colour removal

Three-dimensional surface graphs showing (a) magnetite load vs hydraulic retention time, (b) pH versus hydraulic retention time, and (c) pH versus magnetite load on colour removal effectiveness are shown in Figure 4-30, Figure 4-31 and Figure 4-32. The three-dimensional graph in Figure 4-30 shows a slight concave downward surface. The optimal colour removal was obtained at a magnetite load of approximately 0.5 g/L and a retention time of 27 days, with a removal efficiency of 98.52%. In Figure 4-31, the maximum colour removal of 98.83% was attained at maximum pH (9) and maximum hydraulic retention time (30 days). In Figure 4-32, the percentage removal rose steadily at the lowest magnetite load, peaked at roughly 0.45 g/L, and then progressively dropped as the load grew to its maximum. With a pH of 8 and a magnetite load of 0.55 g/L, the best removal rate of 98.20% was attained.

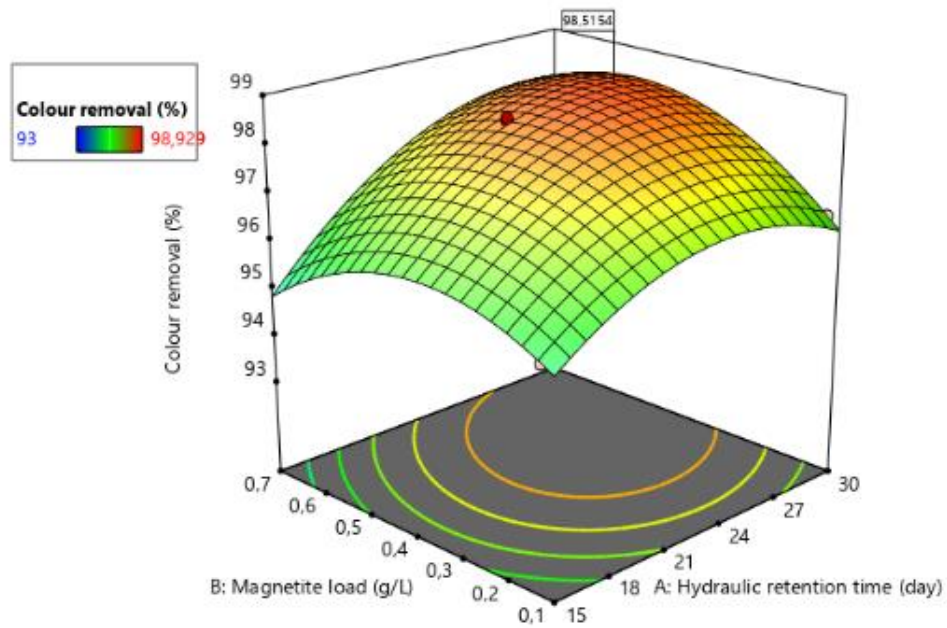


Figure 4-30: Surface plot showing the effect of the interaction of HRT versus magnetite load on colour removal

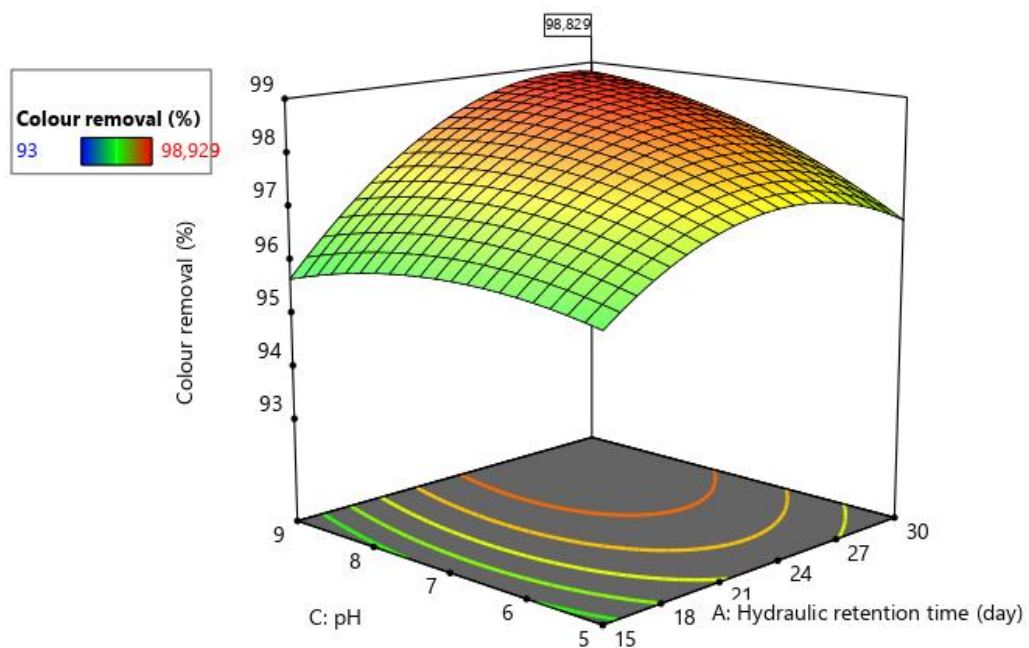


Figure 4-31: Surface plot showing the effect of the interaction of pH versus HRT on colour removal

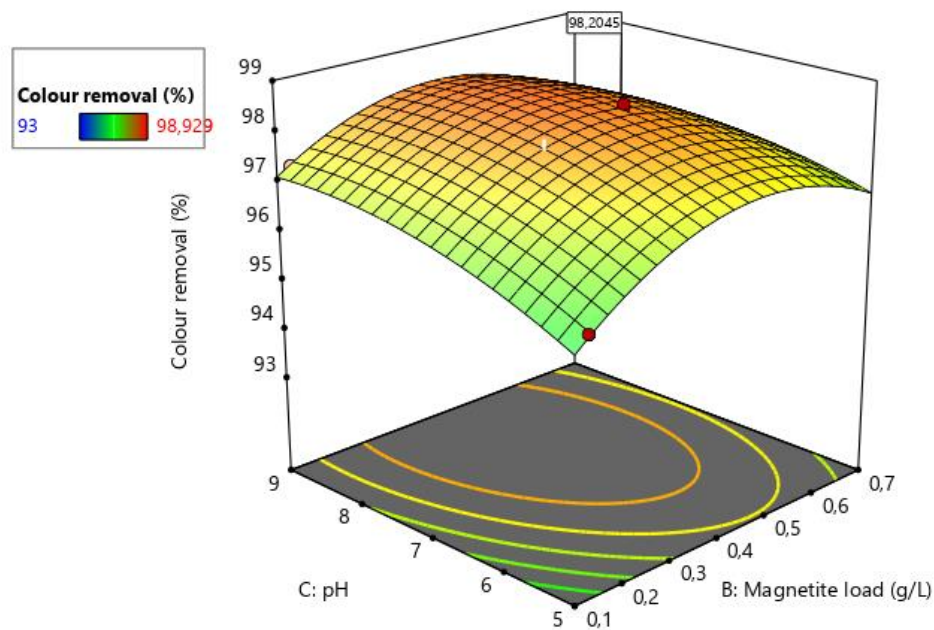
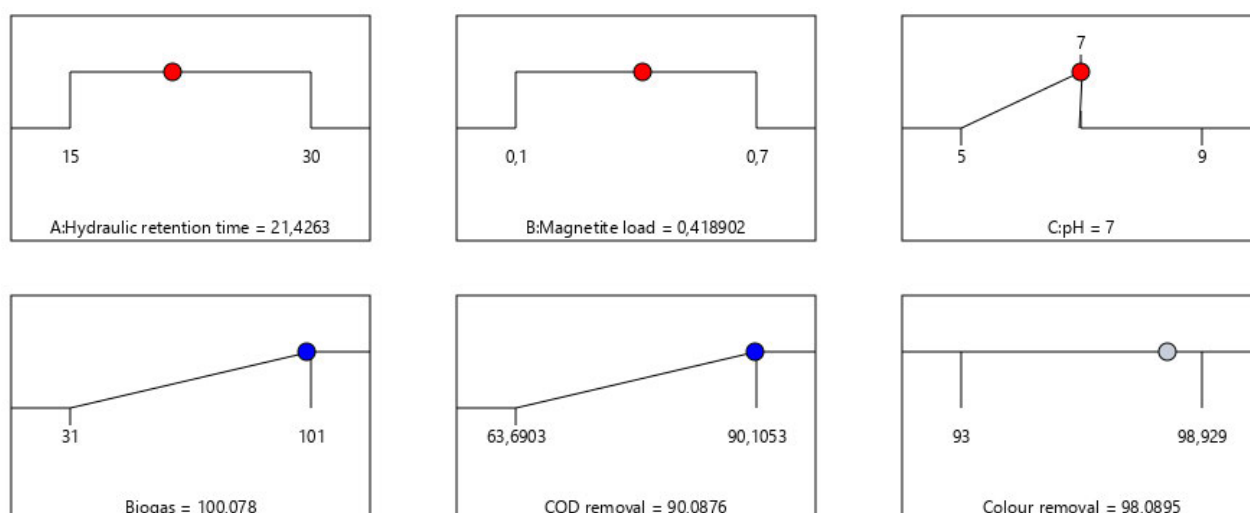


