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Effect of Substrate Improvement on Optimising Biogas Yield from Anaerobic Digestion

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This Master's Thesis is carried out as part of the education at the University of Agder and is therefore approved as a part of this education. However, this does not imply that the University answers for the methods that are used or conclusions drawn

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Abstract

The anaerobic digestion process involves the decomposition of the organic matter to yield biogas; gas that is mainly composed of methane and carbon dioxide with traces of some other gases. This process is used for different purposes including municipal waste treatment, wastewater treatment, fertilizer production, and farm wastes disposal as well as energy production. It is accomplished in three main stages; hydrolysis, acidogenesis and methanogenesis with each of the stages achieved by specific microorganisms that are active in limited ranges of operational and environmental conditions. These conditions are controlled to achieve an optimal output of the process. For example for the energy production purposes, the energy output needs to be optimised in terms of operating conditions. To produce an optimal amount of biogas from a certain substrate, the latter should be analysed for its nutritional composition and if some of the nutrients are not in appropriate amounts they are adjusted by mixing more than one type of substrates.

The purpose of this study is to investigate the effect of improving substrate nutritional composition on the optimal yield of the anaerobic digestion process. In other words, we are looking at a way to increasing the biogas yield by improving the qualitative composition of the substrates. To achieve this objective, a thorough literature review was done to be aware of the effect of various environmental and operational parameters on the yield of the anaerobic digestion. These parameters include the temperature, the pH of the substrates, the carbon to nitrogen ratio, total solids content and substrates composition. To study the effect of the latter, we carried out an inventory of the mostly used substrates for the production of biogas. These include agricultural residues, animal manures, municipal solid wastes and food wastes.

Food wastes collected from the kitchen and exclusively fruit wastes were digested at 55 ± 2^{o} C and at 37 ± 2^{o} C. Digestion of food wastes characterised by a total solids content of 10.423 % and volatile solids to total solids content of 89.145 % produced 95mL/g VS of biogas at thermophilic temperature, fruit wastes with a total solids content of 8.253 % and volatile solids to total solids content of 91.495 % produced 30mL/g VS. Mixed together making total solids content of 12.493 % and volatile solids to total solids content ratio of 86.988 % food and fruit wastes produced 110mL/g VS. Results of this study show that mixing food wastes with fruit wastes improved the biogas yield by 13.67 %. The same increase was observable at mesophilic temperature. This increase in biogas yield in this study shows that optimal biogas yield is produced by improving the nutritional composition of substrates.

Keywords: Anaerobic Digestion, Biogas Yield, Substrates, Food wastes, Fruit wastes, Characterisation.

Preface

This thesis is submitted to the Faculty of Engineering and Science of the University of Agder, in partial fulfilment of the requirements for the degree of Master of Science in Renewable Energy. The purpose of this study is to investigate the effect of the improvement of substrate on optimising the biogas yield from the anaerobic digestion. It includes results of a series of experiments carried out in Biomass and Fuel Cell Technology laboratories at the University of Agder. This work was conducted under the supervision of Professor Peter Hugh Middleton and Nicholas Michael Abson.

My sincere gratitude goes to the supervisors, Professor Hugh Middleton and Nicholas Michael Abson for their encouragement, advice and technical support in the course of this work. I am very grateful to Professor Henrik Kofoed Nielsen for his support during the analysis of the substrates in the Biomass laboratory.

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Contents

List of Figures

List of Tables

List of Abbreviations

AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model No.1
BW	Biodiesel Waste
CGW	Cotton Gin Waste
CM	Cow Manure
COD	Chemical Oxygen Demand
CPMF	Chemically Precipitated Manure Fibre
CSTR	Continuously Stirred Tank Reactor
DM	Dry Matter
FM	Fish Manure
FPMF	Filter Pressed Manure Fibre
LCFAs	Long Chain Fatty Acids
MSW	Municipal Solid Waste
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
PM	Pig Manure
PTS	Percentage Total Solid
SBP	Specific Biogas Production
sCOD	Soluble Chemical Oxygen Demand
SRT	Solid Retention Time
tCOD	Total Chemical Oxygen Demand
TDS	Total Dissolved solid
TOC	Total Organic Carbon
TS	Total Solids
TSS	Total Suspended Solid
VFAs	Volatile Fatty Acids
VS	Volatile Solids
VTS	Volatile Total Solids
WAS	Waste Activated Sludge

1 Introduction

This chapter gives the background information of the anaerobic digestion and presents the motivation for optimising the biogas production focusing on the effect of substrate composition. In this part of the report we start by expressing the motivation that leads us to the investigation of the effect of substrate composition on the improvement of the yield of the anaerobic digestion. The chapter continues by stating the goals and objectives of this study, discussing the research methods through which the study was carried out, providing the assumptions and limitations and it ends in giving the outlines of the report.

1.1 Motivation for Optimizing biogas production

During the anaerobic digestion (AD) process microorganisms break down organic material. The organic material can be food waste, wastewater, municipal solid waste, cattle manure, agricultural residues, etc. This digestion results in biogas, composed of methane and carbon dioxide with traces of other gases like hydrogen sulphide, ammonia and water vapours.

AD takes place in an air-tight anaerobic digester of which different types exist. There are various types of digester systems; continuous, batch, single-stage, two- or multi-stage and finally wet and dry systems. These types can be combined into four principal types; single-stage wet, single-stage dry, multi-stage wet and multi-stage dry digester systems. Based on the operating temperature, AD process is classified as psychrophilic, mesophilic or thermophilic process. Prominent full-scale plants currently in operation are single-stage systems, both wet and dry in relatively equal numbers, as these types are simple to fabricate requiring relatively small investment and maintenance costs. Multi-stage systems present improved performance but have not attracted attention given their complexity in construction and their high investment cost.

For energy production purposes, studies are continuously done to efficiently produce energy from anaerobic digestion by also minimising overall investment and maintenance costs for the technology to be economically viable. By thoroughly studying the dependence of the energy output of anaerobic digester on the operating parameters, one can figure out how same of the parameters can best be improved to optimise the output of the digester.

1.2 Research Goals and Objectives

The overall goal of this project is to optimise the biogas yield of an anaerobic digester in terms of substrate nutritional composition. For this purpose, the following objectives are set:

- Detailed description of the digestion steps in classical anaerobic digestion processes
- Study of the factors affecting the anaerobic digestion process
- Review of existing types of anaerobic digesters
- Study of the mostly used types of substrates and their optimal biogas yield
- Identify mixing regimes that can lead to optimal biogas yields that couldn't be produced by single substrates
- Carry out laboratory experiments using the selected substrates.
- Analysis of the biogas produced to determine its fractional composition.
- Compile the project work in a written report

1.3 Research Methods

To achieve the optimal production of biogas in terms of substrate composition, this research study was done in the following complementary steps:

1.3.1 Potential Substrates for Biogas Production

Different types of substrates are used for biogas production producing different quantities and qualities of biogas. Some substrates are loaded to the digesters under their pure nature without any improvement or pre-treatment, others go through composition improvements and/or pre-treatment. The choice of substrate to be loaded to a particular type of digester and its treatment has a great effect on the biogas produced.

In this section of work, different types of substrates will be explored to get knowledge about their chemical composition and their biogas potential. This helps in figuring out substrates that are most likely to produce a better biogas yield and methods that can be used to improve needy substrates to come out with optimal biogas yield.

1.3.2 Substrate Selection and Improvement

After a review of the potential substrates, substrates will be selected to be experimentally studied and be subjected to characterisation. Based on the results of the characterisation, biogas potential of elementary substrates will be estimated and where improvement is needed substrates will be mixed together in calculated proportions in the purpose of optimising biogas production.

1.3.3 Experimental Set up and Analysis

The selected substrates will be loaded to laboratory-based digesters under thermophilic conditions to study their gas outputs. The biogas produced will be analysed with gas chromatography to identify its chemical composition. We will compare the results to standard AD outputs to find out whether the intended yield optimisation was achieved.

1.4 Key Assumptions and Limitations

- By characterising the substrates, nutrients that need to be improved are determined
- The appropriate mixing of two types of substrates helps in increasing the biogas yield compared to single substrate digestion.
- Thermophilic digestion helps in increasing the biogas production rate as a result of increased organic matter decomposition rate.
- Thermophilic range, though the best in terms of degradation rate is coupled with destabilization of digestion reactions resulting in compromised optimal biogas yield production.

Using multi-stage anaerobic digestion leads to increased parameters control and separation of the phases of the anaerobic digestion. Unfortunately, this was not achieved due to its complexity that couldn't be achieved in the laboratory; we only used batch systems.

1.5 Thesis Outline

For easy and comprehensive communication of the results of this study, the rest of this thesis is organised as follows:

Chapter two discusses the AD process by describing its three main stages. In Chapter three the operating parameters of the anaerobic digestion are discussed where focus is put on the effect of each operating parameter on the overall process and how its variation would affect the yield of the digestion. Chapter four covers a description of different types of anaerobic digesters by reviewing their structures and their working principles. Chapter five continues by explaining the experimental set up that was used during this study. Chapters six and seven give and discuss the results of the experiments and finally in Chapter eight conclusions are drawn from the results of the study.

2 Anaerobic Digestion Process

This chapter describes the process of anaerobic digestion focusing on three stages through which it is completed. Biochemical transformations that take place in each step of the process are explained and the general description of anaerobic digestion is given by establishing the interconnection between the three stages.

