OPTIMISATION OF BIOGAS PRODUCTION USING SOME PROCESS PARAMETERS IN A FIXED DOME LABORATORY BIOREACTOR

BARASA HENRY MASINDE

A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Doctor of Philosophy Degree in Agricultural Engineering of Egerton University

EGERTON UNIVERSITY

JANUARY 2021

DECLARATION AND RECOMMENDATION

Declaration

I declare that this Thesis is my original work and has not been presented for the award of a degree in any University.

Signature  Date 07.01.2021

Barasa Henry Masinde

BD11/0401/13

Recommendation

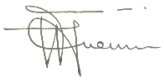
This thesis has been submitted with our approval as University supervisors.

Signature  Date 09.01.2021

Prof. Daudi M. Nyaanga, PhD.

Department of Agricultural Engineering, Egerton University

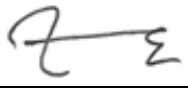
Egerton, Kenya.

Signature  Date 09.01.2021

Dr. Musa R. Njue, PhD.

Department of Agricultural Engineering, Egerton University

Egerton, Kenya.

Signature  Date 09.01.2021

Prof. Joseph W. Matofari, PhD.

Department of Dairy and Food Science and Technology, Egerton University

Egerton, Kenya.

DEDICATION

This work is dedicated to my wife Christine, and my children Andrew, Eric, Margaret, Richard, Moses, and Florence.

ACKNOWLEDGEMENTS

I give thanks to the Almighty God for giving me the life, strength, determination, and vision to undertake and complete this work. I am very grateful to my supervisors Prof. Daudi M. Nyaanga, Dr. Musa R. Njue, and Prof. Joseph W. Matofari for the constant guidance and encouragement throughout this work.

It would not have been possible to perform this work without the funding from the National Commission for Science, Technology and Innovation (NACOSTI) for fabricating a laboratory bioreactor in which the experiments were done, and consequently the documentation of this work. Many thanks to Egerton University for providing a secure and a conducive environment for my studies; Graduate School under the leadership of Prof. Nzula Kitaka, Dean of Faculty of Engineering and Technology Prof. Japheth Onyando, Chairman of Agricultural Engineering (AGEN) Department Dr. Raphael Wambua, the Chairman of Electrical Control and Instrumentation Engineering (ECIEN) Department Dr. Franklin Manene, Dr. Peter Kundu, and Dr. Booker Osodo. I also appreciate the support from the following staff: Mrs. Bernadette Misiko (Dairy, Food Science and Technology Department), Meshack Korir, Peter Mecha, Patrick Wamalwa, A. Muyera, W. Tallam, and Napoo (all from AGEN).

My thanks also go to the Vice Chancellor of Masinde Muliro University of Science and Technology (MMUST) for granting me study leave to enable me complete this research work. This was possible through the efforts made by Mrs. Brenda Simiyu of Mechanical and Industrial Engineering (MIE) Department, and the Dean of the School of Engineering and Built Environment (SOBE) Dr. Bernadette Sabuni. Encouragement by the late Prof. C K Ndiema and Dr. Joel Chirchir (of MIE) is highly appreciated. A special mention goes to Dr. Peter Cherop (MIE) for reading my work thereby assisting me to make appropriate improvements.

Lastly thanks go to my family for allowing me to use their resources, and to my friends for encouraging me to finish this work.

ABSTRACT

Biogas is a renewable energy that has many applications including cooking, lighting households among others. It is produced through the breakdown of organic matter in an air tight compartment through a biochemical process which is generally termed digestion. This technology involves various techniques through use of digesters or bioreactors and operational parameters which could be predicted and must be optimised. A 0.15m^3 capacity fixed dome laboratory bioreactor was used to determine the effect of total solids, temperature, and substrate retention time on biogas production rate. The feedstock was cow dung from dairy cows managed under a free-range system during the day but held under a shed overnight at Egerton University, Kenya. Three different experiments were conducted in a batch feeding regime of the bioreactor. In the first one, the substrate at total solids of 6%, 7%, 8%, 9%, and 10% was digested at a constant temperature of 35^{0}C (using auto control system). The second experiment was conducted at mesophilic temperatures of 25^{0}C, 30^{0}C, 35^{0}C, 40^{0}C, and 45^{0}C using a cow dung substrate at total 8% solids. An evaluation of existing biogas production prediction models was done. A third model (named the fixed dome temperature model) was developed and tested. Biogas production rate was optimised with the help of response surface methodology – in which a central composite design was applied. An interaction of three variables namely total solids, temperature, and substrate retention time were tested at five different levels. The highest average biogas production rate was 0.48 $m^3$of biogas per m^3 of digester volume per day (m^3/m^3d) at 8% total solids. The highest average result of 0.52 m^3/m^3d occurred at 40^{0}C. Lastly substrate retention time was observed while the cow dung was at 8% total solids and 35^{0}C; and the highest average output was 0.68 m^3/m^3d at 11 days. Low Temperature Lagoon model and Toprak model suited the results obtained in this research. The optimum output of 0.50 m^3/m^3d was achieved at a level of 8% total solids, 43.41^{0}C, and 15 days. The optimal values were verified and found to be in agreement with experimental results at an admissible tolerance of 6.6-10.7%. The above conclusions can be transferred for adoption for field and industrial fixed dome digesters for biogas production into operational guidelines for biogas stakeholders including designers and operators.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

Symbol	Meaning
Al	Aluminium
Ca	Calcium
Cd	Cadmium
CH_2COOH	Acetic acid
CH_3CH_2COOH	Propionic acid
$CH_3CH_2CH_2COOH$	Butyric acid
CH_4	Methane
cm	Centimetre
cm^2	Square centimetre
cm^3	Cubic centimetre
CO_2	Carbon dioxide
Co	Cobalt
Cu	Copper
d	Day
g	Gram
H_2	Hydrogen
H_2O	Water
H_2S	Hydrogen sulphide
Hg	Mercury
kg	Kilogram
km	Kilometre
km^2	Square kilometer
l	Litre
m	Metre
m^2	Square metre

m^3	Cubic metre
Mg	Magnesium
ml	Millilitre
Na	Sodium
^0C	Degree Celsius
Zn	Zinc

LIST OF ACRONYMS/ABBREVIATIONS

Abbreviation	Meaning
a	Mass of dry can
AD	Anaerobic Digestion
ANN	Artificial Neural Networks
ASBR	Anaerobic Sequencing Batch Reactors
b	Mass of dry can and fresh dung
BBD	Box-Behnken Design
c	Mass of dry can and oven dried dung
CCD	Central Composite Design
CI	Computational Intelligence
CSTR	Continuous Stirred Tank Reactors
DACE	Design and Analysis of Computer Experiments
DOE	Design of Experiment
EFB	Empty Fruit Bunch
EPA	Environmental Protection Agency
FID	Flame Ionisation Detector
GAs	Genetic Algorithms
GC	Gas Chromatograph
GHG	Green House Gases
HRT	Hydraulic retention time
IPCC	Inter-governmental Panel for Climate Change
LCFA	Long Chain Fatty Acids
NPRSM	Non-Parametric and Semi-Parametric Response Surface Methodology
PIDs	Proportional Integral Differentials
PLCs	Program Logic Controls
POME	Palm Oil Mill Effluent
PSO	Particle Swarm Optimisation
RSM	Response Surface Methodology
SRT	Substrate retention Time
TS	Total Solids

UASB	Up-Flow Anaerobic Sludge Blanket Reactors
VFA	Volatile Fatty Acids
VS	Volatile Solids

CHAPTER ONE
GENERAL INTRODUCTION

1.1 Background

Biogas is a renewable and an environmentally friendly form of energy which can substitute wood and fossil fuels in a number of applications and thus mitigate the rising costs of petroleum products and deforestation (Deublein and Steinhauser, 2008). Biogas is a combination of gases produced during anaerobic decomposition of organic materials of plant origin. It is produced from the organic wastes by a concerted action of various groups of anaerobic bacteria (Boe, 2006). The main gaseous by-product is methane, with relatively less carbon dioxide, ammonia and hydrogen sulphide (Saleh *et al.*, 2012). Methane is the principal constituent of natural gas and ranks first in the series of saturated hydrocarbons known as alkanes (Khanal, 2008; Monnet, 2003). It is a light, colourless, odourless and highly inflammable gas, second only to hydrogen in the energy released per gram of fuel burnt, hence its potential as a household energy source (Nijaguna, 2006).

Biogas production was introduced in developing countries as a low-cost alternative source of energy to alleviate acute energy shortage for households (Parawira and Mshandete, 2009). However, few households currently use biogas. The poor adoption of this technology is associated with the high cost of the digesters, lack of knowledge in installing and maintaining them and frequent microbial failures (Ho *et al.*, 2015). In Kenya, implementation of foreign biogas systems has not only led to lower performance but also hampered local innovativeness and scientific advancement in the field of renewable energy based on the local resources (Nzila *et al.*, 2012). Biogas technology provides an alternate source of energy in rural areas, and is an appropriate technology that meets the basic need for cooking and lighting. The biogas technology is adaptive and cheap because the gas burns clean, has high calorific value and can be virtually produced anywhere using any locally available biodegradable material like cattle waste, farm crop residues and other organic wastes (Bond and Templeton, 2011; Nzila *et al.*, 2012). A small household can use biogas for lighting and cooking without causing air pollution, while large industrial systems can utilize it to generate electricity to run their establishment and even sell the surplus to the National Grid (Rajendran *et al.*, 2012). The use of biogas offers a great opportunity towards the reduction in global warming gases and climate change (Lippmann *et al.*, 2003). Cooking with biogas can save women time spent in harvesting wood and instead engage in other economic empowering activities, reduces smoke which is a major cause of lung diseases and poor eye sight for rural women

and children who cook with firewood in poorly lit and ventilated spaces (Nzila *et al.*, 2015). Other applications include gas-powered refrigerators and chicken incubators than run on biogas in Kenya (Laichena and Wafula, 1997; Sibisi and Green, 2005). In India and Nepal, biogas is connected to toilets for lighting (Batzias *et al.*, 2005).

Manure that is left to decompose releases two main gases that cause global climate change: nitrous dioxide warms the atmosphere three hundred and ten (310) times more than carbon dioxide (CO_2), and methane (CH_4) warms the atmosphere twenty one (21) times more than CO_2; therefore by converting cow manure into biogas instead of letting it decompose, it would be able to reduce global warming gases by 99 million metric tons or 4% (EPA, 2005; Saleh *et al.*, 2012). Cattle dung is a complex and naturally occurring polymeric substrate which consists of soluble and insoluble matter which can be used as a source of renewable energy (Garcia-Ochoa *et al.*, 1999). Improper management of this waste leads to many environmental hazards including water pollution and greenhouse gases (GHS) emissions (Kobayashi and Li, 2011). United States of America exclusively produces approximately two hundred and thirty (230) million tonnes of dry matter of animal waste that cannot be applied as local fertilizer (Karim *et al.*, 2007), United Kingdom produces eighty eight (88) million tonnes annually (Phillips *et al.*, 2008), while China produces 1.07 billion tonnes of livestock waste yearly (Chen *et al.*, 2009). Anaerobic digestion (AD) has been found to be an efficient, cheap and easy method to manage livestock waste (Nasir *et al.*, 2014).

A bioreactor (also termed as a digester) is any manufactured device or installed or constructed structure with associated facilities (henceforth termed system) in which a biologically active biological cum chemical process (digestion and sometimes fermentation) is carried out which involves microorganisms or biochemically active substances derived from such organisms is supported (IUPAC, 2006). Bioreactors are commonly cylindrical, with a capacity ranging from 10 millilitre (ml) bottles to several cubic metres (m^3), and are often made of stainless steel and are mainly small and precise for experimental laboratory studies while digesters are larger field or industrial biomass digestion systems used to produce biogas for use in the household, farm or industry (Arthur *et al.*, 2011).

There are many designs of biogas plants but the most common ones in Kenya include the lagoon, floating drum, fixed dome and flexible structure bio-digester models (Schön, 2010). These designs have evolved over the years since the first digester was installed in Kenya around the year 1959 (Nyaanga *et al.*, 2015). The first field biogas systems were based on the

floating drum originating from India, and fixed Chinese systems which have since been modified and advanced to meet the local conditions (Rupfa *et al.*, 2017) by local engineers, technologists, technicians and masons and accompanied by the introduction of portable flexible bag digesters, being installed underground or above ground (Nyaanga *et al.*, 2015). Rupfa *et al.* (2017) suggests that the most suitable biodigester design for different applications in Kenya should be based on user defined inputs, including energy and fertiliser requirements; feedstock (type, amount, and rate of supply); water supply; land use (area, soil type, ground water level); climate (temperature and rainfall); construction materials available locally; and the priorities (based on sustainability criteria) of the intended biogas user. There are many factors and hence there is need for long term and multi-agency corroboration. The environmental, management, and civil designs including sizing of the digesters with the idea of optimising the biogas production can be studied and decided on using appropriately designed laboratory bioreactors that replicate the field systems (Nyaanga *et al.*, 2015).

A fixed dome reactor has been used in this study. A fixed dome is a bioreactor which consists of a digester with fixed, non-movable gas holder, which sits on the digester – it is the most commonly used digester in China (Santerre and Smith, 1982) and also in Kenya. In terms of absolute numbers, the fixed dome is by far the most common digester type in developing countries (Gunaseelan, 1997). The Chinese fixed dome is the most popular and used most in developing countries because of its reliability, low maintenance costs, a long lifespan, and relatively minimal loss of the biogas yield (Ghimire, 2013; Huba *et al.*, 2013).

A parameter is any of the factors that limit the way in which something can be done. In this study, the parameters that have been considered are total solids, temperature, and substrate retention time; and their effect on biogas production rate in a fixed dome bioreactor under mesophilic laboratory conditions. Bioreactors operate at different environmental and management conditions. There are three possible ranges of temperature in which the anaerobic digestion (AD) process can be carried out. According to Comino *et al.* (2010), psychrophilic temperature ranges from 15^0C to 25^0C, mesophilic temperature ranges from 30^0C to 40^0C, and thermophilic temperature ranges from 50^0C to 60^0C. Temperature and substrate concentration may be the most important parameters determining the performance and stability of the AD process (Chae *et al.*, 2008). Together, they influence the microbial community structure, the biochemical conversion pathways, the kinetics and thermodynamic balance of the biochemical reactions, and the stoichiometry of the products formed (Arikan *et*

al., 2015). Because the formation and consumption of products can occur at different rates, transient accumulation of potentially inhibitory substances is possible, particularly with complex substrates (Labatut *et al.*, 2014). Consequently, temperature is a critical factor affecting anaerobic digestion because it influences both system heating requirements and methane production (Ramaraj and Unpaprom, 2016). Other factors that affect the efficient production of biogas include lack of feedstock, appropriate design of digesters, development of inoculums, pH, organic loading rate, hydraulic retention time (HRT), Carbon to Nitrogen (C:N) ratio, and volatile fatty acids (Nzila *et al.*, 2010). Also defects in digester construction and microbiological failure are the major areas of concern and are crucial for the optimisation of biogas production technologies and their economic viability (Nijaguna, 2006).

The hydraulic retention time (HRT) refers to the duration that the substrate or the organic matter compounds take to be digested or get bio-chemically decomposed in the absence of oxygen, as they pass or traverse and move through the digester from the inlet (as influent) to the exit (as effluent) (Nyaanga *et al.*, 2015). Complex organic compounds require longer retention times than simple compounds since the former are harder to breakdown (Singh *et al.*, 2017).

The retention time of the solids, which can also be termed as substrate retention time (SRT), has been associated with the ability of a biological system (including fermentation and digestion of organic matter) to reduce complex harmful compounds to safe levels and hence meet the effluent standards or the allowed pollutants' biodegradability levels for complete the production of biogas (Nyaanga *et al.*, 2015; Singh *et al.*, 2017). Substrate retention time in biogas production systems depends on the amount of substrate and nutrients available for methanogenic bacteria to consume and complete to generate methane (Masinde *et al.*, 2020). Using this understanding, substrate retention time, can be defined as the time taken from loading the digester or bioreactor with the influent and inoculum, to the time the substrate stops yielding biogas. The SRT will be influenced by the given biogas system (size or volume), type of substrate, prevailing operational conditions such as temperature and agitation. In most cases, lay biogas stakeholders use the terms HRT and SRT interchangeably despite the difference and similarity. Both SRT and HRT are used in the design of bio-chemical reactors including biogas production systems which use bacteria and enzymes. Stirring or agitation and feeding regime (frequency and amount fed into a bioreactor or a digester) may lead to longer or shorter HRT or SRT or wash out (where excess microbial

mass is moved out of digester or digesters), respectively. Hence HRT and SRT depend on digester volume or size, prevailing conditions, material type especially with respect to digestibility in addition to factors such as pH, carbon to nitrogen ratio, microbial growth inhibitors, among others. The anaerobic digesters, which are capable of owning prolonged solid or substrate retention times (SRT) because of immobile or congested bacterial biomass, operate rapidly with smaller hydraulic retention time (HRT) and decreased expenses (Singh *et al.*, 2017).

The biogas quantity and quality are greatly influenced by the range of temperature in the process of anaerobic digestion. A sudden drop or increase of temperature causes temperature shocks to the bacteria which might inhibit their performance or cause their death (Patharwat *et al.*, 2016). The same can happen in the event of insufficient or excess supply of their specific food or nutrients. Singh *et al.* (2017) reported that naturally, the microorganisms (specifically, the methanogenic kind of bacteria) that take part in anaerobic digestion are largely categorized into three types as mesophiles, thermophiles and cryophiles or psychrophiles. The elevated temperature of the thermophilic regime induces more biochemical processes, causing massive production of methane (Leenawat *et al.*, 2016). Thermophilic regime consumes a large quantity of energy, and this is counterbalanced by the huge biogas production.

A number of semi theoretical and empirical models have been proposed and used in the mathematical estimation of the amount of biogas produced from a given biogas setup and prevailing conditions. They include Plug Flow Digester, Lagoon Low Temperature, Toprak, Chen and Hashimoto, and Scoff and Minott which involve hydraulic retention time, volatile solids concentrations, bacteria growth rate, digester temperature, and daily substrate flow rate. These are described and tested in later chapters in this thesis. A few other mathematical models have been proposed. Delgadillo *et al.* (2018) proposed the model for the simulation of biogas production using model parameters obtained by performing a sensitivity analysis, using a sequential quadratic programming algorithm. They calibrated and validated the model using experimental data obtained from a pilot-scaled plant and concluded that the model was able to correctly predict the methane production dynamics from few key measurements. Korolev and Maykov (2019) optimized a two-stage methanogenesis regime based on the theory of the Pontryagin's maximum principle and concluded that optimal control of the biogas process can be estimated using a controlled algorithm.

Optimisation is the act of achieving the best possible result under given circumstances. The aim is either to minimise the effort or to maximise benefit. The effort or benefit can be expressed as a function of certain design variables. Hence optimisation is the process of finding conditions that give the minimum or the maximum value of a function (Astolfi and Praly, 2006). In this study, biogas production was maximised while the parameters were kept in range. Some optimisation techniques associated with anaerobic digestion including basic designs of single-stage or two-stage systems, environmental conditions within the reactors such as temperature, pH and buffering capacity have been applied in Nigeria, Tanzania and Zimbabwe among others in sub-Saharan Africa (Parawira and Mshandete, 2009). Response surface methodology (RSM) is one of the most effective approaches for designing experiments, for building models, and for determining optimal conditions on responses which are influenced by several independent variables (Kang *et al.*, 2016). Apart from defining the influences of independent variables on the responses, RSM also determines the effect of interaction between parameters to obtain the best performance on a system (Belwal *et al.*, 2016). Other optimisation techniques include Artificial Neural Networks (ANN) (Ghatak and Ghatak, 2018), Genetic Algorithm (GA), and Taguchi.

1.2 Statement of the Problem

Biogas production is influenced by a number of process parameters including substrate retention time, total solids, and temperature for different digester designs and management regimes (including batch, semi continuous and continuous feeding of the organic matter) in the field, and industrial, household and experimental or laboratory digesters or bioreactors. The common biogas systems in Kenya including the fixed dome digesters are likely to perform differently as per the manure characteristics and the management of some of the operational parameters including dilution levels, retention time and digester temperature. The dilution of feedstock to water used in Kenya is 1:1, leading to a variation of the total solids in the influent due to the inherent amount of water in the feedstock including cow dung or manure.

The effect of varying substrate retention time, total solids, and temperature on biogas production in a batch bioreactor is not clearly articulated. The applicability of some of the simpler empirical existing biogas production prediction models have not been tested on this type fixed dome laboratory bioreactor for adoption. Optimisation using substrate retention time, total solids, and temperature to maximise biogas production in a fixed dome bioreactor

using response surface method on the biogas production in a fixed dome lab bioreactor has not been done. Therefore, there was need to carry out this study in order to fill these gaps in the advancement of biogas technology in Kenya and other parts of the world.

1.3 Objectives

1.3.1 Broad Objective

The broad objective of this research was to optimise biogas production using some process parameters in a fixed dome laboratory bioreactor.

1.3.2 Specific Objectives

The specific objectives were to:

i. Determine the effect of different total solids, temperature and substrate retention time on biogas production for the fixed dome laboratory bioreactor.

ii. Evaluate existing biogas production prediction models that relate to total solids, temperature and substrate retention time.

iii. Optimise biogas production based on total solids, temperature and substrate retention time for the fixed dome laboratory bioreactor.

1.4 Research Questions

a) How is biogas production affected by total solids, temperature and substrate retention time?

b) How do the existing biogas production prediction models that relate total solids, temperature, and substrate retention time to biogas production with respect to the data collected?

c) Has optimisation using total solids, mesophilic temperature, and substrate retention time been employed to maximise biogas production for the fixed dome laboratory bioreactor?

1.5 Justification

The need to relate the field medium and small scale fixed dome digesters common in most institutions such as universities, slaughter houses, large scale farms and small scale households, has been identified that led to the development of a laboratory bioreactor representing the fixed dome digesters by Nyaanga *et al.* (2015), however, the functioning of

the bioreactor has not been done. It is on this basis that this research's objectives were formulated.

The effect of varying the levels of total solids, temperature, and substrate retention time on biogas production gives the optimal point at which each factor gives the highest amount of biogas. This enables the application of the appropriate levels of each parameter by the biogas producer. The relationship from such an evaluation can be adapted by biogas stakeholders and producers to improve the technology and enhance its adoption.

Evaluation of models of biogas production is important because it helps to understand how a system behaves when a parameter is varied. Models can be used to predict the level of input at which maximum biogas can be produced. This helps in reducing the cost of production while maximising on the output by identifying the appropriate settings of values of the concerned parameters for different sized fixed dome digesters operated at varied conditions.

Optimisation of factors that affect biogas production helps in giving the level of combining the inputs in order to achieve the best desired output. This knowledge assists in easing the production process and the associated costs. In this particular case, the optimum temperature, total solids and substrate retention time for the laboratory fixed dome bioreactor could be scaled up to the field or industrial digesters.

1.6 Scope and Limitations

1.6.1 Scope

The cattle dung herein termed as the cow manure (used as a feedstock) was sourced from semi free range cattle rearing system (where the animals are allowed to graze in the fields during the day and come to the shed for milking and overnight) of Tatton Agricultural Park (Farm), Egerton University, Kenya. Tap water at room temperature, was used to dilute the manure to the required total solids before loading into the $0.15m^3$ fixed dome batch laboratory reactor, designed and fabricated in the Agricultural Engineering workshop at Egerton University. The factors considered in the research were temperature, total solids and substrate retention time and their effects on biogas production and quality. The substrate retention time of 11 to 18 days, a mesophilic temperature range of 25^0C to 45^0C at intervals of 5^0C, and a total solids range from 6% to 10% were proposed for the research. Biogas

volume was measured by the water displacement method and validated by bag storage, while the quality of the gas was verified by a Gas Chromatograph and visual blue flame.

The flow chart (Figure 1.1) below outlines the input parameters or variables and outputs (parameters) that constitute the process conditions that were researched on in this study.

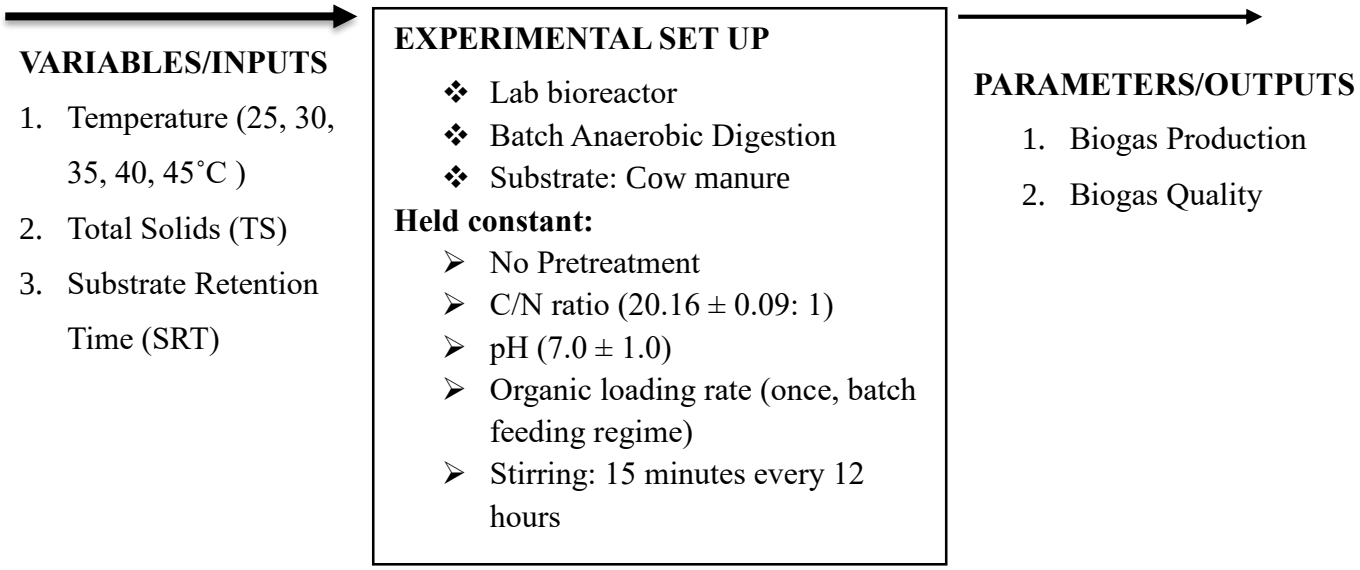


Figure 1.1: Variables, experimental unit and parameters

1.6.2 Limitations

The initial manure handling involving no pretreatment, carbon to nitrogen ratio, pH, and organic loading rates were predetermined and held constant throughout the experiments. Effluent from anaerobic digestion of cow dung at Egerton University biogas plant was used as the initial inoculum for each experiment.

Electric power outages, at times, disrupted the smooth running of the experiments. Whenever a power outage occurred, the affected experiment was restarted afresh in order to get credible results. Biogas quality analysis had a challenge because the Gas Chromatograph (GC) was old and it did not have a protocol. The protocol for methane content analysis was adopted from a Hewlett Packard 5890plus GC with a flame ionization detector (FID) and a Supelco Carboxen1006 Plot Column that is 30 m long with 0.53 mm internal diameter (Walsh and McLaughlan, 1999). The manpower to assist in running the experiment was a challenge. The visual estimation of a bright blue flame was (albeit, subjectively) used as a judgement of good quality biogas (55 to 70% methane).

References

Arikan, O., Mulbry, W. and Lansing, S. (2015). Effect of temperature on methane production from field-scale anaerobic digesters treating dairy manure. *Waste Management, 43*(2), 108-113.

Arthur, R., Baidoo, M. and Antwi, E. (2011). Biogas as a potential renewable energy source. A Ghanaian case study. *Renewable Energy, 36*(5), 1510-1516. doi:10.1016/j.renene.2010.11.012

Astolfi, A. and Praly, L. (2006). Global complete observability and output-to-state stability imply the existence of a globally convergent observer. *Mathematics of Control, Signals and Systems, 18*(1), 32-65.

Batzias, F., Sidiras, D. and Spyrou, E. (2005). Evaluating livestock manures for biogas production: A GIS method. *Renewable Energy, 30*, 1161-1176.

Belwal, T., Dhyani, P., Bhatt, I. D., Rawal, R. S. and Pande, V. (2016). Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM). *Food Chemistry, 207*, 115-124.

Boe, K. (2006). *On-line monitoring and control of the biogas process.* (PhD), Technical University of Denmark, Lyngby, Denmark.

Bond, T. and Templeton, M. (2011). History and future of domestic biogas plants in the developing world. *Energy for Sustainable Development, 15*(4), 347-354. doi:10.1016/j.esd.2011.09.003

Chae, K., Jang, A., Yim, S. and Kim, I. (2008). The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresource Technology, 99*, 1-6.

Chen, S., Li, R. and Li, X. (2009). Anaerobic co-digestion of kitchen waste and cattle manure for methane production. *Energy Sources, 31*, 1848-1856.

Comino, E., Rosso, M. and Riggio, V. (2010). Investigation of increasing organic loading rate in the co-digestion of energy crops and cow manure mix. *Bioresource Technology, 101*(9), 3013-3019.

Delgadillo, L., Machado-Higuera, M. and Hernandez, M. (2018). Mathematical modelling and simulation for biogas production from organic waste. *International Journal of Engineering Systems Modelling and Simulation, 10*(2), 97-101. doi:10.1504/IJESMS.2018.10013112.

Deublein, D. and Steinhauser, A. (2008). *Biogas from Waste and Renewable Resources: An Introduction*. Weinheim, Germany.

EPA. (2005). Anaerobic Digestion: Benefits for Waste Management, Agriculture, Energy, and the Environment. *U.S. Environment Protection Agency*.

Garcia-Ochoa, F., Santos, V., Naval, L., Guardiola, E. and Lopez, B. (1999). Kinetic model for anaerobic digestion of livestock manure. *Enzyme Microbiology and Techology, 25*, 55-60.

Ghatak, M. and Ghatak, A. (2018). Artificial neural network model to predict behavior of biogas production curve from mixed lignocellulosic co-substrates. *Fuel, 232*, 178-189.

Ghimire, P. (2013). SNV supported domestic biogas programmes in Asia and Africa. *Renewable Energy, 49*, 90-94. doi:10.1016/j.renene.2012.01.058

Gunaseelan, V. (1997). Anaerobic digestion of biomass for methane production: A review. *Biomass and Bioenergy, 13*(1-2), 83-114.

Ho, T. B., Roberts, T. K. and Lucas, S. (2015). Small-scale household biogas digesters as a viable option for energy recovery and global warming mitigation—Vietnam case study. *Journal of Agricultural Science and Technology A, 5*, 387-395.

Huba, E., Cheng, S., Li, Z. and Mang, H. (2013). A review of prefabricated biogas digesters in China. *Renewable and Sustainable Energy Review, 28*, 738-748. doi:10.1016/j.rser.2013.08.030

IUPAC. (2006). *Compendium of Chemical Terminology* (2nd ed.).

Kang, J., Kim, S. and Moon, B. (2016). Optimization by response surface methodology of Lutein recovery from paprika leaves using accelerated solvent extraction. *Food Chemistry, 205*, 140-145.

Karim, K., Klasson, K., Drescher, S., Ridenour, W., Borole, A. and Al-Dahhan, M. (2007). Mesophilic digestion kinetics of manure slurry. *Applied Biochemistry and Biotechnology, 142*, 231-242.

Khanal, S. (2008). *Anaerobic Biotechnology for Bioenergy Production: Principles and Applications* (S.K. Khanal ed.). New York, USA: John Wiley and Sons

Kobayashi, T. and Li, Y.-Y. (2011). Performance and characteriztion of a newly developed self-agitated anaerobic reactor with biological desulphurization. *Bioresource Technology, 102*(10), 5580-5588. doi:10.1016/biortech.2011.01.077

Korolev, S. and Maykov, D. (2019). Optimization of two-stage methanogenesis regime based on the Pontryagin's maximum principle (English Abstract) *https://www.researchgate.net/publication/334126988*.

Labatut, R., Angenent, L. and Scott, N. (2014). Conventional mesophilic vs. thermophilic anaerobic digestion: a trade-off between performance stability? . *Water Research*(53), 249-258.

Laichena, J. and Wafula, J. (1997). Biogas technology for rural households in Kenya. *OPEC Reviews, 21*, 223-244.

Leenawat, A., Pasakorn, J., Atip, L., Rodriguez, J. and Khanita, K. (2016). *Effect of temperature on increasing biogas production from sugar industrial wastewater treatment by UASB process in pilot scale.* Paper presented at the 3rd International Conference on Power and Energy Systems Engineering, CPESE, Kitakyushu, Japan.

Lippmann, M., Frampton, M., Schwartz, J., Dockery, D., Schlesinger, R., Koutrakis, P., Froines, J., Nel, A., Finkelstein, J. and Godleski, J. (2003). The US Environmental Protection Agency Particulate Matter Health Effects Research Centers Program: a midcourse report of status, progress, and plans. *Environmental Health Perspectives, 111*(8), 1074-1092.

Masinde, B. H., Nyaanga, D. M., Njue, M. R. and Matofari, J. W. (2020). Effect of Total Solids on Biogas Production in a Fixed Dome Laboratory Digester Under Mesophilic Temperature. *Annals of Advanced Agricultural Sciences, 4*(2), 26-33. doi:10.22606/as.2020.42003

Monnet, F. (2003). *An Introduction to Anaerobic Digestion of Organic Wastes*. Scotland.

Nasir, I., Ghazi, T., Omar, R. and Idris, A. (2014). Bioreactor performance in anaerobic digestion of cattle manure: A Review. *Energy Source: Part A, 36*, 1476-1483. doi:10.1080/15567036.2010.542439

Nijaguna, B. (2006). *Biogas Technology*. New Delhi, India: New Age International.

Nyaanga, D., Barasa, H. and Gisemba, J. (2015, 11-15 May). *Current and future of biogas contribution to the energy needs of Kenya.* Paper presented at the NACOSTI Proceedings of Conference, University of Nairobi.

Nzila, C., De Wulf, J., Spanjers, H., Kiriamiti, H. and Langenhove, H. (2010). Biowaste energy potential in Kenya. *Renewable Energy, 35*(12), 2698-2704.

Nzila, C., De Wulf, J., Spanjers, H., Tuigong, D., Kiriamiti, H. and Van Langenhove, H. (2012). Multi criteria sustainability assessment of biogas production in Kenya. *Applied Energy, 93*, 496-506.

Nzila, C., Njuguna, D., Madara, D., Muasya, R., Githaiga, J., Muumbo, A. and Kiriamiti, H. (2015). Characterization of agro-residues for biogas production and nutrient recovery in Kenya. *Journal of Emerging Trends in Engineering and Applied Sciences, 6*(5), 327-334.

Parawira, W. and Mshandete, A. (2009). Biogas technology research in selected sub-Saharan African countries–A review. *African Journal of Biotechnology, 8*(2), 102-111.

Patharwat, I., Kumbhar, S., Hingalaje, P., Khurpe, A., Goudgaon, J. and Dhamangaonkar, P. (2016). Analysis of optimum temperature and validity of biogas plant. *International Journal of Industrial Electronics and Electrical Engineering, 4*(4), 76.

Phillips, K., Silgram, M., Newell-Price, P., Warret, M., Provey, G., Cottril, B. and Newton, J. (2008). *The environmental impact of livestock production*. Report ADAS 2007, Deffra FFG, 1-96.

Rajendran, K., Aslanzaden, S. and Taherzadeh, M. J. (2012). Household Biogas Digesters - A Review. *Energies, 5*, 2911-2942. doi:10.3390/en5082911

Ramaraj, R. and Unpaprom, Y. (2016). Effect of temperature on the performance of biogas production from Duckweed. *Chemistry Research Journal, 1*(1), 58-66.

Rupfa, G., Bahri, P., de Boer, K. and McHenry, M. (2017). Development of an optimal biogas system design model for Sub-Saharan Africa with case studies from Kenya and Cameroon. *Renewable Energy, 109*, 586-601. doi:10.1016/j.renene.2017.03.048

Saleh, A., Kamarudin, E., Yaacob, A., Yussof, A. and Abdullah, M. (2012). Optimization of biomethane production by anaerobic digestion of palm oil mill effluent using response surface methodology. *Asia-Pacific Journal of Chemical Engineering*(7), 353-360.

Santerre, M. and Smith, K. (1982). Measures of appropriateness: the resource requirements of anaerobic digestion (biogas) systems. *World Development, 10*, 239-261.

Schön, M. (2010). *Numerical modelling of anaerobic digestion processes in agricultural biogas plants* (Vol. 6): BoD–Books on Demand.

Sibisi, N. and Green, J. (2005). A floating dome biogas digester: perceptions of energising a rural school in Maphephetheni, KwaZulu-Natal. *Journal of Energy in Southern Africa, 16*(3), 45-52.

Singh, G., Jain, V. and Singh, A. (2017). Effect of temperature and other factors on anaerobic digestion process responsible for biogas production. *International Journal of Theoretical and Applied Mechanics, 12*(3), 637-657.

Walsh, K. and McLaughlan, R. (1999). Bubble extraction of dissolved gases from ground water samples. *Water, Air, and Soil Pollution, 115,* 525-534.

CHAPTER TWO
LITERATURE REVIEW

2.1Biogas

With an overall human population growth of 70% between 1970 and 2004, the largest contribution to global greenhouse gas (GHG) emissions has come from the energy supply sector (EPA, 1992). Thus, innovations and improvements in this field can have major effects on this issue and contribute to mitigate climate change and its accompanying effects. Among other advantages, energy recovery from renewable sources can help to reduce GHG emissions since - unlike combustion of natural gas, liquefied gas, oil and coal - energy generation from biogas is an almost carbon-neutral way to produce energy from regional available raw materials (Saleh *et al.*, 2012).

Biogas comprises of gases that are produced during anaerobic digestion of organic materials that originate from plants. It is produced from the organic wastes by a concerted action of various groups of anaerobic bacteria (Boe, 2006). The main gaseous by-product is methane, with relatively less carbon dioxide, ammonia and hydrogen sulphide (Saleh *et al.*, 2012). Methane is the principal constituent of natural gas and ranks first in the series of saturated hydrocarbons known as alkanes (Khanal, 2008; Monnet, 2003). It is a light, colourless, odourless and highly inflammable gas, second only to hydrogen in the energy released per gram of fuel burnt, hence its potential as a household energy source (Nijaguna, 2006).

Besides hydro power, solar energy, biomass energy and wind energy, biogas plants are important producers of electricity and heat from renewable energy sources (Lippmann *et al.*, 2003). However, there are some shortcomings. Major benefits of energy production with biogas plants include utilisation of locally available, renewable resources; no supply costs in the case of agricultural waste products utilisation; almost carbon-neutral energy supply; local energy supply – no overland lines required; controllable performance – adjustable to demand; capability to provide base load electricity; and improved fertilization quality compared to raw agricultural wastes (EPA, 2005; Lippmann *et al.*, 2003). Bio-slurry is used as a fertilizer to promote the growth of crops and improve the crop yield (Gisemba and Barasa, 2019). Biogas is used mainly for lighting, cooking and heating. Cooking with biogas can save women time spent in harvesting wood and instead engage in other economic empowering activities, reduces smoke which is a major cause of lung diseases and poor eye sight for rural women

and children who cook with firewood in poorly lit and ventilated spaces (Nzila *et al.*, 2015). Other applications include gas-powered refrigerators and chicken incubators than run on biogas in Kenya (Laichena and Wafula, 1997; Sibisi and Green, 2005). In India and Nepal, biogas is connected to toilets for lighting (Batzias *et al.*, 2005).

Manure that is left to decompose releases two main gases that cause global climate change: nitrous dioxide warms the atmosphere three hundred and ten (310) times more than carbon dioxide (CO_2), and methane (CH_4) warms the atmosphere twenty one (21) times more than CO_2; therefore by converting cow manure into biogas instead of letting it decompose, we would be able to reduce global warming gases by 99 million metric tons or 4% (EPA, 2005; Saleh *et al.*, 2012). Cattle dung is a complex and naturally occurring polymeric substrate which consists of soluble and insoluble matter which can be used as a source of renewable energy (Garcia-Ochoa *et al.*, 1999). Improper management of this waste leads to many environmental hazards including water pollution and greenhouse gases (GHS) emissions (Kobayashi and Li, 2011). United States of America exclusively produces approximately two hundred and thirty (230) million tonnes of dry matter of animal waste that cannot be applied as local fertilizer (Karim *et al.*, 2007), United Kingdom produces eighty eight (88) million tonnes annually (Phillips *et al.*, 2008), while China produces 1.07 billion tonnes of livestock waste yearly (Chen *et al.*, 2009). Anaerobic digestion (AD) has been found to be an efficient, cheap and easy method to manage livestock waste (Nasir *et al.*, 2014).

2.2 Types of Biogas Systems

Digesters provide anaerobic conditions for biogas generation from biomass. The digester design and size vary depending on specific geographical conditions, substrate type, quantity of substrate available, and availability of construction materials (Rajendran *et al.*, 2012). The main digester designs used in developing countries are; fixed dome, floating drum and plug flow digesters.

Fixed dome digesters are non-portable two tank systems, usually built underground to protect them from temperature fluctuations and to save space (Vögeli *et al.*, 2014). Digester feeding is through an inlet pipe that reaches the bottom level of the digester chamber, gas produced is accumulated at the gas collection chamber just above before piping to a separate chamber, while slurry is collected through the expansion chamber (Rajendran *et al.*, 2012). Gas pressure is created by level differences between the slurry in the digester and that in the

expansion chamber; this helps push the digested substrate out. This study employed the use of a fixed dome laboratory batch digester. A fixed dome is a bioreactor which consists of a digester with fixed, non-movable gas holder, which sits on the digester – it is the most commonly used digester in China (Santerre and Smith, 1982) and also in Kenya. In terms of absolute numbers, the fixed dome is by far the most common digester type in developing countries (Gunaseelan, 1997). The Chinese fixed dome is the most popular and used most in developing countries because of its reliability, low maintenance costs, a long lifespan, and relatively minimal loss of the biogas yield (Ghimire, 2013; Huba *et al.*, 2013).

Floating drum digesters may have a well-shaped underground digester unit with a movable inverted drum acting as a gas holder or gas storage tank (Regattieri *et al.*, 2018). They produce gas at constant pressure and variable volume whereby the movable drum moves up and down depending on the amount of gas generated in the digester (Green and Sibisi, 2002; Rajendran *et al.*, 2012). The drum's weight also helps to pressurize gas flow through pipelines for conveyance, distribution and use; its position above the digester also helps indicate the amount of biogas held (Green and Sibisi, 2002). This design was developed in India by Khadi and Village Industry Commission, generally referred to as the KVIC design (Sooch and Singh, 2004). The mixing of the substrate is achieved in the digester during the feeding time whereby the substrate moves along the wall, the digester is easy to operate, and it has constant gas pressure because of the weight of the floating drum (Buysman, 2009). The main disadvantage of this system is the high cost of the steel drum, and the corrosion of steel caused by sulphide ions (Balasubramaniyam *et al.*, 2008).

Plug flow digesters are constant volume portable digesters but produce biogas at variable pressure (Green and Sibisi, 2002). They consist of a long, narrow, heated and insulated cylindrical tank whereby substrates are fed from one end while the gas and digestate are collected from the other end (Neibling and Chen, 2014); they may be partially or fully built below the ground and covered by a flexible or rigid roof. They are inclined to produce a two-phase system by facilitating the separation of acidogenesis from methanogenesis longitudinally (Rajendran *et al.*, 2012). This design is capable of achieving high temperatures during the day due to the thin covering of the digester body because it is exposed to solar radiation; but the digester experiences high heat loss at night and in the winter season (Daxiong *et al.*, 1990).

It was found that for the production of biogas by anaerobic digestion processes, residues from livestock farming, food processing industries, waste water treatment sludge, and other organic wastes can be utilised (Schön, 2010). Anaerobic digesters can be designed and engineered to operate using a number of different variants and process configurations. Anaerobic digestion processes can be classified according to the total solids content of the slurry in the digester and categorized further on the basis of number of reactors used, into single stage and multi stage (Monnet, 2003). In single stage reactors, the different stages of anaerobic digestion occur in one reactor while multi stage processes make use of two or more reactors that separate the steps in space.

Eder and Schulz (2006) have established that biogas reactors can either be designed to operate at a high total solids content (greater than 20%), or at a low solids concentration. Plants treating substrates with high solids content are referred to as dry fermentation reactors, those with low solids content are called wet fermentation systems (Gray, 2004). Also, there are combinations of both semi-dry and wet-dry. Low-solids digesters can transport material through the system using standard pumps with a significantly lower energy input but require more volume and area due to an increased liquid-to-feedstock ratio (Grady *et al.*, 1999). The dry fermentation process utilizes solid, stackable biomass and organic waste, which cannot be pumped, and it is mainly based on a batch-wise operation with a high TS content ranging from 20 to 50% at mesophilic temperatures (Schön, 2010). Dry fermentation systems are operated in a variety of specifications with and without percolation in digesters having a box or container shape accessible for loading machinery as well as in digesters formed by an air-tight plastic sheeting filled with substrate without any further conditioning. Koettner (2002) observed that digesters which solely work on the dry system with very little or no additional liquid are inoculated with a digested substrate and thus, inoculants and fresh material have to be mixed in suitable ratios beforehand. In dry–wet fermentation systems, the substrates don't need to be mixed or inoculated as bacteria rich percolation liquid re-circulated from the digester effluent takes over the role of the bacterial inoculation and process starting. The liquid that is heated in a heat exchanger, is either sprayed over the biomass from nozzles on top of the tank or flooded into the reactor (Eder and Schulz, 2006).

Regarding the flow pattern of anaerobic digesters, two basic types can be distinguished: batch and continuous. In continuous flow reactors the processes involved in anaerobic digestion

proceed spatially as well as temporarily in parallel steps whereas batch reactors exhibit temporarily staggered sequences (Jegede *et al.*, 2019). The operation of batch-type digesters consists of loading the digester with organic materials and allowing it to digest; once the digestion is complete, the effluent is removed and the process is repeated (Eder and Schulz, 2006). For example, covered lagoons and anaerobic sequencing batch reactors (ASBR) are operated in batch mode.

A covered lagoon consists of a pond containing the organic wastes which is fitted with an impermeable cover that collects the biogas. The cover can be placed over the entire lagoon or over the part that produces the most methane. The substrate enters at one end of the lagoon and the effluent is removed at the other. Cover lagoons are not heated and operate at ambient temperature which implies seasonal variations in reaction and conversion rates, and have the advantage of relatively low costs which are partly offset by lower energy yields and poor effluent quality (Schön, 2010).

Anaerobic sequencing batch reactors (ASBR) are discontinuously operated in a fill and draw mode. Filling of the tank is followed by a reaction period yielding biogas. During this stage the substrate is allowed to settle to the bottom of the tank and the solids separate from the effluent liquor. After that the supernatant and the digested substrate are withdrawn except a small portion which is retained in the tank in order to inoculate the incoming feed with active microorganisms.

In a continuous or quasi-continuous digester, organic material is constantly or regularly fed into the digester where it is moved forward either mechanically or by the force of the new feed pushing out digested material. Continuous digesters, unlike batch-type digesters, produce biogas without the interruption of loading material and unloading effluent (Schön, 2010). Continuous digesters include plug-flow systems, continuous stirred tank reactors (CSTR), and high-rate bio-film systems such as up-flow anaerobic sludge blanket reactors (UASB).