Figure 4-32: Surface plot showing the effect of the interaction of pH versus magnetite load in COD removal

4.10 Numerical optimization and desirability

The final step in statistical analysis is to obtain the optimum conditions and responses via the numerical optimization technique. Numerical optimization ramps were exercised to attain the hydraulic retention time, magnetite load, and pH to optimize the biogas yield, COD removal, and colour removal. The goals were set to maximize the output variables. As observed in Figure 4-33, at optimum input variables of hydraulic retention time (21 days), magnetite load (0.41 g/L), and pH (7.0), Design Expert indicated that the optimum output variables for biogas yield, COD removal, and colour removal were respectively equal to 100.078 mL/g TDS, 90.1%, and 98.1%. Overall, the outcome of this study indicated the maximum combined desirability of 0.995 with 27 possible solutions at a hydraulic retention time of 21.4 days, magnetite load of 0.42 g/L, and pH of 7.0. The first 10 possible solutions of optimum conditions are presented in Table 4-9.



Desirability = 0,995
Solution 1 out of 27

Figure 4-33: Ramp plots showing optimum conditions with predicted outputs

Table 4-9: First 10 out of 27 optimum solutions generated

Number	HRT	Magnetite load	pH	Biogas	COD removal	Colour removal	Desirability	
1	21,426	0,419	7	100,078	90,088	98,09	0,995	Selected
2	21,405	0,417	7	100,075	90,089	98,085	0,995	
3	21,439	0,422	7	100,079	90,087	98,092	0,995	
4	21,472	0,418	7	100,084	90,085	98,097	0,995	
5	21,365	0,421	7	100,066	90,091	98,079	0,995	
6	21,426	0,424	7	100,074	90,087	98,09	0,995	
7	21,296	0,418	7	100,053	90,094	98,067	0,995	
8	21,592	0,436	7	100,053	90,071	98,118	0,995	
9	21,531	0,397	7	100,025	90,071	98,097	0,995	
10	21,604	0,399	7	100,035	90,067	98,11	0,995	

4.11 Experimental validation of the RSM optimum conditions

After the model was analyzed and validated, numerical optimization was conducted to obtain optimum operating conditions for the anaerobic treatment of the local wastewater. The goal was to optimize the removal of color and COD and improve the recovery of biogas while operating within the range of the investigated input parameters. The response surface methodology was utilized to

optimize the AD and 27 possible solutions with a desirability of 0.99 were obtained. The predicted optimum conditions for the input variables namely HRT, magnetite (MNP) load and pH were found to be 21 days, 0.42 g/L and 7.04, respectively. Table 4-10 summarizes and compares the predicted optimum conditions (inputs and outputs) and optimum outputs achieved during experimental validation of the predicted optimum conditions using the industrial sewage stream. The difference between predicted outputs and validated outputs was found to be below 2% for all responses, which suggests that the predicted optimum conditions can be applicable during AD of municipal wastewater.

Table 4-10: Validation of optimum conditions in the industrial sewage stream

Inputs			Outputs			
Factor	Optimum	Response	Predicted	1 L	5 L	10 L
HRT (days)	21.4	%COD removal	90.98	89.41	89.25	88.74
MNP load (g/L)	0.419	%Colour removal	98.09	96.79	92.78	91.28
pH	7.0	Biogas (mL/gTDS)	100.08	108.2	526.34	986

The optimum was further validated in the treatment of sugar refinery stream to evaluate the feasibility of producing biogas and presented in Table 4-11. The results show a significant increase in biogas production, indicating that this stream is suitable for anaerobic treatment and the optimum conditions are applicable. However, a decrease in contaminant removal suggests that this stream may require other post-treatment processes before it can be discharged or reused in order to comply with wastewater discharge standards.

Table 4-11: Validation of optimum conditions in the sugar refinery stream

Inputs			Outputs			
Factor	Optimum	Response	Predicted	1 L	5 L	10 L
HRT (days)	21.4	%COD removal	90.98	69.46	61.47	64.38
MNP load (g/L)	0.419	%Colour removal	98.09	70.14	65.8	60.19
pH	7.0	Biogas (mL/gTDS)	100.08	112.6	687.4	1252

4.12 Evaluation of biogas production in a 50 L pilot system

The optimum operating conditions were employed at an upscale AD system to improve overall wastewater treatability efficiencies and biogas yield. The influence of pH, temperature, magnetite load and residence time was studied on biogas production and contaminant removal. Magnetite load and residence time were kept constant whereas control for pH and temperature was automated based on the signal received from the sensors in the reactor. The effectiveness of automated sensors and controls for pH and temperature was monitored over the first week. It took about five days for pH and temperature to reach the desired range and thereafter stabilized for the duration of the process. The pH was initially very low because the effluent was strongly acidic, nutrient-rich with high organic content. Table 4-12 summarises the changes on the automated input parameters at the beginning of the process prior to reaching anaerobic conditions, the desired HRT of 21 days was considered from day 6 after the system had reached desired anaerobic conditions.

Table 4-12: Automated monitoring of the controlled inputs

Day	1	2	3	4	5
pH	3.13	3.02	3.69	5.92	6.78
Temperature	22.59°C	23.1°C	31.4°C	36.87°C	37.1°C

After achieving anaerobic conditions, the responses (biogas production and contaminants removal) were monitored over the HRT of 21 days (3 weeks). During the first week, it is evident that the anaerobic bacteria were still trying to adapt to the environment with COD removal of less than 10%. In the second week, the system had reached stability and there was an exponential growth in both biogas production and COD reduction. This suggests that the microbial activity was activated using magnetite and controlled operating conditions, allowing microorganisms to convert organic content to biogas. Figure 4-34 shows a direct-proportional relationship between organic content degradation (COD removal) and biogas production.

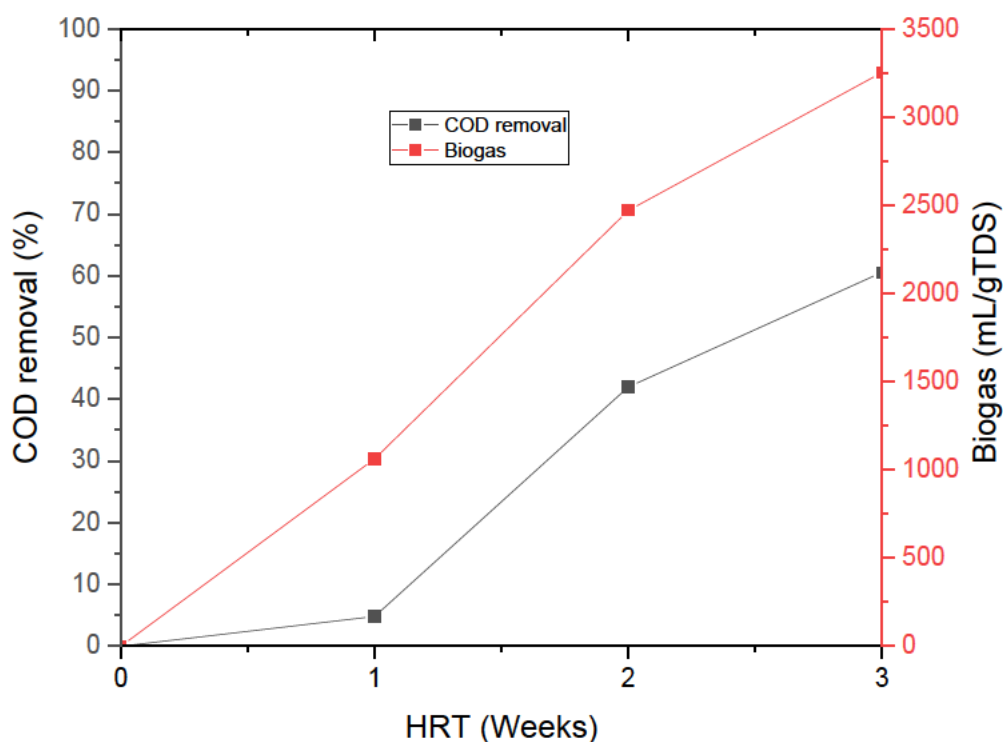


Figure 4-34: COD removal and biogas production for the 50 L up-scale AD system

In anaerobic digestion (AD), the bacterial community removes contaminants via biodegradation of organic content to produce methane-rich biogas and reduce costs for effluent and sludge discharge (Lee *et al.* 2017). Biogas was produced over 21 days, and the quality of biogas reached a maximum of 92% methane content within the first week of production as shown in Figure 4-35. High methane content indicates efficient conversion of organic content into methane by microorganisms. This is a result of optimal anaerobic conditions with close monitoring of operating conditions and balanced solid-liquid ratios. The achieved biogas quality indicates a low concentration of impurities and may not require further upgrading before converting to electricity. The energy value of high-quality biogas averages at 13.4 kWh based on methane content (Garcia *et al.* 2019). Therefore, the energy value of the 92% methane-rich biogas is estimated to be 12.27 kWh.