2.1 Hydrolysis

Hydrolysis is the initial stage of the AD in which complex organic polymers are degraded to simple soluble molecules by extracellular enzymes; proteins are hydrolysed to amino-acids, lipids to long-chain fatty acids and carbohydrate polymers to simple chain sugars [1]. Depolymerisation of organic polymers and fermentation to organic acids, alcohols and methanogenic substrates are done by hydrolytic bacteria. In fact, these bacteria secrete extracellular enzymes mainly, hydrolases and lyases responsible for the depolymerisation giving out small soluble molecules which can be assimilated by microbial cells and metabolised.

For complex substrates with a high solids content, hydrolysis is the slowest step and hence the rate-limiting step in the overall digestion process [2]. In fact, during depolymeristion the main substrates broken down are cellulose, hemicellulose, starches, proteins, lipids and lignin. Lignin is quite resistant to degradation and if it is abondant in the substrates it will limit the reaction rate. However, complete modelling of hydrolysis does not limit to lignin content, it also involves overall substrate concentration, product concentration, surface kinetics, temperature and toxicity [3]. Hydrolysis products are volatile fatty acids, amino-acids, simple sugars, carbon dioxide and hydrogen.

2.2 Acetogenesis

Acetogenesis is the second stage of the anaerobic digestion. The intermediate products that were generated from hydrolysis such as propionate, butyrate, lactate and ethanol are converted to acetate by acetogenic bacteria. These bacteria produce hydrogen which in turn is utilised by hydrogen utilising bacteria keeping hydrogen concentration in an acceptable range, the failure to do so will lead to longer chain fatty acids lowering the pH of the process that leads to inhibition of acetogenesis.

2.3 Methanogenesis

Methanogenesis is the third and final stage of the anaerobic process. During this stage methanogens which are methane forming bacteria produce methane gas from the products of the acetogenesis which are the volatile fatty acids, hydrogen and carbon dioxide. This is done in two ways; either by cleavage of acetic acid into methane and carbon dioxide or by reduction of carbon dioxide by hydrogen. It is important to mention that this stage of the anaerobic digestion is the most sensitive to the change of environmental conditions inside the digester as it will be explained later in this document.

2.4 General Process Description

During the anaerobic digestion, polymeric organic matter is degraded in methane and carbon dioxide by the action of several consortia of microorganisms. This degradation is completed into three complementary and interdependent steps. In the first step hydrolytic bacteria hydrolyse complex organic matter and ferment the hydrolysis products to acetate, alcohols, longer chain fatty acids, amino-acids, carbon dioxide and some other products. In the next step acetogenic bacteria degrade fatty acids, alcohols and aromatic compounds to methanogenic substrates, H_2 and acetate. In the final steps two types of microorganisms are involved; hydrogenotrophic methanogens which use hydrogen to reduce CO_2 to CH_4, and acetotrophic bacteria which induce the cleavage of acetate to CO_2 and CH_4 [4].

2.5 Mathematical Modelling of the Anaerobic Digestion

The anaerobic digestion process involves complex processes that need to be understood to be able to achieve efficient and economically viable systems. To achieve this prior analysis should be carried out to predict the biogas yield from any potential substrates. With traditional designing methods, it would require construction of different prototypes and carrying out many lab-scale experiments and measurements before a practical anaerobic digestion is undertaken, requiring more time and investment cost. Today the design and optimisation of the anaerobic digestion are facilitated by validated mathematical models that avoid the development of expensive prototypes and carrying out time consuming measurements.

Mathematical models are used for deeper understanding of transport phenomena, flow phenomena, microbial kinetics and the stoichiometry of the AD. Different models have been invented over years but their uses by engineers and anaerobic digestion operators were limited mainly due to their wide variety and their specific nature. To solve this problem, the international water association (IWA) task force for mathematical modelling in 2002 published a generalised and sophisticated anaerobic digestion model No1

now commonly known as ADM1 [5]. This model takes into consideration two of the most important processes that are undertaken in the anaerobic digestion; biochemical processes and physico-chemical processes. Biochemical processes include the disintegration of particulate matter to carbohydrates, proteins and lipids whereas physico-chemical equations describe ion association-dissociation and gas-liquid transfer.

The implementation of this model will depend on whether the liquid phase physico-chemical processes are implemented as algebraic or kinetic rate equations. In any case for each state component the mass balance is as shown in Equation 2.1

$$\frac{dS_{liq,i}}{dt} = \frac{q_{in}}{V_{liq}} S_{in,i} - \frac{S_{liq,i}}{V_{liq}} q_{out} + \sum_{j=1}^{19} \rho_j \nu_{i,j} \tag{2.1}$$

where:

ρ_j: the specific kinetic rate for process j

$\nu_{i,j}$: Stoichiometric coefficient

V_{liq}: Liquid reactor volume

q: flow into or out of the reactor

$S_{in,i}$: input concentration of the soluble component

Since the publication of ADM1 different researchers and industries use it to model their anaerobic systems. Boubakar Fezzani and Ridha Ben Cheikh adapted the model to replicate the thermophilic anaerobic digestion of olive mill wastewater with olive mill solid wastes. Simulation results showed that the modified ADM1 was able to predict well the steady-state results of gas flows, methane and carbon dioxide contents, pH and total volatile fatty acids [6]. Zaobo Chen *et al*. built a mathematical model on the basis of ADM1 to model a two-phase anaerobic process treating traditional Chinese medicine wastewater. Implemented with the simulation software MATLABTM/Simulink, model was able to predict the system performance in terms of COD removal, volatile fatty acids accumulation and pH fluctuations [7].

3 Important Operating Parameters

The steps of the anaerobic digestion are interdependent with the effect on one of them affecting the overall output of the process. Microorganisms that are involved in the process need different environmental requirements to be fulfilled for their survival and optimal activity. Biochemical transformations that take place in AD need to be achieved under certain conditions for optimal biogas yield. Therefore, it is important to control the AD process to make sure that all of the parameters which affect the process are optimised. This part of the thesis discusses major operating parameters; the impact of temperature, carbon to nitrogen ratio, the retention time, substrates composition and pH value is explained.

3.1 Temperature

There exist 3 temperature ranges in which anaerobic digestion is achieved; psychrophilic (15 to 20^{o}C), mesophilic (35 to 37^{o} C) and thermophilic range (50 to 60^{o}C) with two biogas production optima, one in mesophilic range (35^{o} C) and another in thermophilic range (55^{o} C) [8]. Studies have been done on the influence of temperature on biogas production, concluding that biogas production is higher in thermophilic than in psychrophlic and mesophilic processes.

H. Bouallagui *et al.* compared the performance of the anaerobic digestion of fruits and vegetable wastes in thermophilic (55^{o}C) digesters with those in psychrophilic (20^{o}C) and mesophilic (35^{o}C) digesters. In this study, the experiments showed that biogas production in thermophilic digesters was higher than that in psychrophilic and mesophilic digesters by 144 and 41% respectively [8]. This difference in biogas yield was due to the improved anaerobic biodegradation of complex organic matter. During the co-digestion of waste activated sludge Gou C. *et al*. found that the average gas production rate of the thermophilic (55^{o}C) process was 1.6 times higher than that of mesophilic (35^{o}C) process [9]. During the digestion of dewatered-sewage sludge, I. A. Nges and J. Liu [10] compared the performance of thermophilic and mesophilic systems at different solid retention times and reported that a thermophilic system demonstrated a higher treatment performance and biogas production as a result of high specific growth of microorganisms and improved hydrolysis.

These research results show that within an acceptable range of temperatures, biogas production increases with increasing temperature. It is worth noting that thermophilic digestion leads to higher biogas production and production rates, but it is accompanied by some drawbacks such process instability and external energy requirements. Therefore, by adopting to operate in thermophilic conditions to optimise

biogas yield, analysis should be done to predict energy balance to make the process cost effective and minimise instability inside the digester.

Table 3.1 gives the average daily biogas production rate at different temperature ranges for different substrates.

Table: 3.1 Average daily biogas production [11]

Temperature	Manure	Production rate (mL/hr/L digester)
Room temperature (23-28)	Cow	9
	Chicken	3
	Pig	1
Mesophilic	Pig	2
	Cow	4
	Pig+ Cow	12
	Water hyacinth	2
	Kitchen waste	2
	Chicken	13
Thermophilic	Cow	3
	Pig	1

The substrates behave differently in terms of biogas production rate in different temperature ranges.

For cow manure the biogas production rate is higher at room temperatures than at mesophilic and thermophilic temperatures. For pig manure, the rate is the same at room and thermophilic temperatures and is higher at mesophilic temperature range. When mixed together, the rate is far higher at mesophilic temperatures. For chicken manure, the rate is much higher in mesophilic temperature ranges than at room temperatures. Comparatively, chicken manure and pig + cow manure have the highest biogas production rates.

3.2 Carbon to Nitrogen Ratio

Before starting the anaerobic digestion the feedstock must be characterised to determine its composition in carbohydrates, proteins, lipids and fibre. During this characterisation Carbon to Nitrogen (C/N) ratio should be taken into consideration because this parameter has considerable influence on biogas production.