In most cases, a plug-flow digester comprises a stirred and heated horizontal tank which is fed at one end and the emptied at the other. By continuous feeding, a ‘plug’ of substrate is slowly moved through the tank towards the effluent. This mode of operation has various advantages that include the prevention of premature removal of fresh substrate through

hydraulic short-circuiting and a high sanitizing potential. Since the plug flow digester is a growth based system where the biomass is not conserved, it is less efficient than a retained biomass system and inoculation may be required (Eder and Schulz, 2006).

Basically, a CSTR consists of a closed vessel equipped with stirring devices providing mixing of the content. The reactor is continuously fed with substrate and due to the mixing it can be assumed that the concentrations of the compounds inside the vessel equal those at the effluent. Also, there is no liquid-solid separation or stratification and, hence, the substrate retention time (SRT) is the same as the hydraulic retention time (HRT). Since the biomass is suspended in the main liquid and will be removed together with the effluent, relatively long HRTs are required to avoid an outwash of the slow-growing methanogens (Batstone *et al.*, 2002).

Up-flow anaerobic sludge blanket reactors belong to the group of so-called high-rate anaerobic reactors. The term “high-rate” refers to reactor configurations that provide significant retention of active biomass, resulting in large differences between the SRT and the HRT, and operate at relatively short HRTs, often on the order of two days or less (Grady *et al.*, 1999). In an UASB digester the influent is introduced into the bottom of the vessel with a relatively uniform flow across the reactor cross section and distributed such that an upward flow is created. In the upper portion of the tank a cone shape with a widening cross section is introduced reducing the flow as it rises. As a consequence, combined with the flow rising upward from the bottom, gradually descending sludge will be hold in equilibrium forming a blanket which suspends in the tank. Small sludge granules begin to form whose surface area is covered with aggregations of bacteria. Finally the aggregates form into dense compact bio-films referred to as "granules". Substrate flows upwards through the blanket and is degraded and converted to biogas by the anaerobic microorganisms. Treated effluent exits the granular zone and flows upward into the gas-liquids-solids separator. There, the gas is collected in a hood and the supernatant liquid is discharged while separated solids settle back to the reaction zone. The combined effects of influent distribution and gas production result in mixing of the influent with the granules. Some variants of bio-film reactors use up-flow reactors provided with an internal packing to improve sludge blanket stability. The media have a high specific surface and allow for the growth of attached biomass (Grady *et al.*, 1999).

Generally, choice of reactor type is determined by waste characteristics, especially particulate solid contents. Consequently, the process must be able to convert solids to gas without clogging the anaerobic reactor. Solids and slurry waste are mainly treated in CSTRs, while soluble organic wastes are treated using high-rate bio-film systems such as UASB reactors (Boe, 2006). As explained previously, these reactors have very low HRTs and bacteria are retained in these reactors. The bacteria convert the soluble constituents to gas but have little opportunity to hydrolyze and degrade the particulate solids, unless the solids become attached to the biomass (Burke, 2001).

2.3 Anaerobic Digestion

Anaerobic process technologies initially intended for food and beverage production have been developed and applied over many centuries (Batstone *et al.*, 2002). With the employment of anaerobic digestion for treatment of organic waste and biogas production, an environmentally attractive technology has been established (Singh, 2017). Anaerobic digestion has several environmental benefits with regard to waste treatment, pollution reduction, production of CO_2-neutral renewable energy and improvement of agricultural practices by recycling of plant nutrients (Boe, 2006). Anaerobic digestion is a slow process, and it normally takes at least 21 days for the microbes to adapt to a new condition when there is a change of temperature or a substrate (Deublein and Steinhauser, 2008).

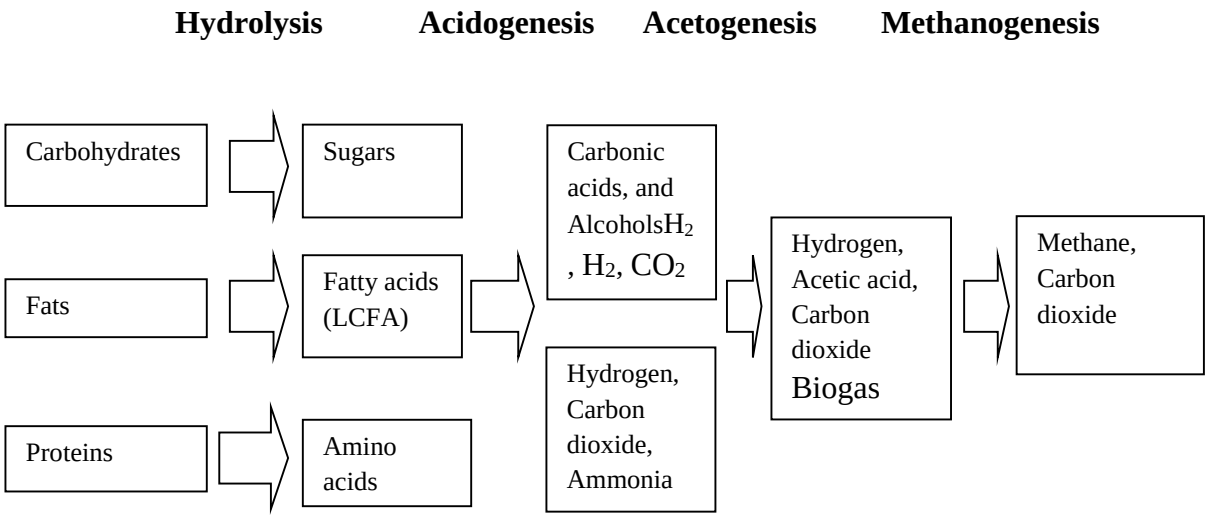
Björnsson *et al.* (2000) reported that the anaerobic degradation of complex organic matter into methane and certain by-products is a complex multi-step process of metabolic interactions performed by a well-organized community of microbial populations. Accordingly, a variety of microorganisms coexist in anaerobic digesters even when a single substrate is utilised, and their concerted activity is necessary for the complete bioconversion of organic materials to methane, carbon dioxide as well as trace gases such as hydrogen sulphide (H_2S) and hydrogen (H_2) (Boe, 2006). Maintaining a healthy bacterial population heavily depends on the microbial status and suitable operating conditions (Björnsson *et al.*, 2000; Lee and Hajela, 1996).

2.3.1 Microbial aspects of the anaerobic process

Anaerobic digestion of organic matter can be subdivided into four phases, termed hydrolysis/liquefaction, acidogenesis, acetogenesis and methanogenesis. These phases are a

series of interlinked reactions proceeding spatially as well as temporarily in consecutive and parallel steps and hence, influence one another (Batstone *et al.*, 2002).

Hydrolysis is a process where complex macromolecular organic matter comprising of carbohydrates, proteins and fats is subjected to enzymatic degradation and transformed to monosaccharides, amino acids and long chain fatty acids. Further anaerobic digestion finally leads from acidogenesis to acetogenesis and then to methanogenesis via intermediates and by-products to biogas production (CH_4, CO_2) (Batstone *et al.*, 2002). Figure 2.1 shows a schematic flow of the stages in an anaerobic digestion process.



Source: Batstone *et al.* (2002)

Figure 2.1: Illustration of the anaerobic process.

Anaerobic degradation starts with the hydrolysis step in which the organic polymers get solubilized into simpler and more soluble intermediates which can then pass the cell membrane of the bacteria that is involved in biogas production (Pavlostathis and Giraldo-Gomez, 1991).

The subsequent step to hydrolysis is referred to as acidogenesis (also termed fermentation) which is generally defined as an anaerobic acid-producing microbial process without an additional electron acceptor or donor (Gujer and Zehnder, 1983). The monosaccharides and amino acids resulting from hydrolysis are degraded to a number of simpler products such as volatile fatty acids (VFA) including propionic acid (CH_3CH_2COOH), butyric acid

($CH_3CH_2CH_2COOH$), and acetic acid (CH_3COOH) (Batstone *et al.*, 2002). These products can not be utilised directly by the methanogens and must be degraded further in a subsequent process that is referred to as acetogenesis (Björnsson *et al.*, 2000). Acidogenesis is often the quickest step in the anaerobic conversion of complex organic matter in liquid phase digestions (Vavilin *et al.*, 1996).

Acetogenesis is the degradation of higher organic acids formed in acidogenesis to yield carbon dioxide and hydrogen (Batstone *et al.*, 2002). This intermediate conversion is crucial for the successful production of biogas. During methanogenesis, the fermentation products such as acetate, H_2 and CO_2 are converted to CH_4 and CO_2 by methanogenic archaea which are strict obligate anaerobes (Björnsson *et al.*, 2000; Pavlostathis and Giraldo-Gomez, 1991).

2.3.2 Factors affecting anaerobic digestion

The metabolic activity involved in microbiological methanation is dependent on the following factors: substrate temperature, available nutrients, retention time (flow-through time), pH level, nitrogen inhibition and carbon to nitrogen ratio, substrate solid content and agitation, and inhibitory factors (Grady *et al.*, 1999). Each of the various types of bacteria responsible for the four stages of the anaerobic digestion is affected differently by the above parameters (Varma *et al.*, 2009). Since interactive effects between the various determining factors exist, no precise quantitative data on gas production as a function of the above factors are available (Gray, 2004). Thus, optimisation was limited to the three selected factors (temperature, substrate concentration and solid retention time) while holding the other factors constant.

a) Temperature of substrate

Anaerobic fermentation is, in principle, possible between 3^0C and approximately 70^0C. Differentiation is generally made between three temperature ranges: the psychrophilic temperature range lies below 20^0C, the mesophilic temperature range lies between 20^0C and 40^0C, and the thermophilic temperature range from 40^0C to 65^0C (Eder and Schulz, 2006; Grady *et al.*, 1999; Patharwat *et al.*, 2016). The best temperatures are 10^0C, 37^0C and 52^0C for psychrophilic, mesophilic, and thermophilic bacteria respectively (Patharwat *et al.*, 2016).

The rate of bacteriological methane production increases with temperature. Since, however, the amount of free ammonia also increases with temperature, the bio-digestive performance

could be inhibited or even reduced as a result (Eder and Schulz, 2006). In general, unheated biogas plants perform satisfactorily only where mean annual temperatures are at least 20^0C. Within the range of 20^0C to 28^0C mean temperature, gas production increases over-proportionally while below 15^0C, gas production is so low that the biogas plant is no longer economically feasible (Eder and Schulz, 2006; Grady *et al.*, 1999).

The process of bio-methanation is very sensitive to changes in temperature; the degree of sensitivity, in turn, is dependent on the temperature range (Leenawat *et al.*, 2016). Brief fluctuations not exceeding the following limits may be regarded as still un-inhibitory with respect to the process of fermentation: psychrophilic range: 2^0C per hour, mesophilic range: 1^0C per hour, and thermophilic range: 0.5^0C per hour, (Eder and Schulz, 2006).

Lettinga *et al.* (1997) studied the rate of growth of methanogens with increasing temperature and came up with the findings as shown in Table 2.1.

Table 2.1: Growth rate of methanogens with increase in temperature of substrate

Temperature (^{0}C)	Growth rate (%)		
	Psychrophiles	Mesophiles	Thermophiles
0	0	0	0
5	5	0	0
10	12	3	0
15	20	5	0
20	4	10	0
25	0	20	3
30	0	30	5
35	0	40	12
40	0	50	25
45	0	40	30
50	0	0	50
55	0	0	60
60	0	0	95
65	0	0	90
70	0	0	25
75	0	0	0
80	0	0	0

Source: Lettinga et al. (1997).

Figure 2.2 shows a graphical representation of the data in Table 2.1.

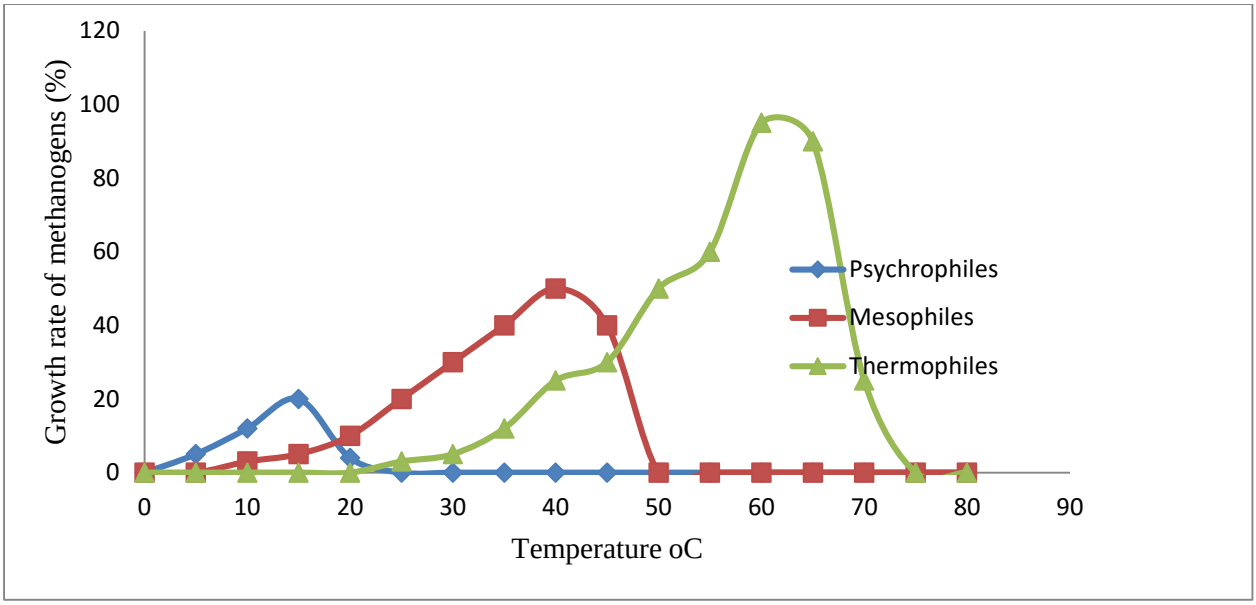


Figure 2.2: Growth rate of methanogens with increase in temperature

Redrawn from Lettinga *et al.* (1997)

Lettinga *et al.* (1997) noted that psychrophiles grew steadily from 0^0C to a maximum of 20% at 15^0C, and then started dying off to zero at 25^0C at a faster rate. Similarly, their data show that mesophiles are dormant between 0 and 5^0C and only start growing gradually at 5^0C, then steadily (to a maximum of about 55% at 40^0C), decline to 35% at 45^0C, and then to zero at 50^0C. Thermophiles start growing at 20^0C, rise steadily up to 35% at 45^0C. Thereafter the growth rate rises exponentially to 95% at 60^0C, and then dies off to zero at 75^0C. It can be seen from their data that the trends obey the biological exponential growth and decay trends but at different rates (gradients) depending on digestion temperatures. It can be concluded that the decay rate is faster (greater negative gradient) increasing from psychrophiles through mesophiles to thermophiles. This has implications on the rate of biogas production and justifies the use of higher digester and bioreactor temperatures.

b) Substrates and available nutrients

In order to grow, bacteria need more than just a supply of organic substances as a source of carbon and energy. They also require certain mineral nutrients. In addition to carbon, oxygen and hydrogen, the generation of bio-mass requires an adequate supply of nitrogen, sulphur, phosphorous, potassium, calcium, magnesium and a number of trace elements such as iron, manganese, molybdenum, zinc, cobalt, selenium, tungsten, and nickel (Bischofsberger *et al.*,

2005). Common substrates such as agricultural residues or municipal sewage usually contain adequate amounts of the mentioned elements (Tchobanoglous *et al.*, 2003). Higher concentration of any individual substance usually has an inhibitory effect, hence the analyses are recommended on a case-to-case basis to determine the amount of which nutrients, if any, still needs to be added (Bischofsberger *et al.*, 2005; Tchobanoglous *et al.*, 2003). Table 2.2 shows characteristics of manure production from some animals.

Table 2.2: Characteristics of manure production from some animals

Kinds	Body weight (kg)	Discharge per day (kg)	TS value of fresh discharge (% by wt.)	Water to be added with fresh discharge to make 8% TS (kg)
Human	50.00	0.50	20.00	0.75
Cow	200.00	10.00	16.00	10.00
Chicken	1.50	0.10	20.00	0.15
Pig	50.00	5.00	20.00	7.50

Source: EPA (1992)

It will be worthy to confirm if the amount of water to be added to make cow dung influent with a total solids (TS) of 8% are 10 kg if the fresh dung has a TS of 16% and as suggested by EPA (1992) since this could be a function of the feed the animals consume.

Table 2.3 gives biogas production in cubic metres (m^3) per cubic metre (m^3) of digester volume per day (m^3/m^3d) for different types of feedstock when digested at experimental temperature of 35^0C as the ambient conditions of temperature varied between 8^0C and 25^0C while the fermentation or digestion period of the excrement materials was 60 days and that of the stalk type feedstock was 90 days, both at a total solid content of 6%.

Table 2.3: Biogas-production (m^3/m^3d) from various materials at medium (mesophilic) and ambient (psychrophilic) temperatures

Materials	Medium temperature (35^0 C)	Ambient temperature (8^0 - 25^0C)	Reference
Pig manure	0.45	0.25 - 0.30	Nijaguna (2006)
Cattle dung	0.30	0.20 - 0.25	Nijaguna (2006)
Human wastes	0.43	0.25 - 0.30	Bolzonella *et al.* (2006)
Rice straw	0.40	0.20 - 0.25	Deublein and Steinhauser (2008)
Wheat straw	0.45	0.20 - 0.25	Deublein and Steinhauser (2008)
Green grass	0.44	0.20 - 0.25	Nijaguna (2006)

The table, generally, shows that the rate of gas production increases with temperature, being 0.2 -0.25 m^3/m^3d and 0.30 m^3/m^3d at 8^0C to 25^0C, and 30^0C respectively for cattle or cow dung according to Nijaguna (2006).

Biogas production from several substances at 30^0C in a batch reactor is presented in Table 2.4, giving the amount of biogas produced with time of digestion in days (as a % of the total yield).

Table 2.4: Biogas production from several substances at 30^0C in a batch reactor

Material	Yield in m^3 / m^3d	Yield in m^3/kgTS	Amount of biogas produced in days (as a % of the total yield)				Reference
			0-15	15-45	45-75	75-130	
Water Hyacinth	0.40	0.16	83.0	17.0	00.0	00.0	Gallert *et al.* (2003)
Alligator Weed	0.38	0.20	23.0	45.0	32.0	00.0	(Bauer *et al.*, 2018)
Water Lettuces	0.40	0.20	23.0	62.0	15.0	00.0	Saint-Joly *et al.* (2000)
Cattle Dung	0.20	0.12	11.0	33.8	20.9	34.3	Nijaguna (2006)
Pig Manure	0.30	0.22	19.6	31.8	25.5	23.1	Nijaguna (2006)
Human Wastes	0.53	0.31	45.0	22.0	27.3	05.7	Bolzonella *et al.* (2006)

The biogas production of 0.02 and 0.03 m^3/m^3d from cow dung when digested at medium temperatures of 30^0C and 35^0C as reported by Nijaguna (2006) and was compared to the results of the present research.

The amount of dung excreted per day and corresponding amount of biogas per animal is presented in Table 2.5. Cattle or cows are represented by an ox that weighs an average of 500kg producing 0.36 to 0.96 cubic metres (m^3) of biogas per day.

Table 2.5: Amount of dung excreted per day by different animals

Kinds	Body weight (kg)	Daily excrement (kg)	Daily urine (kg)	Annual excrement discharged (kg)	Annual excrement collection (kg)	Daily yield of biogas per capita (m^3)
Pig	50.0	6.0	15.0	2190.0	1752.0	0.18 - 0.25
Ox	500.0	34.0	34.0	12410.0	9928.0	0.36 - 0.96
Horse	500.0	10.0	15.0	3650.0	2920.0	
Sheep	15.0	1.5	2.0	548.0	438.0	
Chicken	1.5	0.1	0.0	36.8	29.4	0.0076 - 0.0112
Human	50.0	0.5	1.0	182.5	146.0	0.028

Source: EPA (1992)

EPA (1992) reported that the annual amount of excrements collected accounts for 80% of the discharge from ox (and by inference cattle) manure can produce 0.36 - 0.96 m^3 of biogas per day when the digester temperature is 35^0C, the total length of fermentation period being 60 days for the excrement material and total solid content of the fermentative fluid being 6%.

c) Retention time

Retention time refers to the duration that the substrate or the organic matter compounds take to be digested or get bio-chemically decomposed in the absence of oxygen, as they pass or traverse and move through the digester from the inlet (as influent) to the exit (as effluent) (Nyaanga *et al.*, 2015). The retention time can only be accurately defined in batch-type digestion systems for the production of biogas. For continuous systems, the mean retention time is approximated by dividing the digester volume by the daily influent rate. Depending on the reactor geometry, and the means of mixing among others, the effective retention time

may vary widely for the individual substrate constituents. Selection of a suitable retention time thus depends not only on the process temperature, but also on the type of substrate used (Dong, 2004). Optimising the process parameters (retention time, process temperature, substrate quality, and volumetric loading) ensures the cost efficiency of the biological processes. But as each m^3 digester volume has its price, heating equipment can be costly and high quality substrates may have alternative uses, the cost-benefit optimum in biogas production is almost always below the biological optimum (Nijaguna, 2006).

Gray (2004) reported that the approximate retention time in days for different liquid manures undergoing fermentation in the mesophilic temperature range to produce biogas are 20-30 days for cow manure, 15-25 days for liquid pig manure, 20-40 days liquid chicken manure:, and 50-80 days animal manure mixed with plant material. Another study also reported that if the retention time is too short, the bacteria in the digester are "washed out" faster than they can reproduce, so that the fermentation practically ceases but also notes that this problem rarely occurs in agricultural biogas systems (Hamad *et al.*, 1981; Jash and Gosh, 1990; Marti-Herero, 2011). The current research used a batch reactor, mesophilic temperature range and liquid cow manure, hence the retention time.

d) pH value

The methane-producing bacteria thrive best under neutral to slightly alkaline conditions (Bischofsberger *et al.*, 2005). Once the process of fermentation has stabilized under anaerobic conditions, the pH will normally take on a value of between 7 and 8.5 (Boe, 2006). Due to the buffer effect of Carbon dioxide-bicarbonate (CO_2 - HCO_3^-) and ammonia-ammonium (NH_3 - NH_4^+) compounds, the pH level is rarely taken as an indicator of substrate acids and/or potential biogas yield (Tchobanoglous *et al.*, 2003). A digester containing a high volatile-acid concentration requires a higher-than-normal pH value (Grady *et al.*, 1999). If the pH value drops below 6.2, the medium will have a toxic effect on the methanogenic bacteria (Grady *et al.*, 1999).

d) Nitrogen inhibition, and Carbon to Nitrogen ratio

All substrates contain nitrogen apart from carbon. Table 2.6 presents the nitrogen content of various organic substances and the Carbon to Nitrogen (C:N) ratio. For higher pH values,

even a relatively low nitrogen concentration may inhibit the process of fermentation (Bischofsberger *et al.*, 2005). Noticeable inhibition occurs at a nitrogen concentration of roughly 1700 mg ammonium-nitrogen (NH_4-N) per litre of substrate (Møller *et al.*, 2004). Nonetheless, given enough time, the methanogens are capable of adapting to NH_4-N concentrations in the range of 5000-7000 milligrams per litre (mg/l) of substrate, the main prerequisite being that the ammonia level (NH_3) does not exceed 200-300 mg NH_3-N per litre of substrate (Monnet, 2003). The rate of ammonia dissociation in water depends on the process temperature and pH value of the substrate slurry (Møller *et al.*, 2004).

Table 2.6: Carbon to nitrogen ratios of some fermentation materials

Material	C-content (%)	N-content (%)	C:N ratio
Dry wheat straw	46.00	0.50	87:1
Dry rice straw	42.00	0.50	67:1
Corn stalks	40.00	0.80	53:1
Soybean stalks	41.00	1.30	32:1
Wild grass	14.00	0.50	27:1
Peanut stems/leaves	11.00	0.60	19:1
Fresh sheep manure	16.00	0.60	29:1
Fresh cattle dung	07.30	0.30	25:1
Fresh horse dung	10.00	0.42	24:1
Fresh pig manure	07.80	0.60	13:1
Fresh human wastes	02.50	0.90	29:1

Source: Nijaguna (2006)

Microorganisms need both nitrogen and carbon for cell development. Various experiments have shown that the metabolic activity of methanogenic bacteria can be optimised at a carbon to nitrogen ratio of approximately 8-20, whereby the optimum point varies from case to case, depending on the nature of the substrate (Bischofsberger *et al.*, 2005). A study by Dioha *et al.* (2014) on the effects of carbon to nitrogen ratio to biogas production showed that microorganisms required 20:1 to 30:1 for Carbon to Nitrogen ratio. Nitrogen plays an important role in amino acid synthesis and the formation of ammonia to neutralize volatile acids generated by acid-forming bacteria, and hence it offers suitable pH levels that provide a conducive environment for anaerobic digestion of organic matter (Tufaner and Avşar, 2016).

Cow manure has a C:N ratio of about 22.71:1 (Ardaji *et al.*, 2016) and hence its suitability for this research .

e) Agitation and its effect on total solids content

The mobility of the methanogens within the substrate is gradually impaired by increasing solids content, and the biogas yield may suffer as a result. However, reports of relatively high biogas yields from landfill material with high solids content are found in recent literature (Hansen *et al.*, 2004; Ras *et al.*, 2007). No general valid guidelines can be offered with regard to specific biogas production for any particular total solid percentage (Abbassi-Guendouz *et al.*, 2012).

Substrates and various modes of fermentation require some substrate agitation or mixing in order to maintain process stability within the digester (Bridgeman, 2012; Lemmer *et al.*, 2013). The most important objectives of agitation include removal of the metabolites and gas produced by the methanogens, mixing of fresh substrate and bacterial population (inoculation), preclusion of scum formation and sedimentation, avoidance of pronounced temperature gradients within the digester, provision of a uniform bacterial population density, and prevention of the formation of dead spaces that would reduce the effective digester volume (Halalsheh *et al.*, 2011).

Bridgeman (2012) gives the following points to be considered while selecting or designing a suitable means of agitation:

i. The process involves a symbiotic relationship between various strains of bacteria namely, i.e. the metabolite from one species can serve as nutrient for the next species, among others. Whenever the bacterial community is disrupted, the process of fermentation will remain more or less unproductive until an equivalent new community is formed. Consequently, excessive or too frequent mixing is usually detrimental to the process. Slow stirring is better than rapid agitation.
ii. A thin layer of scum must not necessarily have an adverse effect on the process. For systems in which the digester is completely filled with substrate, so that any scum always remains sufficiently wet, there is little or no danger that the extraction of gas could be impeded by the scum.
iii. Some types of biogas systems can function well without any mechanical agitation at all. Such systems are usually operated either on substrates with such a high solid

content, that no stratification occurs, or on substrates consisting primarily of solute substances.

Karapaju and Rintala (2008) reported biogas production for minimal and intermittent agitation relative to the biogas yield from a continuously mixed system. An evaluation, done by Ghanimeh *et al.* (2012), on the effect of mixing and organic loading rate on anaerobic digestion of source-separated organic fraction of municipal solid waste that was continuously and slowly mixed at 100 revolutions per minute (rpm) showed a superior digestion efficiency.

Since the results of agitation and mixing are highly dependent on the substrate in use, it is not possible to achieve a sufficiently uniform comparative evaluation of various mixing systems and/or intensity levels (Lemmer *et al.*, 2013). Thus, each such system can only be designed on the basis of empirical data. The current research has reported on the form of agitation and mixing that was used.

f) Inhibitory metals

There are light as well as heavy metals. Light metals include sodium (Na), magnesium (Mg), Potassium (K), and Calcium (Ca), while the heavy metals include chromium (Cr), cobalt (Co), copper (Cu), cadmium (Cd), nickel (Ni) and zinc (Zn). The presence of heavy metal ions in the substrate is a major cause of toxicity that inhibits biogas production from anaerobic digestion (Jin *et al.*, 1998; Sterrit and Lester, 1980; Swanwick *et al.*, 1969). Vallee and Ulner (1972) reported that these heavy metal ions disrupt enzymatic functioning by binding the metals with the organic substrate or by replacing naturally occurring metals in enzyme prosthetic groups. Also the presence of antibiotics (this may include bacitracin, flavomycin, lasalocid, monensin, and spiramycin) and detergents used in livestock husbandry can have an inhibitory effect on the process of bio-methanation (Bischofsberger *et al.*, 2005). Table 2.7 presents the limit of concentrations (mg/l) for various metal inhibitors (Møller *et al.*, 2004).

Table 2.7: Limiting concentrations for inhibitors of biomethanation

Substance	mg/l
Copper	10 – 250
Calcium	8000
Sodium	8000
Magnesium	3000
Nickel	100 –1000
Zinc	350 – 1000
Chromium	200 – 2000
Sulphide (as Sulphur)	200
Cynide	2

Source: Møller *et al.* (2004)

In most free range animal husbandry systems as practiced in most African tropical conditions, heavy metals are likely to be low or do not exist and hence this was not a major concern in the current research.

g) Organic loading rate

Organic loading rate is amount of substrate that is fed into 1 m^3 of the digester volume per day, and it is expressed in either total solids (TS) or volatile solids (VS). Total solids are defined as a measure of dry matter left after the moisture has been removed from a moist sample. Total solids is the measurement of dry matter as a percentage, and is determined by drying the sample at 103 – 105^0C in succession until no further change in weight is observed (APHA, 1999; EPA, 2001). Volatile solids content is determined by igniting the substrate at 550^0C in the incinerator and then weighing the remaining contents (EPA, 2001). Household digesters have been reported to use a total solid that varies from 5% to 10% (Bouallagui *et al.*, 2003; Mohammad, 1991; Shyam and Sharma, 1994). Total solids were used as a variable in this study.

The volumetric organic loading rate (OLR) is related to the retention time through the active biomass concentration in the bioreactor and is used to characterize the loading on anaerobic treatment systems (Bauer *et al.*, 2018). The OLR provides useful information for the design and operation of anaerobic processes as its knowledge is used to quantify how effectively the

reactor volume is being utilised (Schön, 2010). It can be expressed in terms of the mass of volatile solids applied and is calculated by equation (2.1):

$$OLR = \frac{QC}{V} = \frac{C}{HRT} \qquad 2.1$$

Where,

OLR = the volumetric organic loading rate (kgVS/m^3.d)

Q = the influent flow rate (m^3/d)

C = the concentration of volatile solids in the substrate (kgVS/m^3)

V = the bioreactor volume (m^3)

HRT = Hydraulic Retention Time (days)

That is, volumetric organic loading rate is a ratio of the product of the influent flow rate in m^3/d, and the concentration of volatile solids in the substrate in kgVS/m^3 to the bioreactor volume (m^3) or the ratio of the concentration of volatile solids in the substrate (kgVS/m^3) to hydraulic retention time in days. The most common organic loading rate is 2-3 kgVS/m^3/day (Subramanian, 1977), while the average biogas production was reported to be 0.26-0.55 m^3/kgVS/day (Gupta and Singh, 1990; Safley and Westerman, 1992; Xavier and Nand, 1990).

Ferguson *et al.* (2016) reported that organic loading rate has an effect on the resilience of microbial functioning in anaerobic digesters. Generally, sudden changes in organic loading rate can cause instability in anaerobic processes (Akunna *et al.*, 2007; Rincon *et al.*, 2008). An understanding of the effects of changes in organic loading rate on the performance of anaerobic digesters gives strategies on mitigation measures to be taken in order to stabilize the process of biogas production (Alvarez *et al.*, 2010; Derbal *et al.*, 2009; Ward *et al.*, 2008). It was, however, observed by Ferguson *et al.* (2014) and Tale *et al.* (2015) that the biotechnological tools for managing a consortium of bacteria and archaea for biogas production had not been exploited.

For completely mixed anaerobic reactors operated without solids recycling, the hydraulic retention time (HRT) and substrate retention time (SRT) are identical. For digestion systems, which incorporate solids recycle, the SRT will be greater than the HRT, and the OLR

indicates both the anaerobic digester volume utilisation efficiency and the overall process loading (Grady *et al.*, 1999).

Retention time and organic loading rate are inversely proportional to each other and thus, have to be aligned when designing the reactor layout. The maximum possible OLR depends on both the process temperature and the retention time: the lower the temperature and the longer the retention time, the higher the OLRs that can be processed (Grady *et al.*, 1999). This maximum value depends also on the specific digester/reactor type. The higher the OLR, the higher the risk to exceed the optimum performance limit of the degrading biomass. Feeding the system above its sustainable OLR results in low biogas yield due to accumulation of inhibiting substances such as volatile fatty acids in the digester slurry (Bauer *et al.*, 2018). Typically, OLR ranges from 2 to 6 kgVS/m^3d (Eder and Schulz, 2006) and these values were compared to the results of the current research.

2.3.3 Methods for enhancing biogas production

Manipulations that the feedstock is subjected to before undergoing anaerobic digestion have an effect of promoting the level of biogas production. The methods for enhancing the production of biogas from biomass waste include co-digestion, pretreatment, and hyperthermophilic anaerobic digestion.

a) Co-digestion

Co-digestion involves the use of two or more different feedstock in a bioreactor to undergo anaerobic digestion process (Pannucharoenwong, 2018). Gashaw and Teshita (2014) define co-digestion as the anaerobic treatment of a mixture of at least two different substrates with the aim of improving the efficiency of an anaerobic digestion process. Some of the advantages of anaerobic co-digestion include increased cost efficiency, increased biodegradation of treated materials, and increased biogas yield (Gashaw and Teshita, 2014). Co-digestion helps to mitigate inhibitory effects of unfavourable substrates, balances nutrients and increases organic loading rate which result in a higher methane yield while diversifying and synergizing the bacterial populations that carry out methanogenesis (Shah *et al.*, 2015). Apart from improving the reliability of the feedstock, co-digestion dilutes toxic substances, synergizes microorganisms in the substrate, increases the load of biodegradable matter, and increases methane yield per unit volume of the digester (Nkemka and Murto,

2010). Co-digestion is one of the methods for enhancing biogas production to as much as 25 - 400% over the mono-digestion of the same substrates (Fayyaz *et al.*, 2015).

Gashaw and Teshita (2014) concluded that organic food wastes co-digested with cattle manure improved the biogas potential compared to cattle manure alone. Neczaj *et al.* (2012) reported anaerobic co-digestion of a mixture of a fraction of municipal solid waste, agricultural residues, organic solid wastes, and sewage sludge. Tamrat *et al.* (2013) did co-digestion of three mix ratios of rumen fluid inoculated cow manure with organic kitchen waste and established a substantial increase in biogas yield of 24 – 47% compared to the control. Biogas production from empty fruit bunches was compared with a co-digestion of palm oil mill effluent, and found out that the two enhanced biodegradability with 25 – 32%, and increased methane production by 98% (Sompong *et al.*, 2012). A feasibility study on anaerobic co-digestion of sewage sludge and sugar beet pulp lixiviation was assessed under mesophilic and thermophilic conditions by Montanes *et al.* (2015) in which they established that the net methane generation was higher in the mesophilic range on the biochemical methane potential test.

Zielinski *et al.* (2017) reported that co-digestion of cow manure with energy rich substrates like pig manure and chicken manure increases biogas yield. Sebola *et al.* (2015) concluded that co-digestion of chicken droppings and cow manure enhanced biogas yield as compared to their mono-digestion, with a maximum increase in biogas production of 50% being attained at a mix ratio of 1:1under mesophilic temperature conditions. Afazeli *et al.* (2014) reported that biogas production from co-digestion of chicken manure and cow manure under continuous anaerobic digestion increased by 69.6%. Böjti *et al.* (2017) co-digested pretreated maize silage with chicken manure in continuous anaerobic digestion at mesophilic conditions of 37°C and recorded an increase of 24% in biogas production.

It was observed by Al Mamun and Torii (2015) that methane biogas yields can be improved through co-digestion because this process is capable of creating a synergy between the digesting medium and the supply of missing nutrients. Dahunsi and Orunsi (2013) did a unique experiment that involved co-digestion of food waste and human excreta for 60 days using a 40 litre digester, and the volume of gas generated from the mixture comprised of 58% methane (CH_4), 24% carbon dioxide (CO_2), 19% hydrogen sulphide (H_2S) and other impurities. An experiment of co-digestion of cheese whey and cattle manure, based on

concentric acidogenesis using an innovative two-stage process and methanogenic phases, was designed for reducing footprint and enhancing performance (Lorenzo *et al.*, 2013). It showed an improved yield; that the highest methane production was achieved when a co-digestion of the above substrates was at a ratio of 1:1. Tong *et al.* (2013) conducted an anaerobic co-digestion of goat manure, rice straw, corn stalks and wheat straw under different mixing ratios at mesophilic temperature (35^0C) with a total solid concentration of 8%. They found out that biogas yields improved significantly at all carbon to nitrogen ratios.

A review by Mata-Alvarez *et al.* (2010) on co-digestion practices and analysis included models to be developed and thereafter standardization from laboratory scale anaerobic digesters to an industrial scale. Full-scale plants with sewage sludge co-digestion cases are worth mentioning. A study by Zipancic *et al.* (2008) at a waste water treatment plant equipped with two digesters (each with a capacity of $2000m^3$), operating at a hydraulic retention time of 20 days, and an average organic loading rate of 0.8 VSS/m^3d and were supplemented with organic fraction of municipal solid waste (OFMSW) to increase the organic loading rate to 25% at Velenje in Slovenia. This resulted in an increased biogas production by 80%, and a specific biogas production increased by over 53%. Another full-scale study was reported by Zitomer *et al.* (2008) in which a waste water treatment plant digester had five co-substrates including yeast waste; the synergistic effect increased biogas production by over 50%.

Anaerobic co-digestion of chicken manure and corn stover was carried out in both a batch reactor and a continuously stirred tank reactor (CSTR) whereby the batch co-digestion experiment was done at an initial volatile solids (VS) concentration of 3g VS/L, carbon to nitrogen ratio of 20 and a retention time of 30 days, whereas in the CSTR experiment, a feeding concentration of total solids (TS) of 12%, a carbon to nitrogen ratio of 20 and organic loading rates of 1-4g VS/L/d (Yeqing *et al.*, 2014). The results showed that in the batch case, the methane yield was 281±12 ml/g of VS added while the CSTR outcome was 223±7 ml/g of VS added (Yeqing *et al.*, 2014). Co-digestion in a batch reactor gives better results than the CSTR by 26%.

b) Pretreatment of feedstock

Pretreatment of feedstock involves the initial manipulations or actions that the material is subjected to facilitate subsequent processes that convert it to a desired product or products. The manipulations may include thermal, biological, chemical, hydro or mechanical actions.

Most of these methods have high energy costs, and are unprofitable (Teghammar *et al.*, 2012). Different feedstocks have different types and degrees of limitations to optimal performance of anaerobic digestion that can be solved by different pre-treatment mechanisms (Carlsson *et al.*, 2012). Conversion of biodegradable material to biogas is limited by the rate and extent of hydrolysis. Animal manure contains a high lignocellulose content and therefore pretreatment is important in increasing carbohydrate, protein and fatty acids accessibility along with hydrolysis efficiency (Rusanowska *et al.*, 2018). Pretreatment reduces feed stock size to increase surface area and reduce cellulose crystals for improved hydrolysis yield by 5-25%, to potentially enhance biogas yield and reduce retention time by about 23-59% (Kratky and Jirout, 2011). Assefa *et al.* (2014) reported that there was no significant difference in pH and organic matter content between poultry substrates subjected to different temperature and sodium hydroxide (NaOH) pretreatments before anaerobic digestion.

i. Mechanical Pretreatment

Mechanical pretreatment involves disintegration, grinding, mincing, and milling or extrusion of solid particles of the substrate into easily fermentable components through reduction in resistance to flow and making mixing within the digester easier, and thus releasing cell compounds and increasing the specific surface area (Elliot and Mahmood, 2012; Skiadas *et al.*, 2005). Knives and mills are used to break open the cellular structure to increase the specific surface area for bacterial attack, especially for lignocellulosic substrates (Bochmann and Montgomery, 2014). Shearing and compressive forces acting on biomass reduces its crystallinity, particle size and increases specific surface area and bulk density (Kratky and Jirout, 2011). Some of the advantages of mechanical pretreatment include better dewaterability of the final anaerobic residue, no generation of odour, moderate energy consumption and an easier implementation of subsequent processes (Toreci *et al.*, 2009). The disadvantages include insignificant effect on removal of pathogens, and the possibility of scaling and clogging of equipment (Perez-Elvira *et al.*, 2006).

Mechanical pretreatment methods include the rotary drum, lysis-centrifuge, liquid shear, collision, high-pressure homogenizer, sonication, liquefaction, and maceration (Esposito *et al.*, 2011; Hartmann *et al.*, 2000). The following discussion highlights findings that have been made in mechanical pretreatment processes.

Rotary drum is one of the mechanical pretreatment methods that was used by Zhu *et al.* (2009) and Subramani and Ponkumar (2012) for organic fraction of municipal solid waste (OFMSW) separation pretreatment before anaerobic digestion, and it enhanced biogas production by 18-36%. Davidson *et al.* (2007) studied the biomethane potential of source-sorted OFMSW, and reported small variations in both methane yield per gram of volatile solids, and content of methane in biogas while using different mechanical methods such as the screw press, disc screen shredder, food waste disposer and piston press. In a similar experiment, Zhang and Banks (2013) found no significant enhancement of biogas production.

Rusanowska *et al.* (2018) reported that mechanical pretreatment of lignocellulosic biomass increased biogas production by about 22% and biomass degradation by about 6%. Izumi *et al.* (2010) studied the effect of size reduction in food waste in anaerobic digestion and observed that biogas production from food waste at sizes less than 0.7mm in mesophilic conditions increased by 28%. A size reduction that is less than 0.7mm causes an accumulation of volatile fatty acids (VFA) (Izumi *et al.*, 2010) whereas methanogens are sensitive to acidic intermediates (Park *et al.*, 2011); this may result in a decreased anaerobic digestion performance. Carlsson and Anox (2008) reported an increase in biogas production of 20-40% when OFMSW was subjected to electroporation pretreatment. Liquifaction of OFMSW was to yield biogas that was 15-26% higher (Carrère *et al.*, 2010), while sonication on the same material gave 16% more cumulative biogas compared to untreated substrates (Cesaro and Belgiorno, 2013). Sonication involves the use of a probe in which the mechanical vibration disrupts the cell structure and floc matrix (Elliot and Mahmood, 2007). The effect of the vibration at low frequencies of 20-40 kHz sound waves is the reduction in particle size (Chua *et al.*, 2002). At high frequency sound waves, there is a formation of radicals such as H*, OH*, and HO_2* that cause oxidation of solid substances (Bougrier *et al.*, 2006). Sonication of the feedstock before anaerobic digestion has been reported to enhance biogas production by 24-140% in batch systems, and 10-45% in continuous or semi-continuous systems (Carrère *et al.*, 2010). Studies on enhancement of volatile solids (VS) destruction and higher biogas production in waste activated sludge (WAS) yielded negligible results (Cesaro and Belgiorno, 2013; Sandino *et al.*, 2005). It was concluded that extruders and colloid mills are more suited to reducing sizes of materials that have over 15-20% moisture content while hammer and knife mills are suited for dry biomass at 10-15% moisture content (Taherzadeh and Karimi, 2008). The effectiveness and uniformity of grinding of a given feed stock depends on its moisture content.

Generally, maceration enhances biogas production by 10-60% (Carrère *et al.*, 2010). Maceration has an effect of shearing rather than the cutting of fibres (Hartmann *et al.*, 2000). Angelidaki and Ahring (2000) macerated fibres in manure upto 2mm and it gave an increase of 16% biogas production, while size reduction of down to 0.35mm yielded 20% increase, and a negligible difference was observed when further reduction in size was made. High-pressure homogenization (HPH) is another method of mechanical pretreatment. Barjenbruch and Kopplow (2003) treated surplus sludge with HPH at a pressure of 600 bar and showed that the filaments were completely disintegrated. A study on the effect of HPH on the anaerobic digestion of sewage sludge showed an increase of 25% volatile solids reduction (Engelhart *et al.*, 2000). This development has necessitated a full scale application of HPH to waste water treatment plants (WWTP), and has achieved 30% biogas enhancement and a reduction in working volume of digesters by 23% (Carrère *et al.*, 2010).

ii. Thermal pretreatment

Thermal pretreatment involves subjecting a given material to heat at given temperatures over a period of time to achieve disintegration of cell membranes and tough bonds holding material fibres together in order to increase solubilization of organic compounds, ease bacterial attack and biodegradation (Ariunbaatar *et al.*, 2014). This method is the most studied, and has been applied at an industrial scale (Carlsson *et al.*, 2012; Carrère *et al.*, 2010; Cesaro and Belgiorno, 2014). Thermal pretreatment helps to remove pathogens, improves dewatering performance, lowers the viscosity of the digestate, and consequently enhance the handling of the digestate (Carlsson *et al.*, 2012; Edelmann *et al.*, 2005; Shen *et al.*, 2012; Val del Rio *et al.*, 2011). Heat helps break down the hydrogen bonds holding together the cellulosic and lignocellulosic complexes, thereby making the feed stocks to increase in specific surface area (Garrote *et al.*, 1999). Pathogen removal and easy handling of the digestate was also reported by Val del Rio *et al.* (2011) and Carlsson *et al.* (2012).

An increase in biogas yield occurs with an increase in temperature of pretreatment up to a certain optimum temperature above which production decreases (Bochmann *et al.*, 2010; Jahng *et al.*, 2011) because of xylose formation and lignin breakdown that become toxic to anaerobic digestion bacteria (Bochmann and Montgomery, 2014). At high temperatures ($>170^0$C), materials form chemical bonds which agglomerate the particles (Bougrier *et al.*, 2006). An example is that of the Maillard reaction in which the carbohydrates and amino

acids form complex substrates that are difficult to biodegrade using anaerobic digestion (Carrère *et al.*, 2010; Elliot and Mahmood, 2012; Hendriks and Zeeman, 2009). Thermal pretreatment is also known to cause a loss of organics and potential biogas production from easily biodegradable substrates; depending on the feedstock and the range of temperature applied (Panaud *et al.*, 1999; Pinnekamp, 1989).

Thermal pretreatment at lower temperatures (<110^{0}C) does not degrade complex molecules but instead cause deflocculation of macromolecules (Protot *et al.*, 2011). Barjenbruch and Kopplow (2003) made a similar conclusion when they observed at 90^{0}C that the filaments were not disintegrated but were only attacked by thermal pretreatment. Chamchoi *et al.* (2011) and Gonzalez-Fernandez *et al.* (2012) pretreated household waste at 70^{0}C for 8 hours and 60 minutes respectively, and reported no enhancement in the production of biogas. Appels *et al.* (2010) reported a negligible increase in biogas production from sludge pretreated at 70^{0}C for 60 minutes.

Biogas production improved 20 times when thermal pretreatment was applied to sludge for 60 minutes at 90^{0}C (Appels *et al.*, 2010). Rafique *et al.* (2010) reported an enhancement of 78% biogas production with 60% methane content by thermal pretreatment at 70^{0}C. Ferrer *et al.* (2008) reported a 30% increase in biogas production with 69% methane content. Ariunbaatar *et al.* (2014) found out that at a temperature <110°C, thermal pretreatment and subsequent anaerobic digestion of feed stocks achieved a more cost-effective performance compared to other methods. Assefa *et al.* (2014) observed that thermally pre-treating poultry litter-cow manure substrate at 80ºC increased gas production and VS removal by 46.3% and 26.1% respectively. Climent *et al.* (2007) reported an increase of 50% in biogas production from organic solid waste subjected to 70°C thermal pretreatment prior to digestion at thermophilic conditions. On a laboratory scale, thermal pretreatment can be done using microwave heaters, pressure cookers and autoclaves (Bochmann and Montgomery, 2014).