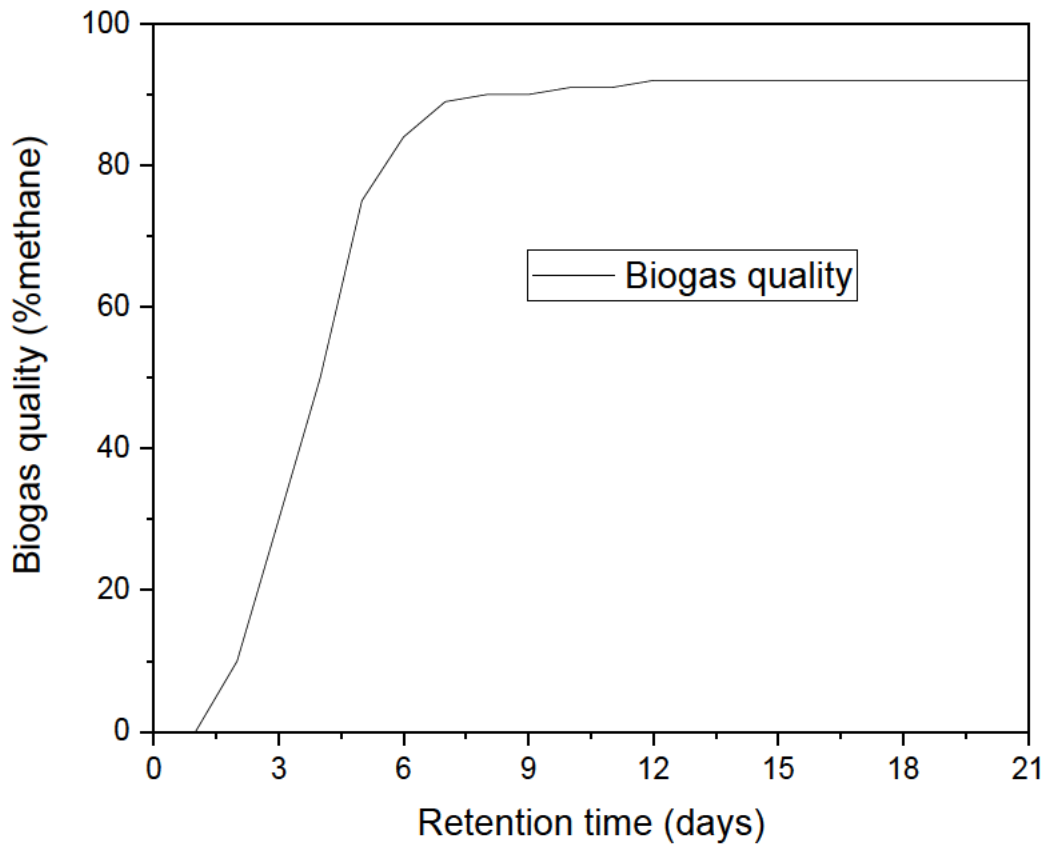


Figure 4-35: Quality of biogas (%methane) produced in the up-scale 50 L AD system

The quality of effluent produced during the degradation process was closely monitored to ensure that it is well within disposal limits or required post-treatment before disposal. Figure 4-36 summarizes the overall removal efficiencies for COD, colour, and turbidity. The removal efficiencies for all three contaminants were between 55-65%. The lower removal efficiencies could be due to poor contact between solids and liquid as there was no sufficient agitation in the tank to promote contact. However, 65% of removals signify that the microorganisms were able to digest most of the organic content. The produced effluent at the end of AD may require post-treatment such as advanced oxidation to further remove these contaminants before discarding to the environment.

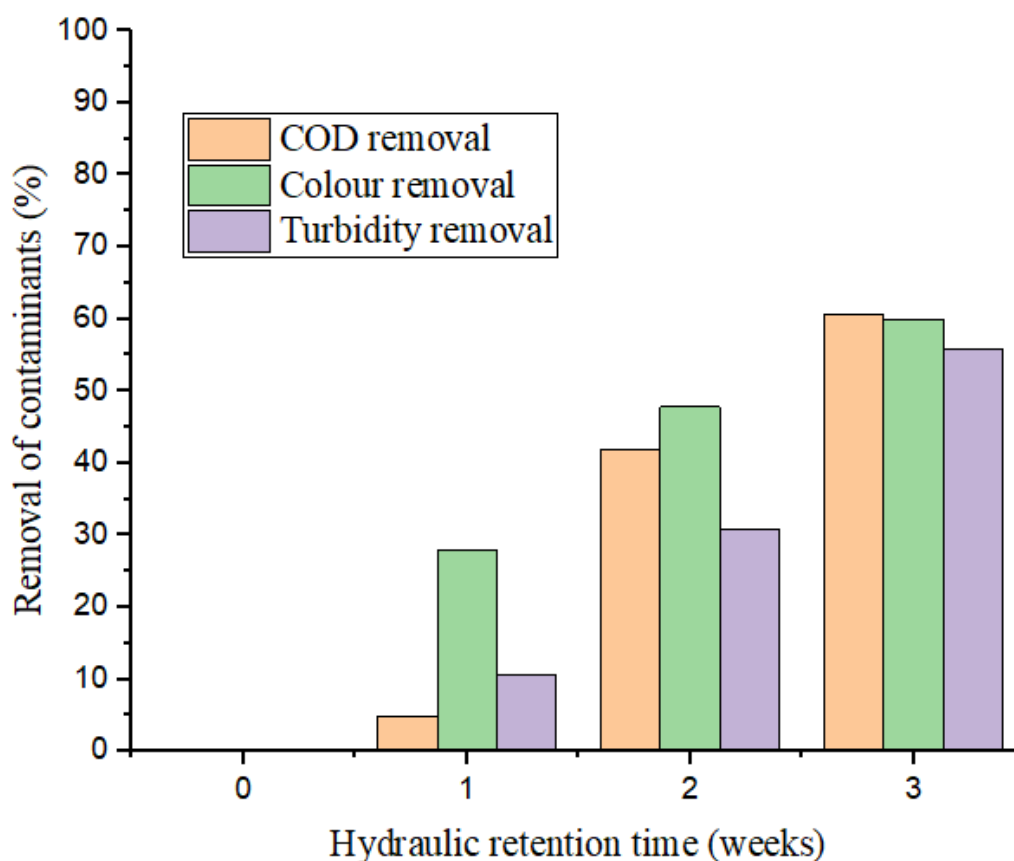


Figure 4-36: Removal of wastewater contaminants in the 50 L AD system

4.13 Analysis of water quality and digestate post anaerobic digestion

The quality of water post-anaerobic digestion was characterized and compared to South African wastewater discharge limits that were sourced from Agoro *et al.* (2018) as presented in Table 4-13. The results show that the concentration of contaminants in the treated effluent exceeds the discharge limits, suggesting that the effluent needs to undergo post-treatment before it can be discharged.

Table 4-13: Properties of water after anaerobic digestion

Water pollutant	This study	Discharge limits (Agoro <i>et al.</i> (2018))
Chemical oxygen demand (COD)	4 186.5	30 – 75 mg/L
pH	6.41	5.5 – 9.5
Turbidity	415	10 – 50 NTU
Total dissolved solids (mg/L)	329.8	450 mg/L

The optimization of anaerobic digestion allows for production of clean water and improved biogas. Digested sludge is another product that is also produced and discharged to the environment. Figure 4-37A portrays SEM image for sludge from conventional digestion; Figure 4-38B represents sludge digested with the addition of magnetite and controlled pH. In Figure 4-37A, sludge particles appear to have formed a solid shape or lumps with flat surfaces while in Figure 4-38B it has greater surface area. The increased surface area as a result of magnetic nanoparticles allows for greater contact between solids and liquid during AD, making it easy for microorganisms to access organic matter for digestion, and thus increases the performance of AD. Small surface area shown in Figure 4-37B makes it difficult for microorganisms to access organics and hinders contact between solids and liquid. Additionally, the large surface area allows for the use of sludge material into fertilizers, and it is easy to handle for further processing or drying and disposal. Landfilling and land application of the sewage sludge are suggested to be the most economical sludge disposal methods. The application of municipal sludge to land is highly advantageous due to its fertilizing and soil-conditioning characteristics, provided that it does not contain any hazardous substances (Singh and Agrawal 2008; Usman *et al.* 2012).