Most of the literature suggests a C/N ratio range of 20:1 to 30:1 with an optimal ratio of 25:1 for bacterial growth in AD digester, because inappropriate ratios could result in high total ammonia nitrogen or high Volatile Fatty Acids (VFAs) accumulation leading in inhibition of the process [1] [12] [13]. These ratios are explained by the fact that in anaerobic digestion carbon utilisation is 25 to 30 higher than nitrogen, but optimum C/N ratio will vary depending on the type of the substrate. While investigating a novel method to reduce the ammonia inhibition during the thermophilic anaerobic digestion, X. Jiang *et al.* observed that adjusting C/N ratio of distillery wastewater significantly increased the maximum Organic Loading Rate (OLR) of the system resulting in increased biogas production rate. In fact, when C/N ratio of the distillery wastewater was increased from 9.0 to 11.4 the maximum OLR increased from 3.0 to 7.0g VTS (Volatile Total Solid)/L/d[14]. Zeshan *et al.* studied the effect of ammonia-Nitrogen (ammonia-N) accumulation in dry thermophilic anaerobic digestion using the alteration of C/N ratio between 27 and 32 as a method to vary ammonia-N concentrations in the substrates. They found that the increase of C/N ratio correlated with the decrease of ammonia-N and free ammonia, which corresponds to the increase of biogas production. Therefore, the observed that the feedstock with a C/N ratio of 32 was better than that with a C/N ratio of 27 in terms of reducing ammonia-N concentrations leading to increased biogas production [15]. For example, anaerobic digestion of olive mill wastes fails because of its low ammonium content but its digestion becomes feasible when it is co-digested with other organic wastes containing high level of ammonium nitrogen such as piggery effluents, cattle manure and dairy wastes.

3.3 Retention Time

Hydraulic retention time (HRT) of the anaerobic digestion system is a measure of the average length of time that the liquid substrate remains inside the digester whereas the solid retention time is the average length that the solid substrates spend inside the digester. In other words, the retention time is the average time that a specific substrate spends inside the digester. The retention time depends on the volume of the digester and the flow rate of the influent as it is given by equation 3.1 for the case hydraulic retention time.

$$\mathrm{HRT} = \mathrm{V}/\mathrm{Q} \tag{3.1}$$

where:

V: Volume of the digester in m^3

Q: Influent flow rate in m^3/h.

E.A Salminen and J.A Rintala [16] studied the effect of HRT and loading on anaerobic digestion of poultry slaughterhouse wastes and found that the anaerobic digestion was feasible at an HRT of 50-100

days and a loading rate of 0.8kg volatile solids (VS)/m^3d. In the same study it was observed that the process was inhibited at higher loading rate and a shorter HRT in the range of 25 to 13 days, this inhibition was reported to be a result of the accumulation of VFA and long chain fatty acids (LCFA) and highly depressed yield. In the purpose to observe the effect of HRT on the anaerobic digestion, I. A. Nges and J. Liu [10] loaded the continuously stirred tank reactors (CSTRs) with dewatered-sewage sludge and examined the process at different HRTs. By shortening HRT from 35 to 30 days, biogas yield increased from 0.518 to 0.666Nm3 biogaskg/VS day implying and increase of biogas production rate increase from 0.86 to 1.05m^3/m day. The experiments in this study confirmed that short HRTs lead to increased biogas production rate and a positive energy balance for the anaerobic digestion process; this improves the economy of the process as a result of higher energy gain. D. Bolzonella *et al* [17] studied the performance of four large Italian wastewater treatment plants employing different HRTs and an OLR of 1kgVS/$m^3_{reactor}$ day. It was reported that higher applied SRT in the activated sludge process for wastewater treatment resulted in lower biogas production; increasing SRT in the wastewater treatment line from 8 to 35 days resulted in a decrease of the specific biogas production from 0.18 to 0.07m^3/kg VS_{fed} . From this performance study equation 3.2 was suggested to predict the specific biogas production of a wastewater treatment process based on the solid retention time.

$$\mathrm{SBP} = 0.23\mathrm{e}^{-0.028\mathrm{SRT}} \tag{3.2}$$

where:

SBP: Specific biogas production in m^3/kgVS_{fed}

SRT: Solid retention time in hours.

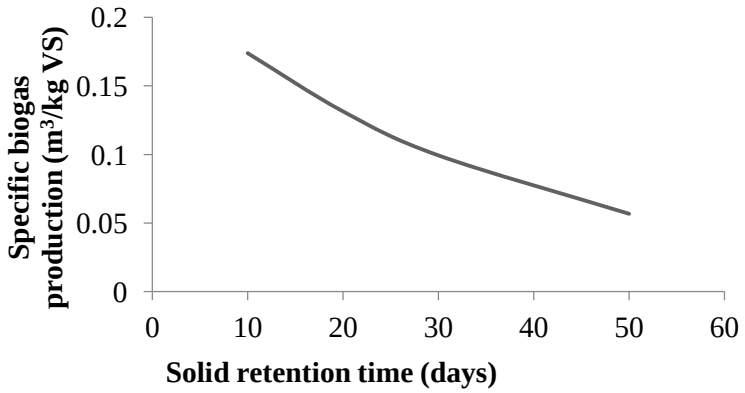


Figure: 3.1 Specific biogas production [17]

During the experiments to study the effect of SRT on the specific biogas production they observed that when the SRT in the activated sludge process increased from 10 to 20 days, the specific biogas production decreased by 20%. The figure 3.2 illustrates that the increase of the solid retention time during the digestion of the activated sludge results in an exponential decrease of the specific biogas production.

3.4 Total Solids Content

Total Solids (TS) of a substrate are the total of all solids in a substrate. It is the sum of the Total Suspended Solid (TSS) and the Total Dissolved Solids.

$$\mathrm{TS} = \mathrm{TSS} + \mathrm{TDS} \tag{3.3}$$

where:

TSS: the amount of filterable solids in the substrates

TDS: the amount of non-filterable solids in the substrates

Total solids concentration is the amount of fermentable material in unit volume of substrate and it influences pH, temperature and effectiveness of the microorganisms in the decomposition. TSS in substrates influences biogas production, time taken for biogas production and the composition of the biogas produced. The knowledge of percentage total solids of a substrate would help in predicting the yield of the anaerobic digestion of a specific feedstock.

A. Igoni *et al* [18] investigated various concentrations of the TS of Municipal Solid Wastes (MSW) in CSTR and the corresponding amounts of biogas produced, in order to determine conditions for optimum gas production. They varied the TS concentration from 4% to 10% and the results showed that the volume Vg of biogas produced was a power function of the Percentage Total Solid (PTS).

$$\mathrm{Vg} = 0.2225\mathrm{PTS}^{2.7717} \tag{3.4}$$

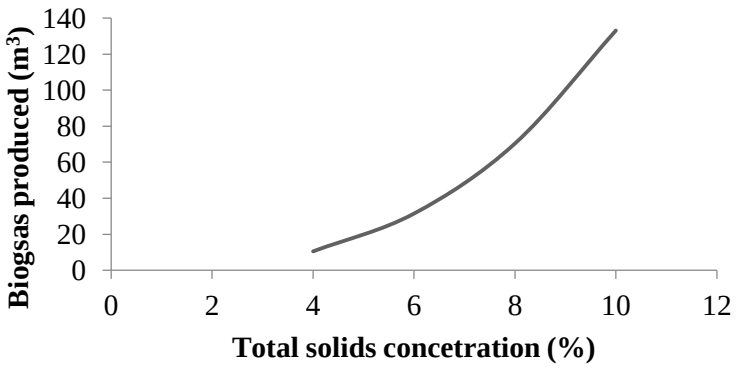


Figure: 3.2 Variations of biogas produced [18]

The figure illustrates that an increased percentage total solids solid content results in a power increase of the volume of biogas produced.

It is also important to mention that this power increase of the volume of the biogas produced with the increase of PTS is not continuous; at a certain level the effect of PTS on biogas produced stops. This is partly due to the fact that the increase of PTS implies the reduction of the dilution level of the substrates reducing the activity of microorganisms. Moreover, changing PTS would go with the change of pH of the substrates which might also have an effect on the quantity of biogas produced. J. Fernandez *et al* [19] studied the effect of substrate concentration on dry mesophilic anaerobic digestion and reported different outputs for 20%TS and 30%TS. In the study, the methanogenic stage started at day 14 for 20%TS and at day 28 for 30%TS and that the digestion with 30%TS produced 17% less biogas than that with 20%TS. Table 2 presents total solids content of different wastes

Table: 3.2 Total solids content of different wastes

Substrates	**Total solid concentration**	**Sources**
Cattle manure	7-9	[20]
Pig manure	5-7	[20]
Fruit and vegetable wastes	4-10	[8]
Waste activated sludge	25	[9]

Table 3.2 shows that substrates have different total solids content which is one of the important characteristics that determine the biogas potential of the substrates.

The table three shows the change in biogas production and cumulative biogas yield in terms of total solids concentration at 35^{o}C during the digestion of fruits and vegetable wastes.

Table: 3.3 Biogas production in terms of total solids content [8]

TS (%)	Biogas yield (L/kg VS)	Production rate (L/L day)
4	695.45	1.41
6	705.90	1.74
8	638.84	2.34
10	183.22	0.83

The cumulative biogas yield is the highest for 6 % TS but the highest biogas production rate is achieved for 8 % TS substrates.