Thermal pretreatment at a temperature of 175^{0}C was studied by Liu et al on food waste and fruit and vegetable waste, in which they reported 7.9% and 11.7% reduction in biomethane yield respectively due to the formation of melanoidins (Shen *et al.*, 2012). Ma *et al.* (2011) pretreated food waste at 120^{0}C and reported an increase in biogas production of 24%. A lower biogas yield was obtained from pig manure that had been pretreated at a temperature >110^{0}C (Rafique *et al.*, 2010).

iii. Hydro pretreatment

In hydro pretreatment, water is the main solution that is applied to the feed stock prior to anaerobic digestion. An increase in biogas yield occurs with an increase in temperature of pretreatment up to a certain optimum temperature above which production decreases (Bochmann *et al.*, 2010; Jahng *et al.*, 2011) because of xylose formation and lignin breakdown that become toxic to anaerobic digestion bacteria (Bochmann and Montgomery, 2014). Hydro pretreatment involves increasing specific surface area to volume ratio of feed stocks prior to their digestion by soaking them in water for a given period of time to help loosen their tough outer coatings. Bolaji *et al.* (2017) found that soaking maize in water for a period up to 36 hours increased the specific surface area and sphericity of the grains. The practice of soaking hard covered feedstocks like sheep, goat and chicken manure by farmers in Kenya has been common in most rural areas although proper process details and procedures of how to approach this pretreatment have not been documented (Smith *et al.*, 2013).

iv. Chemical pretreatment

Chemical pretreatment employs the use of strong alkalis, acids and oxidants to destroy organic bonds in lignocellulosic and cellulosic feed stocks. For the production of biogas, anaerobic digestion requires a pH of near neutral and hence alkali pretreatment is preferred (Li *et al.*, 2012). Pretreatments involving acids and oxidative methods such as ozonation are not suited to feedstock that is easily biodegradable because they lead to an accumulation of volatile fatty acids (VFA) which inhibits the production of biogas during the anaerobic digestion process (Mattsson *et al.*, 2011). However, the application of acids has a positive effect on the substrates that contain lignin and cellulose (Fernandes *et al.*, 2009).

In alkali pretreatment, sodium hydroxide (NaOH) is the most common component that is used. The initial reactions that induce the swelling of solids include solvation and saphonification (Carlsson *et al.*, 2012), and these result in an increase in surface area that enables easy accessibility by anaerobic microbes (Hendriks and Zeeman, 2009; Lopez-Torres and Llorens, 2008; Molenbach and Nokes, 2012). Assefa *et al.* (2014) pretreated cow and chicken manure with 0.45 g, 1.35 g, and 2.25 g of NaOH and recorded respective increase of 0.03%, 21% and 56% in cumulative biogas yield. Bochmann and Montgomery (2014) observed that alkali pretreatment of substrates leads to an increase in system pH and if used

in continuous anaerobic process, this can result in the build-up of salts, mainly ammonium-ammonia imbalance that may inhibit methane production during the anaerobic digestion process.

Acid pretreatment involves use of acids like hydrochloric acid (HCl) and sulphuric acid (H_2SO_4), in combination with heat, to lignocellulosic substrates not only to degrade lignin but also the hydrolytic microbes are capable of acclimating to acidic conditions (Musoline *et al.*, 2012). Strong acidic pretreatments should be avoided because of the by-products that may inhibit biogas production, loss of fermentable sugar, a high cost of acids and the associated additional cost for neutralizing the acidic conditions before commencing the anaerobic digestion (Molenbach and Nokes, 2012; Murphy and Kumar, 2011; Taherzadeh and Karimi, 2008).

Ozonation is an oxidative method that is applied in chemical pretreatment. It involves the use of hydrogen peroxide or ozone that causes swelling of lignocelluloses to increase substrate specific surface area and cause partial lignin solubilization for improved hydrolysis (Kianmahr *et al.*, 2010). Ozonation does not increase salt concentration, nor remainder of chemical residues, but it disinfects pathogens (Weemaes *et al.*, 2000). Ozone (O_3) is a very strong oxidant which decomposes itself into radicals and also reacts with organic substrates (Sri *et al.*, 2011). Ozonation was done on waste water and sludge in a waste water treatment plant (WWTP) and established that the optimal ozone dose for enhancing anaerobic digestion to produce biogas from a WWTP is 0.05-0.5 gO_3/gTS (Carballa *et al.*, 2007; Carrère *et al.*, 2010; Goel *et al.*, 2003; Yoem *et al.*, 2002).

A study by Monlau *et al.* (2012) on pretreatment of sunflower stalks with hydrochloric acid at a temperature of 170°C showed in an increase of 20% methane yield. Cesaro and Belgiorno (2013) reported an optimum ozone dose of 0.16 gO_3/gTS for source-sorted OFMSW which gave an increase of 37% biogas production. Lopez-Torres and Llorens (2008) applied alkaline pretreatment on OFMSW and obtained an increased methane production of 11.5%. Neves *et al.* (2006) pretreated barley waste with an alkaline (0.3 gNaOH/gTS) and reported 100% of the potential production of biogas.

Alkaline pretreatment was done on water hyacinth (which has a lower lignin content compared to other plants) and recorded an insignificant effect relative to mechanical

pretreatment (Patil *et al.*, 2011). Bochmann and Montgomery (2014) concluded that chemical pretreatment should not be applied to substrates that contain low lignin, and that the high costs associated with this pretreatment method is one of the factors preventing its large scale adoption.

v. Biological pretreatment

Biological pretreatment method involves use of micro-organisms to aerobically or anaerobically breakdown feed stock structures to increase their surface area for hydrolysis. Enzymes may also be added to the anaerobic digestion system to enhance biogas production (Ariunbaatar *et al.*, 2014). Anaerobic microbiological pretreatment involves separation of the first and second stages of anaerobic digestion (hydrolysis and acidogenesis) from methanogenesis, also called two stage anaerobic digestion (Ge *et al.*, 2010, 2011a, 2011b), pre-acidification or dark fermentation (Bochmann and Montgomery, 2014). Aerobic microbial pretreatment is done naturally using natural mixed cultures whereby the cultures generate enzymes that help degrade cellulose, lignin and hemicellulose hence increasing the surface area of substrate. A physical separation of acidogenic bacteria from methanogenic bacteria results in a higher biogas yield and a higher efficiency in the removal of chemical oxygen demand (COD) in a shorter hydraulic retention time (HRT) relative to conventional single stage digesters (Hartmann and Ahring, 2006). It has been reported by Parawira *et al.* (2005) that if the hydrolysis stage is optimized, it stimulates acidogenic bacteria to produce more precise enzymes that accelerate the breakdown of the feedstock . Consequently, the first step of the anaerobic digestion is assumed to be a pretreatment method.

In conventional biological pretreatment, the feedstock can undergo an aerobic pretreatment such as composting or micro-aeration before anaerobic digestion in order to get a higher production of hydrolytic enzymes that is induced by increased specific microbial growth (Lim and Wang, 2013). A study by Fdez-Guelfo *et al.* (2011) on composting OFMSW showed that specific microbial growth rate increased between 160-205% relative to thermo-chemical pretreatment of OFMSW . Another observation by Lim and Wang showed that aerobic pretreatment gives an increase in volatile fatty acid (VFA) formation because of the enhanced activities of the hydrolytic and acidogenic bacteria (Lim and Wang, 2013). Brummeler and Koster (1990), however, reported a loss of 19.5% volatile solids (VS) after pre-composting treatment of OFMSW. A loss of potential for methane production when sisal

pulp waste was subjected to a longer duration of aerobic pretreatment was reported by Mshandete A *et al.* (2005).

Miah *et al.* (2005) studied pretreatment of feedstock under aerobic thermophilic bacteria at 65^0C with fungus *Geobacillus thermodenitrificans* and reported the highest biogas production of 70ml/gVS having 80-90% methane content. Melamane *et al.* (2007) pretreated wine distillery wastewater with a fungus and obtained 53.3% removal of COD. Pure cultures of fungus *Trichoderma reseei* aerobically pretreated for four days with sisal leaf decortication residues gave 30-40% cumulative biogas (Muthangya *et al.*, 2009).

vi. A combination of different pretreatments

Every pretreatment method has a specific mechanism of solubilizing organic matter, and hence using a combination of different pretreatment methods can have a significant effect on the biodegradability of biomass. This combination has been studied with an aim of enhancing biogas production during anaerobic digestion (Lu *et al.*, 2008; Valo *et al.*, 2004).
Thermal-chemical pretreatment is one of the combinations. Shahriari *et al.* (2012) pretreated OFMSW using a combination of microwave irradiation at temperatures higher than 145^0C and chemical pretreatments; and reported a decrease in biogas production caused by a large component of refractory material per gram of chemical oxygen demand (gCOD). When pig manure was pretreated with lime and heated to a temperature higher than 110^0C, a similar trend was observed courtesy of the Maillard reaction (Carrere *et al.*, 2009; Rafique *et al.*, 2010).

Thermal-mechanical pretreatment has been studied to enhance anaerobic digestion of OFMSW, although this combination is unpopular (Ariunbaatar *et al.*, 2014). A high enhancement of biogas production of 17% was reported when rice straw was ground to 10mm and heated to 110^0C before undergoing anaerobic digestion (Melamane *et al.*, 2007). A study by Wett *et al.* (2010) (2010) on the disintegration of sludge pretreated at 19-21 bar pressure and 160-180^0C for 1 hour, and found out that biogas production increased by 75% at steady state, dewatering characteristics of the digestate improved, and consequently reduced the disposal cost by 25%.

2.4 Process Optimisation

Optimisation is the act of achieving the best possible result under given circumstances. In design, construction, maintenance and operation, engineers have to make decisions. The aim of such decisions is either to minimise the effort or to maximise benefit. The effort or benefit can be expressed as a function of certain design and operation variables. Hence optimisation is the process of finding conditions that give the minimum or the maximum value of a function (Astolfi and Praly, 2006).

There is no single method available for solving all optimisation problems efficiently. Therefore, a number of methods have been developed for solving different types of problems. Optimum seeking methods are also called mathematical programming techniques, which are a branch of operations research, comprising of the following areas (Du *et al.*, 2008):

i) Mathematical programming methods which are useful in finding the minimum of a function of several variables under a prescribed set of constraints.

ii) Stochastic process techniques that are used to analyse problems which are described by a set of random variables of known distribution.

iii) Statistical methods often used in the analysis of experimental data, and in the construction of empirical models.

The statement of an optimisation problem comprises of a design vector, constraints, and objective functions and may include:

a) Any system is described by a set of quantities, some of which are viewed as variables during the design process, and some are pre-assigned parameters or imposed by the environment. All the quantities that can be treated as variables are called design or decision variables, and are collected in the design vector x (Nocedal and Wright, 1999).

b) Design variables cannot be selected arbitrarily, but have to satisfy certain requirements. These requirements are called design constraints. Design constraints may represent limitation on the performance or behavior of the system. Constraints are side conditions that are used to specify the feasible set C within real numbers R. The constraints can be categorized as (Nocedal and Wright, 1999):

i. Equality constraints: Conditions of the form $f_i(x) = c_i$ for certain functions f_i on R and constant c_i in R.

ii. Inequality constraints: Conditions of the form $f_i(x) \leq c_i$ or $f_i(x) \geq c_i$ for certain functions f_i on R and constants c_i in R.

iii. Range constraints: Conditions restricting the values of some decision variables to within certain closed intervals of R. They are important in many situations, for instance the non-negativity constraints may only allow variables that take values ≥0. Range constraints can also arise from the desire to keep a variable between certain upper and lower bounds.

iv. Linear constraints: Range constraints or conditions of the form $f_i(x) = c_i$, $f_i(x) \leq c_i$ or $f_i(x) \geq c_i$ in which the function is linear in the standard sense of being expressible as the sum of constant coefficients times the variables $x_1 \ldots \ldots x_n$.

v. Data parameters: General problem statements usually involve not only decision variables but symbols designating known coefficients, constants, or data elements. Conditions on such elements, such as the non-negativity of a particular coefficient, are not among the constraints in a problem of optimisation since the numbers in question are supposed to be given and are not subject to choice.

c) The objective function aims at finding a design which satisfies the constraints. In general, there are acceptable designs, and the purpose of optimisation is to single out the best possible design. Thus, a criterion has to be selected for comparing different designs. This criterion, when expressed as a function of the design variables, is called an objective function (Nocedal and Wright, 1999).

An optimisation problem can be classified in several ways (Nocedal and Wright, 1999):

a) Constrained or unconstrained, depending on the presence or absence of constraints.

b) Linear, quadratic, polynomial or non-linear depending on the nature of the objective functions and the constraints. This classification is important because computational

methods are usually selected on the basis of such classification i.e. the nature of the functions involved dictate the type of procedure for finding the solution.

c) Integer or real valued, and deterministic or stochastic; depending upon the values permitted for the design variables.

A number of optimisation methods have been used in biogas studies including techniques such as Design of Experiment (DOE), Response Surface Methodology (RSM) with central composite design (CCD) and Box-Behnken design (BBD), have been used (including in this research) in the optimisation of agricultural and industrial biogas plants with respect to external and internal system variations and their effect on the rate and quality of methane produced from the fermentation and digestion of organic matter. Other techniques including artificial neural networks (ANN), and Taguchi have also been applied.

Park and Lek (2016) conceptualised that artificial neural networks (ANN) are biologically inspired computational networks based on the study of the brain and the nervous system, and are used to solve many real complex problems. These computations are based on multilayer perceptrons that involve a supervised procedure that consists of three layers namely the input, hidden, and output layers. Artificial Neural Network (ANN) coupling Genetic Algorithm (GA) was used by Kana *et al.* (2012) to model the non-linear behaviour of the anaerobic process and optimise biogas production from mixed substrates that included cow dung. An evaluation of the optimal profile showed an increase of 8.64% in biogas production over that predicted by the optimised substrate profile. Production from the non-optimised profile started on the 8^{th} day, compared to that of the 3^{rd} day from the optimised one. Basheer and Hajmeer (2000) and Thuiller (2003) found the limitations of ANN that include lack of fixed guidelines for optimal ANN architecture, its "black-box model" behavior, and insufficient concepts of ecology and relations.

The optimisation and control of systems such as the biochemical digestion of organic matter involving the use of microbial population with differing successions, poses challenges due to the underlying highly non-linear and complex processes. However, the flexibility and power of computational intelligence (CI) methods such as Genetic Algorithms (GAs) and Particle Swarm Optimisation (PSO) have been employed beyond the simpler empirical models based on accurate measurements and observations for modelling and simulation techniques.

2.5 Response Surface Methodology for Optimisation

Response surface methodology (RSM) is a combination of techniques used to statistically design data and numerically optimise designs of processes and products (Sinclair and Myers, 2004). This design technique was first applied by Box and Wilson (1951). Most of the underlying concepts had already been in use and had been discussed earlier in the 1930s (Mead and Pike, 1975). The problem in planning and analysis of experiments in a bid to find conditions desirable for a group of design variables was the motivation behind the work by Box and Wilson (Myers, 1989). Since then, there has been a rise and continuous evolution of the application of RSM in the research sphere (Sinclair and Myers, 2004).

Response surface methodology is one of the best techniques used to empirically study the relationship between one or more estimated response functions (Voznesensky, 1974). RSM makes use of statistical and mathematical methods to depict the domain of all practicable solutions for a process model, and once the model development is completed, process optimisation can be conducted free of a trial and error procedure (Box *et al.*, 1978). RSM comprises of selected statistical and mathematical methods used for analysis of problems in which the input variables influence the responses (Montgomery *et al.*, 2009). Regression analysis, based on polynomials, is used to define the relationship between the input variables and the responses.

RSM deals with surface estimation from initial preliminary observations, with the primary aim of establishing the input variable levels that can have the response maximised (Balkin and Lin, 2000). RSM is based on the assumption that the response Y has a relationship with a cluster of design variables x_1, x_2,….,x_n in addition to the fact that the approximation of the relationship can be done (using a polynomial model) in a certain section of inputs (Myers, 1989). The first order and second order models are the most common polynomial functions for approximation (Montgomery *et al.*, 2009). Equations 2.2 and 2.3 present the first order and second order polynomial functions respectively.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n + \epsilon \qquad 2.2$$

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j>i} \beta_{ij} X_i X_j + \epsilon \qquad 2.3$$

In general, the common error variance, σ^2 assumption is made and the ordinary least squares aids in the estimation of coefficients (Myers, 1989). According to Montgomery *et al.* (2009), the effective estimation of model parameters depends on the correct use of experimental designs for data collection. Response surface design is the terminology for designs used to fit response surfaces. The most common response surface designs are the central composite design (CCD), Box-Behnken design, and orthogonal design, which incorporates the fractional and 2^k factorial design points (Box and Behnken, 1960). The CCD was used during this research study. Polynomial models generally give a sensible true functional relationship approximation for a particular sub-area owing to its focus in a confined search space area. The assumption is that if the true response function can adequately be approximated by the fitted surface, then there is very little difference between the analysis of the fitted surface and that of the actual system (Montgomery *et al.*, 2009).

Response surface methodology has a sequential procedure and its aim is to quickly and efficiently guide the experimenter to the overall optimum domain. During the initial stages, a first-order model is fitted due to the fact that there is a likelihood that the domain being investigated could be far from the optimum, and consequently there is small curvature detection in the system. Consequently, the approach of steepest ascent is applied in order to provide guidance in the search for an operability region that is most favourable.

The steepest ascent is in the direction that is perpendicular to the response which is fitted, and matches with the direction which produces the most rapid increase in the fitted response. The stages along the course are proportionate to the approximated regression coefficients. The iteration of the procedure is carried out until the first order model lack of fit shows the optimum condition domain (Montgomery *et al.*, 2009).

After the location of the optimum region, the fitting of the second order model is conducted before the analysis for location of the optima is carried out. In the course of the entire procedure, the subsequent stage is planned using the information from the previous stage. There may be need to conduct extra runs to get additional information about the response in some sections of the domain that the initial design may have inadequately covered (Myers, 1989). Sequential design together with extra experimental runs fall in the design augmentation concept (Myers, 1989).

Design and analysis of computer experiments (DACE) is a statistical technique that was established by Sacks *et al.* (1989). DACE has found its application in the field of engineering as a solution to problems associated with expensive optimisation of designs such as electronic circuits design, computational fluid dynamics and analysis of finite elements (Bates, 1996; Buche *et al.*, 2005; Grierson and Pak, 1993; Lee and Hajela, 1996). Where processes are complex such that physical experimentation is extremely expensive, time consuming, or even impossible to conduct, then simulation of the system through mathematical modelling comes in handy (Sacks *et al.*, 1989). Although advances have been made in terms of computer capacity improvement, exorbitant computational cost renders complex engineering simulations impractical.

In DACE, data from an experimental design is used to build a surrogate or a metamodel (also known as model of model) before being optimised. Consequently, a larger simulator is used to model the physical process, and the computer code aids to directly conduct the experiments (Bates, 1996). The behaviour of the system can either be approximated by a deterministic or a stochastic model. For a stochastic simulation model, the experimentation, presents a behaviour similar to that of a physical system (Montgomery *et al.*, 2009). Conversely, there is a difference between experimentation using a deterministic model and experimentation of the physical system due to the fact there is no random error linked with the output. Ultimately, when the code is run with unchanged inputs, identical response values will be achieved (Sacks *et al.*, 1989). Sacks *et al.* (1989) were among the first scholars to introduce and pursue deterministic models, and they put forth the idea of using a statistical model that views the response as if it were a stochastic process realisation.

Gaussian stochastic process model is the name given to the statistical model which Sacks *et al.* (1989) adopted, with an advantage of providing fundamental statistics for computation of an efficient response predictor at untried inputs, and hence it permits the estimation of the uncertainty of the predictor. According to the proposal by Sacks *et al.* (1989), the concept is to select a design with high response predictability at untried inputs of the experimental domain and supplement it by use of extra design points. The aim of augmenting the design is to lay emphasis on sampling that has high uncertainty of the predictor so that scrutiny can be dedicated to the space parts that are not adequately explored (Jones *et al.*, 1998). Computer experiments, in contrast with conventional RSM, aims at finding a model for approximation of true response surface in a much broader span (in some cases extending over the whole

operability domain) of the design parameters. This consequently requires models that have more complexity in comparison with the first and second order polynomial models.

Non-parametric and semi-parametric response surface methodology (NPRSM) is best suited to situations in which the polynomial models in the domain of operability do not accommodate the response (Myers, 1989; Sinclair and Myers, 2004). This happens where there is need to fit a model that covers a section of the space of the factor, which happens to be bigger than the portion considered in the conventional RSM techniques (Sinclair and Myers, 2004). 'Non-parametric response surface methodology' means that there is an absence of a particular model, and that the analysis mainly focuses on prediction. This, therefore, leads to the application of kernel-based methods of regression. Conversely, Semi-parametric response surface methodology implies that there is the use of a model, but not of a standard polynomial nature (Sinclair and Myers, 2004). According to Myers (1989), the situations where Non-parametric and semi-parametric response surface methodology (NPRSM) can be applied include:

i. Where optimum conditions are not required

ii. If the interest in the response surface appearance is greater than the interest in the interpretative function

iii. Where the function exhibits nonlinearity, but not certainly well-behaved

iv. Where the designs must originate from a space-filling grid and do not have to honour the 'form of the model'

There exists a big difference in terms of various aspects (modelling, design and optimisation approaches) between RSM and NPRSM. However, the difference between NPRSM and Design and analysis of computer experiments (DACE) is not as clear. This is because the development of DACE as one of the modelling techniques was to help address the issue of high simulation costs of complex engineering processes (Sacks *et al.*, 1989). NPRSM has been used mainly in biotechnology and biopharmacy (Sinclair and Myers, 2004). At first, sequential sampling and deterministic stochastic Gaussian process were used in DACE but later, models like thin Plate splines and neural networks were developed. Henceforth, DACE may be seen as a component of NPRSM.

Developments have been made with regard to NPRSM and DACE, in the field of optimisation of algorithm parameters. Major techniques that have been introduced in this field include sequential optimisation of parameters (Bartz-Beielstein *et al.*, 2005) and effective global optimisation (Jones *et al.*, 1998). The main objective of these techniques is to help to quantify the significance of each variable and interactions between variables. In the case of multiple instances, these techniques provide a clear view of the interaction between instance properties and the parameters. These techniques also support the performance of interpolation within the variable settings as well as the extrapolation to parameter space domains that were hitherto undetected. These techniques play an important role as far as algorithm interpretation and enhancement is concerned (Hennink *et al.*, 2010; Hutter *et al.*, 2009).

In NPRSM, conventional RSM designs like CCD and factorial designs are commonly used (Myers et al., 2004). It is important, however, to note that conventional RSM designs are suitable in cases where the design domain is regular or if the experimenter is interested in a polynomial model. A regular domain of design can be depicted as a space surrounded by the p-dimensional hypersphere or hypercube, where every point within or on the sphere or cube is considered a possible design point. Due to practical problems associated with the operability, availability and feasibility of the system under study, there may be need to create holes in the design domain, slice portions of the sphere, or cut up the cube corners (Kennard and Stone, 1969). Moreover, when the experiments are time-consuming or expensive, the number of runs that can be achieved is reduced. This number of trials is relatively smaller than the number of conceptually possible experiments.

In the cases where there is a fixed model as well as the number of achievable trials, most RSM designs result in a big number of required replicates (Kennard and Stone, 1969). If there is an occurrence of nonstandard conditions like unusual requirements of the sample size or an irregular experimental domain, then computer-generated designs are preferable (Montgomery *et al.*, 2009). Computer-generated designs are commonly referred to as optimal designs because of their optimality with respect to a particular criterion. G-optimality, A-optimality and D-optimality are the commonly used optimality criteria (Montgomery *et al.*, 2009). D- and A-optimality focus on estimating the regression coefficients and use the minimisation of a function of the covariance matrix of the parameters' least squares estimates (Montgomery *et al.*, 2009; Santner *et al.*, 2003). G-optimal designs focus on response

prediction and their aim is to reduce the variance of the maximum scaled prediction in the design domain (Montgomery *et al.*, 2009).

The common procedure in computer-generated designs is to define the model (usually a polynomial model), establish the interest region, choose the number of runs to conduct, define the criterion of optimality, and finally select the points of design (that would interest the experimenter) from a group of preferred points. The preferred points are a network of points that are spaced over the practicable design domain (Montgomery *et al.*, 2009). With the development of DACE, there has been introduction of other criteria-founded designs. They are more complex to achieve in comparison with the designs mentioned earlier. Typically, the Gaussian stochastic model is presumed and the design criteria has a functional relationship with its unknown variables (Santner *et al.*, 2003). For a set number of trials and for a particular structure of correlation, these criteria of design aims at selecting a design that will give the best response prediction at untried inputs in the experimental domain.

2.6 Biogas Production Prediction Models

A model is a simplified abstract view of a complex reality. A scientific model represents empirical objects, phenomena, and physical processes in a logical way. For the scientist, a model is also a way in which the human thought processes can be amplified (Stockburger, 2006). MacKay (2004) defined a mathematical model as a representation of the essential aspects of an existing system which presents knowledge of that system in a usable form. Mathematical models are used not only in the natural sciences (such as physics, biology, earth science, meteorology) and engineering disciplines, but also in the social sciences (such as economics, psychology, sociology and political science); physicists, engineers, computer scientists, and economists use mathematical models most extensively.

A crucial part of the modeling process is the evaluation of whether or not a given mathematical model describes a system accurately. Rolland and Pernici (1998) evaluated a model by its consistency to empirical data and concluded that any model that is inconsistent with reproducible observations must be modified or rejected. They argued that a fit to empirical data alone was insufficient for a model to be accepted as valid; other factors included ability to explain past observations, ability to predict future observations, cost of use – especially in combination with other models, refutability, enabling estimation of the degree of confidence in the model, and simplicity, or even aesthetic appeal.

Assessing the scope of a model, that is, determining the situations in which the model can be applied can be straightforward. If the model was constructed based on a set of data, one must determine for which systems or situations the known data is a 'typical' set of data (MacKay, 2004). It has been observed that biogas models have been formulated for specific designs of digesters and conditions, and cannot be applied across board. Hence there is need to perform tests in order to adapt the models to various working conditions.

Some of the mathematical models used to relate the inputs (factors) to the output in a biogas system include:

a) Plug Flow Model
b) Chen and Hashimoto Model
c) Low Temperature Lagoon Digester Model
d) Toprak Model
e) Scoff and Minott Model

The application of some of these models was discussed and tested using available statistical indicators in Chapter 4 of this thesis.

References

Abbassi-Guendouz, A., Brockmann, D., Trably, E., Dumas, C. and Delgenès, J. (2012). Total solids content drives high solid anaerobic digestion via mass transfer limitation. *Bioresource Technology, 111*, 55-61.

Afazeli, H., Jafari, A., Rafiee, S. and Nosrati, M. (2014). An investigation of biogas production potential from livestock and slaughterhouse wastes. *Renewable and Sustainable Energy Reviews, 34*, 380-386.

Akunna, J., Abdullahi, Y. and Stewart, N. (2007). Anaerobic digestion of municipal solid wastes containing variable proportions of waste types. *Water Science Technology, 50*, 143-149.

Al Mamun, M. and Torii, S. (2015). Enhancement of production and upgrading of biogas using different techniques - A Review. *International Journal of Earth Sciences and Engineering, 8*(2), 877-892.

Alvarez, J., Otero, L. and Lema, J. (2010). A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. *Bioresource Technology, 101*, 1153-1158.

Angelidaki, I. and Ahring, B. (2000). Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water Science Technology, 41*, 189-194.

APHA. (1999). Standard Methods for the Examination of Water and Wastewater. *American Public Health Association*.

Appels, I., Degreve, J., Bruggen, B., Impe, J. and Dewil, R. (2010). Influence of low temperature thermal pretreatment on sludge solubilization, heavy metal release and anaerobic digestion. *Bioresource Technology, 101*, 5743-5748.

Ardaji, V., Radnezhad, H. and Nourouzi, M. (2016). Improving biogas production performance from pomegranate waste, poultry manure and cow dung sludge using thermophilic anaerobic digestion: Effect of total solids adjustment. *Journal of Earth, Environment and Health Sciences, 2*(3), 97-109.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F. and Lens, P. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy, 123*, 143-156.

Assefa, A., Egigu, M. and Kebede, A. (2014). Thermal and chemical pre-treatments of cow dung and poultry litter enhance biogas production in batch fermentation. *International Journal of Scientific and Technology Research, 3*, 165-170.

Astolfi, A. and Praly, L. (2006). Global complete observability and output-to-state stability imply the existence of a globally convergent observer. *Mathematics of Control, Signals and Systems, 18*(1), 32-65.

Balasubramaniyam, U., Zisengwe, L., Meriggi, N. and Buysman, E. (2008). Biogas production in climates with long winters. *WECF*.

Balkin, S. D. and Lin, D. K. (2000). A neural network approach to response surface methodology. *Communications in Statistics-Theory and Methods, 29*(9-10), 2215-2227.

Barjenbruch, M. and Kopplow, O. (2003). Enzymatic, mechanical and thermal pretreatment of surplus sludge. *Advanced Environmental Resources, 7*, 715-720.

Bartz-Beielstein, T., Lasarczyk, C. W. and Preuß, M. (2005). *Sequential parameter optimization.* Paper presented at the 2005 IEEE congress on evolutionary computation.

Basheer, I. and Hajmeer, M. (2000). Artificial neural networks: fundamentals, computing, design, and application. *Journal of Microbiological Methods, 43*(1), 3-31.

Bates, D. S. (1996). Jumps and stochastic volatility: Exchange rate processes implicit in deutsche mark options. *The Review of Financial Studies, 9*(1), 69-107.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S., Pavlostathis, S., Rozzi, A., Sanders, W., Siegrist, H. and Vavilin, V. (2002). The IWA anaerobic digestion model no 1 (ADM1). *Water Science and Technology, 45*(10), 65-73.

Batzias, F., Sidiras, D. and Spyrou, E. (2005). Evaluating livestock manures for biogas production: A GIS method. *Renewable Energy, 30*, 1161-1176.

Bauer, A., Moeller, L., Wedwitschka, H., Stinner, W. and Zehnsdorf, A. (2018). Anaerobic digestion of mixed silage of waterweed biomass and wheat straw in a long-term semi-continuous biogas production process. *Energy, Sustainability and Society, 8*(4). doi:10.1186/s13705-017-0145-9

Bischofsberger, W., Dichtl, N., Rosenwinkel, K., Seyfried, C. and Böhnke, B. (2005). A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Bioresource Technology, 56*(2-3), 229-237.

Björnsson, L., Murto, M. and Mattiasson, B. (2000). Evaluation of parameters for monitoring an anaerobic co-digestion process. *Applied Microbiology and Biotechnology, 54*(6), 844-849.

Bochmann, G., Drosg, B., Ortner, M., Schonlieb, M., Andres-Lainez, S., Kirchmayr, R. and Braun, R. (2010). *Influence of thermal pre-treatment to increase digestibility of brewers' spent grains.* Paper presented at the 12th World Congress on Anaerobic Digestion, Guadalajara.

Bochmann, G. and Montgomery, L. (2014). *Pretreatment of Feedstock for Enhanced Biogas Production*. Retrieved from Paris, France.

Boe, K. (2006). *On-line monitoring and control of the biogas process.* (PhD), Technical University of Denmark, Lyngby, Denmark.

Böjti, T., Kovács, K., Kakuk, B., Wirth, R., Rákhely, G. and Bagi, Z. (2017). Pretreatment of poultry manure for efficient biogas production as monosubstrate or co-fermentation with maize silage and corn stover. *Anaerobe, 46*, 138-145.

Bolaji, O., Awonorin, S., Shittu, T. and Sanni, L. (2017). Changes induced by soaking period on the physical properties of maize in the production of Ogi. *Cogent Food and Agriculture, 3*(1), 132-157.

Bolzonella, D., Pavan, P., Mace, S. and Cecchi, F. (2006). Dry anaerobic digestion of differently sorted organic municipal solid waste: a full-scale experience. *Water Science Technology, 53*, 23-32.

Bouallagui, H., Ben, C., Marouani, L. and Hamdi, M. (2003). Mesophilic biogas production from fruit and vegetable waste in a tabular digester. *Bioresource Technology, 86*, 85-89.

Bougrier, C., Albasi, C., Delgenes, J. and Carrere, H. (2006). Effect of ultrasonic, thermal and ozone pretreatments on waste activated sludge solubilization and biodegradability. *Chemical Engineering Process, 45*, 711-718.

Box, G. and Behnken, D. (1960). Simplex-sum designs: a class of second order rotatable designs derivable from those of first order. *The Annals of Mathematical Statistics, 31*(4), 838-864.

Box, G., Hunter, W. and Hunter, J. (1978). *Statistics for Experimenters*.

Box, G. and Wilson, K. (1951). On the experimental attainment of optimum conditions. *Journal of the Royal Statistical Society: Series B (Methodological), 13*(1), 1-38.

Bridgeman, J. (2012). Computational fluid dynamics modeling of sewage sludge mixing in an anaerobic digester. *Advances in Engineering Software, 44*(1), 54-62.

Brummeler, E. and Koster, J. (1990). Enhancement of dry anaerobic batch digestion of organic fraction of municipal solid waste. *Biological Wastes, 31*, 199-210.

Buche, D., Schraudolph, N. N. and Koumoutsakos, P. (2005). Accelerating evolutionary algorithms with Gaussian process fitness function models. *IEEE Transactions on Systems, Man, and Cybernetics, Part C (Applications and Reviews), 35*(2), 183-194.

Burke, D. (2001). *Dairy waste anaerobic digestion handbook: Options for recovering beneficial products from dairy manure.*

Buysman, E. (2009). *Anaerobic digestion for developing countries with cold climates.* (MSc), Wageningen, Netherlands.

Carballa, M., Monterola, G., Larrea, I., Temes, T., Omil, F. and Lema, J. (2007). Influence of ozone pretreatment on sludge anaerobic digestion: removal of pharmaceutical and personal care products. *Chemosphere, 67*, 1444-1452.

Carlsson, M. and Anox, K. (2008). *Electroporation for enhanced methane yield from municipal solid waste*. Paper presented at the ORBIT 2008, Wageningen.

Carlsson, M., Lagerkvist, A. and Morgan-Sagastume, F. (2012). The effects of substrate pretreatment on anaerobic digestion: a review. *Waste Management, 32*, 1634-1650.

Carrère, H., Dumas, C., Battimelli, A., Batstone, D., Delgenès, J., Steyer, J. and Ferrer, I. (2010). Pretreatment methods to improve sludge anaerobic degradability: A review. *Journal of Hazardous Materials, 183*, 1-15.

Carrere, H., Sialve, B. and Bernet, N. (2009). Improving pig manure conversion into biogas by thermal and thermal-chemical pretreatments. *Bioresource Technology, 100*, 3690-3694.

Cesaro, A. and Belgiorno, V. (2013). Sonolysis and ozonation as pretreatment for anaerobic digestion of solid waste. *Ultrason Solochem, 20*, 931-936.

Cesaro, A. and Belgiorno, V. (2014). Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. *Chemical Engineering Journal, 240*, 24-37.

Chamchoi, N., Garcia, H. and Angelidaki, I. (2011). Methane potential of household waste: batch assays determination. *Journal of Environmental Resources, 33*, 13-26.

Chen, S., Li, R. and Li, X. (2009). Anaerobic co-digestion of kitchen waste and cattle manure for methane production. *Energy Sources, 31*, 1848-1856.

Chua, C., Lee, D., Chang, B.-V., You, C. and Tay, J. (2002). "Weak" ultrasonic pre-treatment on anaerobic digestion of flocculated activated biosolids. *Water Resources, 36*, 2681-2688.

Climent, M., Ferrer, I., Del Mar Baeza, M., Artola, A., Vázquez, F. and Font, X. (2007). Effects of thermal and mechanical pretreatments of secondary sludge on biogas production under thermophilic conditions. *Chemical Engineering Journal, 133*(1-3), 335-342.

Dahunsi, S. and Orunsi, U. (2013). Co-digestion of food waste and human excreta for biogas production. *British Biotechnology Journal, 3*(4), 485-499.

Davidson, A., Gruvberger, C., Christenssen, T., Hansen, T. and Jansen, J. (2007). Methane yield in source-sorted organic fraction municipal solid waste. *Waste Management, 27*, 406-414.

Daxiong, Q., Shuhua, G., Baofen, L. and Gehua, W. (1990). Diffusion and innovation in the Chinese biogas programme. *World Development, 18*(4), 555-563. doi:10.1016/0305-750X(90)90071-5

Derbal, K., Bencheikh-Lehocine, M., Ceecchi, F., Meniai, A. and Pavan, P. (2009). Application of the IWA ADM1 model to simulate anaerobic co-digestion of aorganic waste with waste- activated sludge in mesophilic condition. *Bioresource Technology, 100*, 1539-1543.

Deublein, D. and Steinhauser, A. (2008). *Biogas from Waste and Renewable Resources: An Introduction*. Weinheim, Germany.

Dioha, I., Ikeme, C., Nafi'u, T., Soba, N. and Yusuf, M. (2014). Effect of carbon to nitrogen ratio on biogas production. *International Research Journal of Natural Sciences, 1*(3), 1-10.

Dong, R. (2004). *Energy – The most important resource for social development.* Paper presented at the International Course on New Technology Popularizing Agricultural Machinery, China Agricultural University, Beijing.

Du, D., Pardalos, P. and Wu, W. (2008). History of Optimization. In C. Floudas & P. Pardalos (Eds.), *Encyclopedia of Optimization* (pp. 1538-1542). Boston: Spinger.

Edelmann, W., Baier, U. and Engeli, H. (2005). Environmental aspects of the anaerobic digestion of organic fraction of municipal solid waste and agricultural waste. *Water Science Technology, 52*, 553-559.

Eder, B. and Schulz, H. (2006). *Biogas Praxis [in German]* (3rd ed.).

Elliot, A. and Mahmood, T. (2007). Pretreatment technologies for advancing anaerobic digestion of pulp and paper biotreatment residue. *Water Resources, 41*, 4273-4286.

Elliot, A. and Mahmood, T. (2012). Comparison of mechanical pretreatment methods for the enhancement of anaerobic digestion of pulp and paper waste. *Water Science Technology, 84*, 497-505.

Engelhart, M., Kruger, M., Kopp, J. and Dichtl, N. (2000). Effects of disintegration on anaerobic digestion of sewage excess sludge in down-flow stationary fixed film digesters. *Water Science Technology, 41*, 171-179.

EPA. (1992). Global methane emissions from livestock and poultry manure. *United States Environment Protection Agency, 400*(1091).

EPA. (2001). Method 1684: Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids. *U.S. Environmental Protection Agency*.

EPA. (2005). Anaerobic Digestion: Benefits for Waste Management, Agriculture, Energy, and the Environment. *U.S. Environment Protection Agency*.

Esposito, G., Frunzo, I., Panico, A. and Pirozzi, F. (2011). Modeling the effect of organic loading rate and organic fraction of municipal solid waste particle size on performances of an anaerobic co-digestion reactor. *Process Biochemistry, 46*, 557-565.

Fayyaz, A., Qaisar, M., Naim, R., Arshid, P., Iftikhar, R. and Mohammad, M. (2015). Co-digestion, pretreatment and digester design for enhanced methanogenesis. *Renewable and Sustainable Energy Reviews, 42*, 627-642.

Fdez-Guelfo, L., Alvarez-Gallego, C., Sales, M. and Romero, G. (2011). The effect of different pretreatments on biomethanation kinetics of industrial organic fraction of municipal solid wastes (OFMSW). *Chemical Engineering Journal, 171*, 411-417.

Ferguson, R., Coulon, F. and Villa, R. (2016). Organic loading rate: A promising microbial management tool in anaerobic digestion. *Water Research, 100*, 348-356.

Ferguson, R., Villa, R. and Coulon, F. (2014). Bioengineering options and strategies for optimization of anaerobic digestion processes. *Environmental Technology, 3*, 1-14.

Fernandes, T., Klaasse Bos, B., Zeeman, G., Sander, J. and Lier, J. (2009). Effects of thermal-chemical pretreatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass. *Bioresource Technology, 100*, 2575-2579.

Ferrer, I., Ponsá, S., Vázquez, F. and Font, X. (2008). Increasing biogas production by thermal sludge pre-treatment prior to thermophilic anaerobic digestion. *Biochemical Engineering Journal*(42), 186-192.

Gallert, C., Henning, A. and Winter, J. (2003). Scale-up of anaerobic digestion of the biowaste fraction from domestic wastes. *Water Resources*(37), 1433-1441.

Garcia-Ochoa, F., Santos, V., Naval, L., Guardiola, E. and Lopez, B. (1999). Kinetic model for anaerobic digestion of livestock manure. *Enzyme Microbiology and Technology*, *25*, 55-60.

Garrote, G., Dominguez, H. and Parajo, J. (1999). Hydrothermal processing of lignocellulosic materials. *Holz als Roh-und Werkstoff, 57*(3), 191-202.

Gashaw, A. and Teshita, A. (2014). Co-digestion of Ethiopian food waste with cow dung for biogas production. *International Journal of Research, 1*(7), 475-500.

Ge, H., Jensen, P. and Batstone, D. (2010). Pre-treatment mechanisms during thermophilic–mesophilic temperature phased anaerobic digestion of primary sludge. *Water Resources*(44), 123-130.

Ge, H., Jensen, P. and Batstone, D. (2011a). Increased temperature in thermophilic stage in temperature phased anaerobic digestion (TPAD) improves degradability of waste activated sludge. *Hazardous Material, 187,* 355-361.

Ge, H., Jensen, P. and Batstone, D. (2011b). Temperature phased anaerobic digestion increse apparent hydrlysis rate for waste activated sludge. *Water Resources, 45*, 1597-1606.

Ghanimeh, S., Fadel, M. and Saikaly, P. (2012). Mixing effect on thermophilic anaerobic digestion of source-sorted organic fraction municipal solid waste. *Bioresource Technology*, *117*, 63-71.

Ghimire, P. (2013). SNV supported domestic biogas programmes in Asia and Africa. *Renewable Energy, 49*, 90-94. doi:10.1016/j.renene.2012.01.058

Gisemba, J. and Barasa, M. (2019). Effect of bioslurry utilization and application methods on the African stalk borer (Buseiola fusca) and yield of maize. *International Journal of Science and Nature, 10*(3), 125-131.

Goel, R., Tokutomi, T., Yasui, H. and Noike, T. (2003). Optimal process configuration for anaerobic digestion with ozonation. *Water Science Technology, 8*, 85-96.

Gonzalez-Fernandez, C., Salve, B., Bernet, N. and Steyr, J. (2012). Thermal pretreatment to improve methane production of Scenedesmus biomass. *Biomass Bioenergy, 40*, 105-111.

Grady, C., Daigger, G. and Lim, H. (1999). Biological wastewater treatment: Principles and practice. New York: Marcel Dekker, New York.

Gray, N. (2004). *Biology of waste water treatment* (2nd ed.). London: Imperial College Press.

Green, J. and Sibisi, M. (2002). *Domestic biogas digesters: a comparative study*.

Grierson, D. and Pak, W. (1993). Optimal sizing, geometrical and topological design using a genetic algorithm. *Structural Optimization, 6*(3), 151-159.

Gujer, W. and Zehnder, A. (1983). Conversion processes in anaerobic digestion. *Water Science and Technology, 15*, 127-167.

Gunaseelan, V. (1997). Anaerobic digestion of biomass for methane production: A review. *Biomass and Bioenergy, 13*(1-2), 83-114.

Gupta, R. and Singh, N. (1990). Community biogas plants in India. *Biological Wastes, 32*, 149-153.

Halalsheh, M., Kassab, G., Yazajeen, H., Qumsieh, S. and Field, J. (2011). Effect of increasing the surface area of primary sludge on anaerobic digestion at low temperature. *Bioresource Technology, 102*(2), 748-752.

Hamad, M., Abdel-Dayem, A. and El-Halwagi, M. (1981). Evaluation of the performance of two rural biogas units of Indian and Chinese design. *Energy Agriculture, 1*, 235-250.

Hansen, C., Schmidt, T., Angelidaki, J., Marca, I., Jansen, J., Mosbaek, H. and Christensen, T. (2004). Method for determination of methane potentials of solid organic waste. *Waste Management, 24*, 393-400.

Hartmann, H. and Ahring, B. (2006). Strategies for the anaerobic digestion of the organic fraction of municipal solid waste: an overview. *Water Science Technology, 53*, 7-22.

Hartmann, H., Angelidaki, I. and Ahring, B. (2000). Increase of anaerobic degradation of particulate organic matter in full-scale biogas plants by mecanical maceration. *Water Science Technology, 41*, 145-153.

Hendriks, A. and Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology, 100*, 10-18.

Hennink, M., Hutter, I. and Bailey, A. (2010). *Qualitative Research Methods*: Sage.

Huba, E., Cheng, S., Li, Z. and Mang, H. (2013). A review of prefabricated biogas digesters in China. *Renewable and Sustainable Energy Review, 28*, 738-748. doi:10.1016/j.rser.2013.08.030

Hutter, F., Hoos, H. H., Leyton-Brown, K. and Stützle, T. (2009). ParamILS: an automatic algorithm configuration framework. *Journal of Artificial Intelligence Research, 36*, 267-306.

Izumi, K., Okishio, Y., Nagao, N., Niwa, C., Yamamoto, S. and Toda, T. (2010). Effects of particle size on anaerobic digestion of food waste. *International Journal of Bio-deterioration and Biodegradation, 64*(7), 601-608.

Jahng, D., Lee, Y. and Zhang, L. (2011). Anaerobic co-digestion of food waste and piggery wastewater: focusing on the role of trace elements. *Bioresource Technology, 102*(8), 5048-5059.

Jash, T. and Gosh, D. (1990). Studies on residence time distribution in cylindrical and rectangular biogas digesters. *Energy, 15*, 987-991.

Jegede, A., Zeeman, G. and Bruning, H. (2019). A review of mixing, design and loading conditions in household anaerobic digesters. *Critical Reviews in Environmental Science and Technology, 49*(22), 2117-2153. doi:10.1080/10643389.2019.1607441

Jin, P., Bhattacharya, S., Williama, C. and Zhang, H. (1998). Effects of sulphide addition on copper inhibition in methanogenic systems. *Water Resources, 32*, 977-988.

Jones, D., Schonlau, M. and Welch, W. (1998). Efficient global optimization of expensive black-box functions. *Journal of Global Optimisation, 13*(4), 455-492.

Kana, E., Oloke, J., Lateef, A. and Adesiyan, M. (2012). Modeling and optimization of biogas production on saw dust and other co-substrates using artificial neural network and genetic algorithm. *Renewable Energy, 46*, 276-281.

Karapaju, P. and Rintala, J. (2008). Effect of solid-liquid separation on recovering residual methane and nitrogen of a digested dairy cow manure. *Bioresource Technology, 99*, 120-127.

Karim, K., Klasson, K., Drescher, S., Ridenour, W., Borole, A. and Al-Dahhan, M. (2007). Mesophilic digestion kinetics of manure slurry. *Applied biochemistry and biotechnology, 142*, 231-242.