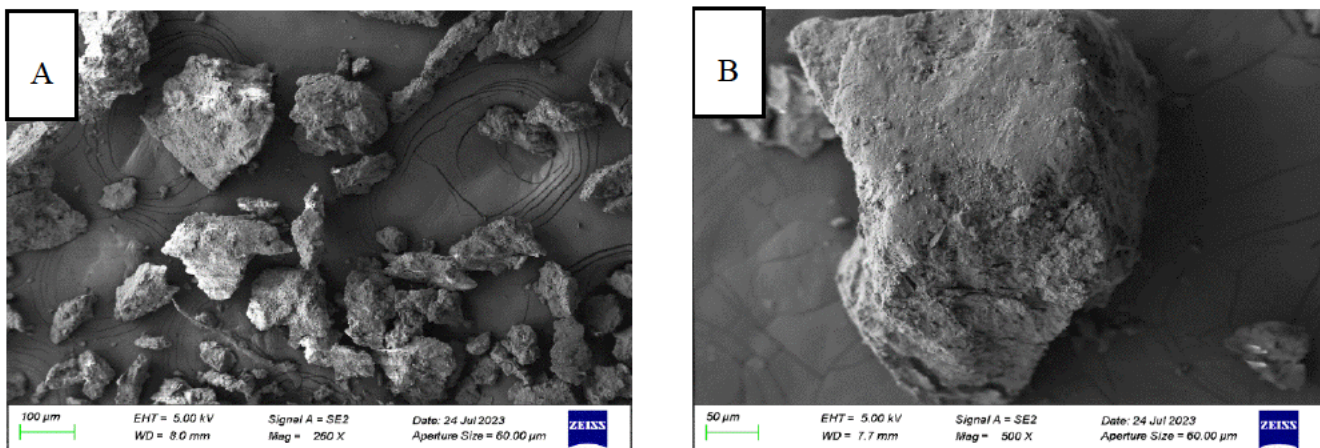


Figure 4-37: SEM images showing the surface area of the digested sludge without magnetite

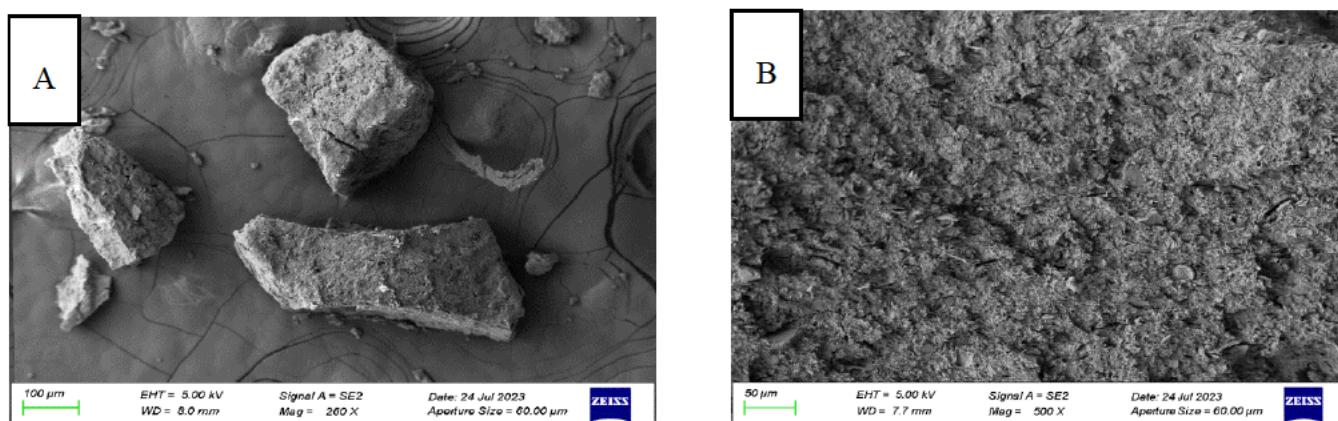


Figure 4-38: SEM images showing the surface area of the digested sludge with magnetite

4.14 Summary of the optimised up-scale AD system

The changes in AD performance were studied over four different AD reactor sizes using the sugar refinery stream. From Table 4-14, it can be noted that the performance of the digesters varies slightly based on reactor size. Apart from reactor size, the design of the reactor and its operation plays a key role in its performance. For a small reactor (1 L), the heat transfer was faster as the reactors were immersed in hot water, providing direct heat to the mixed liquor while in a larger reactor (50 L), there was poor heat transfer due to heat losses from the water bath to the heating jacket and finally to the mixed liquor, hence the temperature took longer to reach the desired range (or set point). In addition, agitation is critical as it warrants contact between solids and liquid. It is easy to achieve proper mixing in a small reactor whereas in a larger reactor, it is more difficult hence pH took longer to stabilize and there was a delay in biogas production.

Table 4-14: Summary of the performance of all digesters in the upscaling work based on sugar refinery stream

AD size	Biogas (mL/gTDS)	%COD removal	%Colour removal	%Turbidity removal
1 L	112.6	69.46	70.14	74.48
5 L	687.4	67.47	65.80	64.57
10 L	1252	64.38	60.19	61.92
50 L	4930	61.40	60.89	64.80

4.15 Evaluating cost benefits analysis for the up-scaled AD system

This section presents the summary of costs associated with the design and operation of the up-scaled biogas recovery AD system. The costs of different AD sizes and revenue generation based on biogas produced will be assessed and compared and conduct the profitability analysis for the four different AD systems.

4.15.1 Capital costs for the up-scaled AD system

The capital costs for the design, commissioning, and operation of the 50 L smart anaerobic digestion system are presented in Table 4-15. The total investment cost that is required for the smart AD system amounts to R 119 048, with approximated annual costs of R8 566 for basic maintenance.

Table 4-15: Capital costs for the biogas recovery plant

Items	Cost	Average life span (Years)	Approximate, Cost/yr
50 L PVC tank (Anaerobic digester)	R 30 617	20	R1 688
Feed pump	R 1 850	20	R92
Water bath	R 0 – Available	20	R1 294
Controller components	R 25 876	20	R1 284
Sensors • pH • Temperature	R 26 803	5	R1 960
Mounting hardware and accessories	R 9 874	10	R619
Installation	R 8 500	20	R510
TOTAL	R 103 520		R7 449
TOTAL (INCLUDING VAT)	R 119 048		R8 566

4.15.2 Evaluating costs for operating the up-scaled 50 L AD system

The operating cost for the system were estimated from electricity costs, chemical requirement, and fuel costs for transportation of wastewater to the system. The fuel cost comes from the distance travelled for sample collection multiplied by the cost of fuel per kilometre. Table 4-16 presents the operating costs for electricity consumption by the electrical appliances on the system. The power usage for each unit was obtained from the supplier description in kW. Each unit has a different operating period per day based on the demand and operating conditions. The feed pump only runs for 0.17 hours per day during system charging and circulation of effluent and the acid/base pump's run period was estimated at 0.25 hours per day as the pumps run only to charge acid or base to balance pH to the desired range. The current cost of electricity in South Africa averages at R3.76/kWh (NRClitchi, 2024), therefore the annual operating cost is calculated as follows using equation (4.6).

$$\text{Annual operating costs per unit} = \text{Power usage} * \text{Operating hours} * \text{Unit costs} * \text{HRT (21 d)} \dots\dots\dots (4.6)$$

Table 4-16: Operating costs - Electricity

Item	Power usage (kW)	Operating hours per day	Unit cost	Monthly costs (21 days)	Annual costs
Feed pump	0.37	0.17	R3.76 per kWh	R4.97	R60
Acid/base dosing pump	0.2	0.25	R3.76 per kWh	R3.95	R47
Water bath	1.5	6.5	R3.76 per kWh	R770	R9 238
Gas analyser	0.25	2	R3.76 per kWh	R39	R474
Total					R9 819

One of the optimum operating conditions found in this study for AD was catalyst loading rate of 0.41 g/L. It is synthesised in-house using chemicals purchased from the external labs. The cost of chemicals required for synthesising the catalyst is summarised in Table 4-17. Sodium hydroxide pellets and Fe²⁺/Fe³⁺ granules are purchased monthly and 500 mL of the acid would be purchased annually as the amount required for synthesis is very low. Therefore, the total operating costs based on chemical requirement was estimated as R2 712 per annum.