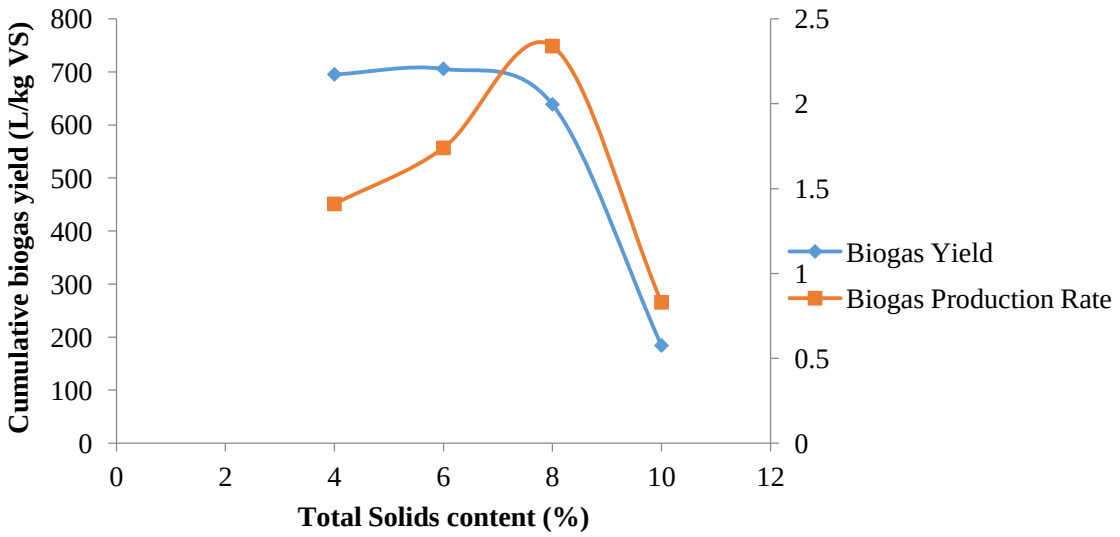


Figure: 3. 3 Biogas yield and production rate

From the figure 2 we can see that if an optimal biogas yield is desired at the highest biogas production rate, 8 % TS would be ideal for the anaerobic digestion of fruits and vegetable wastes.

3.5 Substrate Composition

The substrates that can be subjected to the anaerobic digestion in the purpose to produce biogas are diversified with different level of nutrients susceptible of producing the desired biogas. Experiments showed that for different substrates, different quantities and qualities are produced. Prior to loading any substrate to an anaerobic digester, characterisation should be done to analyse the ability of the substrate to produce the desired yield. In this section of the thesis the influence of the substrates' nutritional composition on the biogas yield is revised and later on the possible improvements on the substrate composition are presented.

3.5.1 Influence of Substrate Composition

During the anaerobic digestion for biogas production, it is important to analyse the composition of substrates that are fed to the digester to ensure optimal biogas production. There exist different types of organic matter that can be treated under anaerobic environment but do not have the same biogas potential and biodegradability. Many research findings have shown different biogas yields for different substrate composition.

Thomas A. *et al.*, in a research aiming at optimising methane production from maize and dairy cattle manure stated that "Composition and biodegradability are key factors for the methane yield from energy crops and animal manure"[21]. After a deep experimental analysis they found that manures with higher crude protein levels gave higher methane yields and that lignin in the manures reduced the specific methane yield. M. Macias-Carral *et al.* investigated single waste anaerobic digestion and co-digestion of municipal solid wastes and agricultural wastes. In this research, single waste digestion of cow manure (CM) resulted in $62m^3$ methane/ton of CM on dry weight basis; single waste digestion of organic fraction of municipal solid waste (OFMSW) gave $37m^3$methane/ton of OFMSW of dry waste. Co-digestion of OFMSW and CM resulted in $172m^3$ methane/ton of dry waste. Then it was shown that co-digestion of OFMSW and CM promotes the synergetic effects which overcome the imbalance in nutrients and improves biodegradation [22]. When studying how bio-methane production can be improved per unit of feedstock in biogas plants, Z.-u.-Z. Asam *et al.* compared methane productivity of raw pig slurry, Filter Pressed Manure Fibre (FPMF), Chemically Precipitated Manure Fibre (CPMF), maize silage and grass silage. They found that the ultimate methane yield per kg Volatile Solids (VS) is lower for FPMF and CPMF than for raw pig slurry. This yield was lower for maize silage than for grass silage [23]. However, the volumetric methane yield of CPMF was lower than that of FPMF because of lower dry matter content in the former.

All the above mentioned studies revealed that substrate composition has a considerable influence on the yield of the AD system. Therefore, it is important to analyse the substrates to determine their biogas potential and degradability. Depending on the findings of the analysis, decision can be made on which type of substrate to be used in a specific digester and on which treatment can be made on the substrate to optimise the gas yield.

3.5.2 Improvement of Substrate Composition

To know the nutritional composition of substrate, one should determine the fraction of the organic matter in the substrates. The organic matter can be determined by several methods, the most used of the methods

is the measurement of VS, but also biogas yield can be estimated from the measurement of COD in which 1g of COD has maximum methane potential of 0.35L of CH_4. To get optimal yield from the anaerobic digestion, the substrate composition should be optimised in terms of nutrients content and biodegradability. For that purpose, the substrates to be supplied to the digester should be characterised to help in identifying the optimisation technique that could be used. To completely characterise the feedstock for anaerobic digestion, specific parameters are determined for both inoculums and substrates. These parameters include volatile solids content (VS), pH, total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), Total organic carbon (TOC), dry matter (DM) content and volatile fatty acids. Experimentally, DM is determined by weighting the sample before and after drying the substrate sample at an elevated temperature and calculating it using the equation 3.5.

$$\%\mathrm{DM} = (\mathrm{W_{DM}}/\mathrm{W_S}) * 100 \tag{3.5}$$

where:

$\mathbf{W_{DM}}$: Weight of dry matter

Ws: Weight of the fresh sample.

The percentage volatile solids content is calculated by the equation 3.6.

$$\%\mathrm{VS} = (\mathrm{W_{ash}}/\mathrm{W_S}) * 100 \tag{3.6}$$

where:

W_{ash}: Weight of ash obtained by burning the dried sample.

Table 3.4 gives the characteristics of some of the substrates that are commonly used for biogas production.

Table: 3.4 Substrates characteristics [24]

Parameter	Pig manure	Fish manure	Biodiesel waste
Liquid fraction (%)	98.3	63.1	100
pH	6.9	Not determined	Not determined
Density (kg /L)	1.0	1.1	1.0
TS (g TS/kg)	17.3	369	0
VS (g SV/kg)	11.7	270	0
Soluble COD (gO2/kg)	15.3	Not determined	1390
TKN-N (g N/kg)	3.3	33.6	0.2
Total alkalinity (g CaCO3/L)	7.7	0.3	32
VFA-COD (g VFA-COD/kg)	12.2	0	0
Proteins (g prot/kg WW)	1.1	205.8	1.2
Lipids (g/kg)	1.5	28	77.3
Carbohydrates (g /kg)	9.2	36.2	921.5
COD/N ratio	8.9	12.2	7315

In terms of TS and VS pig manure has reasonable values. It is clear that fish manure has higher TS and VS making it difficult for the microorganisms to digest. For the digestion of fish manure therefore, further dilution would be a necessity.

The reduction in VS of a substrate together with the biogas production potential will determine whether a particular substrate will be a better choice for the biogas production. The table 3.5 gives the biogas potential for mostly used substrates in terms of VS content illustrating that biogas yield varies among

substrates and it can be a tool to select the most practical substrate to be used a study project like this thesis work.

Table: 3. 5 Biogas production potential [25]

Substrate	Biogas yield (m3/kgVS)
Fruits and vegetable wastes	0.4985
Agricultural wastes	0.730
Municipal wastes	0.564
Cattle dung	0.252
Food wastes	0.540

The table shows that under the same digestion conditions, different substrates have very different biogas yields.

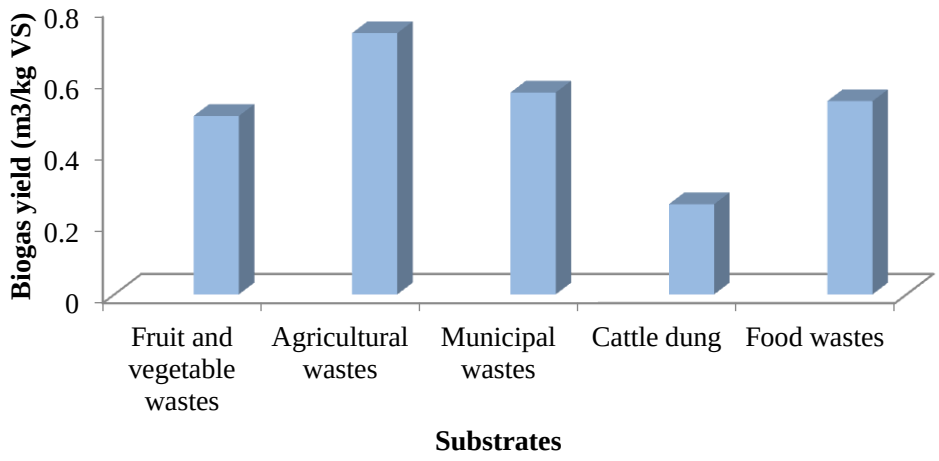


Figure: 3.3 Biogas potential of different substrates

From the figure 3.4, we see that the agricultural wastes have the highest biogas production potential followed by municipal wastes and food wastes. However, food waste is known to have high potential of producing VFAs at the beginning of the anaerobic digestion negatively affecting the methanogenesis stage. In practice, this effect is minimised by using a multi-stage digester to be able to minimise VFAs accumulation in the first phase.

After the characterisation of substrates and determination of their biogas potential, it can be decided whether the substrates need to be improved to optimise the biogas production in terms of nutrients composition. The question here is how this substrate improvement is achieved.