Kennard, R. W. and Stone, L. A. (1969). Computer aided design of experiments. *Technometrics, 11*(1), 137-148.

Khanal, S. (2008). *Anaerobic Biotechnology for Bioenergy Production: Principles and Applications* (S.K. Khanal ed.). New York, USA: John Wiley and Sons

Kianmahr, P., Parker, W. and Seto, P. (2010). An evaluation of prtocols for characterization of ozone impacts on waste agricultural solids properties and digestibility. *Bioresource Technology, 101*, 8565-8572.

Kobayashi, T. and Li, Y.-Y. (2011). Performance and characteriztion of a newly developed self-agitated anaerobic reactor with biological desulphurization. *Bioresource Technology, 102*(10), 5580-5588. doi:10.1016/biortech.2011.01.077

Koettner, M. (2002, 18-20 Sept 2002). *Biogas and fertilizer production from solid waste and biomass through dry fermentation in batch method.* Paper presented at the 3rd

International Symposium on Anaerobic Digestion of Solid Wastes, Technical University of Munich, Munich, Germany

Kratky, L. and Jirout, T. (2011). Biomass size reduction machines for enhancing biogas production. *Chemical Engineering and Technology, 34*(3), 391-399.

Laichena, J. and Wafula, J. (1997). Biogas technology for rural households in Kenya. *OPEC Reviews, 21*, 223-244.

Lee, J. and Hajela, P. (1996). Parallel genetic algorithm implementation in multidisciplinary rotor blade design. *Journal of Aircraft, 33*(5), 962-969.

Leenawat, A., Pasakorn, J., Atip, L., Rodriguez, J. and Khanita, K. (2016). *Effect of temperature on increasing biogas production from sugar industrial wastewater treatment by UASB process in pilot scale*. Paper presented at the 3rd International Conference on Power and Energy Systems Engineering, CPESE, Kitakyushu, Japan.

Lemmer, A., Naegele, H.-J. and Sondermann, J. (2013). How Efficient are Agitators in Biogas Digesters? Determination of the Efficiency of Submersible Motor Mixers and Incline Agitators by Measuring Nutrient Distribution in Full-Scale Agricultural Biogas Digesters. *Energies*(6), 6255-6273. doi:10.3390/en6126255

Lettinga, G., Field, J., Van Lier, J., Zeeman, G. and Pol, L. H. (1997). Advanced anaerobic wastewater treatment in the near future. *Water Science and Technology, 35*(10), 5-12.

Li, H., Li, C., Liu, W. and Zou, S. (2012). Optimized alkaline pretreatment of sludge before anaerobic digestion. *Bioresource Technology*(123), 189-194.

Lim, J. and Wang, J.-Y. (2013). Enhanced hydrolysis and methane yield by applying micro aeration pretreatment to anaerobic co-digestion of brown water and food waste. *Waste Management, 33*, 813-819.

Lippmann, M., Frampton, M., Schwartz, J., Dockery, D., Schlesinger, R., Koutrakis, P., Froines, J., Nel, A., Finkelstein, J. and Godleski, J. (2003). The US Environmental Protection Agency Particulate Matter Health Effects Research Centers Program: a midcourse report of status, progress, and plans. *Environmental Health Perspectives, 111*(8), 1074-1092.

Lopez-Torres, M. and Llorens, M. (2008). Effect of alkaline pretreatment on anaerobic digestion of solid wastes. *Waste Management, 28*, 2229-2234.

Lorenzo, B., Selene, G., Alessandro, S. and Fabio, F. (2013). Innovative two-stage anaerobic process for effective co-digestion of cheese whey and cattle manure. *Bioresource Technology, 128*, 779-783.

Lu, J., Gavala, H., Skiadas, I., Mladenovska, Z. and Ahring, B. (2008). Improving anaerobic sewage sludge digestion by implimentation of hyper-thermophilic pre-hydrolysis step. *Journal of Environmental Management, 88*, 881-889.

Ma, J., Duong, T., Smits, M., Vestraete, W. and Carballa, M. (2011). Enhanced biomethanation of kitchen waste by different pretreatments. *Bioresource Technology, 102*, 592-599.

MacKay, D. (2004). *Information Theory, Inference, and Learning Algorithms*. Cambridge: Cambridge University Press.

Marti-Herero, J. (2011). Reduced hydraulic times in low-cost tabular digesters: Two issues. *Biomass Bioenergy, 35*, 4481-4484.

Mata-Alvarez, J., Dosta, J., Mace, S. and Astals, S. (2010). Co-digestio of solid wastes: A review of its uses and perspectives including modeling. *Critical Reviews in Biotechnology, 1*, 1-13. doi:10.3109/07388551.2010.525496

Mattsson, M., Wang, L., Rundstedt, J. and Karlson, N. (2011). *Different pretreatments to enhance biogas production.* (Master of Science), Halmstad.

Mead, R. and Pike, D. (1975). A biometrics invited paper. A review of response surface methodology from a biometric viewpoint. *Biometrics, 31*(4), 803-851.

Melamane, A., Tandlich, R. and Burgess, J. (2007). Anaerobic digestion of fungally pretreated wine distillery waste water. *African Journal of Biotechnology, 6*, 1990-1993.

Miah, M., Tada, C. and Yang, Y. (2005). Aerobic thermophilic bacteria enhance biogas production. *Journal of Material Cycles Waste Management, 7*, 48-54.

Mohammad, N. (1991). Biogas plants construction technology for rural areas. *Bioresource Technology, 35*, 283-289.

Molenbach, A. and Nokes, S. (2012). The use of high solids loading in biomass pretreatment - A review. *Biotechnology Bioenergy, 109*, 1430-1442.

Møller, H., Sommer, S. and Ahring, B. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy, 26*, 485-495.

Monlau, F., Barakat, A., Steyer, J. and Carrere, H. (2012). Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. *Bioresource Technology, 120*, 241-247.

Monnet, F. (2003). *An Introduction to Anaerobic Digestion of Organic Wastes*. Scotland.

Montanes, R., Soleria, R. and Perez, M. (2015). Anaerobic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: Effect of temperature. *Bioresource Technology, 180*, 177-184.

Montgomery, D. C., Runger, G. C. and Hubele, N. F. (2009). *Engineering Statistics*: John Wiley and Sons.

Mshandete A, Björnsson, L., Kavaisi, A., Rubindamayugi, S. and Mattiasson, B. (2005). Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pretreatment. *Water Resources, 39*, 1569-1575.

Murphy, G. and Kumar, D. (2011). Impact of pretreatment and downstream processing technologies on economics and energy in cellulosic ethanol production. *Biotechnology for Biofuels, 4*, 27-34.

Musoline, W., Esposito, G., Giordano, A. and Lens, P. (2012). Anaerobic digestion of rice straw - a review. *Critical Review in Environmental Science Technology, 12*, 2605-2611. doi:10.1080/10643389.627018

Muthangya, M., AM, M. and Kavaisi, A. (2009). Enhancement of anaerobic digestion of sisal leaf decortication residues by biological pretreatment. *APRN Journal of Agricultural and Biological Sciences, 4*, 66-73.

Myers, G. (1989). The pragmatics of politeness in scientific articles. *Applied Linguistics, 10*(1), 1-35.

Nasir, I., Ghazi, T., Omar, R. and Idris, A. (2014). Bioreactor performance in anaerobic digestion of cattle manure: A Review. *Energy Source: Part A, 36*, 1476-1483. doi:10.1080/15567036.2010.542439

Neczaj, E., Bien, J., Grosser, A., Worwag, M. and Kacprzak, M. (2012). Anaerobic treatment of sewage sludge and grease trap sludge in continuous co-digestion. *Global NEST Journal, 14*(2), 142-148.

Neibling, H. and Chen, L. (2014). *Anaerobic Digestion Basics*. (6). Moscow.

Neves, I., Ribeiro, R., Oliveira, R. and Alves, M. (2006). Enhancement of methane production from barley waste. *Biomass Bioenergy, 30*, 599-603.

Nijaguna, B. (2006). *Biogas Technology*. New Delhi, India: New Age International.

Nkemka, V. and Murto, M. (2010). Evaluation of biogas production from seaweed in batch tests and in UASB reactors combined with the removal of heavy metals. *Journal of Environmental Management, 91*, 1573-1579.

Nocedal, J. and Wright, S. (1999). Numerical optimization. New York: Springer.

Nyaanga, D., Barasa, H. and Gisemba, J. (2015, 11-15 May). *Current and future of biogas contribution to the energy needs of Kenya.* Paper presented at the NACOSTI Proceedings of Conference, University of Nairobi.

Nzila, C., Njuguna, D., Madara, D., Muasya, R., Githaiga, J., Muumbo, A. and Kiriamiti, H. (2015). Characterization of agro-residues for biogas production and nutrient recovery in Kenya. *Journal of Emerging Trends in Engineering and Applied Sciences, 6*(5), 327-334.

Panaud, V., Delgenes, J. and Moletta, R. (1999). Thermal-chemical pretreatment of a microbial biomass: influence of sodium hydroxide addition on solubilization and biodegradability. *Enzyme Microbiology and Techology, 25*, 258-263.

Pannucharoenwong, N. (2018). Optimization of Bio-Methane Production from Mesophilic Anaerobic Co-digestion of Pig Manure and Vegetable Residue. *International Journal of Applied Engineering Research, 13*(4), 1988-1995.

Parawira, W., Murto, M., Read, J. and Mattiasson, B. (2005). Profile of hydrolyses and biogas production during two stage mesophilic anaerobic digestion of solid potato waste. *Process Biochemistry, 40*, 2945-2952.

Park, Y. and Lek, S. (2016). Artificial Neural Networks: Multilayer Perceptron for Ecological Modeling. In Developments in environmental modeling. *Elsevier., 28*, 123-140.

Park, Y., Li, Y. and Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Review, 15*, 821-826.

Patharwat, I., Kumbhar, S., Hingalaje, P., Khurpe, A., Goudgaon, J. and Dhamangaonkar, P. (2016). Analysis of optimum temperature and validity of biogas plant. *International Journal of Industrial Electronics and Electrical Engineering, 4*(4), 76.

Patil, J., AntonyRaj, M. and Gavimath, C. (2011). Study on effect of pretreatment methods on biomethanation of water hyacinth. *International Journal of Advanced Biotechnology Resources, 2*, 143-147.

Pavlostathis, S. and Giraldo-Gomez, E. (1991). Kinetics of anaerobic treatment. *Water Science and Technology, 24*(8), 35-59.

Pavlostathis, S. and Giraldo- Gomez, E. (1991). Kinetics of anaerobic treatment: a critical review. *Critical Reviews in Environmental Science and Technology, 21*(5-6), 411-490.

Perez-Elvira, S., Nieto, D. and Fdz-Palanco, F. (2006). Sludge minimization technologies. *Reviews in Environmental Science and Biotechnology, 5*, 375-398.

Phillips, K., Silgram, M., Newell-Price, P., Warret, M., Provey, G., Cottril, B. and Newton, J. (2008). *The environmental impact of livestock production*. Report ADAS 2007, Deffra FFG, 1-96.

Pinnekamp, J. (1989). Effects of thermal pretreatment of sewage sludge on anaerobic digestion. *Water Science Technology, 21*, 97-108.

Protot, A., Julien, I., Christopher, D. and Patrick, I. (2011). Sludge disintegration during heat treatment at low temperature: a better understanding of involved mechanisms with a multi-parametric approach. *Biochemical Engineering Journal, 54*, 178-184.

Rafique, R., Poulse, T., Nizami, A.-S., Assam, Z., Murphy, J. and Kiely, G. (2010). Effect of thermal, chemical, thermal-chemical pretreatments to enhance methane production. *Energy, 35*, 4556-4561.

Rajendran, K., Aslanzadeh, S. and Taherzadeh, M. (2012). Household biogas digesters—A review. *Energies, 5*(8), 2911-2942.

Ras, S., Veijanen, A. and Rintala, J. (2007). Trace compounds of biogas from different biogas production plants. *Journal of Energy, 32*(8), 1375-1380.

Regattieri, A., Bortolini, M., Ferrari, E., Gamberi, M. and Piana, F. (2018). Biogas micro-production from human organic waste—A research proposal. *Sustainability, 10*(2), 330-336.

Rincon, B., Borja, R., Gonzalez, J., Portillo, M. and Saiz-Jimenez, C. (2008). Influence of organic loading rate and hydraulic retention time on the performance, stability, and microbial communities of one-stage anaerobic digestion of a two-stage olive mill solid residue. *Biochemical Engineering Journal, 40*, 253-261.

Rolland, C. and Pernici, T. (1998). *A Comprehensive View of Process Engineering.* Paper presented at the 10th International Conference CAiSE'98, Pisa Italy.

Rusanowska, P., Zieliński, M., Dudek, M. and Dębowski, M. (2018). Mechanical pretreatment of lignocellulosic biomass for methane fermentation in innovative reactor with cage mixing system. *Journal of Ecological Engineering, 19*(5), 219-224.

Sacks, J., Welch, W. J., Mitchell, T. J. and Wynn, H. P. (1989). Design and analysis of computer experiments. *Statistical Science*, 409-423.

Safley, L. and Westerman, P. (1992). Performance of a low temperature lagoon digester. *Bioresource Technology, 41*, 167-175.

Saint-Joly, C., Desbois, S. and Lotti, J. (2000). Determinant impact of waste collection and composition on anaerobic digestion performance: industrial results. . *Water Science Technology*(41), 291-297.

Saleh, A., Kamarudin, E., Yaacob, A., Yussof, A. and Abdullah, M. (2012). Optimization of biomethane production by anaerobic digestion of palm oil mill effluent using response surface methodology. *Asia-Pacific Journal of Chemical Engineering*(7), 353-360.

Sandino, J., Santha, H., Rogowiski, S., Anderson, W., Sung, S. and Isik, F. (2005). Applicability of ultrsound pre-conditioning of waste activated sludge to reduce foaming potential in mesophilic digesters. *Residuals Biosolids Management, 17*, 819-835.

Santerre, M. and Smith, K. (1982). Measures of appropriateness: the resource requirements of anaerobic digestion (biogas) systems. *World Development, 10*, 239-261.

Santner, T. J., Williams, B. J. and Notz, W. (2003). *The design and analysis of computer experiments* (Vol. 1): Springer.

Schön, M. (2010). *Numerical modelling of anaerobic digestion processes in agricultural biogas plants* (Vol. 6): BoD–Books on Demand.

Sebola, M., Tesfagiorgis, H. and Muzenda, E. (2015). *Methane production from anaerobic co-digestion of cow dung, chicken manure, pig manure and sewage waste*.

Shah, F., Mahmood, Q., Rashid, N., Pervez, A., Raja, I. and Shah, M. (2015). Co-digestion, pretreatment and digester design for enhanced methanogenesis. *Renewable and Sustainable Energy Reviews*(42), 627-642.

Shahriari, H., Warith, M., Hamoda, M. and Kennedy, K. (2012). Anarobic digestion of organic fraction of municipal solid waste combining two pretreatment modalities: high pressure microwaves and hydrogen peroxide. *Waste Management, 32*, 41-52.

Shen, R., Wang, W., Liu, X., Gao, X. and Zhou, Y. (2012). Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. *Waste Management, 32*, 249-255.

Shyam, M. and Sharma, P. (1994). Solid-state anaerobic digestion of cattle dung and agro-residues in small-capacity field digesters. *Bioresource Technology, 48*, 203-207.

Sibisi, N. and Green, J. (2005). A floating dome biogas digester: perceptions of energising a rural school in Maphephetheni, KwaZulu-Natal. *Journal of Energy in Southern Africa, 16*(3), 45-52.

Sinclair, S. L. and Myers, J. E. (2004). The relationship between objectified body consciousness and wellness in a group of college women. *Journal of College Counseling, 7*(2), 150-161.

Singh, M. (2017). Metal ion inhibitors and other inorganic toxicants in anaerobic biological process of industrial effluents: A critical review. *International Journal of Science and Research, 6*(5), 2353-2363.

Skiadas, I., Gavala, H., Lu, J. and Ahring, B. (2005). Thermal pre-treatment of primary and secondary sludge at 70 0C prior to anaerobic digestion. *Water Science Technology*(52), 161-166.

Smith, J., Apsley, A., Avery, L., Baggs, E., Balana, B., Bechtel, K. and Moris, K. (2013). *The potential of small scale biogas digesters to improve livelihoods and long term sustainability of ecosystem services in Sub-Saharan Africa*. Retrieved from Department of International Development, United Kingdom.

Sompong, O., Boe, K. and Angelidaki, I. (2012). Thermophilic anaerobic co-digestion of oil palm empty fruit bunches with palm oil mill effluent for efficient biogas production. *Applied Energy, 93*, 648-654.

Sooch, S. and Singh, J. (2004). Comparative study of economics of different models of family size biogas plants for state of Punjab, India. *Energy Conversion and Management, 45*(9-10), 1329-1341.

Sri, B., Kalyaraman, C. and Thanasekaran, K. (2011). Effect of ozonation and ultrasonication pretreatment processes on co-digestion of tannery solid wastes. *Clean Technology Environment Policy, 13*, 517-525.

Sterrit, R. and Lester, J. (1980). Interaction of heavy metals with bacteria. *Science Total Environment, 14*(5), 1-17.

Stockburger, D. (2006). *Introductory Statistics: Concepts, Models and Applications*.

Subramani, T. and Ponkumar, S. (2012). Anaerobic digestion of aerobic pretreated organic waste. *International Journal of Modern Engineering Resources, 2*, 607-611.

Subramanian, S. (1977). *Bio-Gas Systems in Asia*. (11). New Delhi, India.

Swanwick, J., Shurben, D. and Jackson, S. (1969). A survey of sewage sludge digesters in Great Britain. *Journal of Water Pollution Control Federation, 68*, 639-653.

Taherzadeh, M. and Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences, 9*(9), 1621-1651.

Tale, V., Maki, J. and Zitomer, D. (2015). Bioaugimentation of overloaded anaerobic digesters restores function and archaeal community. *Water Resources, 70*(1), 138-147.

Tamrat, A., Mebeaselassie, A. and Amare, G. (2013). Co-digestion of cattle manure with organic kitchen waste to inrease biogas production using rumen fluid as innoculums. *International Journal of Physical Science, 8*(11), 443-450.

Tchobanoglous, G., Burton, F. and Stensel, H. (2003). *Waste Water Engineering: Treatment and Re-use* (4th ed.): Tata McGraw-Hill Publishing Company Ltd.

Teghammar, A., Karimi, K., Horváth, I. and Taherzadeh, M. (2012). Enhanced biogas production from rice straw, triticale straw and softwood spruce by NMMO pretreatment. *Biomass and Bioenergy, 36*, 116-120.

Thuiller, W. (2003). BIOMOD–optimizing predictions of species distributions and projecting potential future shifts under global change. *Global Change Biology, 9*(10), 1353-1362.

Tong, Z., Linlin, L., Zilin, S., Guangxin, R., Yongzhong, F., Xinhui, H. and Gaihe, Y. (2013). Biogas production by co-digestion of goat manure with three crop residues. *PLoS ONE, 8*(6), 1-7.

Toreci, I., Kennedy, K. and Droste, R. (2009). Evaluation of continuous mesophilic anaerobic sludge digestion after high temperature microwave pretreatment. *Water Resources, 43*, 1273-1284.

Tufaner, F. and Avşar, Y. (2016). Effects of co-substrate on biogas production from cattle manure: a review. *International Journal of Environmental Science and Technology, 13*(9), 2303-2312.

Val del Rio, A., Morales, N., Isanta, E., Mosquera-Corral, A., Campos, J. and Steyer, J. (2011). Thermal pretreatment of aerobic granular sludge: impact on anaerobic biodegradability. *Water Resource, 45*, 6011-6020.

Vallee, B. and Ulner, D. (1972). Biochemical effects of mercury, cadmium and lead. *Annual Reviews in Biochemistry, 41*, 91-128.

Valo, A., Carrere, H. and Delgenes, J. (2004). Thermal, chemical and thermo-chemical pretreatment of waste activated sludge for anaerobic digestion. *Journal of Chemical Technol0gy and Biotechnology, 79*, 1197-1203.

Varma, R., Vesvikar, M., Karim, K., Hoffman, R., De Paoli, D., Klasson, K., Winterberg, A. and Alexander, C. (2009). *Bioenergy production from anaerobic digestion of animal and farm wastes.* Paper presented at the Energy Summit, Missouri University of Science and Technology.

Vavilin, V., Lokshina, L., Rytov, S., Kotsyurbenko, O. and Nozhevnikova, A. (1996). Modeling methanogenesis during anaerobic conversion of complex organic matter at low temperatures. *Water Science and Technology, 36*(6-7), 531-538.

Vögeli, Y., Lohri, C., Gallardo, A., Diener, S. and Zurbrügg, C. (2014). *Anaerobic digestion of biowaste in developing countries*. Retrieved from Dübendorf:

Voznesensky, V. (1974). Statistical Methods of Planning Experiment in Technicomechanical Investigations. *Moscow Izdatelstvo Statistic*.

Ward, A., Hobbs, P., Holliman, P. and Jones, D. (2008). Optimising the anaerobic digestion of agricultural resources. *Bioresource Technology, 99*, 7928-7940.

Weemaes, M., Grootaerd, H., Simens, F. and Verstraete, W. (2000). Anaerobic digestion of ionized biosolids. *Water Resources, 34*, 2330-2336.

Wett, B., Phothilangka, P. and Eladawy, A. (2010). Systematic comparison of mechanical and thermal sludge disintegration technologies. *Waste Management, 30*, 1057-1063.

Xavier, S. and Nand, K. (1990). A preliminary study on biogas production from cowdung using fixed-bed digesters. *Biological Wastes, 34*, 161-165.

Yeqing, L., Ruihong, Z., Yanfeng, H., Chnyu, Z., Xiaoying, L. and Chang, C. (2014). Anaerobic co-digstion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR). *Bioresource Technology, 156*, 342-347.

Yoem, I., Lee, K., Ahn, K. and Lee, S. (2002). Effects of ozone pretreatment on the biodigestibility of sludge from municipal waste water treatment plants. *Water Science Technology, 46*, 421-425.

Zhang, Y. and Banks, C. (2013). Impact of different particle side distributions on anaerobic digestion of organic fraction of municipal solid waste. *Waste Management, 33*, 297-307.

Zhu, B., Gikas, P., Zhang, R., Lord, J., Jenkins, B. and Li, X. (2009). Characteristics and biogas production potential of municipal solid waste pretreated with rotary drum reactor. *Bioresource Technology, 100*, 1122-1129.

Zielinski, M., Dębowski, M., Kisielewska, M., Nowicka, A., Rokicka, M. and Szwarc, K. (2017). Comparison of ultrasonic and hydrothermal cavitation pretreatments of cattle manure mixed with straw wheat on fermentative biogas production. *Waste and Biomass Valorization*. doi:10.1007/ s12649–017–9977-y

Zipancic, G., Uranjek-Zevert, N. and Ros, M. (2008). Full-scale anaerobic co-digstion of organic waste and municipal sludge. *Biomass Bioenergy, 32*, 162-167.

Zitomer, D., Adhikari, P., Heisel, C. and Dineen, D. (2008). Municipal anaerobic digesters for co-digestion, energy recery, and greenhouse gas reductions. *Water Environment Research, 80*, 229-237.

CHAPTER THREE

EFFECT OF TOTAL SOLIDS, TEMPERATURE AND SUBSTRATE RETENTION TIME ON BIOGAS PRODUCTION

Abstract

Biogas production is influenced by a number of factors including total solids, temperature, and substrate retention time, among others. The exact effect of these variables when digesting Kenyan cow dung or liquid cattle manure was the aim of this study. Three experiments were set-up to investigate the effect of total solids, temperature, and substrate retention time on biogas production, respectively. Experiments were done on a laboratory scale in a batch reactor of 0.15 m^3 (or 150 litre) capacity. The substrate was dung from dairy cows managed under a free-range system with an overnight holding yard. The substrate at total solids of 6%, 7%, 8%, 9%, and 10% were investigated at a constant temperature of 35^0C; and the mean biogas production was 37.46, 45.67, 73.12, 42.94, and 36.66 litres per day (l/d), respectively. Mesophilic temperature at 25^0C, 30^0C, 35^0C, 40^0C, and 45^0C were subjected to the substrate having total solids of 8%; and the mean results were 25.52, 51.98, 73.12, 79.21, and 49.41 l/d, respectively. Substrate retention time observed while the dung was at 8% total solids and 35^0C; and the biogas production were 23.64, 61.38, 63.09, 79.88, 88.00, 102.00, 90.37, 86.00, 72.85, and 64.00 l/d for day 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15, respectively. The highest biogas production was attained at total solids of 8%, a temperature of 35^0C, and a substrate retention time of 11 days. It was evident that total solids, temperature and retention time had effect on the rate of biogas production. Generally, it was observed that the biogas production increased with increase total solids, temperature and retention time until a maximum but before the onset of a decrease.

3.1 Introduction

Biogas is an important form of renewable energy. It is stored in biological materials such as straw, manure and other agricultural products; and it is one of the key options for mitigating Green House Gas (GHG) emissions to replace fossil fuels (Elaiyaraju and Partha, 2016; Sovacool, 2012). It can be used to generate heat, electricity, and produce transport fuel (Taherzadeh and Karimi, 2008; Tricase and Lombardi, 2009). Each year, 590-880 million tonnes of methane are exhausted worldwide into the atmosphere through microbial activity and 90% of this comes from biogenic sources (Charlton *et al.*, 2010).

Anaerobic digestion is the process by which organic matter is broken down in the absence of oxygen to produce biogas, carbon dioxide and other traces of gases. The process of anaerobic digestion takes place through four successive stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis; and it is dependent on the interaction between diverse microorganisms that are able to carry out the four aforementioned stages (Verma, 2002). In single stage batch reactors, the substrate is loaded and the four processes are allowed to occur in the same reactor sequentially; then the slurry is emptied after a predetermined retention time or the cessation of biogas production (Verma, 2002).

Working under the assumption that all the substrate is converted to methane (CH_4) and carbon dioxide (CO_2), and that the carbon (C), hydrogen (H_2) and oxygen (O_2) composition of the substrate are known, one can use equation (3.1) and the general gas equation to find a theoretical molar and volumetric output of CH_4 (Buswell and Mueller, 1952).

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \qquad 3.1$$

Under actual operating conditions, equation 3.1 was modified to account for the presence of ammonia (NH_3) and hydrogen sulphide (H_2S) in the waste as given in equation 3.2 (Achinas and Euverink, 2016).

$$C_nH_aO_bN_xS_y + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3x}{4} + \frac{y}{2}\right)H_2O \rightarrow \left(n - \frac{a}{4} + \frac{b}{2} + \frac{3x}{4} + \frac{y}{2}\right)CO_2 + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3x}{4} - \frac{y}{2}\right)CH_4 + xNH_3 + yH_2S \qquad 3.2$$

Parameters that affect biogas production include substrate concentration, temperature, substrate retention time, carbon to nitrogen ratio, pH, organic loading rate, nutrients, and toxicity (Parawira *et al.*, 2005). The effects of these parameters are different on different microbial groups as each microbial group has different physiological and nutritional needs; and their imbalances cause instability (Dolud *et al.*, 2005). An imbalance in the process caused due to the disturbance in the hydrolysis stage will limit the activities in the subsequent stages thereby reducing biogas production; a disturbance in methanogenesis will bring about an accumulation of acids that have been formed in previous stages; changes in the process such as reduced biogas production, accumulation of volatile fatty acids, decrease in pH, and alkalinity are indicators of process instability (Barnes *et al.*, 1989). In this study the effects of

substrate concentration, temperature, and substrate retention time were investigated in a laboratory fixed dome batch reactor.

There are three main temperature regimes for anaerobic digestion: psychrophilic (< 25^0C), mesophilic (30-40^0C) and thermophilic (50-70^0C) (Leenawat *et al.*, 2016; Meegoda *et al.*, 2018; Patharwat *et al.*, 2016). Mesophilic digestion operates in a lower temperature, is slower and yields less biogas. However, mesophilic digesters remain attractive because of their lower heater energy costs compared to thermophilic digesters (Moset *et al.*, 2015). The mesophilic range was used in this study. Thermophilic digestion, on the other hand, operates at a higher temperature with a consequent increase in reaction rates leading to increased biogas production (Franke-Whittle *et al.*, 2014; Lettinga *et al.*, 1997).

Substrate concentration can be determined in terms of volatile solids or total solids. Volatile solids content is determined by igniting the substrate at 550^0C in the incinerator and then weighing the remaining contents (EPA, 2001). Total solids is the measurement of dry matter as a percentage, and is determined by drying the sample at 103 – 105^0C in succession until no further change in weight is observed (APHA, 1999; EPA, 2001). Total solids were used as a variable in this study.

Substrate retention time refers to the mean length of time that the material fed into the batch digester takes before it is emptied (Kircher *et al.*, 2014). Shorter retention times are known to be associated with acidification especially fatty acid and can cause inhibitory effects (Hwu *et al.*, 1996). Nonetheless, shorter substrate retention times allow for increased process efficiency and decreased capital costs, although longer substrate retention times are necessary for the digestion of lignocellulosic wastes (Shi *et al.*, 2017). Generally, mesophilic digestion can be accomplished within 15 – 30 days (Mao *et al.*, 2015).

3.2 Materials and Methods

3.2.1 Experiment Set-up

Figures 3.1 and 3.2 give a schematic sketch and drawing are cross section views of the experiment set-up and the engineering cross section drawing of the system used in the research. The latter figure was drawn including dimensions using AutoCAD 2017 software.

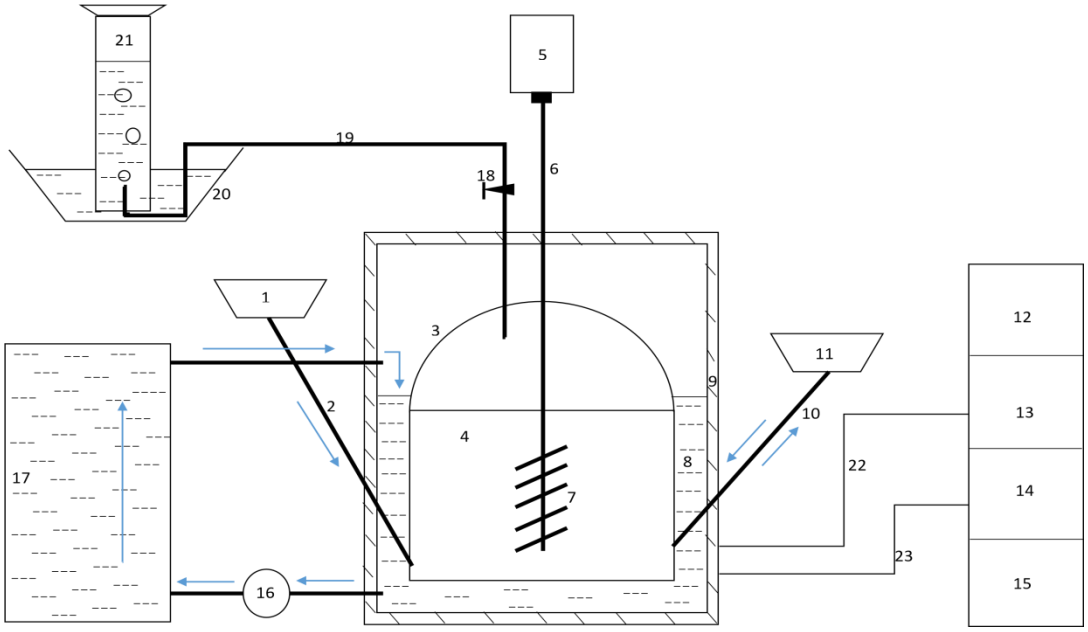


Figure 3.1: Bioreactor schematic sketch

Key to parts of the Fixed Dome Lab Bioreactor

1.	Feeding hopper	9.	Insulation	17.	Water tank
2.	Inlet pipe	10.	Outlet pipe	18.	Gate valve
3.	Bioreactor	11.	Expansion chamber	19.	Delivery pipe
4.	Substrate	12.	Control unit	20.	Water bucket
5.	Electric Motor	13.	Power supply	21.	Inverted graduated flask
6.	Stirring rod	14.	Data Logger	22.	Power supply cable
7.	Stirrer	15.	Computer	23.	Thermocouple wire
8.	Water bath	16.	Centrifugal pump		

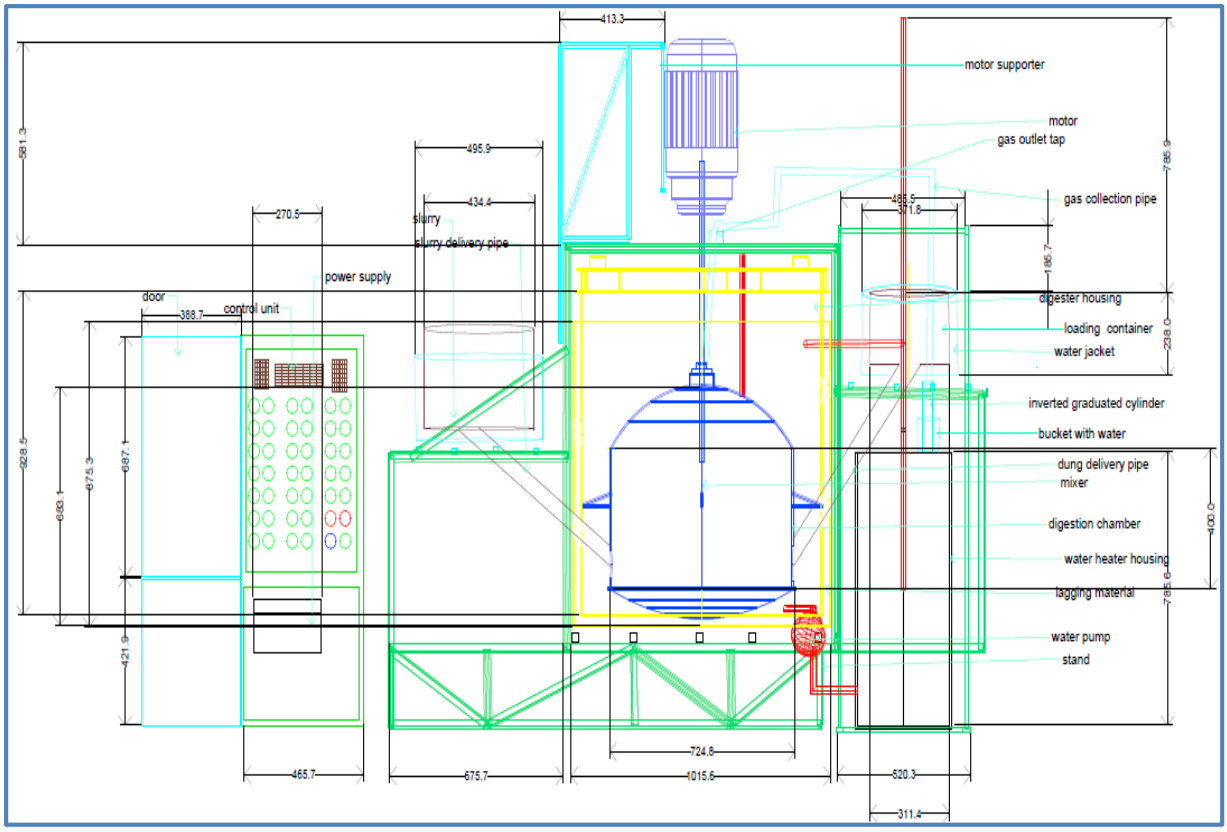


Figure 3.2: Cross-section of laboratory biodigester system

The fixed dome laboratory bioreactor was designed and fabricated at Egerton University. It is a scale model of the 124 m^3 fixed dome underground digester at the University Farm. The laboratory model is surrounded by an automatically controlled temperature and circulated water jacket and automated stirring system so that it can maintain the required temperature and stirring frequency that can imitate of the field fixed dome digester (Nyaanga *et al.* 2015).

The influent was fed into the bioreactor from the feeding hopper through the inlet pipe until it rose to a level in the expansion chamber that closed the opening of the outlet pipe to minimise loss of biogas produced from the bioreactor during the anaerobic digestion process. The substrate was stirred after every four hours by the stirrer attached on the stirring rod with the help of an electric motor. Stirring was essential to break the scum, release the metabolites, and mix the substrate to acquire uniform distribution of temperature and bacteria. When gas was produced, it increased the pressure in the bioreactor and this forced the substrate to be displaced into the expansion chamber. To reduce the pressure, a gate valve was opened to allow the gas to escape through the gas delivery pipe. The gas ended up in an inverted graduated cylinder filled with water; and the water was displaced into the bucket.

Temperature was regulated by a water bath that surrounded the bioreactor. Water was heated by the electric immersion heaters in the hot water tank. Circulation of water was enabled by a centrifugal pump. A power supply provided electricity to the system, through the power cable, for heating the water and monitoring the temperature. A control unit had the instrumentation that allowed water to be heated to a predetermined temperature only. A computer was attached to the control unit via a digital data logger. Thermocouple wires from the substrate in the bioreactor, water bath that surrounded the bioreactor, and the outside of the experiment set-up were connected to the data logger. The data was downloaded by the computer, and also real-time temperature was read on the screen of the computer. Foam mattress pellets were used as an insulation material; they were placed in a casing that surrounded the water bath to minimise heat loss.

Gas collection was by the water displacement method. A gas pipe was connected to an inverted graduated cylinder filled with water, and placed in a bucket of water. A gate valve was opened to allow the gas to displace water in the 500 millilitre capacity cylinder. This was done several times until the gas was exhausted for the duration of production. The volume of gas collected was then calculated to give biogas production rate in cubic metres per cubic metre of digester volume per day (m^3/m^3d) (Mudhoo *et al.*, 2012).

Biogas for analysis of methane content was collected in sampling bags and taken to a Gas Chromatograph (Acer Varian 3400CX model). The model uses a flame ionisation detector (FID). The protocol for methane content analysis was adopted from a Hewlett Packard 5890plus GC with an FID and a Supelco Carboxen1006 Plot Column that is 30 m long with 0.53 mm internal diameter (Walsh and McLaughlan, 1999). This protocol was adjusted to suit the Acer Varian 3400CX model. The injector port was maintained at 200^0C and 80kPa, while the FID was maintained at 200^0C. The oven program started with 35^0C for 1 minute followed by a ramp of 20^0C per minute to 180^0C, and the gas was held for 2 minutes. Manual gas injection into the FID port was done using a tight 1μL syringe (Plate 3.11). The retention time for methane was 1.5 minutes, with an analytical detection limit set at 0.1μg/L (Walsh and McLaughlan, 1999). The carrier gas was nitrogen, and the combustion gases were hydrogen and oxygen. The methane content was read from a print-out having a graph showing the peaks of gas retention per minute. The results were then analysed for percentage methane content in the sample.

3.2.2 Material Preparation

Total solids are defined as a measure of dry matter left after the moisture has been removed from a moist sample. Dung from dairy cows, managed under a free-range system, was used as a feedstock. Fresh cow dung was collected from Tatton Agricultural Park (teaching farm) and Ngongogeri (commercial farm), all of Egerton University, and taken to the laboratory for determining the total solids by percentage. At first, each can was cleaned and weighed while empty by using an electronic balance. Then every can was loaded with fresh dung, weighed, and then put in an air oven that was set at 105^0C to heat the contents for 12 hours to attain a constant weight. Thereafter, each can and its content was removed and taken to a desiccator to cool down to room temperature. The desiccator had silica gel to ensure the hot dried dung did not re-absorb moisture from the atmosphere during the cooling process. After the dry samples attained room temperature, they were weighed again to determine their masses.

Three sets of experiments were done to take care of the variation in total solids content because of the change from the dry season to the wet season. Each set had 11 samples. The first two sets were done at the end of the dry season in the month of March, and the last set was done during the wet season in month of May 2018. Then an average value of total solids was used in subsequent calculations to prepare the influents.

Table 3.1 shows the data that was obtained and the associated calculations.

Table 3.1: Total solids of cow dung samples

Sample No.	Total solids (%)		
	Replica 1 (19/3/2018)	Replica 2 (20/3/2018)	Replica 3 (15/5/2018)
1	18.248	16.232	18.487
2	19.514	17.698	18.616
3	20.373	17.206	18.288
4	17.568	16.915	17.603
5	17.978	17.496	18.185
6	17.304	16.802	19.416
7	19.680	16.964	19.693
8	17.180	35.100	19.152
9	19.406	16.959	18.688
10	17.892	16.618	16.991
11	20.721	17.057	19.640
Mean	18.723	18.641	18.610
Grand mean	18.658		

The total solids (TS) was determined using equation (3.3).

$$TS = \left(\frac{c-a}{b-a}\right) \times 100 \qquad 3.3$$

where

TS = total solids (which is the ratio of the difference in mass of can and dried dung minus mass of dry can to difference between the mass of dry can and fresh dung and mass of dry can times 100.

a = mass of dry can

b = mass of dry can and fresh dung

c = mass of dry can and oven dried dung (at 105^{o}C to constant weight)

The preparation of the influent was done for every total solids value. The prototype laboratory reactor had a fermentation chamber of 120 litres. Consequently, 120 litres of influent was prepared based on the designed TS percentage by diluting a predetermined amount of fresh cow dung (manure) with a predetermined (calculated) amount of clean water from the tap at room temperature.

For example, to prepare an influent having 7% TS; the following calculations were done.

$$\text{The average TS of cow dung } = 18.658\%$$

$$\Rightarrow 1 \text{ kg of cow dung contains } \left(\frac{1 \times 18.658}{100}\right) = 0.18658 \text{ kg of TS}$$

$$\text{But for a 7\% TS influent,}$$

$$\Rightarrow 7 \text{ kg of TS are contained in } 100 \text{ kg of influent}$$

$$\therefore 0.18658 \text{ kg are contained in } \left(\frac{0.18658 \times 100}{7}\right) = 2.665 \text{ kg of influent}$$

$$\text{This means that the water in the influent} = 2.665 - 1 = 1.665 \approx 1.7 \text{ kg}$$

$$\text{It implies that cow dung: water ratio} = 1:1.7$$

From this ratio, 120 litres of influent containing 7%TS was prepared as follows:

$$\text{Cow dung} = \frac{1}{2.7} \times 120 = 45.03 \text{ kg}$$

$$\text{Water} = \frac{1.7}{2.7} \times 120 = 74.97 \text{ kg}$$

The amounts of manure in kg, total solids in the manure (TS, in percent, %), and water required to dilute the manure to the required TS (%) are presented and shown in Table 3.2 below.

Table 3.2: Substrate to water ratios

TS No.	Influent TS (%)	Manure (kg)	TS in Substrate (kg)	Water required (kg)	Manure to Water ratio
1	**6.0**	38.7	7.2	81.3	1: 2.1
2	**6.3**	41.4	8.3	78.6	1:1.9
3	**7.0**	44.4	8.9	75.6	1:1.7
4	**8.0**	52.2	10.4	67.8	1:1.3
5	**9.0**	57.1	11.4	62.9	1:1.1
6	**9.3**	60.0	11.2	60.0	1:1.0
7	**9.7**	63.2	12.6	56.8	1:0.9
8	**10.0**	64.5	12.0	55.5	1:0.9
Average	8.2	52.7	10.3	67.3	1:1.2

From these samples (Table 3.2), the average TS is 8.2% giving a manure to water dilution ratio of 1 to 1.2 (fairly close to 1:1, the generally recommended dilution ratio in Kenya).

A 20 litre bucket was used to prepare the substrate (influent). The dung and water were placed in the bucket, in which a piece of wood was used for mixing until a homogenous mixture was attained. The mixture was then emptied into the bioreactor through the feeding hopper. Table 3.3 shows total solids of different influents that were fed into the digester.

Table 3.3: Amounts of manure and water required per influent (substrate)

TS No.	Influent TS (%)	Manure (kg)	TS of Manure (%)	TS of Manure (kg)	Total Influent (kg)	Water required (kg)
1	6.00	1.00	18.60	0.20	3.10	2.10
2	6.31	1.00	18.60	0.20	2.90	1.90
3	7.00	1.00	18.60	0.20	2.70	1.70
4	8.00	1.00	18.60	0.20	2.30	1.30
5	9.00	1.00	18.60	0.20	2.10	1.10
6	9.68	1.00	18.60	0.20	1.90	0.90
7	10.00	1.00	18.60	0.20	1.90	0.90
Average	8.00	1.00	18.60	0.20	2.41	1.41

It can be noted that the manure samples handled had 1 kg of these Egerton cattle manure with a TS of 18.6% ; had total solids amounting to 0.2 kg which would be digested by the bacteria and hopefully converted to biogas in a given retention time in the batch laboratory digester.

Tchobanoglous *et al.* (2003) gave total solids (TS) range of 7 to 9%. Accordingly, 8% TS is the average value. The step change is 1%. It is logical to apply the step change above and below the range to obtain star values for investigation. Therefore, the effect of total solids on biogas production was investigated by running the experiment at 6%, 7%, 8%, 9% and 10% for 18 days each. All the results were recorded and analysed.

3.2.3 Temperature control

The reactor was put in a water bath of warm water. Water was heated in a water tank using immersion heaters, and circulated by a centrifugal pump to ensure that the heated water releases heat to the reactor until the set temperature was achieved. The immersion heaters were regulated by a thermostat, which stopped the heating when the pre-set temperatures were attained. The temperature control was enabled by use of the proportional differential integrals (PIDs) and program logic controls (PLCs).

In the control experiment, the same batch system was used. The substrate to water dilution was 1:1 as is usually done by biogas producers in Kenya (Nyaanga, 2011). The temperature was left at room conditions. Stirring the substrate was done for two minutes after every four hours at a speed of 15 revolutions per minute (Lemmer *et al.*, 2013). The biogas volume produced was recorded.

Mesophilic temperature ranges from 30^0C to 40^0C (Patharwat *et al.*, 2016). The average temperature of the range is 35^0C, and this gives a step change of 5^0C. A step change was applied below and above the range to get the star values for investigation. Effect of temperature was investigated by varying the runs at 25^0C, 30^0C, 35^0C, 40^0C, and 45^0C for 18 days each. Total solids were kept constant at 8% for all runs. The data was analysed and results discussed in the relevant section below.

3.2.4 Substrate retention time

The effect of substrate retention time was investigated by making three runs while keeping the concentration of the substrate at total solids of 8%, while the temperature was maintained at 35^0C. The 8%TS and 35^0C were chosen because they were at the centre of the design space in their respective ranges. An average value of biogas production was used for analysis.

3.3 Results and Discussions

3.3.1. Effect of total solids on biogas production

The data obtained at 35^0C for various total solid levels are presented in Table 3.4. Figure 3.2 shows a graphical presentation of the data in Table 3.4. The Table and Figure show the four phases (1, 2, 3 and 4) of digestion of organic matter to produce biogas. In the first phase, there is no production of biogas from day 1 to day 5 for total solids of 7% and 8%, from day 1 to day 6 for total solids of 6% and 9%, and from day 1 to day 7 for total solids of 10%. In this phase, three main processes are taking place namely hydrolysis, acidogenesis, and acetogenesis. Hydrolysis is where proteins, carbohydrates, and fats are being broken down into amino acids, simple sugars, and long chain fatty acids (LCFA), respectively by the hydrolytic bacteria (Pavlostathis and Giraldo- Gomez, 1991).