Table 4-17: Operating costs - Chemicals

Item	Quantity	Unit price	Monthly cost	Annual cost
NaOH pellets	1 L	R91.2	R91.2	R1 095
Fe ²⁺ & Fe ³⁺	0.5 L	R124.8	R124.8	R1 498
Acid	0.5 L	R118.5	R118.5	R118
Total				R2 712

The additional operating costs include travelling costs for sample collection. The effluent samples were collected from the sugar refinery plant located 14.3 km away from the lab. The average cost of travel in South Africa equals to R4.84 per kilometre travelled. Therefore, the total cost for travelling is calculated using equation (4.7)

$$\begin{aligned} \text{Total travelling costs} &= \text{distance travelled} * \text{cost of travel per km} \dots \dots \dots (4.7) \\ &= R142.30 \text{ per trip} \end{aligned}$$

The total annual cost was calculated as the sum of electricity, chemicals and travelling costs which amounts to **R14 238**.

4.15.3 Evaluation of economic benefits for the AD system

The key indicator to the benefits of an anaerobic digester is the amount of biogas produced that can be converted into electricity. The produced biogas was quantified using the downward displacement cylinder and characterized using the gas analyser for methane content. The biogas quality achieved was 90% methane at optimum operating conditions with a net energy value of 12.27 kWh. Considering the cost of electricity at R3.76 per kWh, the annual energy and revenue generated was calculated as using Eq. (4.8) and Eq. (4.9) and presented in Table 4-18.

Total annual revenue for the 50 L AD:

Biogas volume = 0.493 m³

Net energy value of biogas with 90% methane content = 12.27 kWh

Electricity price: R3.76/kWh

Hydraulic retention time: 21 day per month

$$\text{Annual energy production} = \text{biogas volume} * \text{Net energy value} * \text{HRT} \dots\dots\dots (4.8)$$

$$\text{Annual energy production} = 0.493 \frac{\text{m}^3}{\text{d}} * 12.27 \frac{\text{kWh}}{\text{m}^3} * 21 \frac{\text{d}}{\text{month}} * 12 \frac{\text{months}}{\text{year}} = 1524.4 \frac{\text{kWh}}{\text{year}}$$

$$\text{Annual revenue} = \text{Annual energy production} * \text{electricity price} \dots\dots\dots (4.9)$$

$$\text{Annual revenue} = 1524.4 \frac{\text{kWh}}{\text{year}} * 3.76 \frac{\text{R}}{\text{kWh}} = \text{R}5731.74$$

Therefore, the annual energy production and revenue for the four AD systems is summarised in Table 4-18.

Table 4-18: Total annual revenue for different AD sizes

AD size	Biogas volume (m ³)	Annual energy production (kWh/year)	Annual revenue (R/year)
1 L	0.01126	34.82	131
5 L	0.06874	212.55	799
10 L	0.1252	387.12	1456
50 L	0.493	1524.4	5732

Net Positive Value (NPV):

The net present (NPV) value was calculated to evaluate the profitability of the project. The NPV represents the difference between the value of the cash flow and the total costs over the 30-year period for the project lifespan. The average discount rate used is 5% for the return on investment. To evaluate NPV for the two AD systems, the net cash flow (CF_t) was calculated using Eq. (4.10).

$$\text{Net cash flow (CF}_t\text{)} = \text{Annual revenue} - \text{Operating costs} \dots\dots\dots (4.10)$$

$$\text{CF}_t = \text{R}5\,732 - \text{R}14\,238 = \text{R} - 8\,506$$

The net cash flow for the 50 L system is R – 8 506, suggesting that the system is operating at a loss with operating costs exceeding the revenue generated from biogas. The cash flow for the system was used to calculate the NPV using Eq. (4.11).

$$\text{NPV} = \sum_t^n \frac{\text{CF}_t}{(1+r)^t} - \text{Initial Investment} \dots\dots\dots (4.11)$$

$$NPV = \frac{-8506}{(1+0.05)^{30}} - 119048 = R - 121\,016$$

The negative NPV for this AD system indicates that the capital costs and cost for operating the system exceeds the projected financial benefits from biogas produced. The negative NPV is a result of higher capital cost that is required for commissioning of the AD system. The negative NPV may indicate financial loss based on revenue generated from biogas, however, this AD system comprises of other benefits that can justify the negative NPV. The cost of treating wastewater and discharging sludge can be added to the financial gains for this project. Moreover, the nutrient-rich sludge produced can be sold for use as a bio-fertilizer and generates more revenue. In addition to economic benefits, this technology has significant impact on environmental benefits with a reduction of carbon footprint and replacing chemical synthetic fertilizers with bio-fertilizers, thus preserving the environment.

Payback period and Benefit-cost ratio

The next critical factor on the project's investment is the payback period that is calculated using the initial capital and annual revenue as shown in Eq. (4.12).

$$\text{Payback period} = \frac{\text{Initial investment}}{\text{Annual revenue}} \dots\dots\dots (4.12)$$

$$\text{Payback period} = \frac{109048}{5731} = 19.03 \text{ years}$$

The calculated payback period for a 5 L AD system is 29.4 years while the payback on the 50 L system comes down to 19.03 years. This suggests that upscaling of the AD generates more revenue, and reduces the payback period on investment, thus reducing the risk on investment. Similar studies by Spyridonidis *et al.* (2020) proved that upscaling increases revenue and reduces the payback period if operating costs are reduced with improvements over the lifespan of the project. The Benefit-Cost ratio (BCR) is another indicator of the financial viability of the project and is calculated by dividing the total annual benefits by the annual operating costs in Eq. (4.13).

$$\text{Benefit} - \text{Cost Ratio (BCR)} = \frac{\text{Annual revenue}}{\text{Operating costs}} \dots\dots\dots (4.13)$$

$$BCR = \frac{5731}{14238} = 0.4$$

The BCR of 1 and above indicates that the project is economically viable and may be recommended for investment. In this study, the BCR for the 50 L system is 0.4, indicating that the cost of operation

outweighs the total benefits, however, there are other non-economic benefits to be considered that justifies the importance of up-scaling and industrialization of this AD system.

The cost benefit analysis was conducted for all four AD systems and presented in Table 4-19. The aim was to evaluate the feasibility of up-scaling from the lab scale to a pilot AD system. This study revealed that up-scaling has a positive impact on revenue generation with an increasing amount of biogas generated. The NPV for all the systems remain negative because of high capital costs required for equipment design and installation. The annual cash flow suggests that all the systems would be operating at a loss due to higher operating costs outweighing the revenue generated from biogas. The benefit-cost ratio for all the systems is below 1, suggesting that there is no profit generated, however, the increasing ratio from 0.05 to 0.4 indicates that up-scaling the AD size increases revenue, thus positively impacting the financial status of the system.

Table 4-19: Summary of cost benefit analysis for different AD systems

AD size	Capital costs (R)	Operating costs (R)	Cash Flow (R)	Net Present Value (R)	Benefit-Cost Ratio	Payback period (years)
1 L	3 250	2563	-2432	-3124	0.05	24.8
5 L	17 460	6407	-5608	-18757	0.12	21.9
10 L	36 874	10788	-9331	-39033	0.13	25.3
50 L	109 048	14238	-8507	-121016	0.4	19.03

The payback period and break-even analysis for the four AD systems was conducted to determine how long the project takes to generate cash flow and pay back on the initial investment. In Figure 4-39 for the 10 L system, the cumulative cash flow equates the annual operating costs after 10 years, indicating that the system will only start generating revenue from the 11th year of the project lifespan. However, the project will take 25 years to pay back the initial investment and can only make profit for only one year before the end of the project lifespan. This is due to low levels of biogas produced based on the AD size, and high operating costs. In Figure 4-40, the 50 L AD system starts generating cash flow after 1.5 years, indicating that the amount of biogas generates revenue that will surpass operating costs at a faster rate. The project for the 50 L AD takes only 19 years to pay back the initial investment based on cumulative cash flow and therefore can generate profit for the remaining 11 years of the project lifetime. This analysis suggests that the upscaling of a biogas production unit helps with generating more revenue and reducing payback period on the initial investment if the operating cost are kept constant or reducing over time due to improvements and stability in the process. The break-even analysis for the 1 L and 5 L systems is presented in Appendix A as Figure I-7 and Figure I-8.