One way of improving substrates is co-digestion of more than one type of substrates to optimise the nutrients content. This method was employed by J.A Alvalez *et al.*; they characterised pig manure (PM), fish manure (FM) and biodiesel waste (BW) and studied their digestion processes. When co-digested, each substrate contributed in optimising the feedstock; pig manure contributed the moisture, fish manure contributed nitrogen and lipids, biodiesel waste contributed COD and glycerol. This research proved that by mixing more than one substrate, nutrients are optimised where each type of substrates contributes in improving a particular nutrient. This optimisation method was also proved to be efficient by M. Macias-Carral *et al.* as it can be observed on figure 3.5.

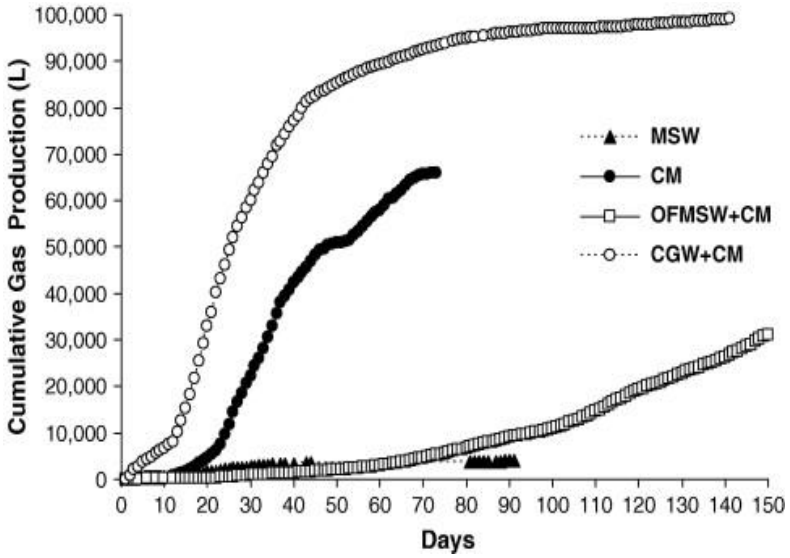


Figure: 3.4 Gas production from different substrates [22]

This figure shows that cumulative gas production from MSW digested alone was the smallest of the rest of the substrates followed by the co-digestion of OFMSW and CM. Larger cumulative biogas yield was produced when CM was digested alone and the largest cumulative yield was found when CGW was co-digested with CM.

3.6 pH Value

pH is an important parameter in biochemical processes, the reaction and transformations that take place in the anaerobic digestion are highly influenced by the pH pertaining inside the digester. For the purpose of energy production control should be made on the alkalinity of the reaction medium to assure better biogas yield. In this section of the thesis literature is reviewed of the effect of change in pH on the yield of the

anaerobic digestion. Moreover, review is done on different methods that can be adopted to adjust the pH value of the substrates to keep it in the desired range.

3.6.1 Effect of pH on Anaerobic Digestion

The AD of complex organic substrates requires a consortium of several groups of microorganisms that require various environmental conditions, including pH value of the digestion medium with methanogens being the most sensitive to pH fluctuations. The ideal pH range for anaerobic digestion is between 6.8 and 7.2.

Various studies have been done to explore the effect of pH value and its fluctuations on the AD process. B. Zhang *et al*. carried out experiments to study the effect of pH on hydrolysis and acidogenesis of kitchen wastes. In batch experiments with controlled pH at 7, relatively high hydrolysis and acidogenesis rates were obtained; acidified products with relatively low lactic acid concentration were more favourable to the subsequent methanogenesis. With the same pH value, experimental results of semi-continuous operation showed that the Total Solid (TS) removal rate and production of biological energy were significantly improved [26]. D. Cysneiros *et al*. investigated the effect of pH control and hydraulic flushing on hydrolysis and volatile fatty acids production in anaerobic leach bed reactors digesting maize. They used the addition of a buffer to control the pH of the substrates and hydraulic flushing to wash out intermediate compounds. The results showed that pH control enhanced butyric and acetic acid production and that the acetic acid production was inhibited at lower pH [27]. When studying the hydrolysis and acidification of Waste Activated Sludge (WAS) at different pHs, Y. Chen *et al*. observed that when pH was decreased from 6.0 to 4.0 or increased from 7.0 to 10.0, methane production decreased and there was no methane generated at pH 10.0 and 11.0 [28].

The above research findings show that pH of the substrates residing in the digester has a great influence on the output and performance of AD system. To optimise the biogas production pH needs to be controlled to ensure that each type of microorganism involved in AD acts at its maximum. Therefore, it is crucial that we explore methods that can be used to control pH value of the digestion process.

3.6.2 pH Monitoring and Adjustment

Being aware of the influence that pH value has on the AD process, it is important to keep pH value in an acceptable range to optimise the biogas yield per fed substrates. The pH value of hydrolysis and acidogenesis is different from that of methanogenesis, it is therefore ideal to use systems that will allow hydrolysis and acidification stage to be separated from methanogenesis to control each step's pH. Using a

multi-stage digester will help in achieving this, where the first stage is used as a buffer against the high organic loading rate. Methods used to achieve pH control include the addition of buffering solutions to the substrates increasing the capacity to resist to the change of pH that might result from the gradual production of VFAs or the addition of new organic matter. The gradual removal of effluent and replacing it with fresh substrate is also used. During the addition of a buffer solution care should be taken not to impose an abrupt change of pH, considering that microorganisms require a certain time to adapt to the change in pH of the medium.

Buffering method was applied by B. Zhang *et al.* while studying the effect of pH on the hydrolysis and acidogenesis of kitchen wastes. For this purpose, NaOH and $Ca(OH)_2$ were alternately added to adjust the pH value to 7 as a way to avoid the drop in pH value caused by acidification of the substrates. To avoid the raise in pH value as a result of NH^+_4-N accumulation, effluent was gradually removed and replaced by an equal amount of fresh substrate [26]. Figure 3 gives the impact on the three buffering methods on the amount of VFAs produced in acidogenesis phase.

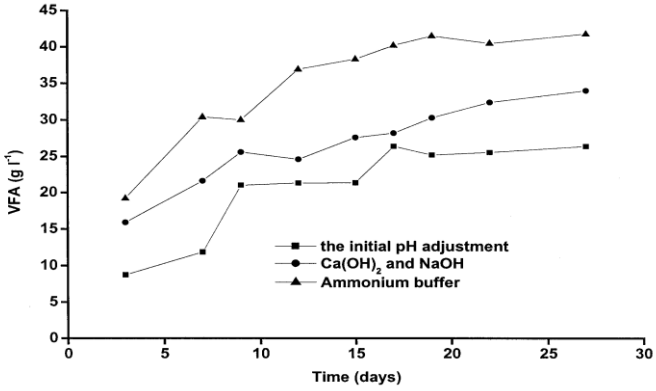


Figure: 3.5 Impact of buffering methods on the acidogenesis [26]

The figure 3.6 shows that without buffering substances, the rate of production of VFAs is smaller compared to the cases when the buffers were applied. It is also clear from the figure that ammonium buffer performs well in terms VFAs production rate.

4 Types of Anaerobic Digestion Systems

There are several types of anaerobic digestion systems in place, though the biochemical processes are the same for all types. Some systems are simple and cheap to construct, operate and maintain; others are complex such as multi-stage systems that might include sensors to help in controlling the operation throughout the process. Anaerobic digestion systems are classified in three main groups: single-stage continuously fed systems, multi-stage continuously fed systems and batch systems. In this part of work, some types of digester systems are reviewed to gain knowledge about their operation and be able to figure out the points that need improvement to optimise biogas yield. Single stage anaerobic digestion systems are explained giving some examples of particular digesters in operation and the multi-stage anaerobic digestion systems are described.

4.1 Single-stage digester systems

In this type of digestion systems all of the stages of the anaerobic digestion take place in one reactor under the same operational conditions. They are simpler to design, build and operate. Some of these digesters are Batch systems for which the substrates are fed and left there to digest for a certain time considered to be the retention time, after this time the effluents are emptied and new feedstock is loaded. During offloading-reloading period the biogas production stops until the new substrates starts to produce biogas. In a situation where continuous production of biogas is necessary, such systems are operated in parallel so that they can be alternately offloaded and reloaded without any biogas production shortages. Other systems are continuous single-stage systems where rectors are continuously fed, effluent is continuously evacuated and biogas is continuously produced. With these digesters care must be taken to ensure proper loading rate of the new substrates which would otherwise acidify the digestion environment, the situation which will negatively affect the methanogenesis stage. In addition, the offloading rate should also be controlled not to remove active microorganisms or undigested substrate. With single-stage systems it is not possible to control and achieve optimal operating conditions for the different stages, resulting in lower biogas yield compared to systems in which the stages of the anaerobic digestion are separated.

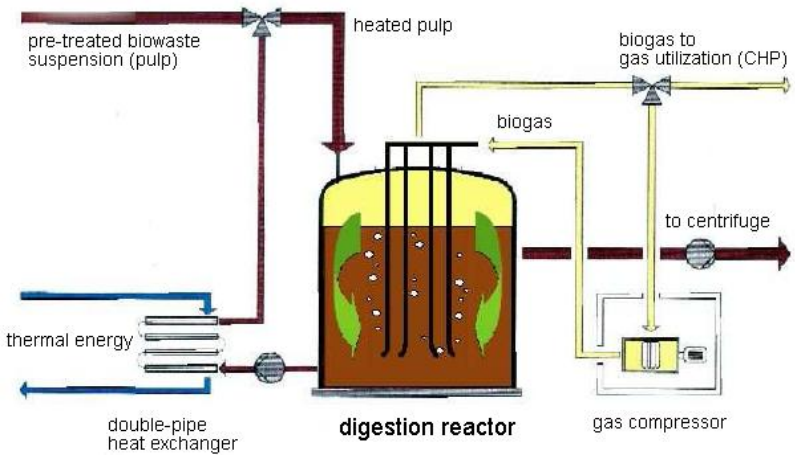


Figure: 4.1 Single-stage anaerobic digester [29]

For the anaerobic digestion of solid substrates, these systems are classified as wet or dry. They are wet when the total solids content of the substrates is less than 15 % and dry when the total solids content is between 22 and 40 %. In-between, they are called semi-dry reactors [30]. Wet systems require higher volume to treat the same amount of substrates as dry systems but wet systems have advantage over dry systems in that dilution of fresh material to achieve 15% total solids reduces the inhibition of methanogenesis. The following are typical examples of single stage anaerobic digesters that are mostly used.