Table 3.4: Biogas production (litres per Day) for various total solids

Day from start	Total solids (TS, %)				
	6%	7%	8%	9%	10%
1	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00
6	0.00	15.81	23.64	0.00	0.00
7	11.03	27.53	61.38	18.15	0.00
8	34.96	44.17	63.09	30.76	11.47
9	40.52	53.39	79.88	47.71	27.93
10	56.07	67.08	88.00	51.63	51.40
11	60.23	74.96	102.00	70.46	55.06
12	41.43	61.20	90.37	63.92	59.31
13	38.91	45.32	86.00	48.14	43.89
14	29.83	38.79	72.85	32.75	24.70
15	24.13	28.41	64.00	22.90	19.52
Average	37.46	45.67	73.12	42.94	36.66

Figure 3.3 below shows the trends of biogas (giving the amounts in litres per day), produced as the laboratory fixed dome cow manure digestion and gas production proceeds for different total solids (6 % to 10 %) of the Egerton free range cattle.

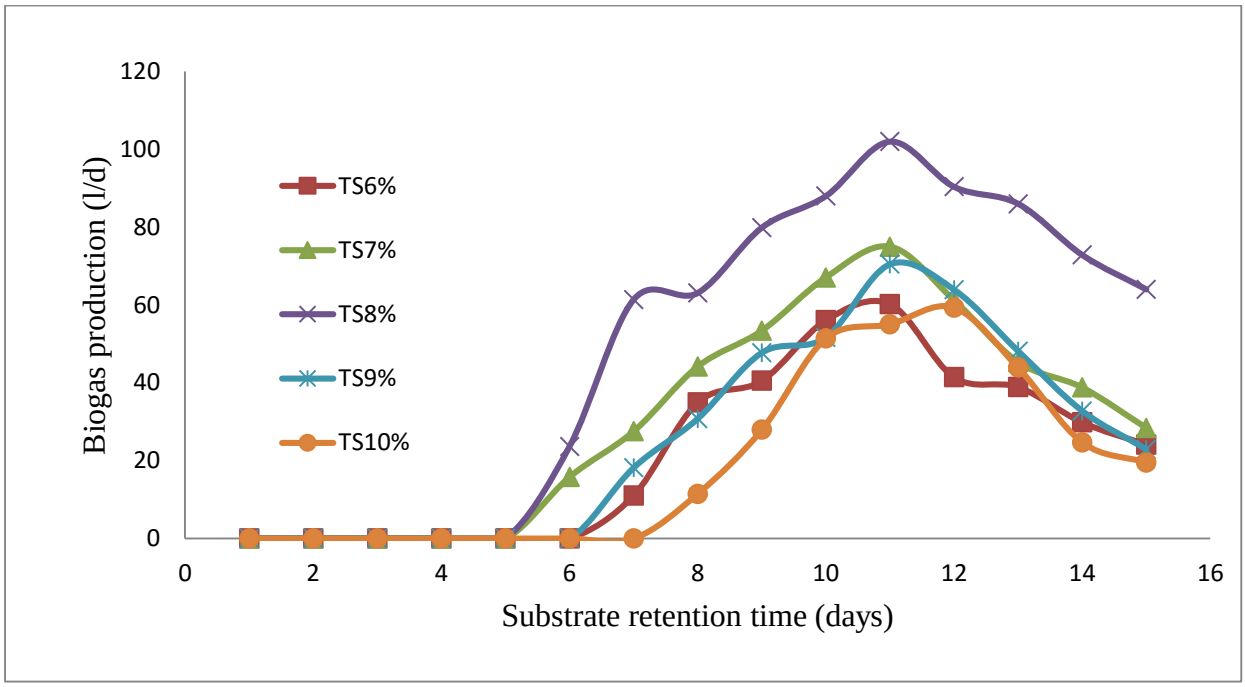


Figure 3.3: Biogas production trends for various total solid levels

The biogas production trends seem to differ for the different total solids (TS) levels. The TS that gave the highest production rate was 8% which was evidently higher than the other three TS levels of 6%, 7%, 9% and 10% in all the days of biogas production. This could be attributed to the dynamics and size hence volume of substrate available for the bacteria in the 0.15m^3 digester. Using the basic statistics (such as error bars in the graphical presentation of the data – see Appendix A2.2) the 8% TS had a significantly higher biogas yield per day in all days than the other TS levels of 6%, 7%, 9% and 10%. However, the rate of biogas production among the 6%, 7%, 9% and 10% TS levels was not significantly different, statistically.

The trend of the biogas yield for all the TS levels, depict a four stage trend as the days of retention increase from zero (day of loading) to the day the experiment ended (when the gas production declined to near zero and was no longer burning, being the 15th day for all the total solids levels tested). The four stage production trends were confirmed by the 4th order polynomial that fitted the data with R^2 values around 0.95 (see Fig A1.4 in the Appendix) as presented in equations 3.4 and 3.5 below,

$$Y_{8\%} = 0.015x^4 - 0.71x^3 + 9.97x^2 - 38.24x + 34.96;\ R^2 = 0.96 \quad 3.4$$

$$Y_{6,7,9,10\%} = 0.0219x^4 - 0.856x^3 + 10.413x^2 - 38.621x + 35.616,\ R^2 = 0.93 \quad 3.5$$

where

Y= biogas yield or production rate (litres per day) for given TS % and x = day from start of the digestion process (days).

Using equations 3.4 and 3.5, the gas production beyond the 15th day (the day when the experiment was stopped due low production and poor quality biogas which could not burn), the gas production rate from the system was as presented in Table 3.5 below and Figure A2.5 in the Appendix.

Table 3.5: Empirically predicted average biogas production with retention time

	Biogas Produced in litres per day		
Retention Time (Days)	Avg $Yield_{Obs}$* for 6 - 10%TS	Avg $Yield_{Pred}$ for 6 - 10%TS	$Yield_{Pred}$ for 8% TS
1	0	6.6	6.0
2	0	-6.5	-7.1
3	0	-7.9	-7.9
4	0	-1.4	0.0
5	4	9.5	13.8
6	14	22.2	30.8
7	30	34.5	48.6
8	42	44.5	65.3
9	57	51.1	79.4
10	65	53.7	89.5
11	56	52.1	94.8
12	44	46.6	94.8
13	32	38.2	89.2
14	24	28.3	78.3
15	26	18.9	62.5
16		12.5	42.7
17		12.0	20.2
18		21.0	-3.4

*Avg $Yield_{Obs/Pred}$ = average biogas production Observed or measured Data and Predicted or simulated values using the empirical polynomial models.

The four stages in the digestion and gas production with time were evident in all levels of total solids tested. Stage 1 had no gas produced for all levels of total solids being 5 to 7 days depending on the TS used. The substrate of 8% and 7% total solids (TS) started producing

biogas after 5 days, while that with 6% and 9% started after 6 days and finally the 10% TS influent produces biogas after 7 days. This difference can be explained by breakdown of complex molecules of sugar and amino acids and increase in the number of anaerobic acid-producing microbial bacteria for the production of biogas.

Stage 2 was fairly different for the varying TS levels being between 5^{th} and 7^{th} day (lasting 2 days), 5^{th} and 8^{th} day (3 days), 6^{th} and 9^{th} day (3 days), 6^{th} and 8^{th} (2 days) and 7^{th} and 10^{th} day (3 days) for the 8%, 7%, 9%, 10% and 6%, respectively. Generally, using the average TS of 8.2% (for the TSs tested), the 2^{nd} stage took 2.17 days starting the 6^{th} day (on average) for this system and at the set conditions of temperature, stirring and Tropical cattle manure from the free range animal production system. The 3^{rd} stage, on average occurred between the 6-7^{th} day and the 11^{th} day for all the TS levels. The mid-point and transition between stage 2 and 3 are not very obvious especially for 7% and 10% TS. It is also worth to note that the rate of biogas production (and gradient of the graph, approximately, less than 10 l/d) is generally the same, especially for the non-optimal TS of 6, 7, 9 and 10%. Similarly, the gradient is higher for the optimal TS of 8% (being about 20 l/d). The science involved in these stages is described in the following paragraphs.

Acidogenesis is a process during which simple sugars and amino acids resulting from hydrolysis are degraded to a number of simpler products such as volatile fatty acids including propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), and acetic acid (CH_3COOH) (Batstone *et al.*, 2002). Acidogenesis is generally defined as an anaerobic acid-producing microbial process without an additional electron acceptor or donor (Gujer and Zehnder, 1983). Acidogenesis is often the quickest step in the anaerobic conversion of complex organic matter in liquid phase digestions (Vavilin *et al.*, 1996).

Acetogenesis is the degradation of higher organic acids formed in acidogenesis to yield CH_3COOH, CO_2 and H_2 (Batstone *et al.*, 2002). This intermediate conversion is crucial for the successful production of biogas.

Biogas production is very slow at the beginning due to the lag phase of microbial growth (Budiyono *et al.*, 2010). The biogas production in batch condition is directly proportional to the specific growth rate of methanogenic bacteria in the bioreactor (Nopharatana *et al.*, 2007).

In the second phase there is an exponential production of biogas from day 5 up to day 11 and day 12. During this phase, the process of methanogenesis is taking place. The fermentation

products such as acetate, H_2 and CO_2 are converted to methane (CH_4) and CO_2 by methanogenic archaea (Björnsson *et al.*, 2000; Pavlostathis and Giraldo-Gomez, 1991). This can be summarized by equations (3.6) and (3.7):

i. Hydrogenotrophic methanogenesis

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (3.6)$$

ii. Aceticlastic methanogenesis

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (3.7)$$

In the third phase there is a steady decline in biogas production from day 11 to day 15. This is due to stationary phase of microbial growth (Castillo *et al.*, 1995). The methanogenic bacteria die due to the depletion of carbon in the substrate.

Figure 3.4 shows the average biogas production rate values for different TS contents.

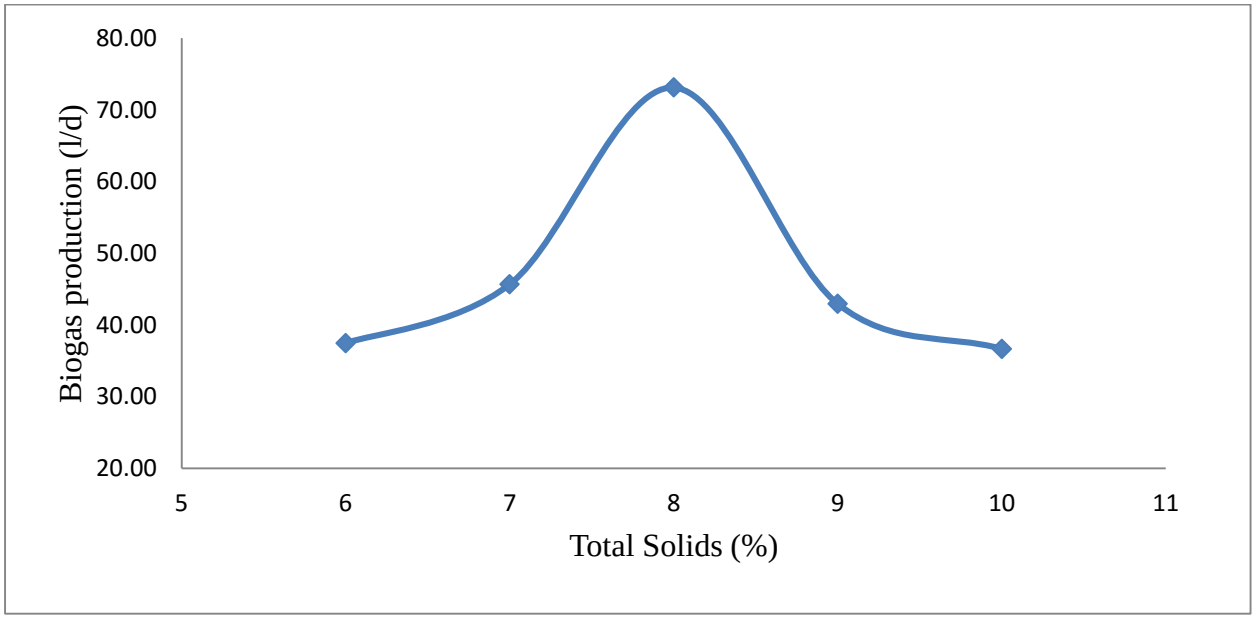


Figure 3.4: Average biogas production rate for various total solid levels

The mean rates of biogas production were 37.5, 45.7, 73.1, 43.0, and 36.7 l/d for 6, 7, 8, 9, and 10% TS, respectively. The rate of gas production for the digestion the fixed dome laboratory system digesting cattle manure at 35^{o}C, was low (37.5 l/d) at the lowest total solids (TS) of 6%, highest at the median TS of 8% and again lowest (36.7 l/d) at the highest TS used of 10%. This could be attributed to the digestion dynamics involving the bacterial succession with respect to the nutrient availability as discussed below.

Anaerobic digestion process increased steadily from total solids of 6% to 8%. At low solids, aceticlastic methanogens called methanosarcina play an important role, followed by hydrogenotrophic methanoculleus and methanomicrobiales species (Yi *et al.*, 2014). Methanosarcina, a typical aceticlastic methanogen, has often been reported to be the dorminant methanogen in anaerobic digestion (Demirel and Scherer, 2008). The ability of genus methanosarcina having high growth rates and forming irregular cell clumps makes them more tolerant to changing pH and high concentrations of toxic ionic agents (Conklin *et al.*, 2006). Methanosarcina produce methane from acetate (although some species are more versatile and can also utilize hydrogen and carbon dioxide), methylated amines and methanol, and also use both the aceticlastic and hydrogenotrophic methanogenesis pathways compared to methanosaeta species (de Vrieze *et al.*, 2012).

As the total solids increase from 8% to 10%, the biogas production rate declines steadily. Increasing the total solids means a higher applied organic loading rate and more volatile solids for microorganisms which results in higher volatile fatty acid concentrations (Yi *et al.*, 2014), and this favours the growth of methanosarcina species (de Vrieze *et al.*, 2012). On the other hand, methanoculleus species population decline in mesophilic anaerobic digesters with an increase in total solids content (Bourque *et al.*, 2008). The changing of microbial communities in mesophilic anaerobic digestion of cow dung was responsible for the performance exhibited in Figure 3.4.

This observation is similar to the work reported by Igoni *et al.* (2008) on a batch reactor. A marginal increase in the percentage of total solids results in a geometric increase in the volume of biogas produced, suggesting therefore (as seen in Figure 3.4) that a continual increase in the percentage of total solids at some point becomes immaterial to the increasing volume of biogas produced. This is possible because when percentage of total solids increases, the amount of water decreases, thus reducing the level of microbial activity, which then affects the amount of biogas, particularly at higher values of the TS% (Igoni *et al.*, 2008).

Itodo and Awulu (1999) showed that slurries of higher total solids concentrations were more acidic than that of lower total solids concentrations, which is an additional reason why higher total solids values would not significantly affect the increasing volume of biogas produced. Abbassi-Guendouz *et al.* (2012) showed that the total methane production decreased with total solids contents increasing from 10% to 25% in batch anaerobic digestion of cardboard

under mesophilic conditions. Forster-Carneiro *et al.* (2008) also showed that the biogas and methane production decreased with total solids contents increasing from 20% to 30% in dry batch anaerobic digestion of food waste.

It can be deduced from Figure 3.3 and Figure 3.4 that generally at low concentrations of total solids, the gas production increases more steadily than at higher concentrations of total solids (Beevi *et al.*, 2015; Liu and Jian, 2016; Tsunatu *et al.*, 2014). It can also be noticed that as the solid concentration increases above 8%, the gas production begins to drop or falls drastically (Eltawil and Belal, 2009).

3.3.2. Effect of temperature on biogas production

Table 3.6 shows the data obtained when the experiment was run at 8% total solids at different temperatures. Figure 3.5 is a graphical presentation of data in Table 3.6.

Table 3.6: Biogas production rate for various temperatures

Day	25^{o}C	30^{o}C	35^{o}C	40^{o}C	45^{o}C
1	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00
6	0.00	11.42	23.64	18.73	9.73
7	0.00	38.27	61.38	57.93	21.94
8	0.00	45.31	63.09	75.60	38.52
9	7.84	54.69	79.88	83.58	51.83
10	21.69	65.83	88.00	97.42	72.96
11	33.47	72.61	102.00	110.81	93.91
12	45.63	80.37	90.37	121.06	69.78
13	36.81	68.74	86.00	90.15	62.71
14	19.25	51.52	72.85	77.87	44.08
15	13.94	31.04	64.00	58.97	28.59
Average	25.52	51.98	73.12	79.21	49.41

The average daily biogas production rates increase with increase in temperature between the 25^{0}C and 40 ^{0}C before it decreased at 45 ^{0}C. These average biogas yields were 25.5 l/d, 52 l/d, 73.1 l/d, 79.2 l/d and 49.4 l/d, for the 25 ^{0}C, 30 ^{0}C, 35 ^{0}C, 40 ^{0}C and 45 ^{0}C, respectively.

Figure 3.5 shows the results of biogas production rate for 8% total solids at 25^0C, 30^0C, 35^0C, 40^0C, and 45^0C. Generally, the production biogas increases with time of digestion and increase in temperature from day zero when the digester is loaded and 25^0C for temperature up to a maximum at 11^{th} or 12^{th} day being varying between 20 l/d to 120 l/d for the different temperatures. Thereafter, the production starts decreasing just as was observed with effect of total solids exhibiting the four phases.

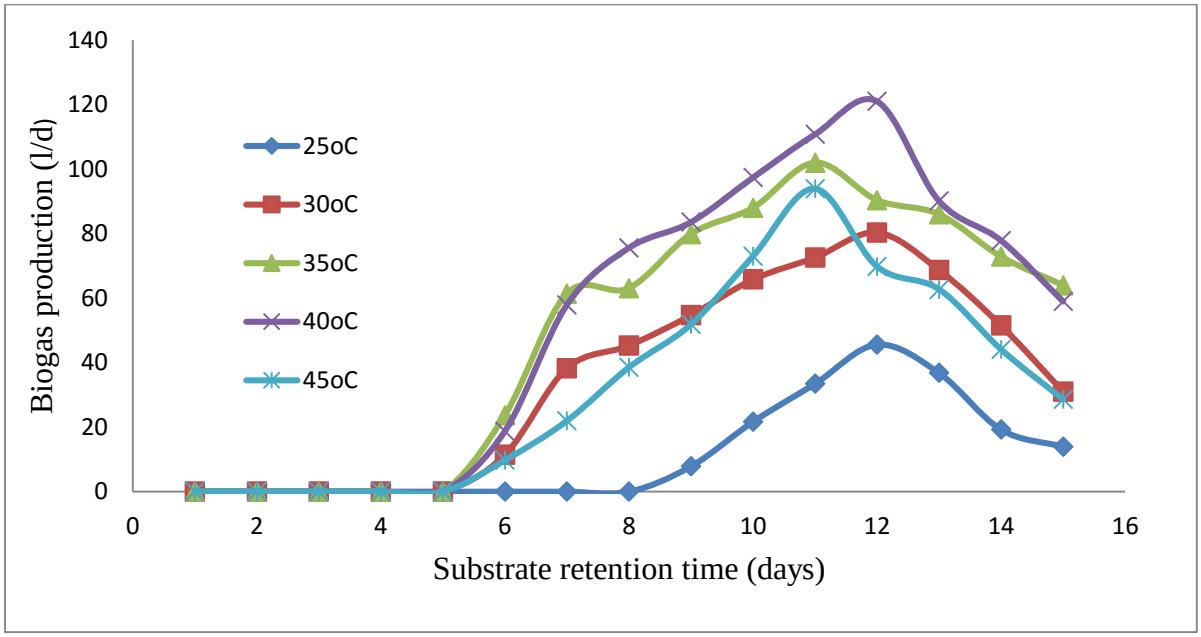


Figure 3.5: Biogas production rate for various temperatures

The first phase ranges from day 1 to day 5 for temperatures 30^0C to 45^0C, and day 1 to day 8 for 25^0C; no biogas is produced. During this phase, hydrolysis is taking place. Proteins, carbohydrates, and fats are being broken down into amino acids, simple sugars, and long chain fatty acids by hydrolytic bacteria (Pavlostathis and Giraldo-Gomez, 1991). This is followed by acidogenesis where the simple compounds are converted into acids such as acetic acid (CH_3COOH) (Batstone *et al.*, 2002). The acids are then converted into CO_2 and H_2 in the process called acetogenesis (Batstone *et al.*, 2002). In this phase, heat is required to facilitate the fermentation process.

In the second phase, there is an exponential biogas production from day 5 to day 11, and day 12 for all the temperatures. The highest biogas production occurs at 40^0C (121.06 l/d on day 12) followed by 35^0C, 30^0C, 45^0C, and lastly 25^0C. The third phase starts from day 12

onwards. Biogas production drops drastically because the feed material is depleted leading to the death of mesophiles in the batch reactor.

There are three temperature ranges within which anaerobic digestion of a substrate takes place. Psychrophilic temperature range lies below 20^0C, mesophilic temperature range between 30^0C and 40^0C, and thermophilic temperature range from 50^0C to 65^0C (Eder and Schulz, 2006; Grady *et al.*, 1999; Patharwat *et al.*, 2016).

Figure 3.6 shows a plot of average biogas production rate against the temperature.

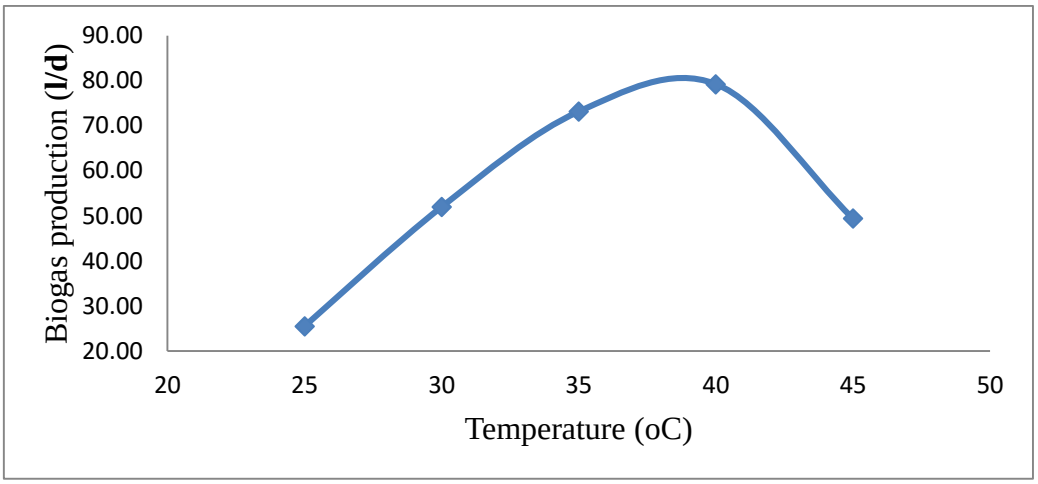


Figure 3.6: Average biogas production for various temperatures

It can be noticed in Figure 3.6 that at 25^0C, biogas production was low due to very little microbial activity because the psychrophilic bacteria had died off while the mesophiles were regenerating to take over the process of methanogenesis (Leenawat *et al.*, 2016; Pandey and Soupir, 2012). There is a steady increase in biogas production in the mesophilic range between 30^0C (51.98 l/d), 35^0C (73.12 l/d), and 40^0C (79.21 l/d) because of the increased regeneration of mesophiles (Lettinga *et al.*, 1997). The observations at 35^0C and 40^0C agree with work reported by Adepoju *et al.* (2016), Hajji *et al.* (2016), and Patharwat *et al.* (2016). An increase in biogas yield occurs with an increase in temperature of pretreatment up to a certain optimum temperature above which production decreases (Bochmann *et al.*, 2010; Jahng *et al.*, 2011) because of xylose formation and lignin breakdown that become toxic to anaerobic digestion bacteria (Bochmann and Montgomery, 2014). The optimal temperature for mesophilic anaerobic digestion is 37^oC (Lettinga *et al.*, 1997; Manjula and Mahanti, 2014; Uzodinma *et al.*, 2007). Although some variation is considered normal, digester temperature should be always maintained between 35^oC and 40^oC (Hajji *et al.*, 2016).

At 45^0C, the biogas production rate is lower than expected. This is so because the enzymes of mesophiles are denatured leading to their death, while the thermophiles regenerate to take over the methane production process (Lettinga *et al.*, 1997). During this transition, the activity of the bacteria is not as intensive as in the mesophilic range. This trend was also reported by Ghatak and Mahanta (2014). The rate of bacteriological methane production increases with temperature as well as the amount of free ammonia; the bio-digestive performance could be inhibited or even reduced as a result (Eder and Schulz, 2006; Grady *et al.*, 1999). There is also inhibition in production of biogas due to the accumulation of ammonia at 45^0C (Møller *et al.*, 2004).

3.3.3. Effect of substrate retention time on biogas production at 8% TS and 35^0C

Table 3.6 shows the results of running the experiment at total solids of 8% and a temperature of 35^0C. Biogas production rate is in litres per day (l/d).

Table 3.7: Biogas production rate (l/d) with time of digestion at 8% TS and 35^0C.

Day	Run 1	Run 2	Run 3	Average
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	22.97	23.25	23.64	23.29
7	62.41	60.86	61.38	61.55
8	62.81	63.15	63.09	63.02
9	79.20	79.52	79.88	79.53
10	89.04	87.93	88.00	88.32
11	100.27	102.51	102.00	101.59
12	89.48	90.79	90.37	90.21
13	87.07	85.96	86.00	86.34
14	71.84	72.62	72.85	72.44
15	60.18	63.70	64.00	62.63

Figure 3.7 shows the results in a graphical form for biogas production rate at total solids of 8% and 35^0C at different retention times.

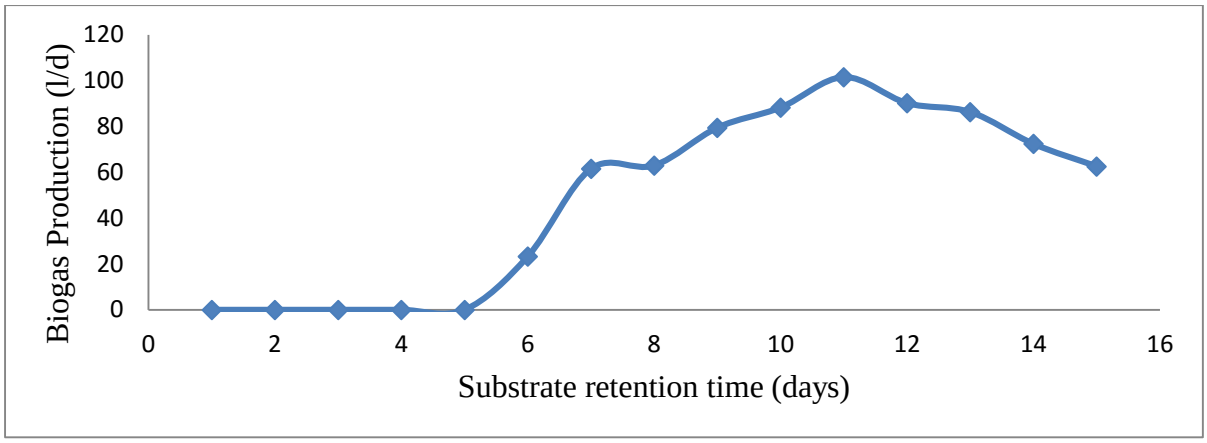


Figure 3.7: Biogas production at various substrate retention times

Assuming a 2 or 3 stage digestion process starting from the onset of quality biogas production, that is from the 5th day, the process can be modelled by second and third order polynomial regression equations as given in Figure 3.8 and equations 3.8 and 3.9.

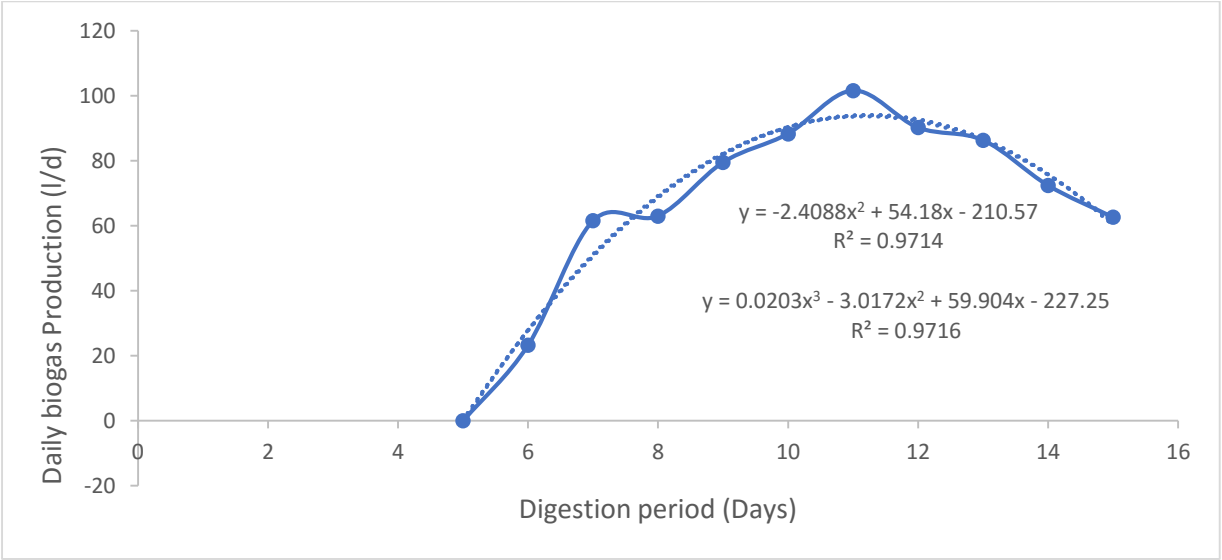


Figure 3.8: Biogas production at 8% TS and 35^0C using 2019 data

$$Y_{2ord} = -2.41x^2 + 54.18x - 210.57 \qquad 3.8$$

$$Y_{3ord} = 0.02x^3 - 3.02x^2 + 59.90x - 227.25 \qquad 3.9$$

The simulations using these equations (3.8 and 3.9) were compared with those previous equations (3.1 and 3.2) using 2018 data as presented in Appendix A2. The simulations depict differences which could be attributed to the assumptions of the 2, 3 or 4 stage digestion processes while the interaction with the total solids and temperature may be difficult to separate. A number of factors may be the cause. For instance, the 1st stage (0-5 days) with zero gas production could rightly be excluded from modelling. Secondly the 2nd and 3rd

stages are difficult to separate since there is only half day difference between the 2. The only model, therefore, suggested for the biogas prediction for the system between day 5 and about day 17 when burnable gas (biogas) ceased, could be a 2nd order polynomial model though its precision and statistical accuracy will require more data to enhance and generalize.

There was no biogas production from day 1 to day 5. This was attributed to three processes of anaerobic digestion taking place: hydrolysis, acidogenesis and acetogensis respectively. The first one was hydrolysis. Organic biomass contains complex polymers which are inaccessible to microorganisms without being further broken down through hydrolysis (Gujer and Zehnder, 1983). As a result, hydrolysis serves the purpose of rendering macromolecules into its smaller components which in turn can be utlised by acidogenic bacteria (Meegoda *et al.*, 2018). During hydrolysis, hydrolytic bacteria are able to secrete extracellular enzymes that can convert carbohydrates, lipids and proteins into simple sugars, long chain fatty acids and amino acids respectively (Park *et al.*, 2011). After enzymatic cleavage, the products of hydrolysis are able to diffuse through the cell membranes of acidogenic microorganisms (van Lier *et al.*, 2008).

The second process was acidogenesis. Acidogenic bacteria are able to produce intermediate volatile fatty acids (VFAs) and other products (Meegoda *et al.*, 2018). Volatile fatty acids constitute a class of organic acids such as acetates, and larger organic acids such as propionate and butyrate (Bergman *et al.*, 1990). As opposed to other stages, acidogenesis is generally believed to proceed at a faster rate than all other processes of anaerobic digestion, with acidogenic bacteria having a regeneration time fewer than 36 hours (Deublein and Steinhauser, 2008). With the rapidity of this stage, the production of volatile fatty acids creates direct precursors for the final stage of methanogenesis; Volatile fatty acid acidification is widely reported to be a cause of digester failure (Akuzawa *et al.*, 2011).

Acetogenesis is the third process during which higher volatile fatty acids (e.g. propionate and butyrate) are converted into acetate and hydrogen (Hansen and Cheong, 2013). At the same time, lipids undergo a separate pathway of acetogenesis through acidogenesis and β-oxidation, where acidogenesis produces acetate from glycerol, while β-oxidation produces acetate from LCFAs (Cirne *et al.*, 2007).

The results and the observation of retention time made in this study may not be in total agreement with (Varma *et al.*, 2009) who reported that hydrolysis takes 2 days while acidogenesis and acetogenesis take 3.6 days. This may be due to a different system (digester) design and management (operational factors such as temperature, feeding and stirring regimes) and substrate characteristics including type of feedstock, total solids among other variables, apart from the data collection precision.

Methanogenesis marks the final stage of anaerobic digestion, where accessible intermediates are consumed by methanogenic bacteria to produce methane (Ferry, 2010). Production of biogas starts from day 5 and increases steadily to day 11 (102.00 l/d). Methanogenic bacteria are confined to a small selection of substrates (Meegoda *et al.*, 2018). Aceticlastic methanogenesis from acetate accounts for nearly two thirds of the methane production while the remaining one third comes from hydrogenotrophic methanogenesis (Belay *et al.*, 1986; Lovley and Klug, 1983).

However, it was noticed that biogas production stagnated from day 7 to day 8. This can be attributed to excessive partial pressure of hydrogen produced by acetogenesis that deleted acetogenic microorganisms (Dinopoulou *et al.*, 1988). But due to the presence of hydrogenotrophic methanogens, especially the methanosarcina species, hydrogen was able to be rapidly consumed while maintaining partial pressure at a level favourable to acetogenesis by creating an exergonic reaction (Stams and Plugge, 2009). During the stagnation period, methanosarcina species, which tend to be robust and are capable of withstanding ammonia, sodium, and acetate concentrations in addition to pH shocks at levels that would otherwise be detrimental to other methanogenic bacteria, kept on producing biogas (de Vrieze *et al.*, 2012). Consequently, the steady production came on course from day 8 to day 11.

Methanogenic bacteria represent a group of obligate anaerobic archaea; as an acute sensitivity, it was found that 99% of methanococcus voltae and methanococcus vannielli cells had been killed within ten hours upon exposure to oxygen (Kiener and Leisinger, 1983). Methanogens have a slower regeneration time than other bacteria in an anaerobic digestion; at least 5 to 16 days (Deublein and Steinhauser, 2008). However, some hydrogenotrophic species, such as methanococcus maripaludis, have a doubling time of only two hours (Richards *et al.*, 2016).

From day 11 onwards, biogas production started declining from 102 l/d to 62.63 l/d on day 15. This was caused by the depletion of nutrients in the substrate and the gradual death of the methanogens. In batch reactors, the end of methanogenesis is determined when biogas production stops, which can take about 40 days (Verma, 2002).

3.4 Conclusions and Recommendation

3.4.1 Conclusions

Biogas production rate varied with different total solid levels increasing from an average of 0.25 m^3/m^3d at 6% total solids (TS), through an average of 0.32 m^3/m^3d for 7% TS to reach a maximum average of 0.48 m^3/m^3d at 8% TS (with a manure to water ratio of 1:1.3), and then started decreasing steadily to 0.24 m^3/m^3d as the total solids increased to 10% through an average of 0.32 m^3/m^3d for the other total solid levels of the cattle manure under free range animal production system in Egerton, Kenya. The optimal TS for the laboratory fixed dome digester is 8% when operation at a digester temperature of 25 to 45^oC.

In the mesophilic range of temperature, biogas production increased with increase in temperature, generally, moving from 0.25 m^3/m^3d at 25^oC through 0.35 m^3/m^3d at 30^0C to 0.53 m^3/m^3d at 40^0C (optimal temperature), and then declined to 0.33 m^3/m^3d at 45^0C.

The biogas production increases with the retention time, being maximum of 0.68 m^3/m^3d from the batch laboratory fixed dome digester at 11^{th} day of digestion, having started biogas production after 5 days for 8% TS cattle manure substrate being maintained at a temperature of 35^0C.

Empirical models to simulate the biogas production with the temperature and total solid ranges used are possible.

3.4.2 Recommendation

The above conclusions can be transferred with appropriate modifications and scale factors for adoption for field and industrial fixed digesters for biogas production into operational guidelines for biogas stakeholders including designers and operators.

Further research is recommended to determine:

1. Effect of feeding regimes (as batch and continuous bioreactor or digester) at varying temperatures and stirring frequencies for different substrates

2. The effect of thermophilic and cryophilic (ambient and below) temperature ranges on biogas production rate and quality for the laboratory fixed dome digester
3. Accuracy and precision of empirical models from experimental and observed data from the biogas system.

References

Abbassi-Guendouz, A., Brockmann, D., Trably, E., Dumas, C. and Delgenès, J. (2012). Total solids content drives high solid anaerobic digestion via mass transfer limitation. *Bioresource Technology, 111*, 55-61.

Achinas, S. and Euverink, G. J. W. (2016). Theoretical analysis of biogas potential prediction from agricultural waste. *Resource-Efficient Technologies, 2*(3), 143-147.

Adepoju, T., Olatunbosun, B. and Olawale, O. (2016). Statistical analysis of biogas production from co-digestion of cornstalk with goat dung using a one factor design. *Chemistry Research Journal, 1*(4), 1-10.

Akuzawa, M., Hori, T., Haruta, S., Ueno, Y., Ishii, M. and Igarashi, Y. (2011). Distinctive responses of metabolically active microbiota to acidification in a thermophilic anaerobic digester. *Microbial Ecology, 61*(3), 595-605.

APHA. (1999). Standard Methods for the Examination of Water and Wastewater. *American Public Health Association*.

Barnes, R., Dhanoa, M. S. and Lister, S. J. (1989). Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy, 43*(5), 772-777.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S., Pavlostathis, S., Rozzi, A., Sanders, W., Siegrist, H. and Vavilin, V. (2002). The IWA anaerobic digestion model no 1 (ADM1). *Water Science and Technology, 45*(10), 65-73.

Beevi, B. S., Madhu, G. and Sahoo, D. K. (2015). Performance and kinetic study of semi-dry thermophilic anaerobic digestion of organic fraction of municipal solid waste. *Waste Management, 36*, 93-97.

Belay, N., Sparling, R. and Daniels, L. (1986). Relationship of formate to growth and methanogenesis by Methanococcus thermolithotrophicus. *Applied Environmental Microbiology, 52*(5), 1080-1085.

Bergman, H., Wichmann, T. and DeLong, M. R. (1990). Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. *Science, 249*(4975), 1436-1438.

Björnsson, L., Murto, M. and Mattiasson, B. (2000). Evaluation of parameters for monitoring an anaerobic co-digestion process. *Applied Microbiology and Biotechnology, 54*(6), 844-849.

Bochmann, G., Drosg, B., Ortner, M., Schonlieb, M., Andres-Lainez, S., Kirchmayr, R. and Braun, R. (2010). *Influence of thermal pre-treatment to increase digestibility of*

brewers' spent grains. Paper presented at the 12th World Congress on Anaerobic Digestion, Guadalajara.

Bochmann, G. and Montgomery, L. (2014). *Pretreatment of Feedstock for Enhanced Biogas Production*. Retrieved from Paris, France.

Bourque, J., Guiot, S. and Tartakovsky, B. (2008). Methane production in an UASB reactor operated under periodic mesophilic-thermohilic conditions. *Biotechnology Bioengineering, 100*, 1115-1121.

Budiyono, Widiasa, I., Johari, S. and Sunarso. (2010). The influence of total solid contents on biogas yield from cattle manure using rumen fluid inoculum. *Energy Research Journal, 1*(1), 6-11.

Buswell, A. and Mueller, H. (1952). Mechanism of methane fermentation. *Industrial and Engineering Chemistry, 44*(3), 550-552.

Castillo, R.-T., Luengo, P. and Alvarez, J. M. (1995). Temperature effect on anaerobic of bedding manure in a one phase system at different inoculums concentration. *Agriculture, Ecosystems and Environment, 54*, 55-66.

Charlton, S., Dollmeyer, T. and Grana, T. (2010). Meeting the US heavy-duty EPA 2010 standards and providing increased value for the customer. *SAE International Journal of Commercial Vehicles, 3*(2010-01-1934), 101-110.

Cirne, D., Paloumet, X., Björnsson, L., Alves, M. and Mattiasson, B. (2007). Anaerobic digestion of lipid-rich waste—Effects of lipid concentration. *Renewable Energy, 32*, 965-975.

Conklin, A., Stensel, H. and Ferguson, J. (2006). Growth kinetics and competition between Methanosarcina and Methanosaeta in mesophilic anaerobic digestion *Water Environment Research, 78*, 486-496.

de Vrieze, J., Hennebel, T., Boon, N. and Verstraete, W. (2012). Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresource technology, 112*, 1-9.

Demirel, B. and Scherer, P. (2008). The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. *Reviews in Environmental Science and Biotechnology, 7*, 173-190.

Deublein, D. and Steinhauser, A. (2008). *Biogas from Waste and Renewable Resources: An Introduction*. Weinheim, Germany.

Dinopoulou, G., Rudd, T. and Lester, J. (1988). Anaerobic acidogenesis of a complex wastewater: The influence of operational parameters on reactor performance. *Biotechnology and Bioengineering, 31*, 958-968.

Dolud, M., Andree, H. and Hügle, T. (2005). Rapid analysis of liquid hog manure using near-infrared spectroscopy in flowing condition. *Precision livestock farming '05. Wageningen Academic Publications, Wageningen, The Netherlands*, 115-122.

Eder, B. and Schulz, H. (2006). *Biogas Praxis [in German]* (3rd ed.).

Elaiyaraju, P. and Partha, N. (2016). Studies on biogas production by anaerobic process using agroindustrial wastes. *Research in Agricultural Engineering, 62*(2), 73-82.

Eltawil, M. and Belal, E. (2009). Evaluation and scrubbing of biogas generation from agricultural wastes and water hyacinth biological engineering. *Miscillenous Journal of Agricultural Engineering, 26*(1), 534-560.

EPA. (2001). Method 1684: Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids. *U.S. Environmental Protection Agency*.

Ferry, J. (2010). The chemical biology of methanogenesis. *Planet and Space Science, 58*, 1775–1783.

Forster-Carneiro, T., Pérez, M. and Romero, L. (2008). Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste *Bioresource Technology*(99), 6994-7002.

Franke-Whittle, I. H., Walter, A., Ebner, C. and Insam, H. (2014). Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. *Waste Management, 34*(11), 2080-2089.

Ghatak, M. D. and Mahanta, P. (2014). Effect of temperature on anaerobic co-digestion of cattle dung with lignocellulosic biomass. *Journal of Advanced Engineering Research, 1*(1), 1-7.

Grady, C., Daigger, G. and Lim, H. (1999). Biological wastewater treatment: Principles and practice. New York: Marcel Dekker, New York.

Gujer, W. and Zehnder, A. (1983). Conversion processes in anaerobic digestion. *Water Science and Technology, 15*, 127-167.

Hajji, A., Rhachi, M., Garoum, M. and Laaroussi, N. (2016). *The effects of pH, temperature and agitation on biogas production under mesophilic regime.* Paper presented at the 2016 3rd International Conference on Renewable Energies for Developing Countries (REDEC).

Hansen, C. and Cheong, D. (2013). *Agricultural Waste Management in Food Processing*. Cambridge, MA, USA: Academic Press.

Hwu, C.-S., Donlon, B. and Lettinga, G. (1996). Comparative toxicity of long-chain fatty acid to anaerobic sludges from various origins. *Water Science and Technology, 34*(5-6), 351-358.

Igoni, A. H., Abowei, M., Ayotamuno, M. and Eze, C. (2008). Effect of total solids concentration of municipal solid waste on the biogas produced in an anaerobic continuous digester. *Agricultural Engineering International: CIGR Journal*.

Itodo, I. and Awulu, J. (1999). Effects of total solids concentrations of poultry, cattle, and piggery waste slurries on biogas yield. *Transactions of the ASAE, 42*(6), 1853-1855.

Jahng, D., Lee, Y. and Zhang, L. (2011). Anaerobic co-digestion of food waste and piggery wastewater: focusing on the role of trace elements. *Bioresource Technology, 102*(8), 5048-5059.

Kiener, A. and Leisinger, T. (1983). Oxygen sensitivity of methanogenic bacteria. *Systematic and Applied Microbiology, 4*, 305-312.

Kircher, M., Witten, D. M., Jain, P., O'Roak, B. J., Cooper, G. M. and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nature Genetics, 46*(3), 310.

Leenawat, A., Pasakorn, J., Atip, L., Rodriguez, J. and Khanita, K. (2016). *Effect of temperature on increasing biogas production from sugar industrial wastewater treatment by UASB process in pilot scale.* Paper presented at the 3rd International Conference on Power and Energy Systems Engineering, CPESE, Kitakyushu, Japan.

Lemmer, A., Naegele, H.-J. and Sondermann, J. (2013). How Efficient are Agitators in Biogas Digesters? Determination of the Efficiency of Submersible Motor Mixers and Incline Agitators by Measuring Nutrient Distribution in Full-Scale Agricultural Biogas Digesters. *Energies*(6), 6255-6273. doi:10.3390/en6126255

Lettinga, G., Field, J., Van Lier, J., Zeeman, G. and Pol, L. H. (1997). Advanced anaerobic wastewater treatment in the near future. *Water Science and Technology, 35*(10), 5-12.

Liu, Z. and Jian, L. (2016). The effect of total solids concentration and temperature on biogas production by anaerobic digestion. *Energy sources, Part A: Recovery, utilization, and environmental effects, 38*(23), 3534-3541. doi:10.1080/15567036.2016.1183064

Lovley, D. and Klug, M. (1983). Methanogenesis from Methanol and Methylamines and Acetogenesis from Hydrogen and Carbon Dioxide in the Sediments of a Eutrophic Lake. *Applied and Environmental Microbiology, 45*, 1310-1315.

Manjula, D. and Mahanti, P. (2014). *Effect of temperature on biogas production from Lignocellulosic biomasses.* Paper presented at the Proceedings of 1st International Conference on non-conventional energy (ICONCE).

Mao, C., Feng, Y., Wang, X. and Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews, 45*, 540-555.

Meegoda, J., Li, B., Patel, K. and Wang, L. (2018). A review of the processes, parameters, and optimization of anaerobic digestion. *International Journal of Environmental Research and Public Health, 15*(10), 2224.

Møller, H., Sommer, S. and Ahring, B. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy, 26*, 485-495.

Moset, V., Poulsen, M., Wahid, R., Højberg, O. and Møller, H. B. (2015). Mesophilic versus thermophilic anaerobic digestion of cattle manure: methane productivity and microbial ecology. *Microbial Biotechnology, 8*(5), 787-800.

Mudhoo, A., Moorateeah, P. and Mohee, R. (2012). Effects of microwave heating on biogas production, chemical oxygen demand, and volatile solids solubilization of food residues. *World Academy of Science, Engineering and Technology, 69*.

Nopharatana, A., Pullammanappallil, P. C. and Clarke, W. P. (2007). Kinetics and dynamic modelling of batch anaerobic digestion of municipal solid waste in a stirred reactor. *Waste Management, 27*(5), 595-603.

Nyaanga, D. (2011). *Performance of Tropical Biogas*. Paper presented at the Egerton University International Conference, Egerton University, Kenya.