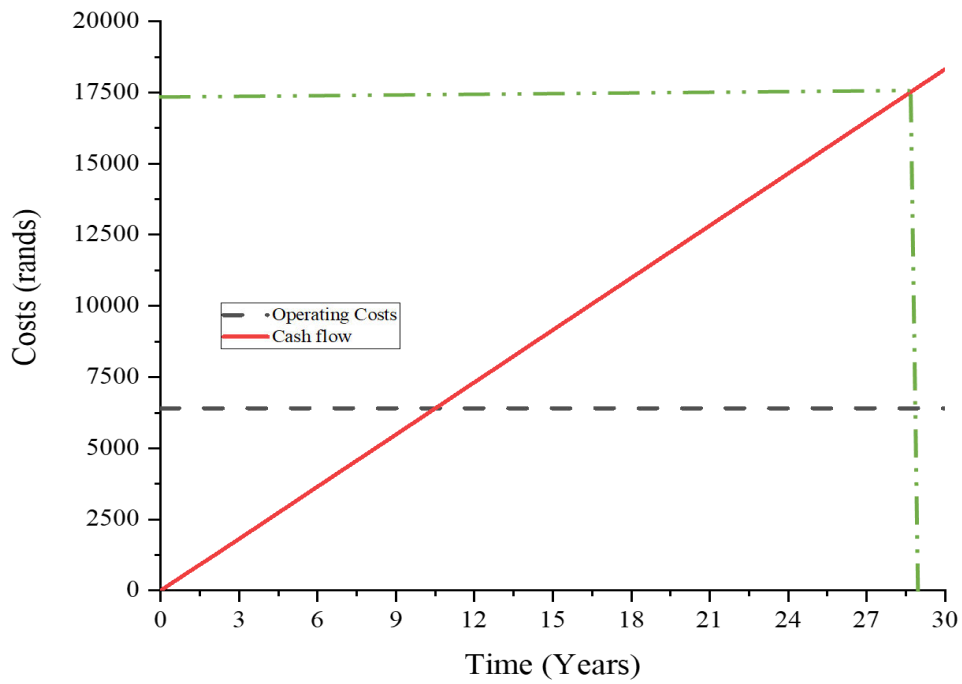


Figure 4-39: Break-even analysis for the 10 L AD system

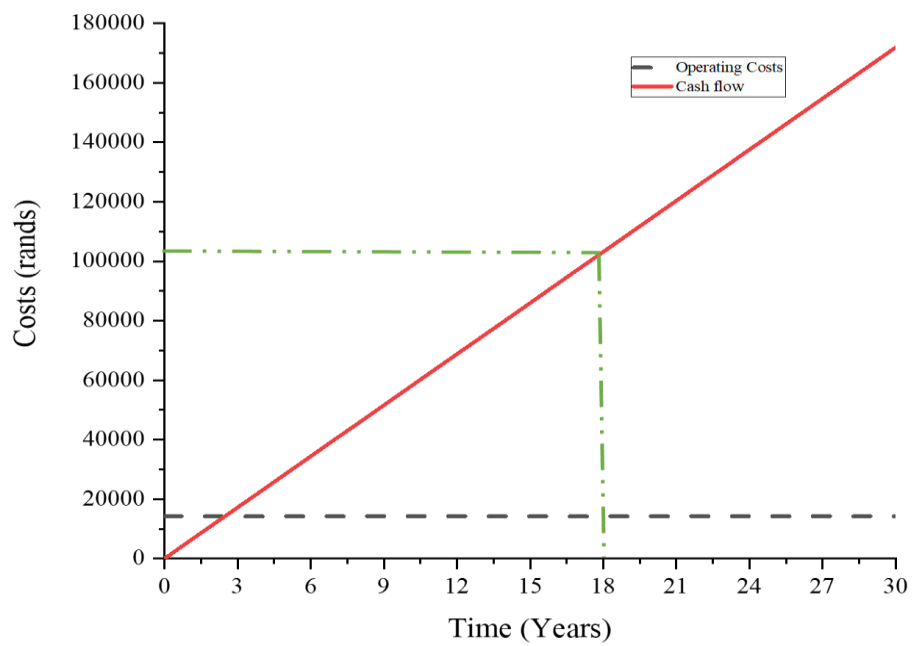


Figure 4-40: Break-even analysis for the 50 L AD system

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

This chapter presents and declares findings from this research study. It also highlights the recommendations that are made for future work in order to improve understanding and operation of anaerobic digestion systems for bioenergy recovery from wastewater. In this study, an upscaling of an anaerobic digester was investigated for biogas recovery from industrial wastewater collected from a local sugar refinery and municipal wastewater treatment plant in Durban, Kwa-Zulu Natal. The specific objectives of this study were as follows.

1. To conduct a feasibility study of producing biogas from four different wastewater streams with high organic content using a biochemical methane potential test.
2. To evaluate biogas production from a characterized wastewater stream with high organic content using 1 L and 10 L lab scale AD systems
3. To design the upscaled 50 L AD and optimize operating conditions (operating temperature, hydraulic retention time, pH and catalyst loading rate) using response surface methodology
4. To conduct a cost-benefit analysis of the optimized AD system with biogas production and its applicability as an alternative energy source

5.1 Conclusions

The characterization of various wastewater streams in this study revealed that the sugar refinery stream had the highest organic content (18 770 mgCOD/L), was rich in nutrients (512 mgNO₃/L, 52 mgPO₄/L) and had high concentration of volatile suspended solids (0.026 mgVSS/L), making it the most suitable stream for biogas production via anaerobic digestion. Additional biochemical methane potential (BMP) tests confirmed that the sugar refinery stream with a maximum average biogas production of 148 mL/day, was best suited for anaerobic treatment. The sugar refinery wastewater also consists of sugars and other biodegradable organics which are easily converted by anaerobic bacteria to methane-rich biogas.

The effect of individual parameters using the 10 L UASB reactor revealed that maximum degradation of organics and biogas production is achievable between 18 – 21 days due to sufficient settleability of solids and increased contact between organic contents and microorganisms promoted. The addition of magnetite with a concentration of 0.4 – 0.5 mg/L reduced the lag phase by stimulating early bacterial activity and increased methane content in biogas by 40%. The iron ions in magnetite plays a significant role in the activity and reproduction of microorganisms. The increase in organic content from 4320 to 18770 mg/LCOD increased biogas production from 210 mL/gTDS to 647 mL/gTDS.

In addition, an increase in methane content from 64% to 71% was achieved. However, a significant decline to 60% in the removal of water contaminants was achieved, indicating the need for a post-treatment step for the production of cleaner and reusable water.

The interactive effect of key parameters (HRT, pH and catalyst load) was studied and analysed using ANOVA. The p-values for all response models (biogas, COD and colour removals) as a function of the input parameters were below 0.05, indicating statistical significance. The difference between the predicted and adjusted R^2 for the three responses (biogas, COD and colour removal) were below 0.2, suggesting good agreement between the models and the experimental data. Response surface methodology (RSM) was applied to optimize HRT, pH and magnetite load for biogas production using the 1 L AD system. The optimal conditions were found to be HRT of 21 days, pH of 7.04 and magnetite load of 0.42 g/L. The interactive effect between pH and catalyst load were found to be the most influential factors and by their control created a favourable environment for microorganisms and enhanced methane production. These optimised conditions were found to be feasible for practical application at the pilot scale for industrial wastewater treatment.

Subsequent evaluation of biogas production volumes from 1 L, 5 L, 10 L and 50 L capacity systems were 108.2 mL, 526.34 mL, 986 mL, and 4930 mL, respectively at optimum conditions. In addition, the biogas quality in the 50 L pilot system was 90% methane, demonstrating the importance of closely monitoring and controlling pH, temperature and catalyst load for maximising the methane content. A cost-benefit analysis was conducted to assess the economic feasibility of up-scaling the AD system for biogas production. Scaling the AD system from 1 L to 50 L increased the annual revenue generation from R126 to R5732 while reducing the payback period of initial investment from 28.6 years to 19.03 years. Although the net present value (NPV) for all four systems remained negative, this indicated the system was not yet economically viable. However, the increase in the benefit-cost ratio from 0.05 (1 L) to 0.4 (50 L) indicated an improvement in the economic viability of the system. Apart from economic benefits, this technology also provides additional advantages such as reduced wastewater treatment costs, and low carbon footprints (methane-rich biogas). It also adds value with the reduction of carbon footprint and the use of sustainable bio-fertilizers, thus preventing soil pollution.

In conclusion, upscaling of an anaerobic digester system for the treatment of sugar refinery wastewater and high-quality biogas production was found economically viable. Optimised operating conditions improved biogas production and were applicable at the up-scale level, suggesting potential

for industrial-scale applications. Further up-scaling may be required to generate more revenue and reduce operating costs, thus improving the economic viability of the system.