4.1.1 Fixed Dome Reactors

These are one of the most common types of digesters used in developing countries. They are single-stage wet anaerobic digesters for which the top and the bottom are hemispherical, the shape that allows the digesters to withstand the pressure that builds-up inside the digesters as the biogas is accumulated. These hemispherical parts are joined by straight wall made up of bricks, stones or poured concrete. To make the digester air-tight, the inside surface is sealed by many thin layers of mortar. They are usually constructed underground which protects them from fluctuations of the operating temperature and helps them to have a longer life span up to 20 years. The feedstock is mixed in an inlet chamber before it is sent through an inlet pipe to the lower fermenting reservoir for digestion.

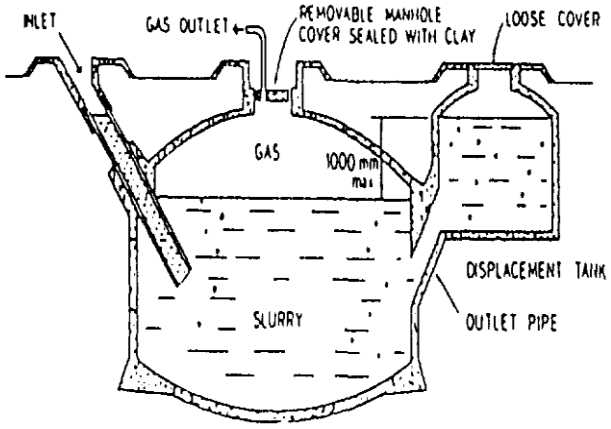


Figure: 4.2 Schematic diagram of fixed dome reactor [31]

Once gas is produced, it is collected in a rigid fixed dome on the upper part of the digestion reservoir from where it is harvested for use through an outlet pipe. It is the increase in pressure inside the digester that forces the biogas on one hand and the effluent on the other hand, to come out of the digester. This dependence on the pressure inside the digester results in fluctuations in gas and effluent flow. Other disadvantages include the leakage of biogas as the digester ages and maintenance difficulties because of its underground construction and immovable parts.

4.1.2 Floating Dome Digester

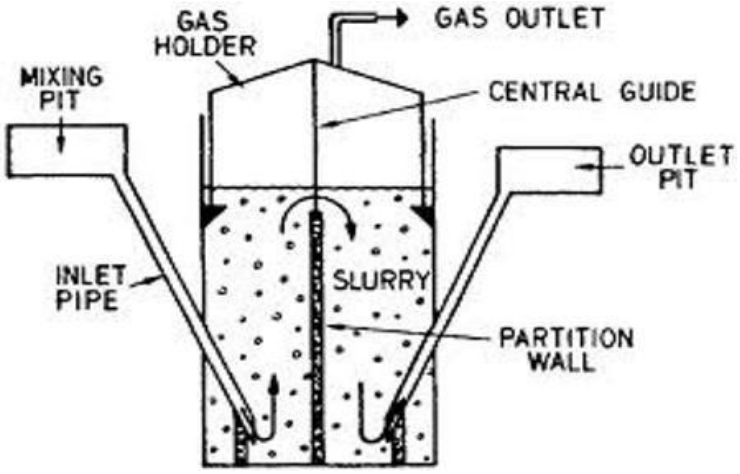


Figure: 4.3 Schematic diagram of floating dome reactor [31]

The digester walls are usually made of brick or reinforced concrete, and the gas holder of fiberglass reinforced plastic. Steel was used in the past, but because of corrosion problems FRP is now more frequently used, even if its costs are higher than for a steel drum. The weight of the gas holder determines

the gas pressure inside the digester As a feed mostly cattle dung is used, sometimes mixed with nightsoil, agricultural residues and other substrates such as water hyacinth. If needed, the influent is diluted to a dry matter content of around 10%.

4.1.3 Covered Lagoon Digester

Covered lagoon digester consists of a lagoon in which the substrate is treated with an impermeable cover which traps the biogas produced. It is operated at room temperature to digest liquid manure with 3 % or less total solids content requiring large volume lagoons. This type of digester is very cheap to construct, operate and maintain but are not suitable for cooler climates as it operates under warm room temperatures.

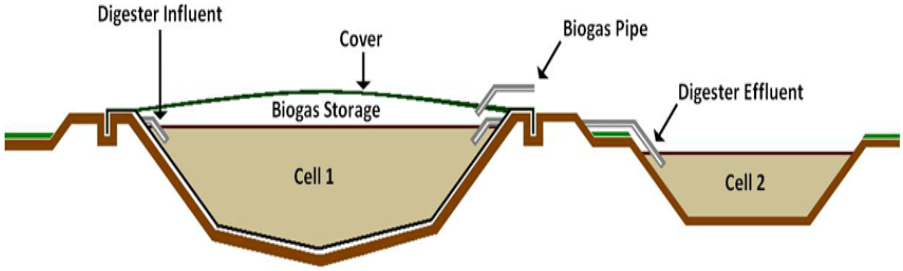


Figure: 4.4 Covered lagoon digester [32]

4.1.4 Complete Mix Digester

Complete mix digester is an anaerobic digester consisting of a steel or poured concrete rector meant to digest high volumes of substrates with solids content between 3 and 10 %. The tank is above or underground and includes a heating system that allows it to operate at higher temperatures and reduce the retention time, making it more expensive to operate and maintain. The digester is named so because during the digestion the substrates are continuously mixed to keep the solids in suspension with the produced biogas accumulating at the top of the digester.

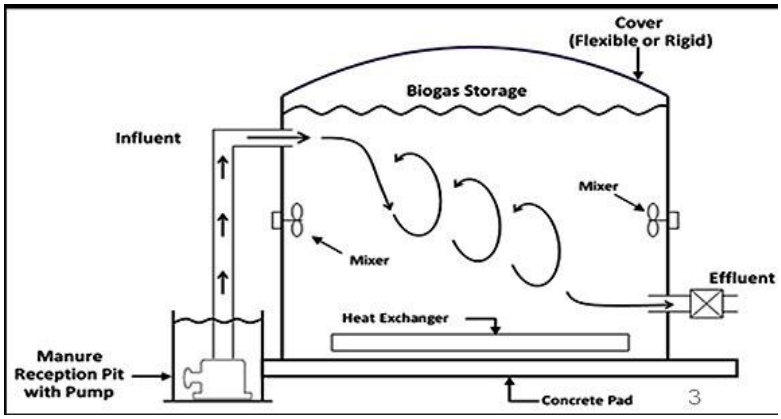


Figure: 4.5 Schematic diagram of complete mix digester [32]

4.1.5 Plug-Flow Digester

Plug-flow digester is a digester that is composed of a mix tank where the substrates are mixed before entering a rectangular tank in which the anaerobic digestion takes place. This digester operates at thermophilic temperatures achieved by the use of hot water that circulates in suspended heating pipes, which serve also for continuously mixing the substrates inside the digester. When a new organic material is added to the digester, the old substrate is pushed towards the discharge end. The biogas is produced as the substrate flows through the digester to finally be trapped by a flexible and impermeable cover. This type of digester is suitable for ruminant animal manures such cattle dung with a total solids content ranging between 11 and 14 % with a retention time ranging between 15 and 20 days. A substrate with lower solids content would reduce the performance of the digester since the solids would not stay in solution, they would rather settle at the bottom of the tank. Plug-flow digester is cheaper to operate and maintain since it has few moving parts.