Pandey, P. and Soupir, M. (2012). Impacts of temperatures on biogas production in dairy manure anaerobic digestion. *International Journal of Engineering and Technology, 4*, 629-631.

Parawira, W., Murto, M., Read, J. and Mattiasson, B. (2005). Profile of hydrolyses and biogas production during two stage mesophilic anaerobic digestion of solid potato waste. *Process Biochemistry, 40*, 2945-2952.

Park, Y., Li, Y. and Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Review, 15*, 821-826.

Patharwat, I., Kumbhar, S., Hingalaje, P., Khurpe, A., Goudgaon, J. and Dhamangaonkar, P. (2016). Analysis of optimum temperature and validity of biogas plant. *International Journal of Industrial Electronics and Electrical Engineering, 4*(4), 76.

Pavlostathis, S. and Giraldo-Gomez, E. (1991). Kinetics of anaerobic treatment. *Water Science and Technology, 24*(8), 35-59.

Pavlostathis, S. and Giraldo- Gomez, E. (1991). Kinetics of anaerobic treatment: a critical review. *Critical Reviews in Environmental Science and Technology, 21*(5-6), 411-490.

Richards, M., Lie, T., Zhang, J., Ragsdale, S., Leigh, J. and Price, N. (2016). Exploring hydrogenotrophic methanogenesis: A genome scale metabolic reconstruction of methanococcus maripaludis. *Journal of Bacteriology, 198*, 3379-3390.

Shi, X., Dong, J., Yu, J., Yin, H., Hu, S., Huang, S. and Yuan, X. (2017). Effect of hydraulic retention time on anaerobic digestion of wheat straw in the semi-continuous continuous stirred-tank reactors. *BioMed Research International*.

Sovacool, B. K. (2012). The political economy of energy poverty: A review of key challenges. *Energy for Sustainable Development, 16*(3), 272-282.

Stams, A. and Plugge, C. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nature Reviews Microbiology, 7*, 568-577.

Taherzadeh, M. and Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences, 9*(9), 1621-1651.

Tchobanoglous, G., Burton, F. and Stensel, H. (2003). *Waste Water Engineering: Treatment and Re-use* (4th ed.): Tata McGraw-Hill Publishing Company Ltd.

Tricase, C. and Lombardi, M. (2009). State of the art and prospects of Italian biogas production from animal sewage: technical-economic considerations. *Renewable Energy, 34*(3), 477-485.

Tsunatu, D., Azuaga, I. and Agabison, J. (2014). Evaluation of the effect of total solids concentration on biogas yields of agricultural wastes. *International Research Journal of Environment Sciences, 3*(2).

Uzodinma, E., Ofoefule, A., Eze, J. and Onwuka, N. (2007). Biogas Production from blends of Agro-industrial wastes. *Trends in Applied Science Research, 2*(6), 554-558.

van Lier, J., Mahmoud, N. and Zeeman, G. (2008). Anaerobic Wastewater Treatment. In Biological Wastewater Treatment: Principles, Modelling and Design. *International Water Association, UK*, 401-442.

Varma, R., Vesvikar, M., Karim, K., Hoffman, R., De Paoli, D., Klasson, K., Winterberg, A. and Alexander, C. (2009). *Bioenergy production from anaerobic digestion of animal and farm wastes.* Paper presented at the Energy Summit, Missouri University of Science and Technology.

Vavilin, V., Lokshina, L., Rytov, S., Kotsyurbenko, O. and Nozhevnikova, A. (1996). Modeling methanogenesis during anaerobic conversion of complex organic matter at low temperatures. *Water Science and Technology, 36*(6-7), 531-538.

Verma, S. (2002). Anaerobic Digestion of Biodegradable Organics in Municipal Solid Wastes.

Walsh, K. and McLaughlan, R. (1999). Bubble extraction of dissolved gases from ground water samples. *Water, Air, and Soil Pollution, 115*, 525-534.

Yi, J., Dong, B., Jin, J. and Dai, X. (2014). Effect of Increasing Total Solids Contents on Anaerobic Digestion of Food Waste under Mesophilic Conditions: Performance and Microbial Characteristics Analysis. *PLoS ONE, 9*(7).

CHAPTER FOUR
EVALUATING BIOGAS PRODUCTION PREDICTION MODELS

Abstract

A number of biogas production prediction models have been proposed but they have not been tested for the common Kenyan organic material, cow or cattle manure used to produce biogas. They include the empirical plug flow digester model at fixed temperature, pH, and loading rate and expressing biogas yield as a function substrate influent and effluent volatile solids and hydraulic retention time. A modification of this model by Chen and Hashimoto Model adds the influence of temperature and bacterial population. A simpler low temperature lagoon model relates temperature to biogas production amount. This model has been advanced to the Toprak model which made yield a function of the exponential of temperature. Chen and Hashimoto Model and Scoff and Minott model are more involving with more variables and hence more since they involve hydraulic retention time, volatile solids concentrations, bacteria growth rate, digester temperature, and daily substrate flow rate. These biogas production prediction models were evaluated using data observed while producing biogas from a fixed dome digester set at selected temperatures and using cow and liquid cattle manure from semi-free range animals of Tatton Farm, at Njoro in Kenya at 6, 8 and 10% total solids. Two (the Low Temperature Lagoon and the Toprak) out of the five models tested, suited a fixed dome batch bioreactor data using a goodness of fit between the predicted data by the models and that obtained from laboratory experiments. A third model (named the Fixed dome Temperature model) relating temperature to the rate of biogas production) was developed from experimental data, and validated using a set of observed data from a different experiment conducted nearly a year later from the data used to develop the model. The similarity among the Low Temperature Lagoon, modified Toprak and Fixed dome Temperature models was observed while noting their simplicity and hence may not fully explain the 3 or 4 stages of the biogas production fluctuations. The other models could be applied in the digester system but with more in depth understanding and determination of the bacterial population dynamics.

4.1 Introduction

Models are developed and used to help scientists, engineers, and decision makers to understand and communicate about a system of interest with the ultimate aim of bringing a positive change to how a system is built and/or managed. First, models must be a simplified

representation of the system and provide a cognitively-mediated environment to explain the systemic behavior (Akbas and Ozdemir, 2018). Second, models are limited to the collective cognitive complexity of the 'mental models' of those involved in model development: some models are an inconsistent representation of a dynamic and complex system (Stockburger, 2006). These challenges are escalated on using models in deep uncertainty situations where stakeholders do not know or cannot agree on the system structure, future scenarios, and model parameters (Kwakkel and Pryut, 2013).

There are four types of models (Ali *et al.*, 2016):

i. Physical models: these are smaller and simpler representations of the thing being studied.
ii. Conceptual models: they tie together many ideas to explain a common phenomenon or event.
iii. Mathematical models: are sets of equations that take into account many factors to represent a phenomenon.
iv. Stochastic models: are tools for estimating probability distributions of potential outcomes by allowing for random variation in one or more inputs over time.

In this study, mathematical models were used because integers and figures were involved in relating the inputs (factors) to the output (biogas production rate).

Models for predicting biogas production include:

a) Plug Flow Model

The model was among the earliest suggested for predicting gas production in a plug flow digester by Jewell *et al.* (1978). It is a simple empirical formula with many parameters like temperature, pH, and organic loading rate remaining constant; and is given as:

$$Y = 0.5\left(\frac{S_{bo} - S_{bi}}{HRT}\right) \qquad 4.1$$

where,

Y = Biogas yield in litres/litre of digester per day

S_{bo} = Influent volatile solids

S_{bi} = Effluent volatile solids

HRT = Hydraulic retention time in days

If any of the fixed parameters changed, the model would not remain valid (Jewell *et al.* (1978).

b) Chen and Hashimoto Model

Chen and Hashimoto (1978) improved on the Plug Flow model by incorporating other parameters in their model to take care of digester temperature, bacterial growth rate and volatile solid concentration of the substrate. The model is given as:

$$Y = \frac{B_0 S_0}{HRT}\left\{1 - \frac{K}{HRT(u_m) - 1 + K}\right\} \qquad 4.2$$

where,

Y = Biogas production in litres/litre of digester per day

B_o = Ultimate yield of gas in litres/gram of volatile solid (VS) added

S_o = Influent VS concentration in grams/litre

K = Kinetic parameter (dimensionless) = $0.6 + 0.0206e^{0.051So}$

HRT = Hydraulic Retention Time

$u_m = 0.013T - 0.129$ = Maximum bacteria growth rate in which T is the temperature in degrees Celsius (^0C)

T = Digester temperature in ^0C

c) Low Temperature Lagoon Digester Model

Safley and Westerman (1992) developed a model to predict gas production from a low temperature lagoon digester or pond digesters. It is based on the temperature of the lagoon, and keeps all other parameters constant including substrate type, total solids, feeding rate and hydraulic retention time among others. The linear Lagoon model was given as

$$Y = 0.0093T + 0.216 \qquad 4.3$$

where,

Y = Biogas production in m^3/kg VS

T = Digester temperature in ^{0}C

The temperature was measured at various points (horizontally and vertically) of the lagoon while observing the ambient temperature.

d) Toprak model

Toprak (1995) developed a model for a pond digester as shown in equation (4.4).

$$Y = 1.241T_a^{1.127} \quad 4.4$$

where,

Y = Biogas production in L/m^3 of digester per day

T_a = Ambient air temperature, ^{0}C

This Pond Model, like the Lagoon model, used temperature (specifically ambient temperature) giving the biogas production as an exponential of the temperature.

The advantages and disadvantages of these models to a laboratory reactor data are likely to be varied and applicability may be low or high and hence the need to test the models using the data from the fixed dome digester.

e) Scoff and Minott model

A more accurate model for plug flow digesters was developed by Minott (2002). It considers hydraulic retention time, volatile solids concentrations, bacteria growth rate, digester temperature, and daily substrate flow rate. It is given as:

$$\mathrm{Y} = \frac{0.5}{\mathrm{HRT}}\left\{\mathrm{C_0} - \mathrm{C_T(x,t)v}\frac{\mathrm{T_a}}{\mathrm{T_0}}\right\} \quad 4.5$$

where,

Y = Gas production in kg/day

HRT = Hydraulic Retention Time (days)

v = Daily flow rate of manure slurry into digester (m^3/day)

C_0 = 0.863TS (which is influent total volatile solids in kg/m^3, TS = total solids)

T_0 = Constant temperature at which the digester is operated

T_a = 273.2 K

C_T = Total substrate degradation in the digester (kg/m^3. Total amount of organic matter digested)

C_T is given as:

$$C_T(x,t) = \frac{C_0 K e^{(-1/K)}}{u_m^2 vt}\left\{(u_m - 2K)\left[1 - e^{(-u_m t/K)}\right] + tu_m\left[1 + e^{(-u_m t/K)}\right]\right\} \quad 4.6$$

where,

C_T = Total substrate degradation

x = at given position

t = at given time

K = Hashimoto ideal plug flow constant = 1.26

u_m= 0.013T_0 − 0.129 (which is the maximum bacterial growth rate)

T = maximum HRT for digestion up to a given point (days).

In this study, some of the above existing mathematical models for predicting biogas production were tested to find out if they could relate the rate of biogas production from a laboratory fixed dome batch bioreactor to some of the selected process or digestion parameters. The tools for testing included:

a) Coefficient of determination, R^2 (Sen, 2008).

$$R^2 = \frac{(\sum_{i=1}^{N} Exp_i Pred_i)^2}{\sum_{i=1}^{N} Exp^2 \sum_{i=1}^{N} Pred^2} \quad 4.7$$

where,

Exp_i = Experimental value

$Pred_i$ = Predicted value of the model

N = Number of observations

R^2 varies from 0 to 1; the closer the value is to 1, the better is the relationship between the experimental and predicted values.

b) Modelling Efficiency, EF (Lahsasni *et al.*, 2004).

$$EF = \frac{\sum_{i=1}^{N}(Exp_i - Exp_{i\,mean})^2 - \sum_{i=1}^{N}(Pred_i - Exp_i)^2}{\sum_{i=1}^{N}(Exp_i - Exp_{i\,mean})^2} \quad 4.8$$

where,

Exp_i = Experimental value

$Exp_{i\,mean}$ = Experimental mean value

$Pred_i$ = Predicted value

N = Number of observations

EF varies from 0 to 1. The best fit comes when EF tends to 1.

c) Root Mean Square Error, RMSE (Eterkin and Firat, 2015).

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}(Exp_i - Pred_i)^2\right]^{0.5} \quad 4.9$$

where,

Exp_i = Experimental value

$Pred_i$ = Predicted value of the model

N = Number of observations

RMSE should tend to zero for a good fit between the experimental and predicted values.

d) Chi Square, X^2 (Hossain *et al.*, 2007).

$$X^2 = \frac{\sum_{i=1}^{N}(Exp_i - Pred_i)^2}{N - n} \quad 4.10$$

where,

Exp_i = Experimental value

$Pred_i$ = Predicted value of the model

N = Number of observations

n = number of constants in the model

The X^2 value tending towards zero gives a better goodness of fit.

Industrial and field biogas plants do not often operate at their optimum, and hence to attain a higher efficiency it is necessary to know the limits for the anaerobic digestion process; these can only be identified in laboratory experiments (Kowalczyk *et al.*, 2011). To use the results of laboratory scale experiments, it is essential to know whether the results are transferable to industrial, institutional or household biogas systems and whether the experiments are reproducible. Experiments at a laboratory scale are the most common way to study the anaerobic digestion process.

Brunn *et al.* (2009) experimented with the reproducibility and transferability of laboratory scale reactors to industrial scale; two identical 120 litre (L) reactors operating under the same conditions were run in parallel, and compared to an industrial scale reactor with a process volume of 4600m^3. The results showed that the industrial scale reactor produced, on average, 36% more gas than the laboratory scale reactors due to the different feeding schedules and substrates; the laboratory scale reactors were fed three times per week while the industrial scale reactor was fed daily, and the substrate for the reactors from different plants. A good reproducibility for reactors of the same scale was found, but not for transferability. This shows the importance of using similar process parameters for tests relating to reproducibility and transferability.

Kowalczyk *et al.* (2011) researched the scalability of anaerobic digestion by analysing the performance of three identical 22 L reactors and a single 390 L reactor, operating under identical process conditions. The goal of the work was to serve as a "pre-study" to determine the transferability of reactors of two different scales, as well as reproducibility between the reactors of the same size. The results of the study showed a high level of correspondence between the four reactors based on the measured parameters (biogas volume, biogas composition, percentage of dry matter, and volatile solids. While studying the transferability of experimental results, there must be a comparison between a laboratory scale reactor and

an industrial scale reactor in such a manner that all process parameters chosen must be as identical as possible for synchronicity.

4.2 Materials and Methods

The Plug Flow, Chen and Hashimoto, and Scoff and Minnott models are only applied to continuously-fed digesters as recommended by (Eder and Schulz, 2006) and hence they cannot be used to analyse the anaerobic digestion process in a batch bioreactor as was the case in this study. Consequently, the tests for goodness of fit were done on a Low Temperature Lagoon Digester, Modified Toprak, and Fixed-dome-lab Temperature models.

4.2.1 Low Temperature Lagoon Digester model

The Low Temperature Lagoon Digester Model (equation 4.3) was tested using data found in this research. The Toprak model was modified and tested to determine if it fitted the situation of a laboratory fixed dome batch reactor; ambient air temperature was replaced with digester temperature with the assumption that the digester could be operated at the stated temperatures as being the prevailing ambient temperatures. The two models were run at 8% total solids. All other factors were assumed to be constant. This was mainly done to test the optimality of performance in terms of biogas yield per day. The Lagoon Temperature model as presented in equation 4.3 after being converting biogas units in the function from m^3/kg VS by multiplying the experimental data gotten in this research with a factor of 1 (since the ratio of TS/VS varies widely depending on the substrate and system digestion conditions varying just below 1 and just above 2) and hence was used without any modification as given in equation. This assumption was used in the evaluation and testing of all the models in this chapter.

4.3.2 Modified Toprak model

Modified Toprak model

$$Y = 1.241T^{1.127} \qquad 4.11$$

where,

Y = Biogas production in m^3/m^3 per day

T = Digester temperature, ^{0}C.

This model was run at 25^{0}C, 30^{0}C, 35^{0}C, 40^{0}C and 45^{0}C.

4.2.3 Fixed-dome-lab Digester Temperature Model

Fixed-dome-lab Digester Temperature (FDT) Model was also developed from the data obtained on temperature, and validated. A test for goodness of fit was conducted using Coefficient of determination, Root Mean Square Error, and Chi Square (Sen, 2008).

4.3 Results and Discussions

4.3.1 Low Temperature Lagoon Digester model

Experimental values for biogas production rate (m^3/m^3d) at 8% total solids at different temperatures, and predicted values from the Low Temperature Lagoon Digester model (m^3/m^3d) as given in equation and given above and they are shown in Table 4.1 and Table 4.2, respectively.

Table 4.1: Actual biogas production rate (m^3/m^3d)

	Temperature (^0C)				
Day	25	30	35	40	45
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0.0761	0.1576	0.1249	0.0649
7	0	0.2551	0.4092	0.3862	0.1463
8	0	0.3021	0.4206	0.5040	0.2568
9	0.0523	0.3646	0.5325	0.5572	0.3455
10	0.1446	0.4389	0.5867	0.6495	0.4864
11	0.2231	0.4841	0.6800	0.7387	0.6261
12	0.3042	0.5358	0.6025	0.8071	0.4652
13	0.2454	0.4583	0.5733	0.6010	0.4181
14	0.1283	0.3435	0.4857	0.5191	0.2939
15	0.0929	0.2069	0.4267	0.3931	0.1906
Mean	0.0794	0.2310	0.3250	0.3521	0.2196

Table 4.2: Low Temperature Lagoon model predicted values

Day	Temperature (^{0}C)				
	25	30	35	40	45
1	0.2160	0.2160	0.2160	0.2160	0.2160
2	0.2160	0.2160	0.2160	0.2160	0.2160
3	0.2160	0.2160	0.2160	0.2160	0.2160
4	0.2160	0.2160	0.2160	0.2160	0.2160
5	0.2160	0.2160	0.2160	0.2160	0.2160
6	0.2160	0.2167	0.2175	0.2172	0.2166
7	0.2160	0.2184	0.2198	0.2196	0.2174
8	0.2160	0.2188	0.2199	0.2207	0.2184
9	0.2165	0.2194	0.2210	0.2212	0.2192
10	0.2173	0.2201	0.2215	0.2220	0.2205
11	0.2181	0.2205	0.2223	0.2229	0.2218
12	0.2188	0.2210	0.2216	0.2235	0.2203
13	0.2183	0.2203	0.2213	0.2216	0.2199
14	0.2172	0.2192	0.2205	0.2208	0.2187
15	0.2169	0.2179	0.2200	0.2197	0.2178
Mean	0.2167	0.2181	0.2190	0.2193	0.2180

The data was tested for goodness of fit using the Coefficient of Determination, Root Mean Square Error, and Chi Square (Devore and Farnum, 2005). Mean values for actual, and predicted biogas production rate (m^3/m^3d) were used.

i. Coefficient of determination, R^2

Table 4.3: R^2 calculations for Low Temperature Lagoon model

Temp (^{0}C)	Yield (m^3/m^3d)		Calculations		
	Exp	Pred	Exp.Pred	Exp^2	$Pred^2$
25	0.0794	0.2167	0.017207	0.006303	0.046976
30	0.2310	0.2181	0.050397	0.053371	0.047589
35	0.3250	0.2190	0.071178	0.105613	0.047971
40	0.3521	0.2193	0.077196	0.123942	0.048081
45	0.2196	0.2180	0.047877	0.048214	0.047542

$\sum(Exp.Pred) = 0.263856, \qquad (\sum(Exp.Pred))^2 = 0.06962$

$\sum Exp^2 = 0.337444, \qquad \sum Pred^2 = 0.238159$

$\sum Exp^2 . \sum Pred^2 = 0.080365$

$\therefore R^2 = \frac{0.06962}{0.080365} = 0.866296$ = 0.87, fair for such research

Generally, this implies that the goodness of fit between the predicted and observed data is between fairly good and good and hence the model for prediction of the system operation can be considered to be good.

ii. Root Mean Square Error, RMSE

Table 4.4: RMSE calculations for Low Temperature Lagoon model

Temp (^{0}C)	Yield (m^3/m^3d)		Calculations	
	Exp	Pred	Exp-Pred	$(Exp-Pred)^2$
25	0.079391	0.216738	-0.13735	0.018864
30	0.231022	0.218149	0.012874	0.000166
35	0.324982	0.219022	0.10596	0.011227
40	0.352053	0.219274	0.132779	0.01763
45	0.219578	0.218042	0.001536	2.36E-06

$$\sum_{i=1}^{5}(Exp_i - Pred_i)^2 = 0.04789$$

$\therefore RMSE = \sqrt{\left(\frac{0.04789}{5}\right)} = 0.009578 = 0.01$, good fit

iii. Chi Square, X^2

Table 4.5: X^2 calculations for Low Temperature Lagoon model

Temp (^{0}C)	Yield (m^3/m^3d)		Calculation	
	Exp	Pred	Exp-Pred	$(Exp-Pred)^2$
25	0.079391	0.216738	-0.13735	0.018864
30	0.231022	0.218149	0.012874	0.000166
35	0.324982	0.219022	0.10596	0.011227
40	0.352053	0.219274	0.132779	0.01763
45	0.219578	0.218042	0.001536	2.36E-06

$\sum_{i=1}^{5}(Exp_i - Pred_i)^2 = 0.04789 = 0.05$, fair

$N - n = 5 - 1 = 4$

$\therefore X^2 = \frac{0.04789}{4} = 0.01197 = 0.01$ indicating good fit

From, the R^2 (0.87), RMSE (0.01) and $X^2(0.01)$, it is clear that the Low Temperature Lagoon Model can be used to predict the biogas yield from a batch bioreactor operating using cow dung at reactor temperatures between 25 and 45^{o}C with some reservations since a R^2 value of 0.87 could be acceptable for systems where R^2 >0.85 is acceptable unlike where most reject good of fitting using R^2 <0.95. This could be attributed to the fact that, even in a low temperature lagoon, once bacterial activity starts, the lagoon temperature soon rises to above body temperature. The major similarity of between the two systems (batch feeding regime) could be the controlling factor due the absence of washout and dilution as can be experienced in a continuous or semi-continuous bioreactor.

4.3.2 Modified Toprak Model

From Table 4.1 that gives the actual values of biogas production rate (m^3/m^3d) and using the Modified Toprak model, Table 4.6 was created, give the predicted biogas production.

Table 4.6: Modified Toprak model predicted biogas values

Day	Temperature (^{0}C)				
	25	30	35	40	45
1	0.046693	0.057344	0.068224	0.079304	0.090561
2	0.046693	0.057344	0.068224	0.079304	0.090561
3	0.046693	0.057344	0.068224	0.079304	0.090561
4	0.046693	0.057344	0.068224	0.079304	0.090561
5	0.046693	0.057344	0.068224	0.079304	0.090561
6	0.046693	0.057344	0.068224	0.079304	0.090561
7	0.046693	0.057344	0.068224	0.079304	0.090561
8	0.046693	0.057344	0.068224	0.079304	0.090561
9	0.046693	0.057344	0.068224	0.079304	0.090561
10	0.046693	0.057344	0.068224	0.079304	0.090561
11	0.046693	0.057344	0.068224	0.079304	0.090561
12	0.046693	0.057344	0.068224	0.079304	0.090561
13	0.046693	0.057344	0.068224	0.079304	0.090561
14	0.046693	0.057344	0.068224	0.079304	0.090561
15	0.046693	0.057344	0.068224	0.079304	0.090561
Mean	0.046693	0.057344	0.068224	0.079304	0.090561

The data was tested for goodness of fit using the Coefficient of Determination, Root Mean Square Error, and Chi Square (Devore and Farnum, 2005). Mean values for actual, and predicted biogas production rate (m^3/m^3d) were used.

i. Coefficient of determination, R^2

Table 4.7: R^2 calculations for Modified Toprak model

Temp (^{0}C)	Yield (m^3/m^3d)		Calculations		
	Exp	Pred	Exp.Pred	Exp^2	$Pred^2$
25	0.079391	0.046693	0.003707	0.006303	0.00218
30	0.231022	0.057344	0.013248	0.053371	0.003288
35	0.324982	0.068224	0.022172	0.105613	0.004654
40	0.352053	0.079304	0.027919	0.123942	0.006289
45	0.219578	0.090561	0.019885	0.048214	0.008201

$\sum(Exp.Pred) = 0.086931$, $(\sum(Exp.Pred))^2 = 0.007557$

$\sum Exp^2 = 0.337444$, $\sum Pred^2 = 0.024613$

$\sum Exp^2 . \sum Pred^2 = 0.008306$

$\therefore R^2 = \frac{0.007557}{0.008306} = 0.909855$ = 0.91, hence a good fit.

ii. Root Mean Square Error, RMSE

Table 4.8: RMSE calculations for Modified Toprak model

Temp (^{0}C)	Yield (m^3/m^3d)		Calculations	
	Exp	Pred	Exp-Pred	$(Exp-Pred)^2$
25	0.079391	0.046693	0.032698	0.001069
30	0.231022	0.057344	0.173678	0.030164
35	0.324982	0.068224	0.256758	0.065925
40	0.352053	0.079304	0.27275	0.074392
45	0.219578	0.090561	0.129017	0.016645

$$\sum_{i=1}^{5}(Exp_i - Pred_i)^2 = 0.188196$$

$\therefore RMSE = \sqrt{\left(\frac{0.188196}{5}\right)} = 0.194$ = 0.2, implying a fairly good fit

iii. Chi Square, X^2

Table 4.9: X^2 calculations for Modified Toprak model

Temp (^{0}C)	Yield (m^3/m^{3}d)		Calculations	
	Exp	Pred	Exp-Pred	(Exp-Pred)^2
25	0.079391	0.046693	0.032698	0.001069
30	0.231022	0.057344	0.173678	0.030164
35	0.324982	0.068224	0.256758	0.065925
40	0.352053	0.079304	0.27275	0.074392
45	0.219578	0.090561	0.129017	0.016645

$$\sum_{i=1}^{5}(Exp_i - Pred_i)^2 = 0.188196 = 0.2$$

$$N - n = 5 - 0 = 5$$

$$\therefore X^2 = \frac{0.188196}{5} = 0.037639 = 0.04, hence\ good\ fit$$

4.3.3 Proposed Fixed-dome-lab Temperature Model

The experimental data of biogas production rate (measured in m^3/m^{3}d and collected in the early part of the year 2018) from fixed-dome-lab bioreactor at Egerton University and digesting at 35ºC using cow dung influent with a total solids content of 8% (presented in Table 4.1) was used to develop the Proposed Fixed-dome-lab Temperature Model. Experiments to collect data to validate the model, were carried out in the year 2019 and obtained results shown in Table 4.10. This data was used for validating the Fixed-dome-lab Temperature Model that was developed from the data of year 2018.

Table 4.10: Biogas production rate (m^3/m^3d) for 2019.

Day	Temperature (^0C)				
	25	30	35	40	45
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	10.620	30.732	17.793	8.465
7	0	35.591	79.794	55.033	19.087
8	0	42.138	82.017	71.820	33.512
9	9.408	50.861	103.844	79.401	45.092
10	26.028	61.221	114.400	92.549	63.475
11	40.164	67.527	132.600	105.269	81.701
12	54.756	74.744	117.481	115.007	60.708
13	44.172	63.928	111.800	85.642	54.557
14	23.100	47.913	94.705	73.976	38.349
15	16.728	28.867	83.200	56.021	24.873
Total(l)	214.356	483.414	950.573	752.514	429.823
Total(m^3/m^3d)	1.429	3.222	6.337	5.016	2.865
Mean(m^3/m^3d)	0.204	0.322	0.633	0.501	0.286

A curve fitting the biogas production rate mean values for 2018 was given by equation 4.12, having an R^2 of 0.9477 as:

$$Y = -0.0024T^2 + 0.1801T - 2.8332 \qquad 4.12$$

where,

T = temperature of the digester (^0C)

This second order polynomial implies a two stage model and in line with the Modified Toprak model (Toprak, 1995) using temperature as the major factor influencing the rate at which biogas is being produced. Both models use degrees Celsius in the computation of gas yield which may need verification if the use of Kelvin was to be applied. These assumptions

may lead to discrepancies that may be noted in the simulation compared to the observed data.

Equation 4.12 was used to produce simulated values as shown in Table 4.11.

Table 4.11: Simulation values of Fixed-dome-lab Temperature model

Sample No.	Year: 2018		
	Temp (^{0}C)	Y.exp*	Y.s**
1	25	0.1701	0.1693
2	30	0.3465	0.4098
3	35	0.4874	0.5303
4	40	0.5280	0.5308
5	45	0.3293	0.4113

*Experimental yields **Simulated yields

Table 4.12: Mean biogas production rate (m^3/m^3d) values for 2018 and 2019

Sample No.	Year	2018	2019
	Temp (^{0}C)	Yexp	Yvd***
1	25	0.1701	0.2041
2	30	0.3465	0.3222
3	35	0.4874	0.6337
4	40	0.5280	0.5016
5	45	0.3293	0.2865

***Data for validation

The data in Table 4.12 gave the graph in Figure 4.1

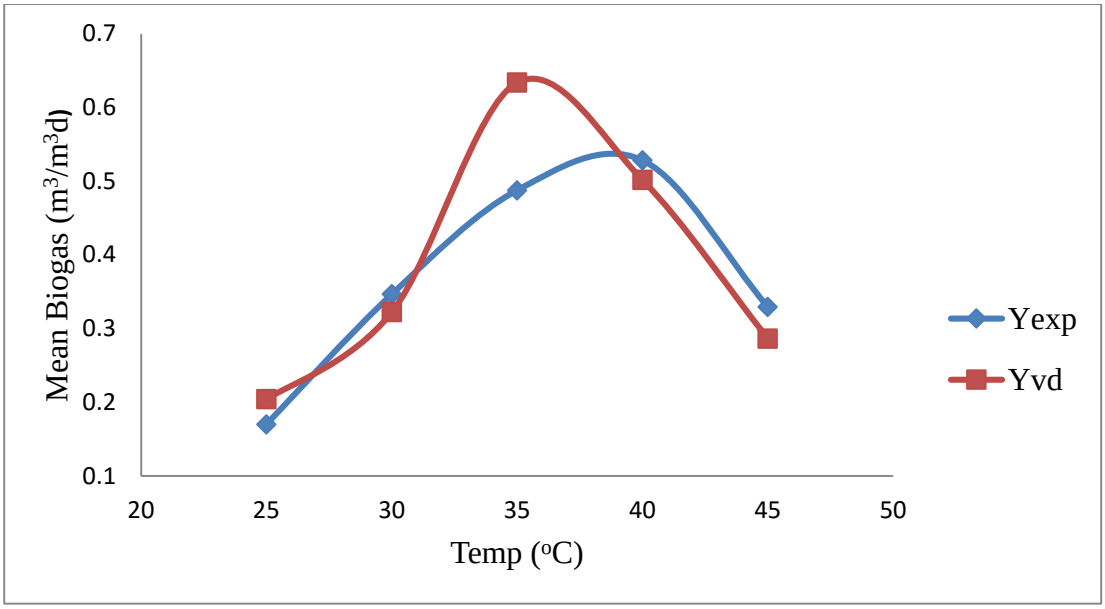


Figure 4.1: Trends of Fixed-dome-lab Temperature model and validation values

The Fixed-dome-lab Temperature model was tested for fitness using R^2, RMSE, and X^2.

i. Coefficient of determination, R^2

Table 4.13: R^2 calculations for Fixed-dome-lab Temperature model

Temp (^{0}C)	Yield (m^3/m^3d)		Calculations		
	Yexp	Yvd	Yexp.Yvd	$Yexp^2$	Yvd^2
25	0.170124	0.204149	0.034731	0.028942	0.041677
30	0.346533	0.322276	0.111679	0.120085	0.103862
35	0.487473	0.633715	0.308919	0.23763	0.401595
40	0.52808	0.501676	0.264925	0.278868	0.251679
45	0.329367	0.286549	0.094380	0.108482	0.082110

$$\sum(Yexp.Yvd) = 0.814634$$

$$(\sum(Yexp.Yvd))^2 = 0.663629$$

$$\sum Yexp^2 = 0.774009\,;\ \sum Yvd^2 = 0.880923$$

$$\sum Yexp^2.\sum Yvd^2 = 0.681842$$

$$\therefore R^2 = \frac{0.663629}{0.681842} = 0.973288$$

ii. Root Mean Square Error, RMSE

Table 4.14: RMSE calculations for Fixed-dome-lab Temperature model

Temp (^{0}C)	Yield (m^3/m^{3}d)		Calculations	
	Yexp	Yvd	Yexp-Yvd	(Yexp-Yvd)^2
25	0.170124	0.204149	-0.034020	0.001158
30	0.346533	0.322276	0.024257	0.000588
35	0.487473	0.633715	-0.146240	0.021387
40	0.52808	0.501676	0.026404	0.000697
45	0.329367	0.286549	0.042818	0.001833

$$\sum_{i=1}^{5}(Yexp_i - Yvd_i)^2 = 0.025663$$

$$N = 5$$

$$\therefore RMSE = \sqrt{\left(\frac{0.025663}{5}\right)} = 0.0716$$

i. Chi Square, X^2

Table 4.15: X^2 calculations for Fixed-dome-lab Temperature model

Temp (^{0}C)	Yield (m^3/m^{3}d)		Calculations	
	Yexp	Yvd	Yexp-Yvd	(Yexp-Yvd)^2
25	0.170124	0.204149	-0.03402	0.001158
30	0.346533	0.322276	0.024257	0.000588
35	0.487473	0.633715	-0.14624	0.021387
40	0.528080	0.501676	0.026404	0.000697
45	0.329367	0.286549	0.042818	0.001833

$$\sum_{i=1}^{5}(Yexp_i - Yvd_i)^2 = 0.025663$$

$$N - n = 5 - 1 = 4$$

$$\therefore X^2 = \frac{0.025663}{4} = 0.006416$$

A summary of model tests is given in Table 4.12

Table 4.16: Summary of model tests

Model	R^2	RMSE	X^2
Low Temperature Lagoon	0.8662	0.0095	0.0119
Modified Toprak	0.9098	0.1940	0.0376
Fixed-dome-lab Temperature	0.9732	0.0716	0.0064

A goodness of fit is achieved when the R^2 value is as close as possible to 1, while the RMSE and X^2 should tend to zero (Eterkin and Firat, 2015). A statistical analysis in Table 4.12 shows that the best goodness of fit is attained by the Fixed-dome-lab Temperature model having a Coefficient of determination of 0.9732, a Root mean square error of 0.0716, and a Chi square of 0.0064.

4.4 Conclusions and Recommendation

4.4.1Conclusions

The Low Temperature Lagoon Digester and Modified Toprak were tested. Consequently, the mathematical models showed relatively high levels of tests for goodness of fit with the observed data from the batch fixed dome lab digester biogas yields for the given temperatures.

The Fixed-dome-lab Temperature (FTP) Model developed for the bioreactor was found good in predicting its performance in terms of gas yield with temperature at set total solids level and could be the be adopted to predict its biogas production rate of the anaerobic digestion process when using cattle manure as a substrate especially at a TS of 8%. It can generally predict and be used with limits of temperature of 25^0C - 45^0C and total solids levels of 6% to 10% using cow manure from free range management system. The Fixed-dome-lab Temperature model is

$$Y = -0.0024T^2 + 0.1801T - 2.8332$$

where,

Y = Mean biogas produced in m^3per $m^3$of digester volume per day (m^3/m^3d)

T = Digester temperature (^0C)

4.4.2 Recommendation

Available mathematical models for the prediction of biogas production rate can be used with a precaution ensuring the initial and final boundary conditions of the model are clear and adhered to. In most cases, the scope and limitations for the models are not easily identified, hence the caution.

It is further recommended that the following research be conducted:

i. Existing models should be validated with huge, replicated and similar experimental data as ones used in their development.
ii. Models relating total solids and substrate retention time to biogas production rate should be developed for a fixed dome laboratory bioreactor as batch and semi continuous system that operates in a psychrophilic, mesophilic and thermophilic temperature ranges.
iii. Compare the lab bioreactor performance with those of the field digester for the same months or operating digestion temperatures.

References

Akbas, H. and Ozdemir, G. (2018). *Prediction Modeling of Biogas Production with Classification and Regression Tree at Wastewater Treatment Plants.* Paper presented at the The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM).

Ali, A., Rundong, L., Shah, F., Mahar, R. and WajidIjaz, M. (2016). Predictive Modeling of Biogas Production from Anaerobic Digestion of Mixed Kitchen Waste at Mesophilic Temperature. *International Journal of Waste Resources, 6*(3), 230. doi:10.4172/2252-5211.1000230

Brunn, L., Dornack, C. and Bilitewski, B. (2009). Application of laboratory scale experiments to industrial scale in case of anaerobic waste treatment. *Fresenius Environmental Bulletin, 18*(2), 196-203.

Chen, Y. and Hashimoto. (1978). *Kinetics of methane fermentation.* Paper presented at the Symposium on Biotechnology in Energy Production and Conservation, New York.

Devore, J. and Farnum, N. (2005). *Applied Statistics for Engineers and Scientists*. Belmont, CA: Curt Hinrichs.

Eder, B. and Schulz, H. (2006). *Biogas Praxis [in German]* (3rd ed.).

Eterkin, C. and Firat, M. (2015). A comprehensive review of thin layer drying model used in agricultural products. *Critical Reviews in Food Science and Nutrition, 57*(4), 701-717.

Hossain, M., Woods, J. and Bala, B. (2007). Single layer drying characteristics and colour kinetics of red Chilli. *International Journal of Food Science and Technology, 42*(11), 1367-1375.

Jewell, W., Carpener, H., Dell'orto, S., Fanfoni, K., Hayes, T., Leuschner, A., Miller, T., Sherman, D., Soest, P., Wolin, M. and Wujcik, W. (1978). *Anaerobic fermentation of agricultural residue: Potential for improvement and implementation - Final Report* (EY-76-S-02-2981-7). Retrieved from Cornell University.

Kowalczyk, A., Schwede, S., Gerber, M. and Span, R. (2011). *Scale up of laboratory scale to industrial scale biogas plants*. Paper presented at the World Renewable Energy Congress, Sweden.

Kwakkel, J. and Pryut, E. (2013). Exploratory Modelling and Analysis, an approach for model-based foresight under deep uncertainty. *Technological Forecasting and Social Change, 80*(3), 419-431.

Lahsasni, S., Kouhila, M., Marhouz, M. and Idlimam, A. (2004). Thin layer convective solar drying and modeling of prickly pear peel. *Energy, 29*, 211-224.

Minott, S. (2002). *Feasibility of fuel cells for energy conversion on the dairy farm*. Cornell University, New York.

Safley, L. and Westerman, P. (1992). Performance of a low temperature lagoon digester. *Bioresource Technology, 41*, 167-175.

Sen, Z. (2008). *Solar Energy Fundamentals and Modeling Techniques: Atmosphere, Environment, Climate Change and Renewable Energy*. Retrieved from London.

Stockburger, D. (2006). *Introductory Statistics: Concepts, Models and Applications*.

Toprak, H. (1995). Temperature and organic loading dependency of methane and carbon dioxide emission rates of a full-scale anaerobic waste stabilization pond. *Water Research, 29*(1), 1111-1119.

CHAPTER FIVE
OPTIMISATION OF BIOGAS PRODUCTION

Abstract

Optimisation was done by investigating the interaction effects of total solids, temperature, and substrate retention time on biogas production in a batch bioreactor. Central composite design (CCD) of Response Surface Methodology (RSM) was used to design the experiment. Total solid levels were varied from 6.31% to 9.68%, temperature was from 26.59^0C to 43.41^0C, and substrate retention time was from 9.95 to 20.04 days. Analysis of results was done using Design Expert software statistical package (version 10.0.0.3). It gave a coefficient of determination of 0.9665 which indicated a high correlation between the variables. All the variables had a significant effect. Contour plots, and three dimensional graphs were discussed. The highest rate of 75.41litres/day was achieved at a level of 8% total solids total solids, 43.41^0C, and 15 days.

5.1 Introduction

Optimisation is the act of achieving the best possible result under given circumstances. In design, construction and maintenance, engineers have to make decisions. The aim of such decisions is either to minimise the effort or to maximise benefit. The effort or benefit can be expressed as a function of certain design variables. Hence optimisation is the process of finding conditions that give the minimum or the maximum value of a function (Astolfi and Praly, 2006). Efforts to maximise biogas production are currently being done by using various feedstocks. Reungsang *et al.* (2012) used Response Surface Methodology (RSM) with a Central Composite Design (CCD) to optimise the key factors affecting methane production from the acidic effluent coming from the sugarcane juice hydrogen fermentation process. The parameters studied were substrate concentration, ratio of $NaHCO_3$ to substrate concentration, and an initial pH. The maximum methane yield of 367 ml CH_4/g-volatile solid (VS) added was obtained at the optimum conditions of 13,823 mg-COD/l, an $NaHCO_3$ to substrate concentration ratio of 3.09, and an initial pH of 7.07. Methane yield was enhanced 4.4-fold in comparison to raw effluent. Sathish and Vivekanandan (2016) studied the optimal conditions for biogas generation from anaerobic digestion of rice straw in a $1m^3$ floating drum anaerobic digester using RSM. The parameters were temperature, pH, substrate concentration, and agitation time. The highest level of biogas of $0.72m^3$ was produced at an optimum temperature of 50^0C, pH 7.5, substrate concentration of 110.70 kg, and an agitation time of 5 seconds, respectively. Saleh *et al.* (2012) investigated the effects of temperature,

palm oil mill effluent (POME) volume, inoculum volume, and co-substrate addition such as oil palm empty fruit bunch (EFB) and palm kernel on the anaerobic digestion process for biogas and methane production. RSM by Box-Behnken design verified that the specific biogas production rate and methane yield were mainly affected by the operating temperature and co-substrate addition. The optimal conditions for the maximum specific biogas production rate (0.0574 m^3/ kg chemical oxygen demand per day) and methane yield (25.6%) were predicted by multiple response optimisation and verified experimentally at 47.8^0C operating temperature, 50.4 ml POME volume, and 5.7 g EFB addition. Other similar works have been reported by Pannucharoenwong (2018) among others.

Response surface methodology (RSM) is one of the most effective approaches for designing experiments, for building models, and for determining optimal conditions on responses which are influenced by several independent variables (Bezerra *et al.*, 2008; Kang *et al.*, 2016). Apart from defining the influences of independent variables on the responses, RSM also determines the effect of interaction between parameters to obtain the best performance on a system (Belwal *et al.*, 2016; Zaroual *et al.*, 2009). RSM is a collection of statistical and mathematical techniques used for developing, improving, and optimising processes (Montgomery *et al.*, 2009). The most extensive applications of RSM are in situations where several input variables potentially influence some performance measure or quality characteristic of a process; most applications of RSM are sequential in nature, and are performed within some region of the independent variable space called the operability region (Carley and Kamneva, 2004).

Two types of RSM are used in design of experiments: Box–Wilson, and Box–Behnken (Jain *et al.*, 2011). The Box–Wilson, which is also called central composite design (CCD), has three different design points: edge points as in two-level designs (±1), star points at ±α that take care of quadratic effects, and centre points (0) (Jiménez *et al.*, 2014). Central composite design does testing at five levels. The edge points are at the design limits, star points are at some distance from the centre depending on the number of factors in the design; the star points extend the range outside the low and high settings for all factors, the centre points complete the design (Leiviskä, 2013). Central composite design provides high quality predictions over the entire design space, and is suitable for fitting a quadratic surface, and usually works well for process optimisation (Kousha *et al.*, 2015).

Box-Behnken design is an independent quadratic design because it does not contain an embedded factorial or fractional factorial design (Kong *et al.*, 2016). In this design, the treatment combinations are at the midpoints of edges of the process space and at the centre, require three levels of each factor: –1, 0, and +1, and has fewer treatment combinations than CCD (Leiviskä, 2013).

In this study, CCD was used. Experiments designed by using CCD have fewer runs, and also give similar results which are comparable to a full-factorial design, and enables an effective evaluation of interaction between individual factors to provide the best combination for optimal performance (Jiménez *et al.*, 2014; Khoobbakht *et al.*, 2016). Central composite design is robust; it makes the process consistent on target and is relatively insensitive to factors that are difficult to control (Taguchi, 1986, 1987). .

5.2 Materials and Methods

All the protocol was the same as in chapter 3 except the experimental design and data analysis.

a) Design of experiment

Central composite design was used to get the matrix of experiments with three factors, each being tested at five different levels. The response was biogas production (Y). The factors were: substrate retention time (x_1), total solids (x_2), and temperature (x_3). Design Expert software (version 10.0.0.3, Stat-Ease, Inc., Minneapolis, United States) statistical package was used to generate Table 5.1 and Table 5.2 for the experiment. The levels of every factor were as indicated in Table 5.1. In the design space, the highest level was coded as +1, the centre point was 0, and the lowest level was coded as –1. The outer design space points corresponding to α were ± 1.68179. $\alpha = 2^{k/4}$, where k is the number of factors (Ahmad, 2009; Aslan, 2007). In this case, k = 3. There was a total of 20 runs of experiments; comprising of 6 centre points and 14 axial points. If a full factorial experiment were done, there would have been 5^3 (or 125) runs of experiments.

Table 5.1: Factors and their coded and actual values

Factor	Symbol	Unit	Coded and Actual Values				
			–1.68	–1.00	0.00	+1.00	+1.68
SRT	x_1	days	9.95	12.00	15.00	18.00	20.04
TS	x_2	%	6.31	7.00	8.00	9.00	9.68
Temp	x_3	^{0} C	26.59	30.00	35.00	40.00	43.41

Actual values (x_i) were found from equation (5.1) (Christakos *et al.*, 2015; Mao *et al.*, 2015; Reungsang *et al.*, 2012) as:

$$x = \frac{x_i - x_0}{\Delta x} \qquad 5.1$$

where:

i = 1, 2, and 3

x_i = the input variable

x_0 = the value of x_i at the centre point

Δx = the step change between input variables

x = the coded value.

Example: to determine the actual value corresponding to a coded value of –1.68 for total solids.

From equation (5.1),

$$\Rightarrow x_i = x.\Delta x + x_0$$

where: x = –1.68, Δx = 8 – 7 = 1, and x_0 = 8

$$\therefore x_2 = -1.68(1) + 8 = 6.31$$

The matrix of experiments is shown in Table 5.2.