5.2 Recommendations and future works

Based on the findings of this study, the following recommendations were made for future improvements on the bioenergy recovery from industrial wastewater.

- ✓ The results from the up-scaling work were promising, however, further up-scaling and optimisation to an industrial 500 L AD can be recommended with a pre-treatment stage of the effluent to ensure efficient conversion of organic content into biogas and minimum operating cost.
- ✓ Quantification of revenue that can be generated from the conversion of nutrient-rich digestate to bio-fertilizers is also recommended to improve the financial viability of this system,
- ✓ A study on the microbial community is necessary for better understanding of their behaviour, activity and reproduction
- ✓ A detailed techno-economic analysis and life cycle assessment (LCA) for better understanding of the economic and environmental aspects of this technology

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APPENDIX A

Statistical analysis post anaerobic digestion in the 1 L system

- 1) This section provides a summary of the removal efficiencies for volatile solids, COD, and colour for the 15 BBD experimental matrix. The aim is to compare performance of different inputs (operating conditions) in biodegradation of volatile solids and removal of wastewater contaminants.

Table I-1: Summary of removal efficiencies for the 15 BBD experimental work

Inputs			Removal efficiencies		
HRT (day)	MNP dose (g/L)	pH	%VSS reduction	COD removal	Colour removal
22,5	0,4	7	93,05	89,78	98,29
30	0,7	7	81,39	78,84	96,76
22,5	0,7	5	78,3	68,62	95,45
22,5	0,1	9	74,77	74,29	97,26
30	0,1	7	76,98	82,56	96,46
30	0,4	5	78,14	63,69	96,93
15	0,7	7	83,26	84,25	93
22,5	0,4	7	87,58	89,51	98,22
15	0,4	9	77,67	70,3	95,4
30	0,4	9	82,09	65,24	98,93
22,5	0,1	5	75,81	64,08	96,02
22,5	0,7	9	75,44	65,31	95,45
15	0,4	5	79,88	65,27	95,81
15	0,1	7	89,53	85,43	95,58
22,5	0,4	7	98,14	90,11	98,13

Correlation graphs for magnetite load:

This section presents graphical analysis for the correlation plots between the input (magnetite load) and the outputs (biogas yield, COD removal, and colour removals). This correlation is represented as a slope of a straight line indicating the direction and strength of the relationship between pH and the outputs.

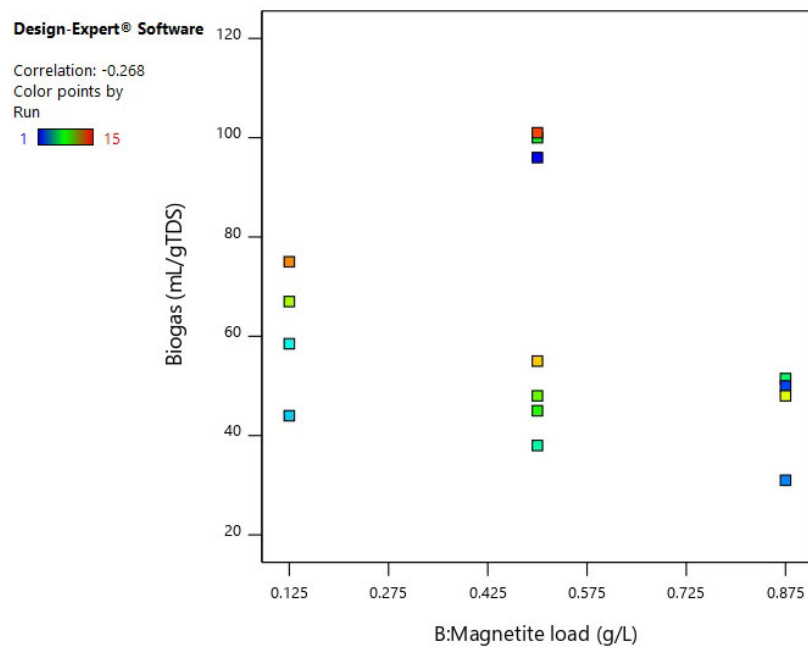


Figure I-1: Correlation graph showing the influence of magnetite load in biogas production

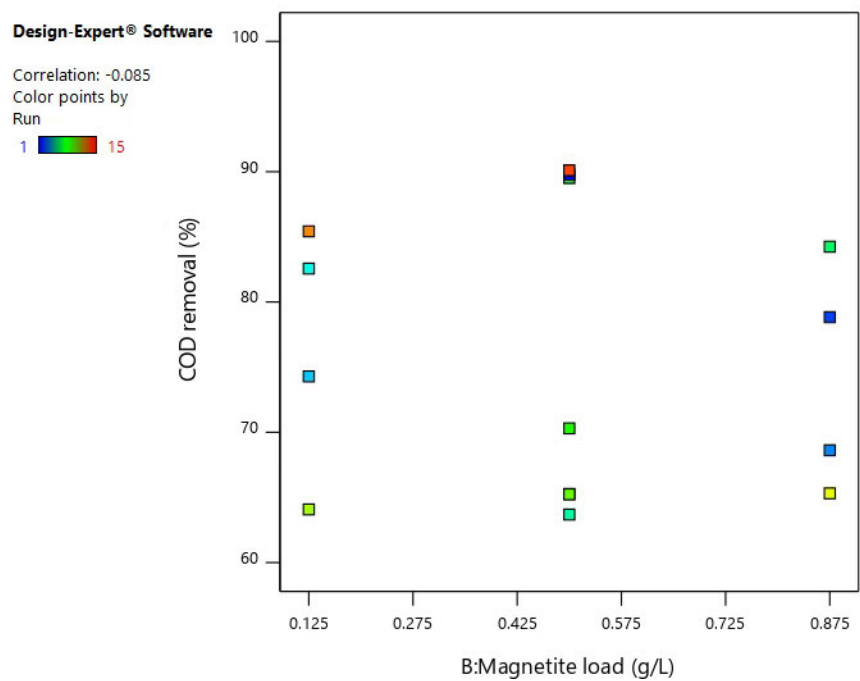


Figure I-2: Correlation graph showing the influence of magnetite load in COD removal

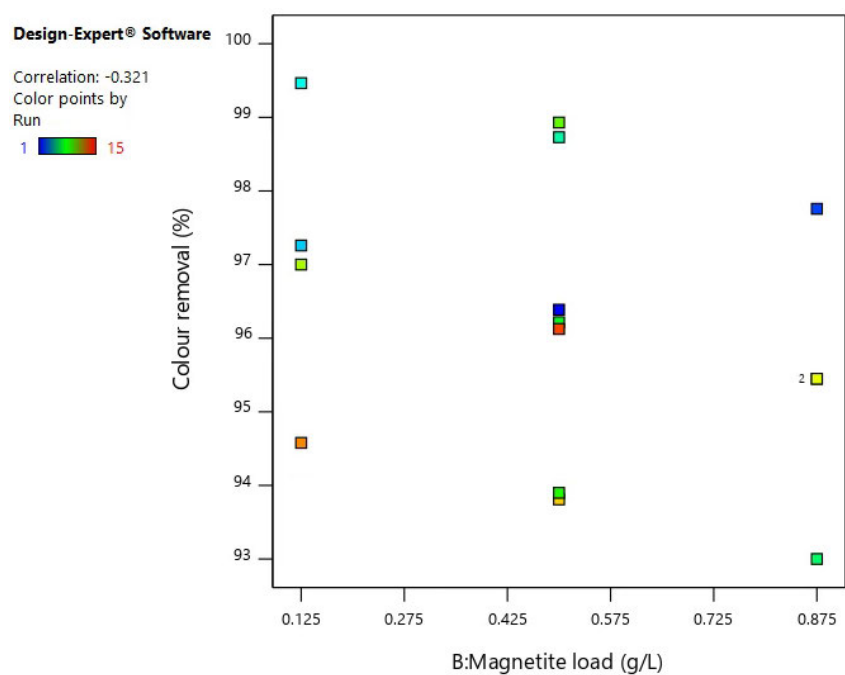


Figure I-3: Correlation graph showing the influence of magnetite load in colour removal

Correlation graphs for pH

This section presents graphical analysis for the correlation plots between the input (pH) and the outputs variables (biogas yield, COD removal, and colour removal).