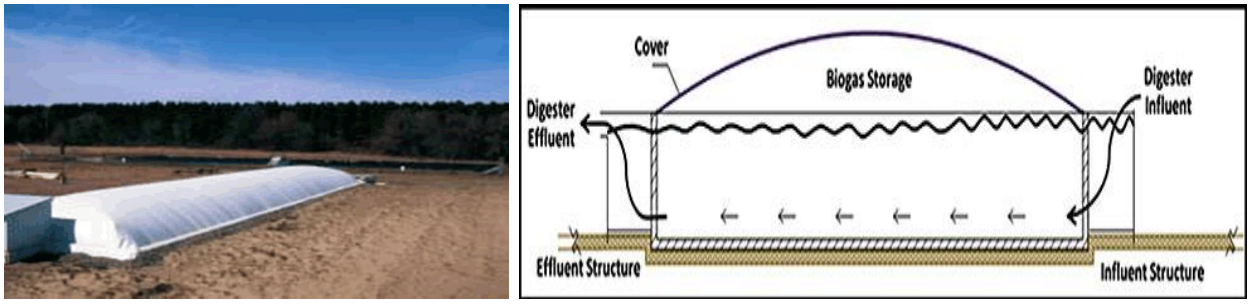


Figure: 4.6 Plug-flow anaerobic digester [33]

4.1.6 Anaerobic Baffled Reactor

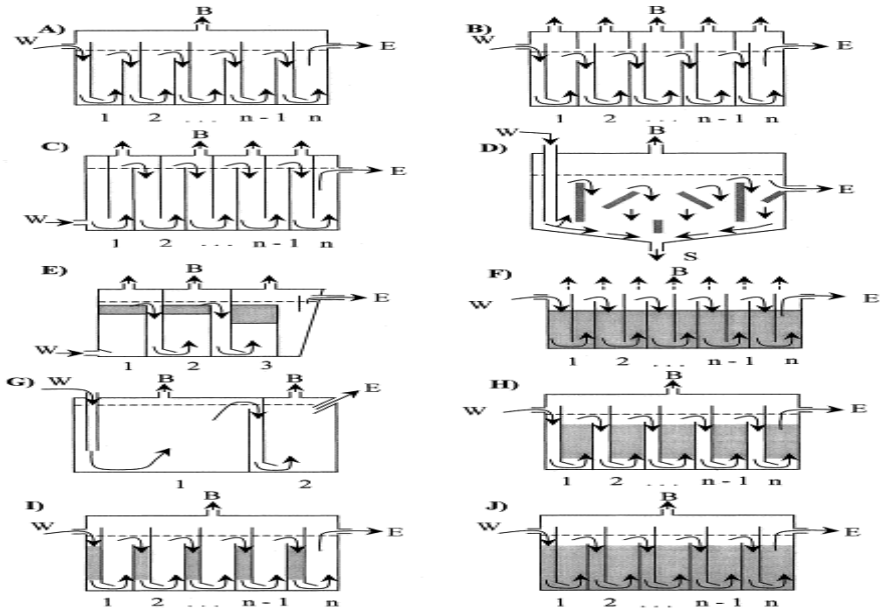


Figure: 4.7 Various configurations of ABR [34]

The anaerobic baffled reactor (ABR) is a digester that has a series of baffles used to force the substrates to flow from the inlet to the outlet. When flowing towards the outlet, the substrates flow under and over the baffles enhancing the solids retention capacity and increasing the capacity to treat higher solids content substrates as well as the digester's performance. The most significant advantage of this type of the anaerobic reactor over other single-stage digesters is its ability to separate the aciodogenesis from the methanogenesis stage longitudinally allowing it to behave like a two-stage digester in terms of performance without control problems associated with the two-stage anaerobic reactor [34].

4.2 Multi-stage Digester Systems

In multi-stage digestion systems, two or more rectors are used to mainly separate the hydrolysis and methanogenesis to be able to optimise the biogas yield in terms of operating conditions by optimising each stage separately. In a two-stage system, hydrolysis and fermentative acidification reactions are optimised in the first stage where the hydrolysis of carbohydrates is the rate-limiting step and in the second stage the methanogenesis is optimised where the reaction rate is limited by methanogens growth. One of the main advantages of multi-stage systems is the ability to overcome the instability that might be caused by fluctuations in substrate loading rate or its heterogeneity. This is because the substrates coming from the first stage reach the methanogenesis stage when they are already homogenised and inhibitors are

reduced during the hydrolysis and acidification phases. But the operational cost of this type of digester systems is higher than single-stage systems.

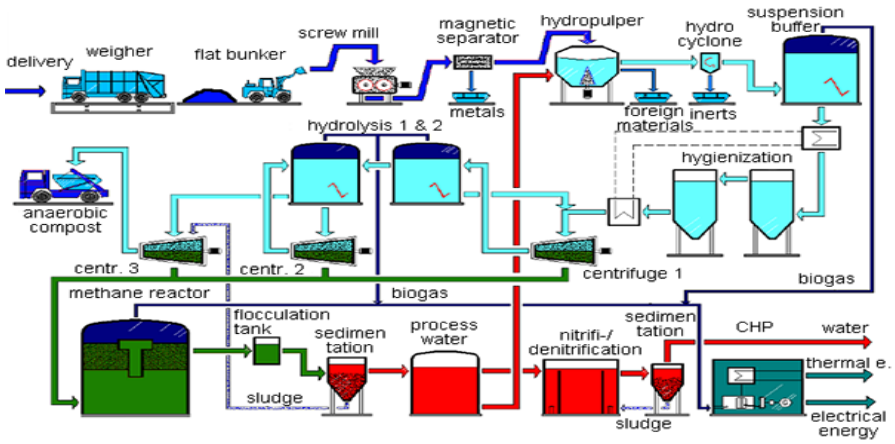


Figure: 4.8 Multi-stage anaerobic digester system [29]

The figure shows how complex multi-stage systems are. They involve many steps before biogas is produced and utilised.

4.3 Comparison of Single-Stage and Multi-stage Systems

As described in the preceding sections in the single-stage anaerobic digestion, all of the digestion steps take place in one reactor under the same conditions whereas for multi-stage anaerobic digestion systems the reactors are made in a way that allows the different stages of the digestion to take place in different reactors. Therefore, they are noticeable advantages of multi-stage systems over the single-stage anaerobic digestion systems. The separation of stages leads to optimising the steps of the digestion by controlling and optimising operating parameters for each step. However, multi-stage systems have higher operating and maintenance requirements than single-stage systems making them more expensive than the latter [35]. Other advantages of multi-stage over single-stage systems include improved odour control, lower retention time and higher loading rates reducing the digester volume requirements, reduced foaming problems and higher performance.

5 Experimental Setup

To study the effect of substrate composition on the improvement of biogas yield from the anaerobic digestion, we experimentally analysed the anaerobic digestion of food wastes and fruit wastes. The choice of these substrates for experimentation is explained by the figure 3.5 which shows that food wastes and fruit wastes have better biogas potential after agricultural wastes and municipal wastes, the latter two being inappropriate in the context of this study.

The substrates were collected and characterised for their compositions before loading them to digesters. To compare the biogas yield, three substrates sample types were digested; food wastes, fruit wastes and the mixture of food wastes and fruit wastes. Furthermore, digestion experiment was carried out to study the impact urine might have on the yield from the digestion of the mixture of food and fruit wastes. In this Chapter we describe the approach with which the experiments were carried out. We start by describing the way the substrates were collected and explain the approach with which the substrates were characterised and prepared for digestion. Finally, we explain the way the digestion was carried out under the appropriate conditions.

5.1 Substrate Collection and Treatment

Food wastes composed of bread, peas, onions, rice, potatoes, salt, tomato sauce, spinach, beans and eggs were collected from the kitchen of the students' hostel at the University of Agder. They were mixed and manually agitated to completely mix and reduce particle size that would facilitate the digestion. To have fruit wastes as substrates, three apples and two oranges were bought from REMA 1000 shop and pressed using a juice master, part of the juice was taken back to the residues and the mixture was manually agitated to completely mix. Both substrates were kept in the fridge at $1^{o}C$ for two days before their use. The inoculum was the granule sludge from a paper industry kept at $1^{o}C$ a long time before use.

5.2 Substrates Preparation and Characterisation

The substrates to be fed to the digesters were prepared in three samples in calculated proportions using laboratory beakers. The first sample was prepared by mixing 49.187g of food wastes, 55.33g of tap water and 15.15g of the inoculum, the second sample was composed of 49.19g of fruit wastes, 55.33g of tap water and 15.15g of inoculum. The third sample was the mixture of 117.247g of food wastes, 60.81g of fruits wastes and 84.78g of tap water.

Table: 5.1 Sample substrates composition

Sample	Food wastes	Fruit wastes	Water	Inoculum	Total
1	49.187g	0	55.33g	15.75g	120.267g
2	0	49.187g	55.33g	15.75g	120.267g
3	117.247g	60.81g	84.78g	62.09g	324.927g

This table shows the proportions of different compositions of the mixtures from which the samples were fed to laboratory digesters.

Substrates were further characterised for their composition in total solids (TS) and volatile solids (VS) contents, these properties being the indicators of the potential of any substrate to produce biogas. There parameters were determined by applying the standard methods for the examination of water and wastewaters.

Figure: 5.1 Crucibles in a desiccator

Crucibles were used as containers of the samples; empty crucibles were weighed and their weight was denoted by W_c, when loaded with fresh substrates, their weight was denoted by W_s. The samples were dried in a laboratory drier at 105^{o}C for three days, cooled in a desiccator for 15 minutes prior to weighing. When weighed, the weight was denoted by W_d; the weight of the crucibles and dried substrates. The TS content was calculated using equation 5.1.

$$TS = \frac{Wd - Wc}{Ws - Wc} * 100 \quad (5.1)$$

where:

TS: total solids content

W_d: weight of the dried sample in g

W_c: weight of empty crucible in g

W_s: weight of fresh sample in g

Figure: 5.2: The drier used to dry the samples

The same dried samples were supplied to a kiln at 550^{o}C for 20 hours to burn to ash, cooled in the desiccator and weighed denoting the weight by W_{ash}. The results of this process were used to calculate VS using equation 5.2.

$$VS = \frac{Wd - Wash}{Wd - Wc} * 100 \quad (5.2)$$

Figure: 5.3 The kiln used to burn the sample to ash

Table: 5.2 Dried and ashed substrates

Crucible	Substrates	W_c (g)	W_s (g)	W_d (g)	W_{ash} (g)
1	Inoculum	24.7009	57.0064	27.8897	25.0973
2	Food wastes	23.2142	55.4347	30.1814	23.5895
3	Fruit wastes	21.9105	50.0284	26.6931	22.0205
4	Food wastes, fruit wastes, water, inoculum	24.57	53.31	28.1606	
5	Food wastes, inoculum, water	23.22	39.78	24.9462	
6	Fruit wastes, inoculum, water	21.94	42.85	23.6659	

This table presents the weights of substrates in different mixtures, illustrating that after drying the weight of the substrates was considerably reduced. Further weight reduction happened when the substrates were burnt to ash.