Table 5.2: Matrix of the experiments

Run	Factors		
	A:SRT (days)	B:TS (%)	C:Temp (^oC)
1	15.00	8.00	35.00
2	15.00	8.00	35.00
3	15.00	6.32	35.00
4	15.00	8.00	35.00
5	18.00	7.00	40.00
6	12.00	7.00	40.00
7	15.00	8.00	26.59
8	18.00	9.00	30.00
9	15.00	8.00	35.00
10	15.00	8.00	35.00
11	15.00	9.68	35.00
12	15.00	8.00	35.00
13	12.00	9.00	30.00
14	20.04	8.00	35.00
15	12.00	7.00	30.00
16	12.00	9.00	40.00
17	9.95	8.00	35.00
18	18.00	7.00	30.00
19	18.00	9.00	40.00
20	15.00	8.00	43.41

b) Statistical analysis and modeling

A second order mathematical model was used to fit the quadratic equation (Beevi *et al.*, 2015; Feng *et al.*, 2017; Mukhopadhyay *et al.*, 2013a; Thanwised *et al.*, 2012) as given below.

$$Y = \beta_0 + \sum_i \beta_i x_i + \sum_{ij} \beta_{ij} x_i x_j + \sum_{ii} \beta_{ii} x_i^2 \qquad 5.2$$

The interpretation of equation 5.2 is as follows:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \qquad 5.3$$

where:

Y is the measured response

x_1, x_2, and x_3 are coded input variables

β_0 is the intercept term

β_1,β_2, and β_3 are coefficients showing the linear effects

β_{12}, β_{13}, and β_{23} are cross-product coefficients showing the interaction effects

β_{11}, β_{22}, and β_{33} are the quadratic coefficients

Design Expert version 10.0.0.3 statistical package was used to analyse the model.

c) Model verification and validation

In this study, optimisation was done with the predictive model for the response as a function of substrate retention time, total solids, and temperature. The model (generated by Design Expert software version 10.0.0.3) was based on results from CCD on biogas production rate. Setting numerical optimisation criteria was by Design Expert software (version 10.0.0.3). This software uses an optimisation method developed by Derringer and Suich (1980), and is described by Montgomery *et al.* (2009). This criteria was also used by Avicenna *et al.* (2015). The objective function was to maximise biogas production rate (Y); subject to the variables (A: substrate retention time, B: total solids, and C: temperature) and their respective constraints as shown below:

Maximise

$$Y = -901.41 + 15.21A + 183.25B + 7.74C - 0.56AB + 0.04AC + 0.06BC - 0.41A^2 - 11.47B^2 - 0.11C^2 \quad 5.4$$

Subject to:

$12 \leq A \leq 18;\ 7 \leq B \leq 9;\ 30 \leq C \leq 40$

Table 5.3: Criteria.

Name	Goal	Lower limit	Upper limit
A: SRT (days)	In range	12.00	18.00
B: TS (%)	In range	7.00	9.00
C: Temp (^{0}C)	In range	30.00	40.00
Y: Biogas production (litres/day)	Maximise	33.01	75.41

This procedure generated Table 5.4.

Table 5.4: Influent preparation

TS (%)	Cow dung : water dilution	Cow dung (kg)	Water (kg)
7.642	1:1.441	49.160	70.840
7.697	1:1.424	49.505	70.495
7.724	1:1.416	49.669	70.331

Influent preparation was done as follows: 1 kg of cow dung contains 0.18658 kg of total solids (TS). In an influent of 7.642% TS, it implies that 7.642 kg of TS are contained in 100 kg of influent. Therefore 0.18658 kg of TS are contained in $\left(\frac{0.18658\times100}{7.642}\right)$ which is equivalent to 2.441 kg of influent. This means that water in the influent = 2.441 – 1 = 1.441 kg. Therefore cow dung : water ratio is 1:1.441

For an influent of 120 kg, Cow dung = $\frac{1\times120}{2.441} = 49.160\ kg$; Water = $\frac{1.441\times120}{2.441} = 70.840\ kg$

Gas collection was by water displacement method. Biogas analysis was done using a Gas Chromatograph. Experiments were done on the first three optimised solutions. The results were compared.

5.3 Results and Discussions

Experimental results as well as the predicted values for biogas production rate are given in Table 5.5.

a) Prediction model

Table 5.5: Actual and predicted biogas yield

Run	Factors			Biogas production rate			
	A:SRT (days)	B:TS (%)	C:Temp (^{0}C)	Actual (l/d)	Predicted (l/d)	Actual (m^3/m^3d)	Predicted (m^3/m^3d)
1	15.00	8.00	35.00	72.11	73.16	0.48	0.48
2	15.00	8.00	35.00	72.90	73.16	0.48	0.48
3	15.00	6.32	35.00	45.73	51.82	0.30	0.34
4	15.00	8.00	35.00	71.56	73.16	0.47	0.48
5	18.00	7.00	40.00	66.91	68.89	0.44	0.45
6	12.00	7.00	40.00	67.48	65.35	0.44	0.43
7	15.00	8.00	26.59	52.71	55.96	0.35	0.37
8	18.00	9.00	30.00	36.40	39.99	0.24	0.26
9	15.00	8.00	35.00	71.80	73.16	0.47	0.48
10	15.00	8.00	35.00	71.96	73.16	0.47	0.48
11	15.00	9.68	35.00	33.01	29.74	0.22	0.19
12	15.00	8.00	35.00	72.30	73.16	0.48	0.48
13	12.00	9.00	30.00	45.88	45.57	0.30	0.30
14	20.04	8.00	35.00	61.52	61.88	0.41	0.41
15	12.00	7.00	30.00	58.00	55.95	0.38	0.37
16	12.00	9.00	40.00	52.65	56.17	0.35	0.37
17	9.95	8.00	35.00	61.25	63.56	0.40	0.42
18	18.00	7.00	30.00	59.14	57.09	0.39	0.38
19	18.00	9.00	40.00	49.17	52.99	0.32	0.35
20	15.00	8.00	43.41	75.41	74.79	0.50	0.49

The combination of factors which yielded the highest biogas production rate of 75.41 litres per day (l/d) was a substrate retention time of 15 days, total solids of 8%, and a temperature of 43.4^0C. This is the optimum combination of factors that gives the highest biogas production rate of 0.50 cubic metres of biogas per cubic metre of biodigester volume per day (m^3/m^3d), which is equivalent to 75.41 l/d.

A regression analysis was done based on the experimental results using the Design Expert 10.0.0.3 statistical package, and it yielded a relationship given by equation 5.4. This equation was used to predict biogas production rate as shown in Table 5.5.

It was observed that the actual biogas production rates were in agreement with $0.4m^3/m^3d$ reported by Nyaanga (2011), and Moog *et al.* (1997). This value also agrees reasonably with 0.42 m^3/m^3d that was observed by Ferrer *et al.* (2011). Consequently, the model can be adopted for use under similar conditions of this experiment.

b) Analysis of variance (ANOVA)

The analysis of variance is necessary in order to determine the significance and adequacy of the model (Thanwised *et al.*, 2012). The statistical significance of the second-order polynomial equation was checked by an F-test (ANOVA). Table 5.6 shows the statistics.

Table 5.6: Analysis of variance

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	3037.53	9	337.50	32.09	< 0.0001
A-SRT	10.43	1	10.43	0.99	0.3428
B-TS	577.69	1	577.69	54.93	< 0.0001
C-Temp	411.52	1	411.52	39.13	< 0.0001
AB	22.88	1	22.88	2.18	0.1710
AC	2.30	1	2.30	0.22	0.6500
BC	0.66	1	0.66	0.062	0.8079
A^2	194.38	1	194.38	18.48	0.0016
B^2	1891.36	1	1891.36	179.83	< 0.0001
C^2	107.16	1	107.16	10.19	0.0096
Residual	105.17	10	10.52		
Cor Total	3142.71	19			

Other Statistics

R^2 = 0.9665; Adjusted R^2 = 0.9364; Predicted R^2 = 0.7442; C.V.% = 5.41; Adequate precision = 19.562; PRESS = 803.97; Standard deviation = 3.24; Mean = 59.89

The ANOVA was used to evaluate the significance of the quadratic polynomial model (Nanduri *et al.*, 2008). For each term in the model, a large F-value and a small P-value would imply a more significant effect on the respective response variable (Quanhong and Caili, 2005). A model F-value of 32.09 was found. There was a chance of less than 0.01% that a value this much could be caused by noise. Hence the model was chosen for the experiment.

Model values whose 'prob>F' are less than 0.05 (i.e. $p < 0.05$) are significant. In this case the linear effects of total solids (B), and temperature (C), were significant. The quadratic effects of substrate retention time (A^2), total solids (B^2), and temperature (C^2) were significant. The interaction effects of AB, AC and BC were insignificant. All the significant factors had an individual effect on biogas production rate.

A coefficient of determination (R^2) is used to measure the variability in the actual response values that can be described by the corresponding independent factors (Mukhopadhyay *et al.*, 2013a). In this case R^2 was 0.9665; it implied that a sample variation of 96.65% of the total

variation could be explained by the model, and only 3.35% could not be explained by the model for this work. For a good statistical model, the R^2 should be in the range of 0.75–1.0 which indicates a good fit of the model (Niladevi *et al.*, 2009).

The adjusted R^2 of 0.9364 was also very high. It indicated the higher significance of the model. The Predicted R^2 value of 0.7442 showed reasonable agreement with the adjusted R^2 value of 0.9364. The threshold is that the difference between the adjusted R^2 and the predicted R^2 should be less than 0.2 (Subha *et al.*, 2015). The difference here is 0.1922. This indicated a good agreement between the observed and the predicted values as demonstrated in Table 4.6.

The percentage of coefficient of variation (CV %) is a measure of residual variation of the data relative to the size of the mean; the higher the value of CV, the lower is the reliability of experiment (Šumić *et al.*, 2016). A lower CV of 5.41% indicated a greater reliability of the experiment. As a general rule, a model can be considered reasonably reproducible if the CV is not greater than 10% (Rasouli *et al.*, 2015). This model is reproducible.

Adequate precision is a measure of signal to noise ratio; a ratio greater than 4 is desirable (Mason *et al.*, 2003). In this case, the ratio was 19.562; which indicated an adequate signal. This model can be used to navigate the design space.

The Predicted Residual Sum of Squares (PRESS) is a measure of how well the model fits each point in the design; the smaller the PRESS statistics, the better would be the model fitting the data points (Rasouli *et al.*, 2015). Here the value of PRESS found was 803.97. This value is small; it shows that the model fits well on each point in the design.

Lack-of-Fit (LoF) F-value of 17.71 implied that it was not significant relative to the pure error. Non-significant lack of fit indicated a good fitness of the model (Kang *et al.*, 2016). There was only 34% chance that this magnitude of LoF F-value could occur due to noise. This is similar to work reported by Cheng *et al.* (2001). The model showed standard deviation and mean values of 3.24 and 59.89 respectively.

c) Diagnostics

i) Predicted results versus actual results

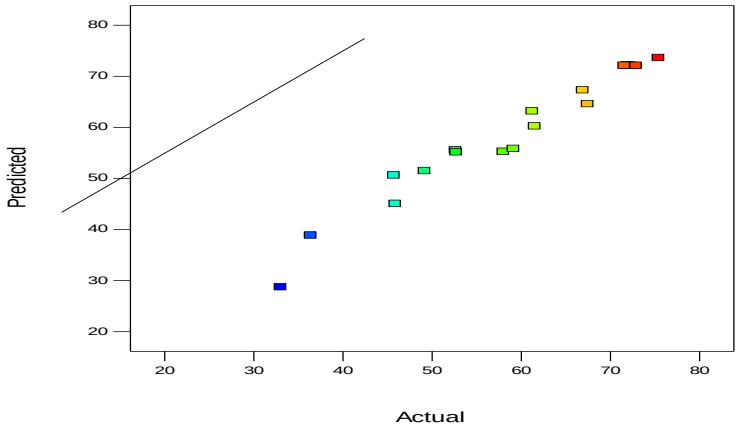


Figure 5.1: Predicted versus actual results

Figure 5.1 shows that the predicted results lie within the normal probability curve. The actual results also exhibit the same behavior. Outliers also exist, implying the errors inherent in the experiment due the losses of biogas and the accuracy of the equipment used to monitor the anaerobic process. However, a high R^2 value that was found emphasizes a high degree of similarity that was observed between the predicted and the experimental values.

ii) Residuals versus Run

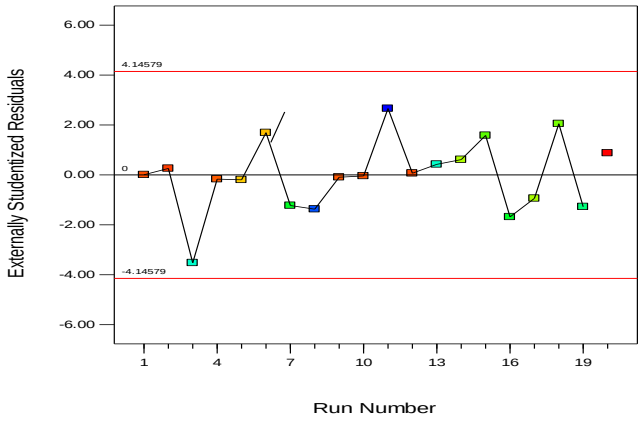


Figure 5.2: Residual versus run

Figure 5.2 shows that the error bars for the runs range from -4.14579 to 4.14579 within which the observed data is statistically the same. It can be seen that all the 20 runs are in this range; hence the observed data for the runs is admissible.

iii) Normal plot of Residuals

The normal probability plot given in Figure 5.3: shows some scatter along the line indicating that the residuals follow a normal distribution.

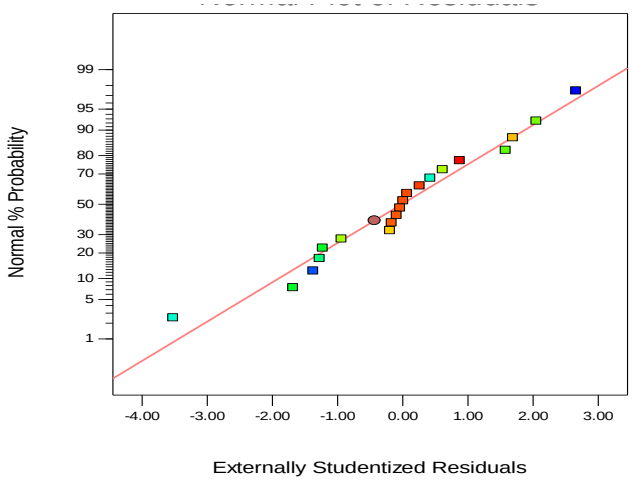


Figure 5.3: Normal plot of residuals

iv) Residuals versus predicted

Figure 5.4 indicates the residuals versus the ascending predicted response values. The plot shows a random scatter; similar to Figure 5.2.

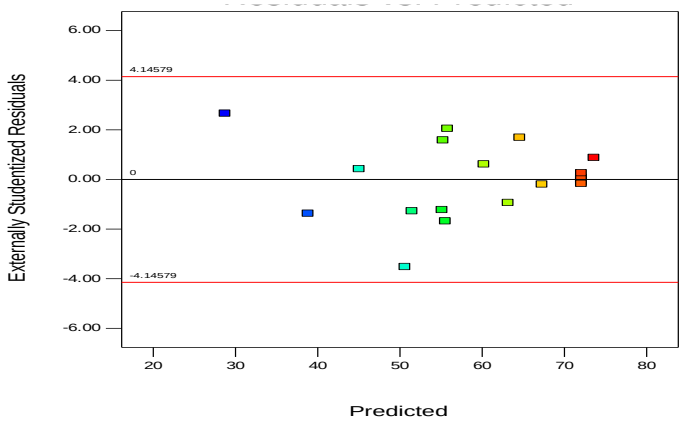


Figure 5.4: Residuals versus predicted

From the four diagnostic plots (Figures 5.1– 5.4), it can be concluded that the model has satisfied the assumptions of the analysis of variance and also reflected the accuracy and applicability of RSM to optimise the process factors for the efficient generation of biogas (Mukhopadhyay *et al.*, 2013a).

d) Model Diagrams

Design Expert software was used to generate contour or two-dimensional (2D) plots and three-dimensional (3D) surface plots to find the optimum operating conditions of the anaerobic digestion process for cow dung (Rasouli *et al.*, 2015). These graphs give an insight view of the behavior of two-factor-interactions (2FI) between factors that influence biogas production in this study. A 2D plot is a graphical representation of a two dimensional response surface as a function of two independent variables, maintaining all other variables at fixed level (Sinha *et al.*, 2013). 2D and 3D surface plots are useful for establishing desirable response values; a contour plot provides a two-dimensional view where all points that have the same response are connected to produce contour lines of constant responses, whereas a 3D surface plot provides a three dimensional view that may provide a clearer picture of the response surface (Rao and Baral, 2011). These plots were helpful in understanding both the main effects and interaction effects of the independent variables on the response (Jain *et al.*, 2011). Figures 5.5, 5.7 and 5.9 are 2D plots, and Figures 5.6, 5.8 and 5.10 are 3D surface plots as given by the Design Expert 10.0.0.3 statistical package.

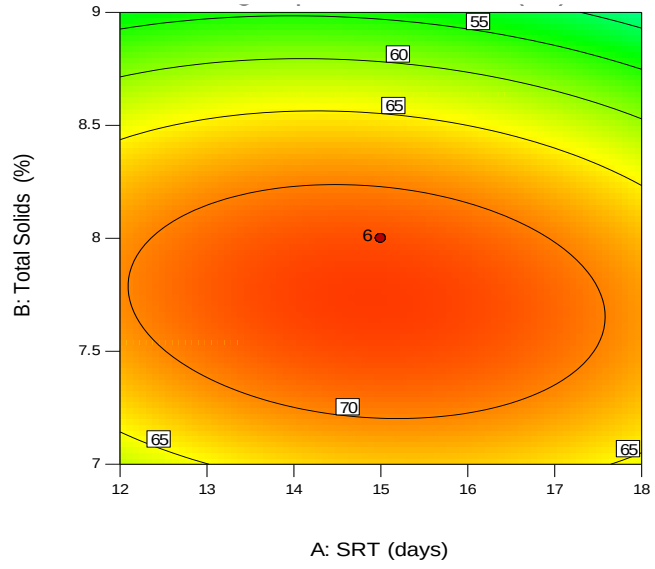


Figure 5.5: AB 2D plot

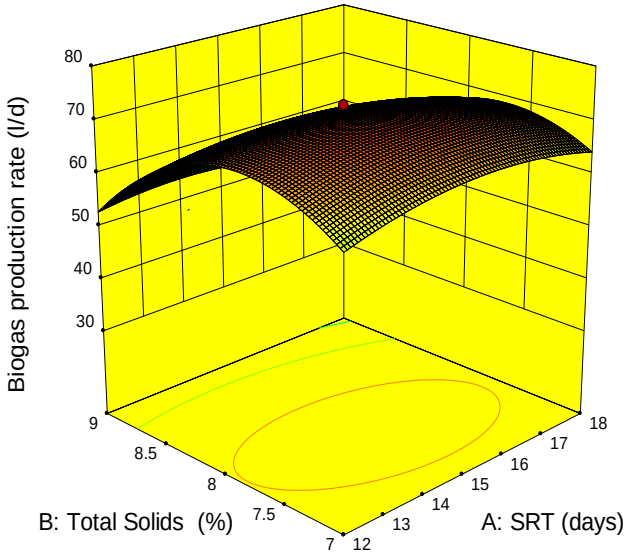


Figure 5.6: AB 3D graph

Figure 5.5 is a 2D plot showing the 2FI between SRT and TS while the temperature is held constant at 35^0C. A maximum biogas production rate of 75 l/d is attained at 7.7% TS and an SRT of 14 days. Above a TS of 7.7%, the biogas production rate starts declining beyond 55 l/d as the TS approaches 9%. It is also observed that as the SRT increases from 14 days, biogas production rate decreases to 65 l/d at 17.5 days.

Figure 5.6 is a 3D surface plot of SRT and TS when the temperature is held constant at 35^0C. It gives a further insight on the interaction effect of SRT and TS on biogas production rate. The rate is 67.6 l/d when SRT is 12 days at a TS of 7%. When SRT is held at 12 days while TS is varied from 7%, the rate increases from 67.6 l/d to 73.3 l/d at 7.78% TS, then declines to 55 l/d at 9% TS. Similarly when the TS is held at 7% while the SRT is varied, biogas production rate increases from 67.6 l/d at 12 days to 68.8 l/d at 15 days. It then declines to 63.8 l/d at 17.9 days. The optimal value, however, is shown at the top of the 3D surface as a biogas production rate of 70.78 l/d at 15.9 days and 8.2% TS.

Figure 5.7 shows a 2D 2FI between SRT and Temperature while TS is held at the centre point (8%). Biogas production rate reaches a maximum value of 74 l/d from 12 days to 17.5 days, and 35.7^0C to 40^0C. The rate then declines to 60 l/d at 18 days.

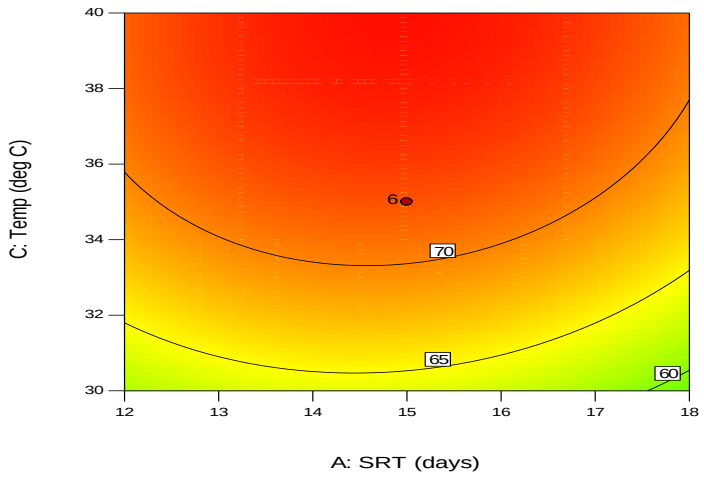


Figure 5.7: AC 2D plot

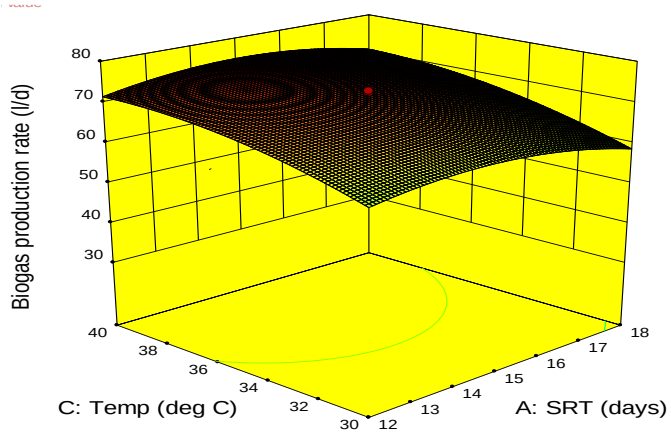


Figure 5.8: AC 3D graph

Figure 5.8 shows the 3D version. At 12 days, the rate increases from 65.7 l/d at 30^0C to 71 l/d at 40^0C. Also at 30^0C, the rate increases from 65.7 l/d at 12 days to 67.3 l/d at 14.7 days; then declines to 58 l/d at 18 days. The optimum biogas production rate shown on top of the 3D surface is 74.07 l/d at 15 days and 34.9^0C.

Figure 5.9 is a 2D 2FI between the TS and Temperature on biogas production rate while SRT is held at the centre point (15 days). A maximum rate of 70 l/d is attained between 7.1 – 8.4% TS, and 33 – 40^0C. The rate declines to 50 l/d at 8.9% TS.

Figure 5.10 is a 3D representation. At 7% TS and 30^0C, the biogas production rate is 35 l/d. When 7% TS is kept constant, the rate increases from 35 l/d at 30^0C to 69 l/d at 40^0C. Similarly when 30^0C is kept constant, the rate increases from 35 l/d at 7% TS to 68 l/d at

8.4% TS; then declines to 45.9 l/d at 9% TS. An optimal rate is 74 l/d at 8.1% TS and 35.2^{0}C. This is similar to an observation reported by Sathish and Vivekanandan (2016) and Beevi *et al.* (2015).

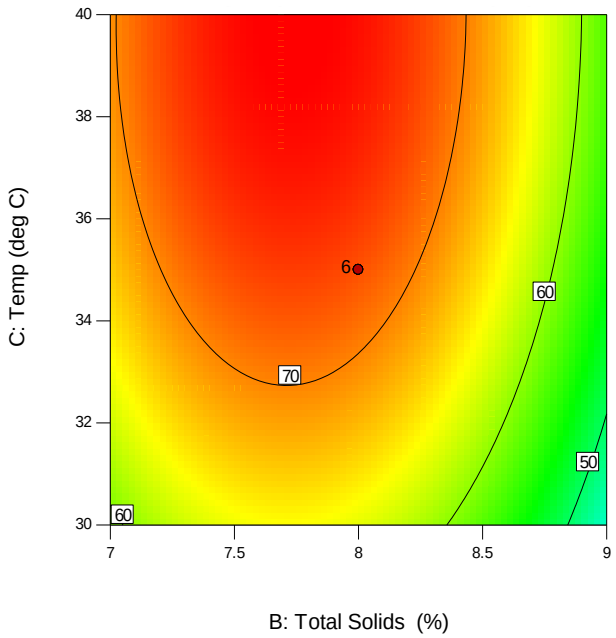


Figure 5.9: BC 2D plot

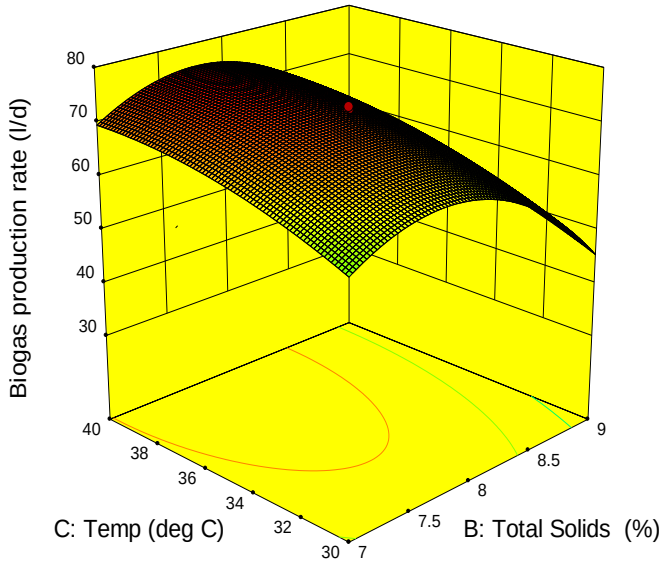


Figure 5.10: BC 3D graph

e) Perturbation

Perturbation is a deviation of a system, moving object, or process from its regular or normal state or path, caused by outside influence. Perturbation plot provides silhouette views of the response surface. For response surface designs, the perturbation plot shows how the response changes as each factor moves from the chosen reference point, with all other factors held constant at the reference value (Mukhopadhyay *et al.*, 2013b). Design Expert sets the reference point default at the middle of the design space (the coded zero level of each factor).Figure 5.11 represents the comparison of the effect of process parameters at the midpoint (coded 0) in the design space.

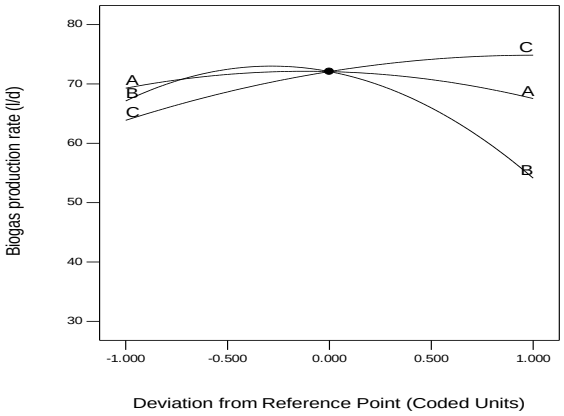


Figure 5.11: Perturbation

When B (TS) and C (Temperature) are held at their respective midpoints, it shows that A (SRT) gives a steadily higher biogas production rate from day 12 up to its midpoint value (day 15), and then the rate starts decreasing. Similarly, for B, the rate increases from 6.31% TS, then starts dropping before the midpoint (8% TS) is reached. Thereafter the rate decreases steadily. For C, the rate increases uniformly from 25^0C to the midpoint (35^0C), then the trend continues up to 40^0C.

f) Verification and validation of the model

The results of the RSM optimised factors of substrate retention time (SRT), total solids (TS) and Temperature (Temp) and their corresponding predicted and observed or measured average biogas production rate (l/d) are given in Table 5.7.

Table 5.7: Optimised and experimental results

Solution/ Expt.* set	Factors			Avg* Biogas production rate (l/d)	
	SRT (days)	TS (%)	Temp (^{0}C)	Optimised/Pred	Experiment/Obs
1	15.339	7.642	39.643	75.578	69.855
2	15.179	7.697	39.202	75.612	70.929
3	15.385	7.724	38.979	75.530	68.247

*Avg = Average, l/d = litres per day, SRT = substrate retention time, TS = total solids, Temp = Temperature, Expt = experiment, pred = predicted, and obs = observed or measured

It was observed that the optimised biogas production rate for solutions 1, 2 and 3 were higher than experimental ones by 8.19%, 6.60% and 10.67% respectively. Gallert *et al.* (2003) made a similar observation while researching on how effective and accurate the data gathered from laboratory scale reactors were at predicting the performance of industrial scale reactors operating under the same basic parameters. For the results to be valid, the difference between the experimental value and the predicted value should be less than 20% (Subha *et al.*, 2015). The differences are within the tolerance limits and hence the optimisation is admissible.

Laboratory scale experimentation is an essential component of anaerobic digestion research and development because it has the ability to simultaneously test multiple variables on a small scale to see their impact on efficiency, and it also helps in reducing the costs associated with optimisation (Gamble *et al.*, 2015). There is a strong correlation between the the optimised values and the experimental values. Kowalczyk *et al.* (2011) observed that such a strong correlation can be used to upscale the laboratory conditions to an industrial scale.

5.4 Conclusions and Recommendation

5.4.1 Conclusions

The optimal biogas production of 75.41 l/d (or 0.50m^3/m^3d) was achieved at an interaction of substrate retention time of 15 days, 8% total solids, and a temperature of 43.41^{0}C using both the various indicators of statistics including those in the analysis of variance (ANOVA) at the 95% significance level while the adequacy of the simulation models for the same was done using all the components of response surface method (RSM) as an optimisation technique. The optimised results were tested using the inbuilt RSM protocols and found to be in

agreement with the experimental results with a tolerance of 6.6 – 10.7 %, hence they can be used.

5.4.2 Recommendation

Analysis of variance (ANOVA) and response surface method (RSM) indicators can be used to determine the optimal points of biogas production when dealing with varying factors; in this case substrate retention time, total solids and temperature. The same can be applied to a combination of other parameters (such as pH, organic loading rate, and agitation) that affect biogas production in the psychrophilic, mesophilic and thermophilic ranges of biomass digestion temperature for biogas production should be investigated to determine their optimal level of biogas production rate.

References

Ahmad, S. (2009). Techniques for inducing accelerated corrosion of steel in concrete. *Arabian Journal for Science and Engineering, 34*(2), 95.

Aslan, N. (2007). Application of response surface methodology and central composite rotatable design for modeling the influence of some operating variables of a multi-gravity separator for coal cleaning. *Fuel, 86*(5-6), 769-776.

Astolfi, A. and Praly, L. (2006). Global complete observability and output-to-state stability imply the existence of a globally convergent observer. *Mathematics of Control, Signals and Systems, 18*(1), 32-65.

Avicenna, Mela, M., Ihsan, S. and Setyobudi, R. (2015). Process Improvement of Biogas Production from Anaerobic Co-digestion of Cow Dung and Corn Husk. *Procedia Chemistry, 14*, 91-100.

Beevi, B. S., Madhu, G. and Sahoo, D. K. (2015). Performance and kinetic study of semi-dry thermophilic anaerobic digestion of organic fraction of municipal solid waste. *Waste Management, 36*, 93-97.

Belwal, T., Dhyani, P., Bhatt, I. D., Rawal, R. S. and Pande, V. (2016). Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM). *Food Chemistry, 207*, 115-124.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S. and Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta, 76*(5), 965-977.

Carley, K. M. and Kamneva, N. Y. (2004). *A network optimization approach for improving organizational design*. Retrieved from Carnigie-Mellon Univ Pittsburg PA.

Cheng, H., Wang, Y. J., Edwards, R. L., An, Z., Wu, J., Shen, C. and Dorale, J. A. (2001). A high-resolution absolute-dated late Pleistocene monsoon record from Hulu Cave, China. *Science, 294*(5550), 2345-2348. doi:10.1126/science.1064618

Christakos, S., Dhawan, P., Verstuyf, A., Verlinden, L. and Carmeliet, G. (2015). Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiological Reviews, 96*(1), 365-408.

Derringer, G. and Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology, 12*(4), 214-219.

Feng, J., Zhang, J., Zhang, J., He, Y., Zhang, R., Chen, C. and Liu, G. (2017). Enhanced methane production of vinegar residue by response surface methodology (RSM). *Amb Express, 7*(1), 89.

Ferrer, I., Garfí, M., Uggetti, E., Ferrer-Martí, L., Calderon, A. and Vel, E. (2011). Biogas production in low-cost household digesters at t he Peruvian Andes. *Biomass and Bioenergy*. doi:10.1016/j.biombioe.2010.12.036.

Gallert, C., Henning, A. and Winter, J. (2003). Scale-up of anaerobic digestion of the biowaste fraction from domestic wastes. *Water Resources*(37), 1433-1441.

Gamble, K. J., Houser, J. B., Hambourger, M. S. and Hoepfl, M. C. (2015). *Anaerobic Digestion from the Laboratory to the Field: An Experimental Study into the Scalability of Anaerobic Digestion*. Paper presented at the ASEE Southeast Section Appalachian State University.

Jain, M., Garg, V. and Kadrivelu, K. (2011). Investigation of Cr(VI) adsorption onto Chemically treated Helianthus annus: optimization using Response Surface Methodology. *Bioresource Technology, 102*, 600-605.

Jiménez, J., Guardia-Puebla, Y., Romero-Romero, O., Cisneros-Ortiz, M., Guerra, G., Morgan-Sagastume, J. and Noyola, A. (2014). Methanogenic activity optimization using the response surface methodology, during the anaerobic co-digestion of agriculture and industrial wastes Microbial community diversity. *Biomass and Bioenergy, 71*, 84-97.

Kang, J., Kim, S. and Moon, B. (2016). Optimization by response surface methodology of Lutein recovery from paprika leaves using accelerated solvent extraction. *Food Chemistry, 205*, 140-145.

Khoobbakht, G., Najafi, G., Karimi, M. and Akram, A. (2016). Optimization of operating factors and blended levels of diesel, biodiesel and ethanol fuels to minimize exhaust emissions of diesel engine using response surface methodology. *Applied Thermal Engineering, 99*, 1006-1017.

Kong, Z., Li, M., Chen, J., Bao, Y., Fan, B., Francis, F. and Dai, X. (2016). Processing factors of triadimefon and triadimenol in barley brewing based on response surface methodology. *Food Control, 64*, 81-86.

Kousha, M., Tavakoli, S., Daneshvar, E., Vazirzadeh, A. and Bhatnagar, A. (2015). Central Composite design optimization of Acid Blue 25 dye biosorption using shrimp shell biomass. *Journal of Molecular Liquids, 207*, 266–273.

Kowalczyk, A., Schwede, S., Gerber, M. and Span, R. (2011). *Scale up of laboratory scale to industrial scale biogas plants*. Paper presented at the World Renewable Energy Congress, Sweden.

Leiviskä, K. (2013). *Introduction to Experiment Design*. University of Oulu: Control Engineering Laboratory.

Mao, C., Feng, Y., Wang, X. and Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews, 45*, 540-555.

Mason, R., Gunst, R. and Hess, J. (2003). *Statistical design and analysis of experiments, eight applications to engineering and science* (2nd ed.). New York: Wiley.

Montgomery, D. C., Runger, G. C. and Hubele, N. F. (2009). *Engineering Statistics*: John Wiley and Sons.

Moog, H., Avilla, A., Agpaoa, E., Valenzuela, F. and Concepcion, F. (1997). Promotion and utilization of polyethylene bio-digester in small-holder farming systems in the Philippines. *Livestock Research for Rural Development, 9*(2).

Mukhopadhyay, D., Sarkar, J. P. and Dutta, S. (2013a). Optimization of process factors for the efficient generation of biogas from raw vegetable wastes under the direct influence of plastic materials using Taguchi methodology. *Desalination and Water Treatment, 51*(13), 2781-2790.

Mukhopadhyay, D., Sarkar, J. P. and Dutta, S. (2013b). Optimization of process parameters for the economical generation of biogas from raw vegetable wastes under the positive influence of plastic materials using response surface methodology. *Journal of Biochemical Technology, 4*(1), 549-553.

Nanduri, J., Yuan, G., Kumar, G. K., Semenza, G. L. and Prabhakar, N. R. (2008). Transcriptional responses to intermittent hypoxia. *Respiratory Physiology and Neurobiology, 164*(1-2), 277-281.

Niladevi, K., Sukumaran, R. K., Jacob, N., Anisha, G. and Prema, P. (2009). Optimization of laccase production from a novel strain—Streptomyces psammoticus using response surface methodology. *Microbiological Research, 164*(1), 105-113.

Nyaanga, D. (2011). *Performance of Tropical Biogas*. Paper presented at the Egerton University International Conference, Egerton University, Kenya.

Pannucharoenwong, N. (2018). Optimization of Bio-Methane Production from Mesophilic Anaerobic Co-digestion of Pig Manure and Vegetable Residue. *International Journal of Applied Engineering Research, 13*(4), 1988-1995.

Quanhong, L. and Caili, F. (2005). Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. *Food Chemistry, 92*(4), 701-706.

Rao, P. V. and Baral, S. S. (2011). Experimental design of mixture for the anaerobic co-digestion of sewage sludge. *Chemical Engineering Journal, 172*(2-3), 977-986.

Rasouli, J., Ciric, B., Imitola, J., Gonnella, P., Hwang, D., Mahajan, K., Mari, E. R., Safavi, F., Leist, T. P. and Zhang, G.-X. (2015). Expression of GM-CSF in T cells is increased in multiple sclerosis and suppressed by IFN-β therapy. *The Journal of Immunology, 194*(11), 5085-5093.

Reungsang, A., Sakchai, P. and Sureewan, S. (2012). Optimization of key factors affecting Methane production from acidic effluent coming from the sugarcane juice Hydrogen fermentation process. *Energies, 5*, 4746-4757;. doi:10.3390/en5114746

Saleh, A., Kamarudin, E., Yaacob, A., Yussof, A. and Abdullah, M. (2012). Optimization of biomethane production by anaerobic digestion of palm oil mill effluent using response surface methodology. *Asia-Pacific Journal of Chemical Engineering*(7), 353-360.

Sathish, S. and Vivekanandan, S. (2016). Parametric optimization for floating drum anaerobic bio-digester using Response Surface Methodology and Artificial Neural Network. *Alexandria Engineering Journal, 55*(4), 3297-3307.

Sinha, K., Chowdhury, S., Saha, P. D. and Datta, S. (2013). Modeling of microwave-assisted extraction of natural dye from seeds of Bixa orellana (Annatto) using response surface methodology (RSM) and artificial neural network (ANN). *Industrial Crops and Products, 41*, 165-171.

Subha, B., Song, Y. C. and Woo, J. H. (2015). Optimization of biostimulant for bioremediation of contaminated coastal sediment by response surface methodology (RSM) and evaluation of microbial diversity by pyrosequencing. *Marine Pollution Bulletin, 98*(1-2), 235-246.

Šumić, Z., Vakula, A., Tepić, A., Čakarević, J., Vitas, J. and Pavlić, B. (2016). Modeling and optimization of red currants vacuum drying process by response surface methodology (RSM). *Food Chemistry, 203*, 465-475.

Taguchi, G. (1986). *Introduction to Quality Engineering*. UNIPUB, White Plains, NY: Asian Productivity Organization.

Taguchi, G. (1987). *System of Experimental Design: Engineering Methods to Optimize Quality and Minimize Cost*. White Plains, NY: UNIPUB/Kraus International.

Thanwised, P., Wirojanagud, W. and Reungsang, A. (2012). Effect of hydraulic retention time on hydrogen production and chemical oxygen demand removal from tapioca wastewater using anaerobic mixed cultures in anaerobic baffled reactor (ABR). *International Journal of Hydrogen Energy, 37*(20), 15503-15510.

Zaroual, Z., Chaair, H., Essadki, A., El Ass, K. and Azzi, M. (2009). Optimizing the removal of trivalent chromium by electrocoagulation using experimental design. *Chemical Engineering Journal, 148*(2-3), 488-495.

CHAPTER SIX
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

The effect of total solids on biogas production rate was investigated in a fixed dome laboratory digester of 0.15m^3. Data was collected at a temperature of 35^0C for total solid levels of 6 – 10%. There was no production of biogas from day 1 to day 5 for total solids of 7% and 8%, from day 1 to day 6 for total solids of 6% and 9%, and from day 1 to day 7 for total solids of 10%. In this phase, three main processes are taking place namely hydrolysis, acidogenesis, and acetogenesis. Hydrolysis is where proteins, carbohydrates, and fats are being broken down into amino acids, simple sugars, and long chain fatty acids, respectively by the hydrolytic bacteria. Acidogenesis is a process during which simple sugars and amino acids resulting from hydrolysis are degraded to a number of simpler products such as volatile fatty acids including propionic acid, butyric acid, and acetic acid. Acetogenesis is the degradation of higher organic acids formed in acidogenesis to yield acetic acid, carbon dioxide and hydrogen. This intermediate conversion is crucial for the successful production of biogas.

In the second phase there was an exponential production of biogas from day 5 up to day 11 and day 12. During this phase, the process of methanogenesis was taking place. The fermentation products such as acetate, hydrogen and carbon dioxide were converted to methane and carbon dioxide by methanogenic archaea.

In the third phase there was a steady decline in biogas production from day 11 to day 15 due to stationary phase of microbial growth. The methanogenic bacteria died due to the depletion of carbon in the substrate. It was generally observed that the highest biogas production rate was achieved by 8% total solids (0.68m^3/m^3d) on day 11, followed by 7%, 9%, 6%, and lastly 10% in a descending order. Anaerobic digestion process increased steadily from total solids of 6% to 8%. At low solids, aceticlastic methanogens called methanosarcina play an important role, followed by hydrogenotrophic methanoculleus and methanomicrobiales species. As the total solids increase from 8% to 10%, the biogas production rate declines steadily. Increasing the total solids means a higher applied organic loading rate and more volatile solids for microorganisms which results in higher volatile fatty acid concentrations. A marginal increase in the percentage of total solids results in a geometric increase in the volume of biogas produced, suggesting therefore that a continual increase in the percentage

of total solids at some point becomes immaterial to the increasing volume of biogas produced. This is possible because when the percentage of total solids increases, the amount of water decreases. This reduces the level of microbial activity which then affects the yield of biogas, particularly at higher values of the TS%.

Effect of mesophilic temperature was investigated. The experiment was run at a constant total solid of 8%. The levels of temperature were 25^0C, 30^0C, 35^0C, 40^0C and 45^0C. The results exhibited three phases. The first phase ranges from day 1 to day 5 for temperatures 30^0C to 45^0C, and day 1 to day 8 for 25^0C; no biogas was produced. During this phase, hydrolysis, acidogenesis, and acetogenesis were taking place. In the second phase, there was an exponential biogas production from day 5 to day 11, and day 12 for all the temperatures. The highest biogas production occurred at 40^0C (0.807 m^3/m^3d on day 12) followed by 35^0C, 30^0C, 45^0C, and lastly 25^0C. The third phase started from day 12 onwards. Biogas production dropped drastically because the feed material was depleted leading to the death of mesophiles in the batch reactor.

There are three temperature ranges within which anaerobic digestion of a substrate can take place. Psychrophilic temperature range lies below 20^0C, mesophilic temperature range between 30^0C and 40^0C, and thermophilic temperature range from 50^0C to 75^0C. At the beginning, biogas production was low due to very little microbial activity because the psychrophilic bacteria had died off while the mesophiles were regenerating to take over the process of methanogenesis. There was a steady increase in biogas production in the mesophilic range between 30^0C (0.346m^3/m^3d), 35^0C (0.487m^3/m^3d), and 40^0C (0.528m^3/m^3d) because of the increased regeneration of mesophiles. At 45^0C, the biogas production rate is lower than expected. This is so because the enzymes of mesophiles are denatured leading to their death. The rate of bacteriological methane production increases with temperature as well as the amount of free ammonia. The accumulation of ammonia inhibited biogas production.

The effect of substrate retention time on biogas production rate was investigated by running the experiment at total solids of 8% and a temperature of 35^0C. There was no biogas production from day 1 to day 5. This was attributed to three processes of anaerobic digestion taking place namely hydrolysis, acidogenesis and acetogenesis, respectively.

Methanogenesis marks the final stage of anaerobic digestion, where accessible intermediates are consumed by methanogenic bacteria to produce methane. Production of biogas starts from day 5 and increases steadily to day 11 ($0.68 m^3/m^3 d$). However, it was noticed that biogas production stagnated from day 7 to day 8. This can be attributed to excessive partial pressure of hydrogen produced by acetogenesis that deleted acetogenic microorganisms. But due to the presence of hydrogenotrophic methanogens, especially the methanosarcina species, hydrogen was able to be rapidly consumed while maintaining partial pressure at a level favourable to acetogenesis by creating an exergonic reaction. During the stagnation period, methanosarcina species, which tend to be robust and are capable of withstanding ammonia, sodium, and acetate concentrations in addition to pH shocks at levels that would otherwise be detrimental to other methanogenic bacteria, kept on producing biogas. Consequently, the steady production came on course from day 8 to day 11.

From day 11 onwards, biogas production started declining from 0.68 to $0.418 m^3/m^3 d$ on day 15. This was caused by the depletion of nutrients in the substrate and the gradual death of the methanogens.

An evaluation of some existing biogas production prediction models was done. The models included the Plug Flow, Chen and Hashimoto, Scoff and Minnot, Low Temperature Lagoon Digester, and Toprak. The first three were found to be applicable to semi-continuously-fed and continuously-fed digesters. The last two models were found to suit the batch-fed digesters, and hence they were evaluated in this work. A test for the goodness of fit between the experimental data and the predicted values from the models was done. The tools for testing included the coefficient of determination (R^2), root mean square error (RMSE), and Chi square (X^2). A Fixed-dome-lab Temperature model was developed and validated.

A goodness of fit is achieved when the R^2 value is as close as possible to 1, while the RMSE and X^2 should tend to zero. A statistical analysis showed that the best goodness of fit was attained by the Fixed-dome-lab Temperature model having R^2 of 0.9732, RMSE of 0.0716, and X^2 of 0.0064.

Optimisation was done by investigating the interaction effects of total solids, temperature, and substrate retention time on biogas production in a batch bioreactor. Central composite design (CCD) of Response Surface Methodology was used to design the experiment.

Experiments designed by using CCD have fewer runs, give similar results which are comparable to a full-factorial design, and enables an effective evaluation of interaction between individual factors to provide the best combination for optimal.

In this study, CCD was used. Design of the experiment was done with the help of Design Expert software (version 10.0.0.3). Total solid levels were varied from 6.31% to 9.68%, temperature was from 26.59^{0}C to 43.41^{0}C, and substrate retention time was from 9.95 to 20.04 days. Analysis of results was done using Design Expert software. It gave a coefficient of determination of 0.9665 which indicated a high correlation between the variables. The highest biogas production rate of 0.502m^3/m^{3}d was achieved at a level of 8% total solids total solids, 43.41^{0}C, and 15 days.