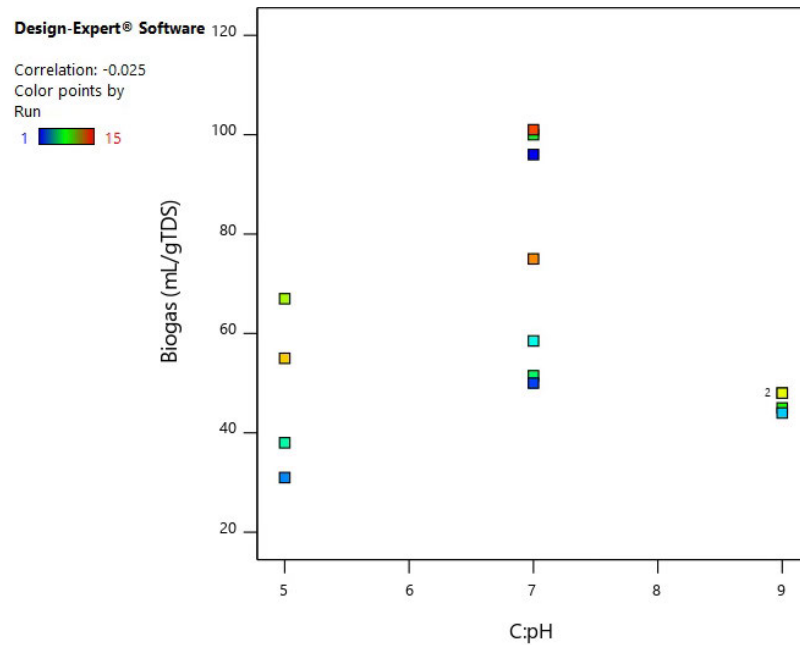


Figure I-4: Correlation graph showing the influence of pH in biogas production

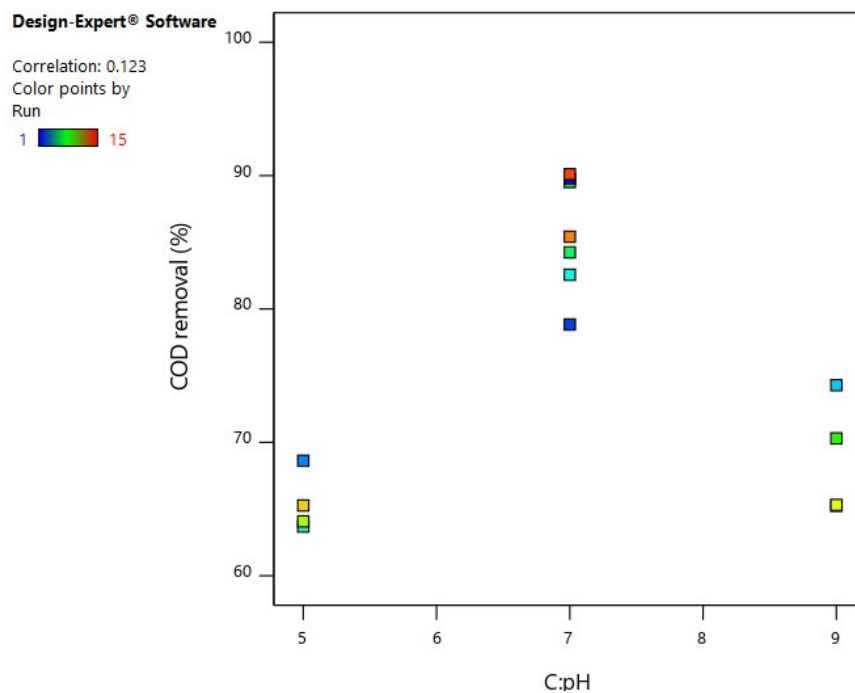


Figure I-5: Correlation graph showing the influence of pH in COD removal

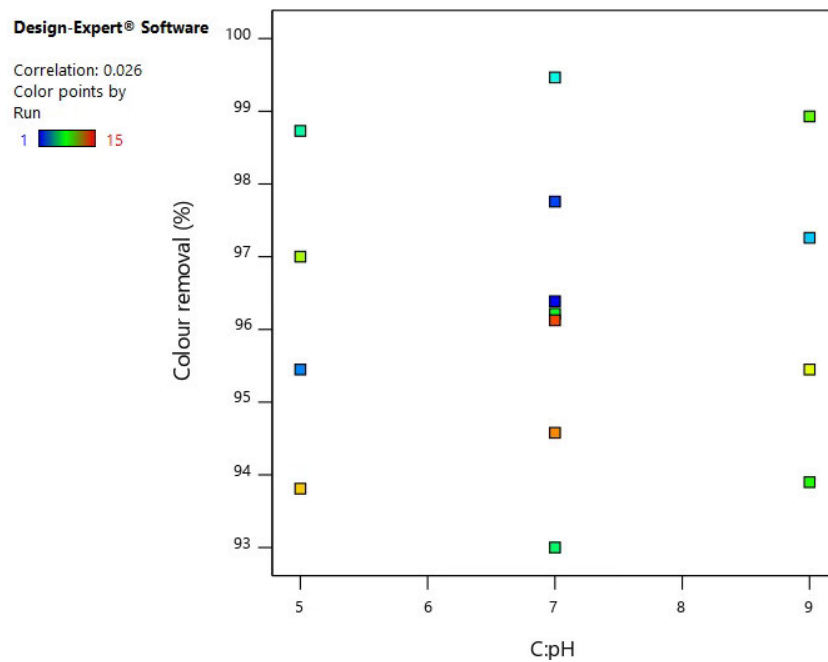


Figure I-6: Correlation graph showing the influence of pH in colour removal

Break-even analysis for the small AD systems

This section presents the break-even analysis for the 1 L and 5 L systems. It is aimed at assessing the economic benefits of the two systems, with graphs showing the period each system takes to start generating revenue and payback to the initial investment.

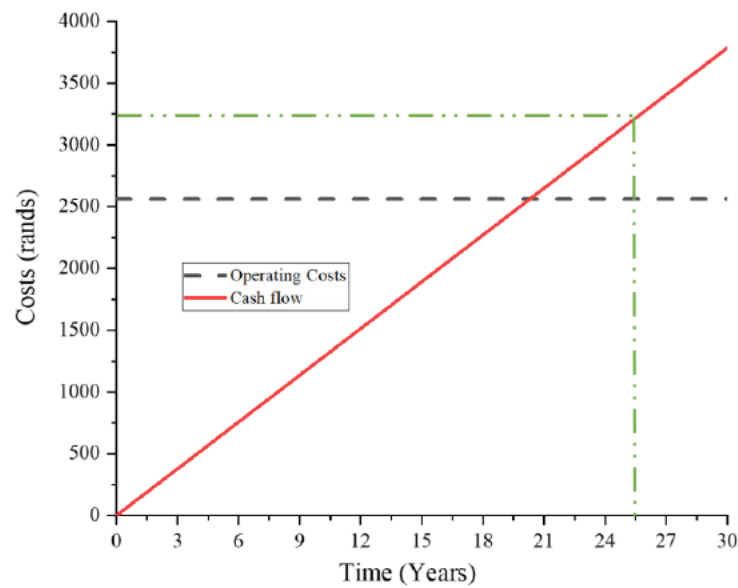


Figure I-7: Break-even analysis for the 1 L AD system

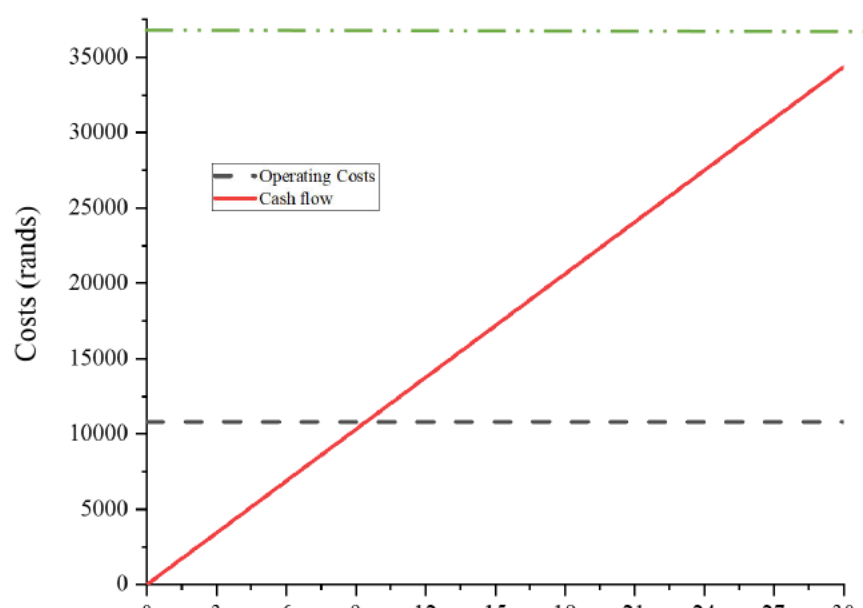


Figure I-8: Break-even analysis for the 5 L system