6.2 General Conclusions

Biogas production rate varied with different total solid levels increasing from an average of 0.25 m^3/m^{3}d at 6% total solids (TS), through an average of 0.32 m^3/m^{3}d for 7% TS to reach a maximum average of 0.48 m^3/m^{3}d at 8% TS (with a manure to water ratio of 1:1.3), and then started decreasing steadily to 0.24 m^3/m^{3}d as the total solids increased to 10% through an average of 0.32 m^3/m^{3}d for the other total solid levels of the cattle manure under free range animal production system in Egerton, Kenya. The optimal TS for the laboratory fixed dome digester is 8% when operating at a digester temperature of 25 to 45^{o}C. In the mesophilic range of temperature, biogas production increased with increase in temperature, generally, moving from 0.25 m^3/m^{3}d at 25^{0}C through 0.35 m^3/m^{3}d at 30^{0}C to 0.53 m^3/m^{3}d at 40^{0}C (optimal temperature), and then declined to 0.33 m^3/m^{3}d at 45^{0}C. The biogas production increases with the retention time, being maximum of 0.68 m^3/m^{3}d from the batch laboratory fixed dome digester at the 11th day of digestion, having started biogas production after 5 days for 8% TS cattle manure substrate being maintained at a temperature of 35^{0}C. Optimised results were in agreement with the experimental results with a tolerance of 6.6 – 10.7 %. Empirical models to simulate the biogas production with the temperature and total solid ranges used are possible.

6.3 General Recommendations

The above conclusions can be transferred (with appropriate modifications and scale factors for adoption for field and industrial fixed digesters for biogas production into operational guidelines for biogas stakeholders including designers and operators. Available mathematical

models for the prediction of biogas production rate can be used with precaution ensuring the initial and boundary conditions of the model are clear and adhered to. In most case the scope and limitations for the models are not easily identified, hence the caution. Biogas production rate was investigated at a constant temperature of 350C while total solids were varied from 6-10%. A maximum yield of 0.48m^3/m^3d was achieved at 8% total solids (or substrate to water dilution of 1:1.3). The effect of mesophilic temperature range on biogas production rate was investigated at total solids of 8%. The highest average yield of 0.52m^3/m^3d was attained at 40^0C. When the experiment was run at a total solids of 8% and a temperature of 35^0C, the highest average biogas production rate of 0.67m^3/m^3d was attained at a substrate retention time of 11 days. A Fixed-dome-lab Temperature model can be adopted to mimic biogas production in a fixed dome batch bioreactor in the mesophilic range. The limits are that the total solids of the substrate should be 8%, temperature should be 25-45^0C. The optimal biogas production rate of 0.50m^3/m^3d was achieved at a substrate retention time of 15 days, 8% total solids, and a temperature of 43.41^0C. Optimised results were in agreement with the experimental results with a tolerance of 6.6 – 10.7%.

Emanating from this research, it is hereby further recommended that the following research be conducted:

i. Existing models should be validated with huge, replicated and similar experimental data as ones used in their development.
ii. Models relating total solids and substrate retention time to biogas production rate should be developed for a fixed dome laboratory bioreactor as batch and semi continuous system that operates in a psychrophilic, mesophilic and thermophilic temperature ranges.
iii. Compare the lab bioreactor performance with those of the field digester for the same months or operating digestion temperatures.
iv. Effect of feeding regimes (as batch and continuous bioreactor or digester) at varying temperatures and stirring frequencies for different substrates.
v. The effect of thermophilic and cryophilic (ambient and below) temperature ranges on biogas production rate and quality for the laboratory fixed dome digester.
vi. Accuracy and precision of empirical models from experimental and observed data from the biogas system.

APPENDICES

Appendix 1: Design Drawings, Calculations, Figures and Plates

A1.1 Design of a bioreactor and figures

The design was done in order to establish the dimensions of the bioreactor.

a) A cross-section of the laboratory bioreactor

Figure A1.1 shows a cross-section of the laboratory bioreactor.

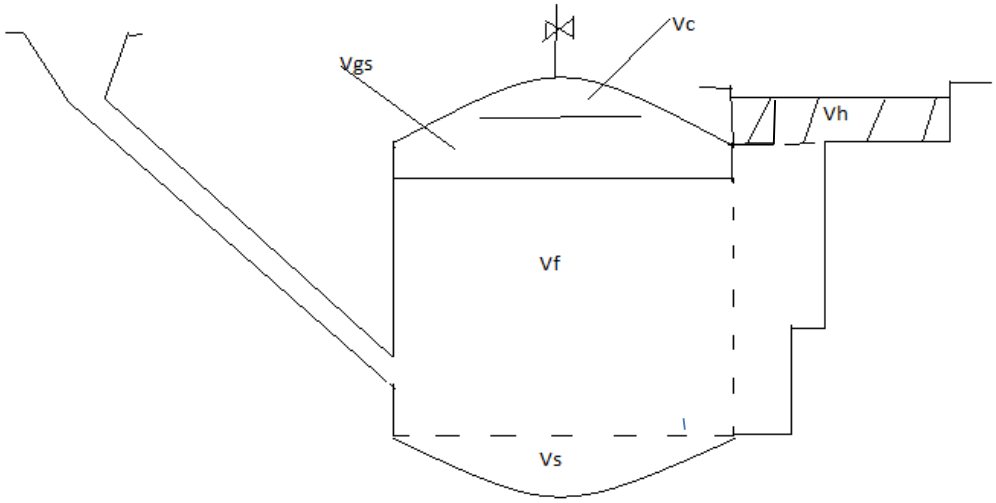


Figure A1.1: Cross-section of a bioreactor

Key:

V_c = Volume of gas collecting chamber

V_{gs} = Volume of gas storage chamber

V_f = Volume of fermentation chamber

V_H = Volume of hydraulic chamber

V_s = Volume of sludge layer

V = Total volume of digester

Assumptions for volume (Nijaguna, 2006) were as given below.

V_c = 5% V

V_s = 15% V

$V_{gs} + V_f$ = 80% V

$V_{gs} = V_H$

V_{gs} = 0.5 ($V_{gs} + V_f + V_s$) K

$V = V_c + V_{gs} + V_f + V_s$

where K = Gas production rate in m^3 per m^3 of digester volume per day.
For Bangladesh K = 0.4 m^3/m^3d (Moog *et al.*, 1997).

b) Geometry of the bioreactor

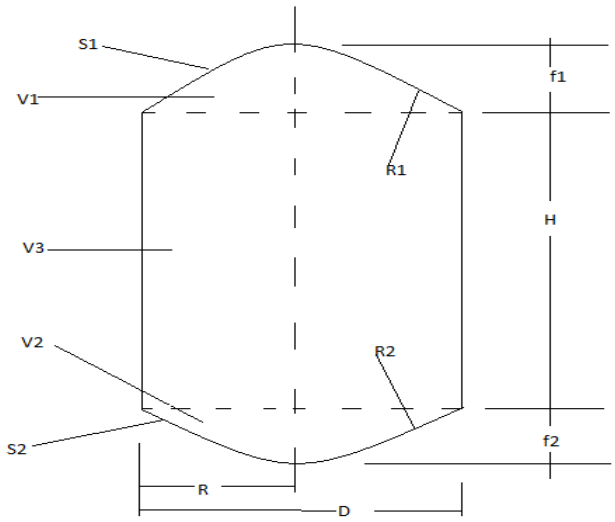


Figure A1.2: Geometry of the reactor

Assumptions of geometrical dimensions (Nijaguna, 2006).

$D = 1.3078V^{1/3}$

$V_1 = 0.0827D^3, V_2 = 0.05011D^3, V_3 = 0.3142D^3$

$R_1 = 0.725D,\ R_2 = 1.0625D$

$f_1 = \frac{D}{5}, f_2 = \frac{D}{8}$

$S_1 = 0.911D^2,\ S_2 = 0.8345D^2$

c) Volume of the bioreactor chamber and its components

The required volume of the biodigester, V

$V = 0.15m^3$ (or 150 litres)

From geometrical assumptions:

Working volume of digester $= V_{gs} + V_f$

$\therefore V_{gs} + V_f = 0.8V = 0.8 \times 0.15 = 0.12m^3$

i. Diameter (D) of the reactor.

$$D = 1.3078V^{1/3} = 0.6948m = 694.8mm$$

ii. Height (H) of the fermentation chamber.

$$V_3 = 0.3142D^3 = 0.1053m^3$$

Also,

$$V_3 = \frac{\pi}{4}D^2H$$

$$\Rightarrow H = \frac{4V_3}{\pi D^2}$$

$$\therefore H = \frac{4 \times 0.1053}{\pi \times 0.6948^2} = 0.2777m = 277.7mm$$

H:D should not exceed 0.5 (Nijaguna, 2006). In this design,

$$H:D \text{ ratio} = \frac{0.2777}{0.6948} = 0.3996 \approx 0.4$$, this is permissible.

iii. Other dimensions

$$f_1 = \frac{D}{5} = \frac{0.6948}{5} = 0.1389m = 138.9mm$$

$$f_2 = \frac{D}{8} = \frac{0.6948}{8} = 0.0868m = 86.8mm$$

$$R_1 = 0.725D = 0.725 \times 0.6948 = 0.5037m = 503.7mm$$

$$R_2 = 1.0625D = 1.0625 \times 0.6948 = 0.7382m = 738.2mm$$

iv. Areas

$$S_1 = 0.911D^2 = 0.911 \times 0.6948^2 = 0.4397m^2$$

$$S_2 = 0.8345D^2 = 0.8345 \times 0.6948^2 = 0.4028m^2$$

v. Volumes

$$V_1 = 0.0827D^3 = 0.0827 \times 0.6948^3 = 0.0277m^3$$
$$V_c = 0.05V = 0.05 \times 0.15 = 0.0075m^3$$
$$V_2 = 0.05011D^3 = 0.05011 \times 0.6948^3 = 0.0168m^3$$

d)Volume of hydraulic chamber (V_H) and its components

Figure A1.3 shows a sketch of the hydraulic chamber and its relative position with the reactor.

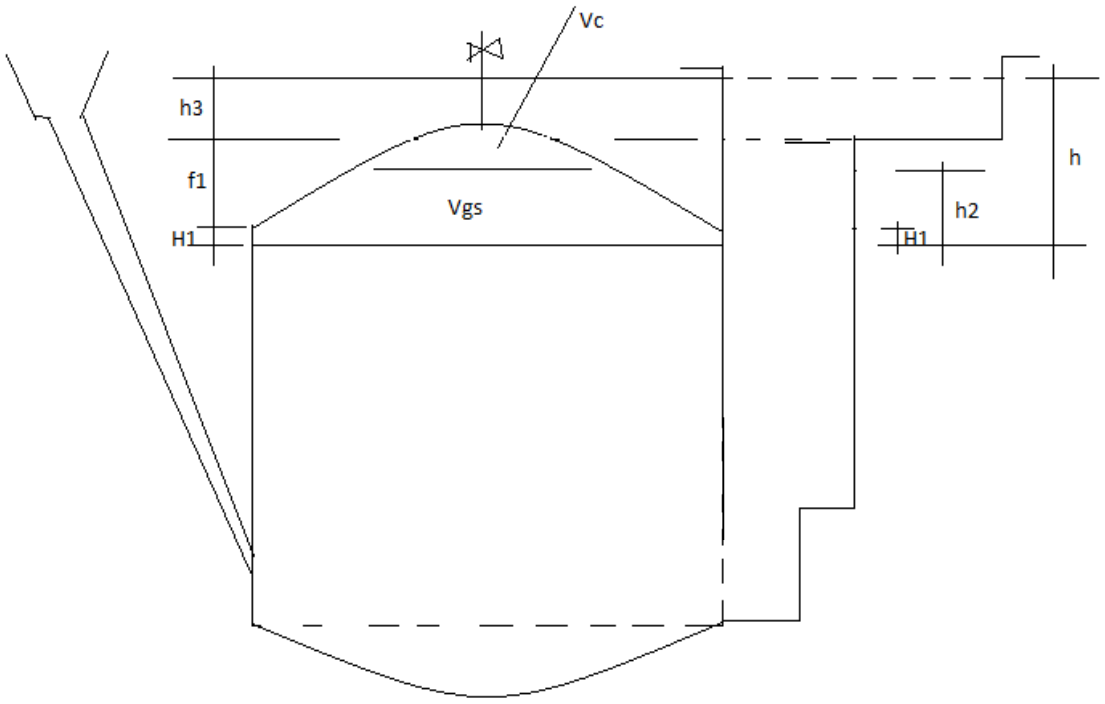


Figure A1.3: Hydraulic chamber in relation to the bioreactor

i)Volume of hydraulic chamber (V_H).

Volume of bioreactor required, V = 150 litres (or 0.15m^3)

From assumptions of volume:

$$V_{gs} = V_H$$

But,

$$V_{gs} = 50\%\ of\ daily\ gas\ produced$$

$$\Leftrightarrow V_{gs} = 0.5(V_{gs} + V_f + V_s)K$$

Where: K = Gas production rate in m^3 per m^3 of reactor volume per day

Again,

$$V_c = 0.05V = 0.05 \times 0.15 = 0.0075m^3$$

$$V_s = 0.15V = 0.15 \times 0.15 = 0.0225m^3$$

$$V_{gs} + V_f = 0.8V = 0.8 \times 0.15 = 0.12m^3$$

Hence,

$$V_H = V_{gs} = 0.5(0.12 + 0.0225) \times 0.4 = 0.0285m^3$$

$$(Note: V_f = 0.8V - V_{gs} = 0.12 - 0.0285 = 0.0915\ m^3 < V_3)$$

ii)Height (h_3) of hydraulic chamber.

From figures A1.2 and A1.3, it can be seen that,

$$V_1 = \left(V_c + V_{gs}\right) - \left(\frac{\pi}{4} D^2 H_1\right)$$

$$\Rightarrow H_1 = \frac{4}{\pi D^2}\left[\left(V_c + V_{gs}\right) - V_1\right]$$

Also,

$$V_c + V_{gs} = 0.0075 + 0.0285 = 0.036m^3$$

$$\therefore H_1 = \frac{4}{\pi \times 0.6948^2}(0.036 - 0.0277) = 0.0218m = 21.8mm$$

Rule:

When H = 1000mm of water, fix h = 800mm ($1mm = 10\ N/m^2$)

By proportion, h = 221mm when H = 277mm,

$$i.e.\ h = \frac{277 \times 800}{1000} = 221.6mm$$

From,

$$h = h_3 + f_1 + H_1$$

$$\Rightarrow h_3 = h - (f_1 + H_1)$$

$$\therefore h_3 = 0.2216 - (0.1389 + 0.0218) = 0.0609m = 60.9mm$$

iii)Diameter of hydraulic chamber (D_H).

V_H can be expressed as,

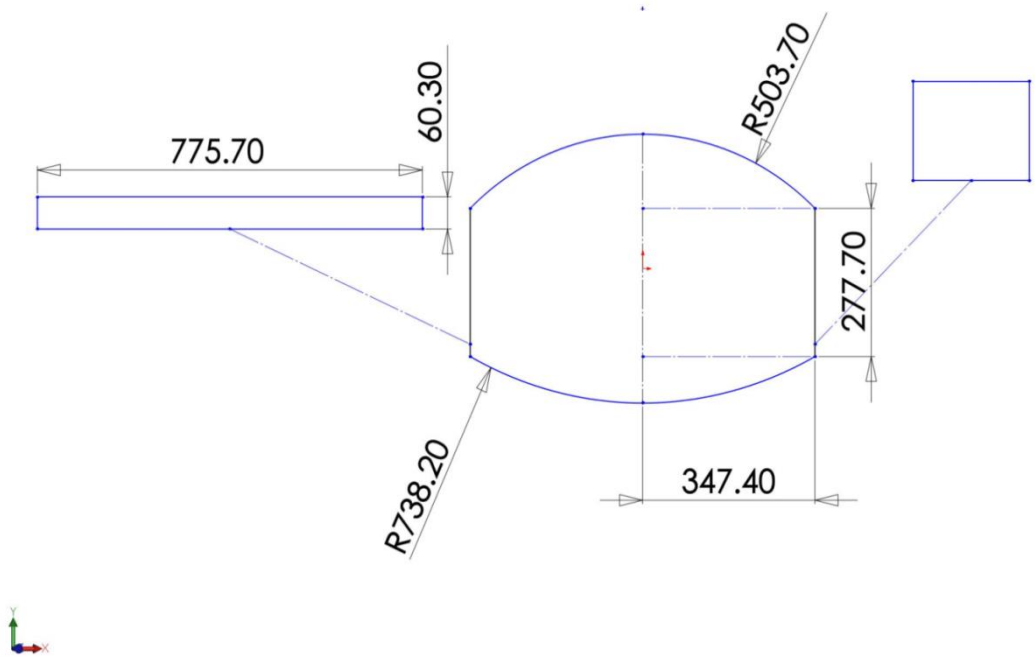
$$V_H = \frac{\pi}{4}(D_H)^2 h_3$$

$$\Rightarrow D_H = \left[\frac{4}{\pi} \times \frac{V_H}{h_3}\right]^{1/2}$$

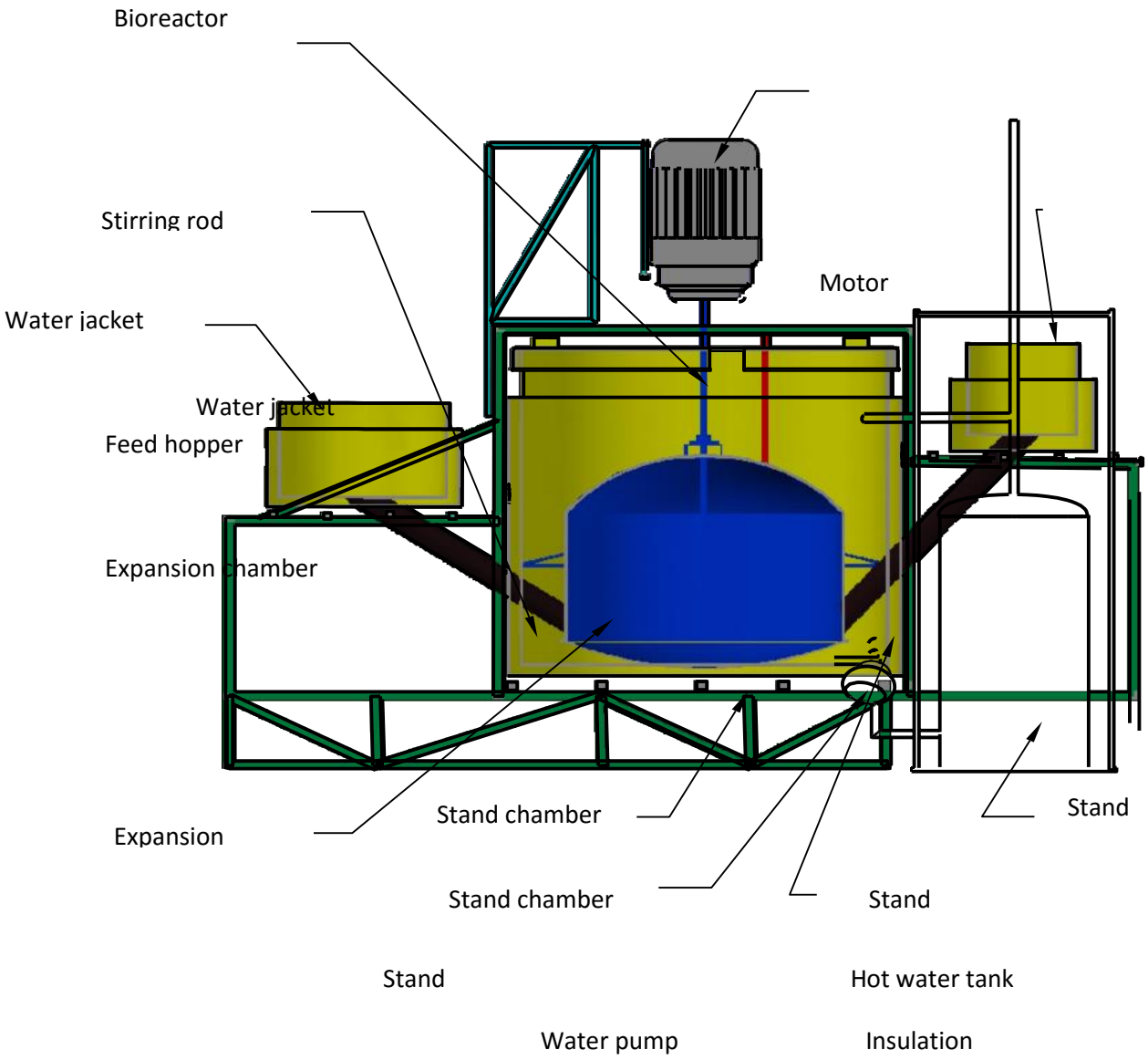
$$\therefore D_H = \left[\frac{4 \times 0.0285}{0.0609\pi}\right]^{1/2} = 0.7757\, m = 775.7mm$$

A1.2: Engineering drawings

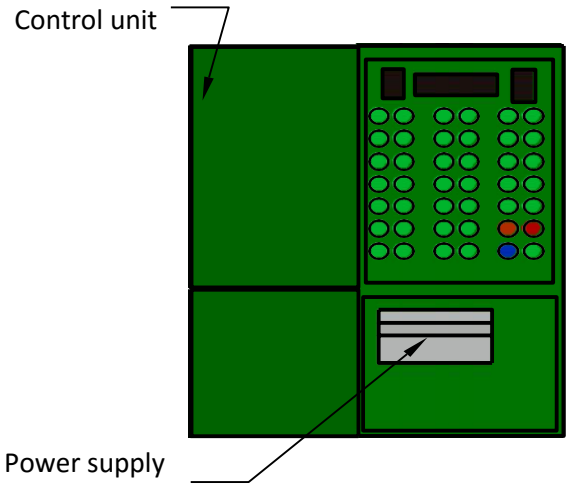
The following drawings were made with the help of an Automatic Computer-Aided Draft software, version 2017 (AUTOCAD 2017).



Drawing A1.1: Expansion Chamber, Bioreactor, and Feed Hopper



Drawing A1.2:Cross-section of the bioreactor system



Drawing A1.3: Cross-section of Electrical control system

A1.3: Plates of types of bioreactors

The following are the photographs of various biogas systems in Kenya.

Plate A1.1: Floating drum

Plate A1.2: Fixed dome

Plate A1.3: Flexible structure

Plate A1.4: Lagoon

Plate A1.5: Lagoon covered with a membrane

Appendix 2: Biogas production data and analysis

A2.1 Biogas Production (litres per day) in the Egerton University Fixed Dome Lab Digester

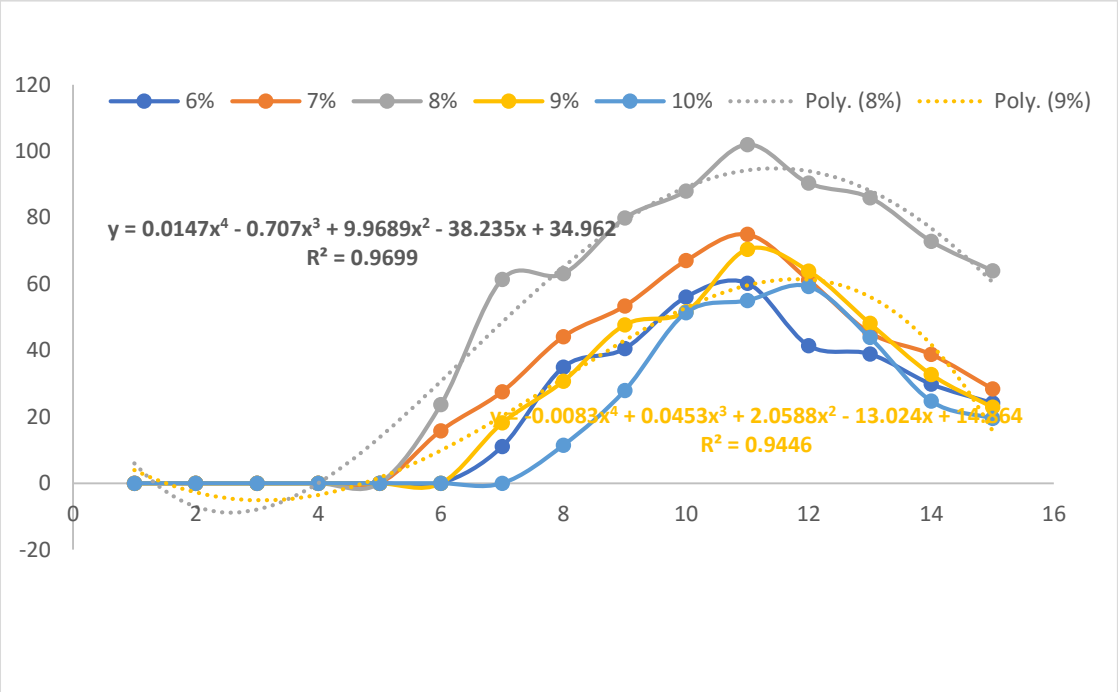
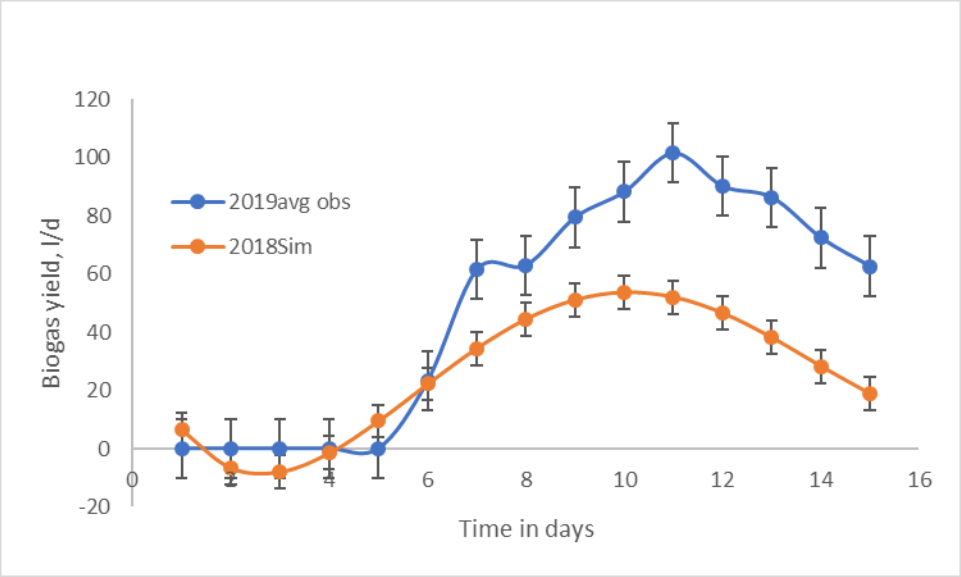


Fig A2.1: Biogas production (on Y-axis in litres per day) with retention time (in days) for various TS and regression models for the optimal TS (8%) and other TSs (6, 7, 9 &10%).

Note:

This for the different total solid (TS) contents of Egerton University (Kenya) cattle manure substrate or influent being digested at 35 degrees Celsius in a Fixed Dome Lab Digester.



Note: Error bars are the same due to insufficient number of replications

Figure A2.2: Average Measured or Observed versus Predicted Biogas yield using the Polynomial

empirical models for 8% and the 6,7,9 and10% Total solids (TS)

The Models used to generate the simulations in Table A2.2.1 are:

$$Y_{2019\_3ord} = 0.0203x^3 - 3.0172x^2 + 59.904x - 227.25 \qquad (R^2 = 0.97)$$

$$Y_{2019\_2ord} = -2.4088x^2 + 54.18x - 210.57 \qquad (R^2 = 0.97)$$

$$Y_{2018\_4ord} = 0.0219x^4 - 0.856x^3 + 10.413x^2 - 38.621x + 35.616 \qquad (R^2 = 0.92)$$

where

Y is gas yield in litres per day, and

x = digestion time from loading in days.

A2.2: Biogas prediction for 8% TS at 35 degrees Celsius for models based on 2018 and 2019 data

Using Observed and simulated Data collected in 2018 a regression model was developed and used to simulate the data that was compared with observed data collected in 2019 as presented in Table and Figure below.

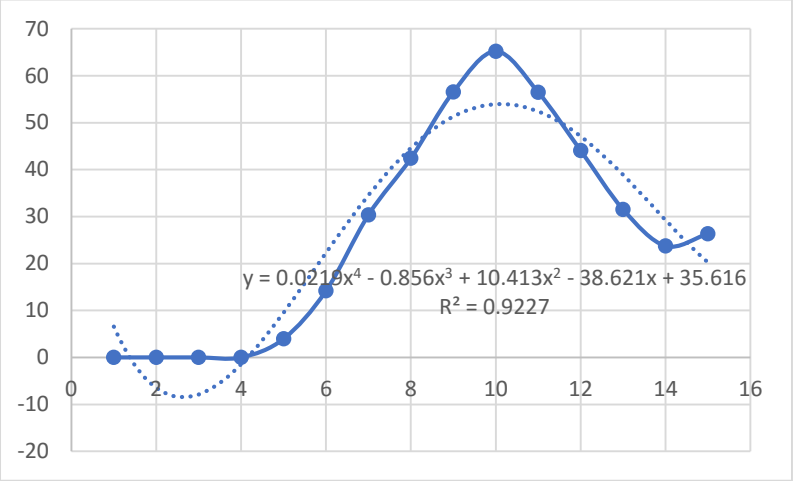


Fig A2.4: Average Biogas (for 8% TS) production (l/d, on Y- axis) with time of digestion (days, X-axis)

The resulting regression line is:

$Y_{8\%TS} = 0.0219x^4 - 0.856x^3 + 10.413x^2 - 38.621x + 35.616$ ($R^2 = 0.9227$)

Table A2.2 Biogas prediction for 8% TS at 35^0 C for models based on 2018 and 2019 data

Day	2019avg obs	2018Sim	2019Sim3ord	2019Sim2ord
1	0	*6.6*	*-290*	*-159*
2	0	*-6.5*	*-359*	*-112*
3	0	*-7.9*	*-434*	*-70*
4	0	*-1.4*	*-514*	*-32*
5	0	*9.5*	*-600*	*0*
6	23	*22.2*	*-691*	*28*
7	62	34.5	*-787*	51
8	63	44.5	*-889*	69
9	80	51.1	*-996*	82
10	88	53.7	*-1108*	90
11	102	52.1	*-1224*	94
12	90	46.6	*-1345*	93
13	86	38.2	*-1471*	87
14	72	28.3	*-1602*	76
15	63	18.9	*-1736*	60

Appendix 3: Copyright Form

This is a copyright form from Isaac Scientific Publishing (ISP) for the first paper.

COPYRIGHT FORM

This form is provided for original papers submitted to the journals of Isaac Scientific Publishing (ISP) and must be received by ISP before the paper is accepted for publication. Please read this form carefully before signing.

Journal name: Annals of Advanced Agricultural Sciences.

Title of Paper: Effect of total solids on biogas production in a fixed dome laboratory digester under mesophilic temperature.

Author(s): Barasa H. Masinde, Daudi M. Nyaanga, Musa R. Njue, and Joseph W. Matofari.

Affiliation(s): Masinde Muliro University of Science and Technology, Egerton University.

Copyright Transfer

The undersigned hereby assigns to the ISP all the rights under copyright that may exist in and to the above Work, and any revised or expanded derivative works submitted to the above mentioned journal by the undersigned based on the Work. The undersigned hereby warrants that the Work is original and that he/she is the author of the Work; to the extent that the Work incorporates passages, figures, data or other materials from the works of others, the undersigned has obtained any necessary permission.

Retained Rights/Terms and Conditions

Authors/employers retain all proprietary rights in any process, procedure, or article of manufacture described in this Work.

Authors/employers may reproduce or authorize others to reproduce the Work, materials extracted verbatim from the Work, or derivative works for the author's personal use or for the company or organizational use, provided that the source and any ISP copyright notice are indicated, the copies are not used in any way that implies ISP endorsement of a product or service of any employer are not offered for sale.

Authors/employers may make limited distribution of all or portions of the Work prior to publication if they inform the ISP in advance of the nature and extent of such limited distribution.

ISP Copyright Ownership

It is the formal policy of the ISP to own the copyrights to all the copyrightable materials in its technical publications and to the individual contributions contained therein, in order to protect the interests of ISP, its authors and their employers, and, at the at the same time, to facilitate the appropriate re-use of this material by others. The ISP distributes its technical publications throughout the world and does so by various means such as hard copy and electronic media. It also abstracts and may translate its publications, and articles contained therein, for inclusion in various compendiums, collective works, databases and similar publications.

Author/Employer Rights

If you are employed and prepared the Work on a subject within the scope of your employment, the copyright in the Work belongs to your employer as a work-for-hire. In that case, the ISP assumes that when you sign this Form, you are authorized to do so by your employer and that your employer has consented to the transfer of copyright, to the representation and warranty of publication rights, and to all other terms and conditions of this Form as the author. In the event the above is not accepted and published by the ISP or is withdrawn by the author(s) before acceptance by the ISP, the foregoing copyright transfer shall become null and void and all materials embodying the Work submitted to the above mentioned journal will be destroyed.

The author signs for and accepts responsibility for releasing this material on behalf of any and all co-authors.

Author/Authorized Agent For Joint Authors Date (dd-mm-yy)

*Signed: BHM 08-02-2020

This is a copyright form from Isaac Scientific Publishing (ISP) for the second paper.

COPYRIGHT FORM

This form is provided for original papers submitted to the journals of Isaac Scientific Publishing (ISP) and must be received by ISP before the paper is accepted for publication. Please read this form carefully before signing.

Journal name: International Journal of Power and Energy Research.

Title of Paper: Optimisation of Biogas Production in a Batch Laboratory Digester using Total Solids, Substrate Retention Time, and Mesophilic Temperature.

Author(s): Barasa H. Masinde, Daudi M. Nyaanga, Musa R. Njue, and Joseph W. Matofari.

Affiliation(s): Masinde Muliro University of Science and Technology, Egerton University.

Copyright Transfer

The undersigned hereby assigns to the ISP all the rights under copyright that may exist in and to the above Work, and any revised or expanded derivative works submitted to the above mentioned journal by the undersigned based on the Work. The undersigned hereby warrants that the Work is original and that he/she is the author of the Work; to the extent that the Work incorporates passages, figures, data or other materials from the works of others, the undersigned has obtained any necessary permission.

Retained Rights/Terms and Conditions

Authors/employers retain all proprietary rights in any process, procedure, or article of manufacture described in this Work.

Authors/employers may reproduce or authorize others to reproduce the Work, materials extracted verbatim from the Work, or derivative works for the author's personal use or for the company or organizational use, provided that the source and any ISP copyright notice are indicated, the copies are not used in any way that implies ISP endorsement of a product or service of any employer are not offered for sale.

Authors/employers may make limited distribution of all or portions of the Work prior to publication if they inform the ISP in advance of the nature and extent of such limited distribution.

ISP Copyright Ownership

It is the formal policy of the ISP to own the copyrights to all the copyrightable materials in its technical publications and to the individual contributions contained therein, in order to protect the interests of ISP, its authors and their employers, and, at the at the same time, to facilitate the appropriate re-use of this material by others. The ISP distributes its technical publications throughout the world and does so by various means such as hard copy and electronic media. It also abstracts and may translate its publications, and articles contained therein, for inclusion in various compendiums, collective works, databases and similar publications.

Author/Employer Rights

If you are employed and prepared the Work on a subject within the scope of your employment, the copyright in the Work belongs to your employer as a work-for-hire. In that case, the ISP assumes that when you sign this Form, you are authorized to do so by your employer and that your employer has consented to the transfer of copyright, to the representation and warranty of publication rights, and to all other terms and conditions of this Form as the author. In the event the above is not accepted and published by the ISP or is withdrawn by the author(s) before acceptance by the ISP, the foregoing copyright transfer shall become null and void and all materials embodying the Work submitted to the above mentioned journal will be destroyed.

The author signs for and accepts responsibility for releasing this material on behalf of any and all co-authors.

Author/Authorized Agent For Joint Authors Date (dd-mm-yy)

*Signed: BHM 28-03-2020

Appendix 4: Abstracts of Publications

Two papers were published from this work:

a) Paper 1: Effect of total solids on biogas production in a fixed dome laboratory digester under mesophilic temperature. Annals of Advanced Agricultural Sciences, Vol. 4, No. 2, May 2020. https://dx.doi.org/10.22606/as.2020.42003
b) Paper 2: Optimisation of biogas production in a batch laboratory digester using total solids, substrate retention time, and mesophilic temperature. International Journal of Power and Energy Research, Vol. 4, No. 2, July 2020. https://dx.doi.org/10.22606/ijper.2020.42001

The abstracts of the papers are as follows.

Annals of Advanced Agricultural Sciences, Vol. 4, No. 2, May 2020
https://dx.doi.org/10.22606/as.2020.42003

Effect of Total Solids on Biogas Production in a Fixed Dome Laboratory Digester under Mesophilic Temperature

Barasa H. Masinde[1*], Daudi M. Nyaanga[2], Musa R. Njue[2] and Joseph W. Matofari[3]

[1] Mechanical and Industrial Engineering Department, Masinde Muliro University of Science and Technology, P.O. Box 190 – 50100, Kakamega, Kenya

[2] Agricultural Engineering Department, Egerton University, P.O. Box 536 – 20115, Egerton, Kenya

[3] Dairy, Food Science and Technology Department, Egerton University, P.O. Box 536 – 20115, Egerton, Kenya

Email: he.masb62@gmail.com

Abstract. An investigation on the effect of total solids on biogas production was done using a laboratory scale batch reactor of 0.15 m^3 capacity. The feedstock was dung from dairy cows managed under a free-range system. Experiments were done on a substrate having total solids of 6%, 7%, 8%, 9%, and 10% at a constant temperature of 35°C; and the mean biogas production was 0.249, 0.304, 0.487, 0.287, and 0.244 m^3 of biogas per m^3 of digester volume per day (m^3/m^3d), respectively. It was concluded that the highest average biogas production of 0.487 m^3/m^3d is attained at total solids of 8%.

Keywords: total solids, biogas production, fixed dome, laboratory digester

1 Introduction

Biogas is an important form of renewable energy. It is stored in biological materials such as straw, manure and other agricultural products; and it is one of the key options for mitigating Green House Gas (GHG) emissions to replace fossil fuels [1, 2]. It can be used to generate heat, electricity, and produce transport fuel [3, 4]. Each year, 590-880 million tonnes of methane are exhausted worldwide into the atmosphere through microbial activity and 90% of this comes from biogenic sources [5].

Anaerobic digestion is the process by which organic matter is broken down in the absence of oxygen to produce biogas, carbon dioxide and other traces of gases. The process of anaerobic digestion takes place through four successive stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis; and it is dependent on the interaction between diverse microorganisms that are able to carry out the four aforementioned stages [6]. In single stage batch reactors, the substrate is loaded and the four processes are allowed to occur in the same reactor sequentially; then the slurry is emptied after a predetermined retention time or the cessation of biogas production [6].

Working under the assumption that all the substrate is converted to CH_4 and CO_2, and that the carbon (C), hydrogen (H_2) and oxygen (O_2) composition of the substrate are known, one can use the following equation and the general gas equation to find a theoretical molar and volumetric output of CH_4 [7].

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \quad (1)$$

Under actual operating conditions, equation 1 was modified to account for the presence of ammonia (NH_3) and hydrogen sulphide (H_2S) in the waste as given in equation 2 below [8].

$$C_nH_aO_bN_xS_y + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3x}{4} + \frac{y}{2}\right)H_2O \rightarrow \left(n - \frac{a}{4} + \frac{b}{2} + \frac{3x}{4} + \frac{y}{2}\right)CO_2 + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3x}{4} - \frac{y}{2}\right)CH_4 + xNH_3 + yH_2S \quad (2)$$

Parameters that affect biogas production include substrate concentration, temperature, substrate retention time, carbon to nitrogen ratio, pH, organic loading rate, nutrients, and toxicity [9]. The effects of these parameters are different on different microbial groups as each microbial group has different physiological and nutritional needs; and it is imbalances between them that cause instability [10]. An imbalance in the process caused due to the disturbance in the hydrolysis stage will limit the activities in the subsequent stages thereby reducing biogas production; a disturbance in methanogenesis will bring

International Journal of Power and Energy Research, Vol. 4, No. 2, July 2020
https://dx.doi.org/10.22606/ijper.2020.42001

Optimization of Biogas Production in a Batch Laboratory Digester Using Total Solids, Substrate Retention Time, and Mesophilic Temperature

Barasa H. Masinde[1*], Daudi M. Nyaanga[2], Musa R. Nju[2] and Joseph W. Matofari[3]

[1] Mechanical and Industrial Engineering Department, Masinde Muliro University of Science and Technology, P.O. Box 190 – 50100, Kakamega, KENYA.

[2] Agricultural Engineering Department, Egerton University, P.O. Box 536 – 20115, Egerton, KENYA.

[3] Dairy, Food Science and Technology, Egerton University, P.O. Box 536 – 20115, Egerton, KENYA.

Email: he.masb62@gmail.com

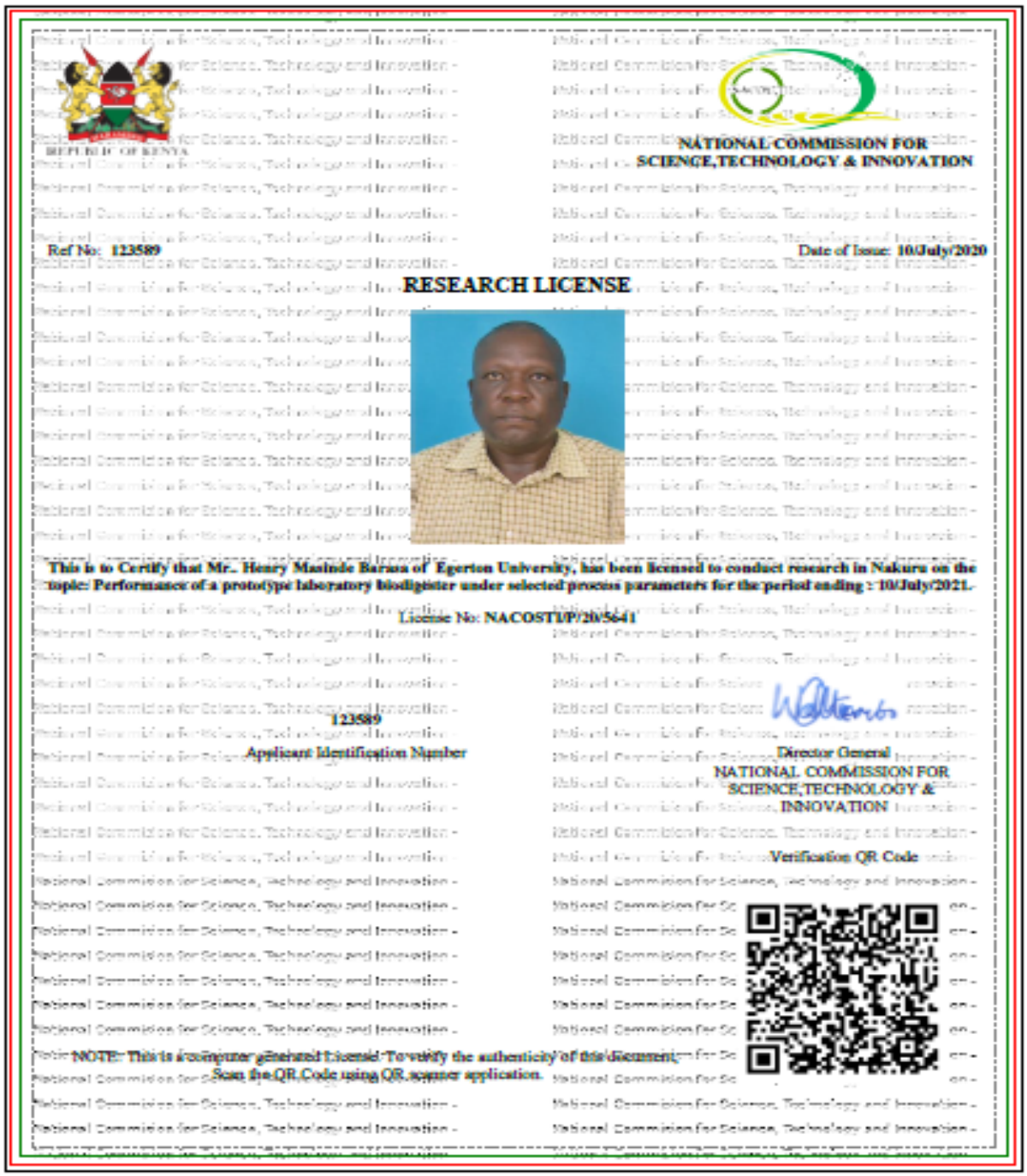
Abstract. Optimization was done by investigating the interaction effects of total solids, mesophilic temperature, and substrate retention time on biogas production in a batch biodigester. The volume of the biodigester was 0.15m³. Central composite design of Response Surface Methodology was used to design the experiment. Total solid levels were varied from 6.31% to 9.68%, temperature was from 26.59°C to 43.41°C, and substrate retention time was from 9.95 to 20.04 days. Analysis of results was done using Design Expert software statistical package (version 10.0.0.3). It gave a coefficient of determination of 0.9665 which indicated a high correlation between the variables. All the variables had a significant effect. The highest biogas production rate of 75.41litres/day (or 0.50 m³ of biogas per m³ of digester volume per day, m³/m³d) was achieved at a level of 8% total solids, a temperature of 43.41°C, and a substrate retention time of 15 days.

Keywords: optimization, biogas production, total solids, mesophilic temperature, substrate retention time.

1 Introduction

Optimization is the act of achieving the best possible result under given circumstances. In design, construction and maintenance, engineers have to make decisions. The aim of such decisions is either to minimize the effort or to maximize benefit. The effort or benefit can be expressed as a function of certain design variables. Hence optimization is the process of finding conditions that give the minimum or the maximum value of a function [1]. Efforts to maximize biogas production are currently being done by using various feedstocks. Reungsang *et al* [2] used Response surface methodology (RSM) with a central composite design (CCD) to optimize the key factors affecting methane production from the acidic effluent coming from the sugarcane juice hydrogen fermentation process. The parameters studied were substrate concentration, ratio of $NaHCO_3$ to substrate concentration, and initial pH. The maximum methane yield of 367 mL CH_4/g-volatile solid (VS) added was obtained at the optimum conditions of 13,823 mg-COD/L, an $NaHCO_3$ to substrate concentration ratio of 3.09, and an initial pH of 7.07. Methane yield was enhanced 4.4-fold in comparison to raw effluent. Sathish and Vivekanadan [3] studied the optimal conditions for biogas generation from anaerobic digestion of rice straw in a 1m³ floating drum anaerobic digester using RSM. The parameters were temperature, pH, substrate concentration, and agitation time. The highest level of biogas of 0.72m³ was produced at an optimum temperature of 50°C, pH 7.5, substrate concentration of 110.70 kg, and an agitation time of 5 seconds, respectively. Saleh *et al* [4] investigated the effects of temperature, palm oil mill effluent (POME) volume, inoculum volume, and co-substrate addition such as oil palm empty fruit bunch (EFB) and palm kernel on the anaerobic digestion process for biogas and methane production. RSM by Box-Behnken design verified that the specific biogas production rate and methane yield were mainly affected by the operating temperature and co-substrate addition. The optimal conditions for the maximum specific biogas production rate (0.0574 m³/ kg chemical oxygen demand per day) and methane yield (25.6%) were predicted by multiple response optimization and verified experimentally at 47.8°C

Appendix 5: Research permit



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 123589

Date of Issue: 10/July/2020

RESEARCH LICENSE

This is to Certify that Mr.. Henry Masinde Barasa of Egerton University, has been licensed to conduct research in Nakuru on the topic: Performance of a proto(ype laboratory biodigister under selected process parameters for the period ending : 10/July/2021.

License No: NACOSTI/P/20/5641

123589

Applicant Identification Number

Director General

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.