The pH values of the substrates were measured by a stick pH & temperature meter after a thorough two-point calibration using pH 10.01 and pH 7.01 buffer solutions.

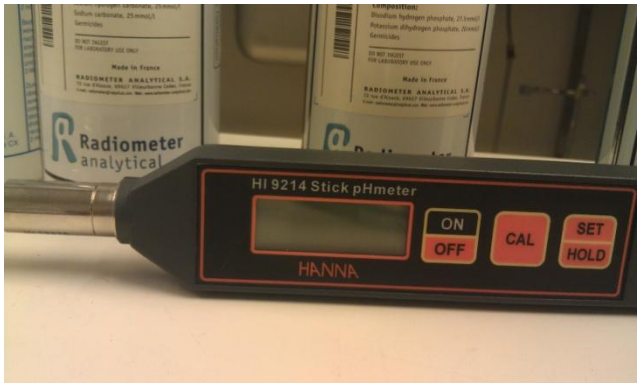


Figure: 5.4: Stick pH meter used to measure the pH values

5.3 Anaerobic Digestion Experiments

After the characterisation for TS, VS and pH, it was time to feed the substrates to the digesters to start the digestion process. Batch digesters were 100 ml plastic syringes fed with 20ml of substrates in triplicates and air-tightness was assured.

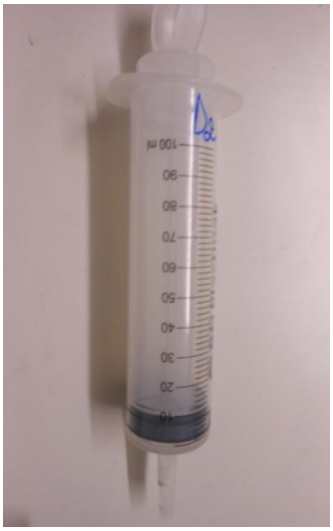


Figure: 5.5 100mL syringe used in the experiments

In addition to these digesters containing food wastes and/or fruit wastes, one syringe was loaded with 10 ml of the mixture of food wastes and fruit wastes and 10 ml of human urine to examine the effect this urine might have on the accumulative biogas production. To ensure thermophilic temperature conditions the digesters were kept in an incubator and the temperature of the incubator was set to 55± 2^{o} C. This temperature was chosen to be used in this study because it is suggested by the literature to be the average thermophilic temperature at which an increased substrate decomposition rate is achieved reducing the retention time.

Figure: 5.6 Digesters in the incubator

Figure: 5.7 Syringe with some biogas formed

When the biogas was formed the piston of the syringe was pushed up by the pressure exerted on it by the biogas. The position of the piston and the scales of the syringe were used to read the volume of the gas produced. Cumulative biogas production records were taken daily at a fix hour of the day over 12 days when the biogas production had stopped or was not significant.

6 Results

In this study a set of works were done to investigate the effect of substrate composition on the biogas yield from the anaerobic digestion. In this part of the report the results of the experiments are presented. The Chapter starts by giving the results of the substrate characterisation process, continues with the results of the digestion process of food wastes and fruit wastes in different mixtures and ends by the results of the anaerobic digestion of food wastes and urine. To ease the analysis of the results, they are accompanied by tables and figures.

6.1 Substrates Characterisation Results

Fresh food wastes, fruit wastes and the inoculum were characterised for their total solids content, volatile solids content before they were prepared for digestion. Food wastes were found to have 21.623 % TS, 94.613 % VS and VS/TS ratio of 77.145 %, fruit wastes had 17.009 % TS, 97.699 % VS and VS/TS ratio of 82.590 %. For the inoculum these parameters were 9.870 %, 87.568 % and 88.728 % respectively. The results of the characterisation are tabulated in table 6.1.

Table: 6.1 Substrates characterisation results

Substrates	TS (%)	VS (%)	VS/TS (%)
Food wastes	21.623	94.613	77.145
Fruit wastes	17.009	97.699	82.590
Inoculum	9.870	87.568	88.728

Food wastes have the highest TS with the lowest value of VS.

For ease of analysis figure 6.1 was plotted clearly showing the differences in the characteristics of the substrates. The literature suggested that biogas production depends extensively on the composition of the substrates, so the figure leads us to believing that a remarkable difference in the quantity of biogas would be produced by different substrates over the course of the experiments.

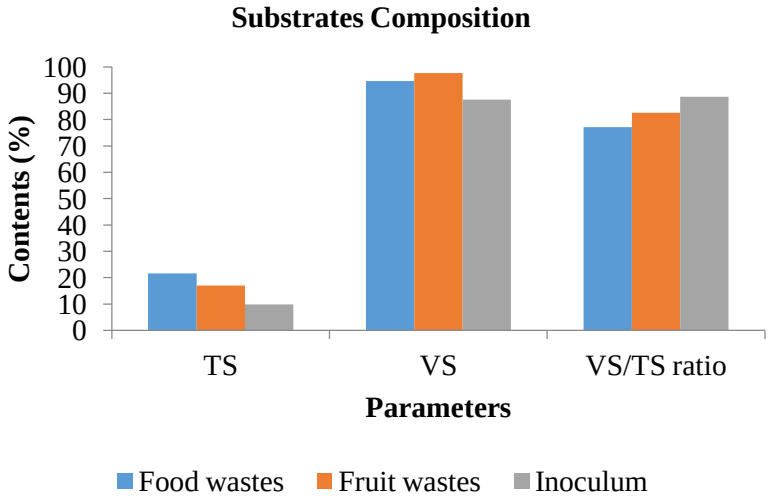


Figure: 6.1 TS, VS and VS/TS ratio for the substrates

VS/TS ratio is higher for fruit wastes and the highest for the inoculum, but for the VS values the order is reversed i.e. it is higher in the fruit wastes and the highest in food wastes. Figure 6.1 shows that the composition of food waste is different from that of the fruit wastes which implies a probable difference in biogas production.

After the preparation of the samples for digestion, the same parameters were measured for food wastes, fruit wastes and for the mixture of both before they were loaded to digesters for digestion. Each substrate was mixed with tap water and inoculum, reducing considerably the TS content and increasing the microorganisms' population which facilitates substrates decomposition. The sample including food wastes presented a TS content of 10.423 %, 96.025 % VS and a VS/TS ratio of 89.145 %, fruit wastes had a TS content of 8.253 %, 97.046 % VS and VS/TS ratio equal to 91.495. The mixture of food wastes and fruit wastes was characterised by 12.493 % TS, 96.014 VS and VS/TS ratio of 86.988. These results are reflected in table 6.2 and figure 6.2.

Table: 6.2 Characterisation results for digestion samples

Substrates	TS (%)	VS (%)	VS/TS (%)	pH
Food wastes	10.423	96.025	89.145	4.91
Fruit wastes	8.253	97.046	91.495	4.52
Food + Fruit wastes	12.493	96.014	86.988	4.65

The dilution and the addition of the inoculum to the substrates reduced the TS content and increased the VS/TS of the substrates. pH of all of the samples were in the acid range.

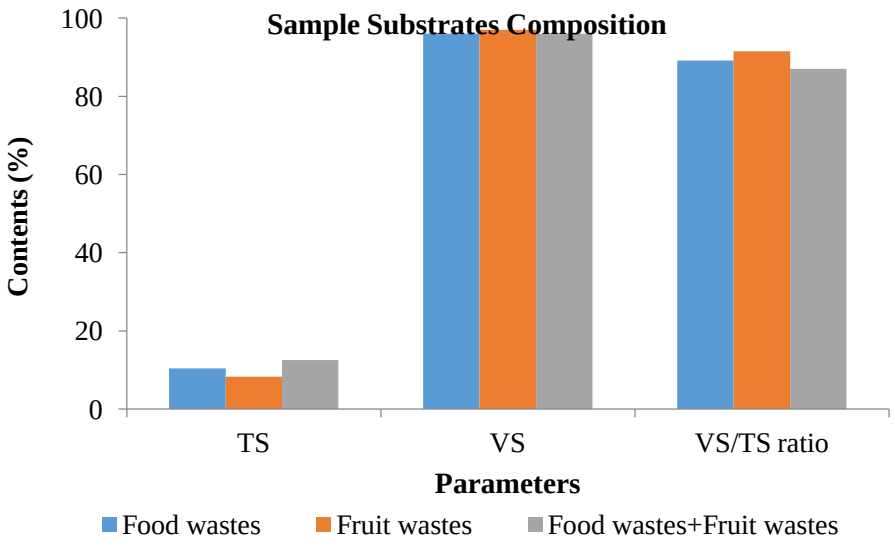


Figure: 6.2 TS, VS and VS/TS ratio of the samples

TS content of the mixture is higher than that of the separate substrates. But VS is quite the same for food wastes and the mixture, a bit lower than that of fruit wastes. A noticeable difference arises in VS/TS ratio; the parameter is higher for fruit wastes and the lowest for the mixture. This parameter shows that the proportion of volatile solids in the mixture in lower implying a higher content in digestible organic matter.

6.2 Anaerobic Digestion Results

Digestion experiments were done at two different temperatures, thermophilic temperature equal to 55±2^{o}C and mesophilic temperature equal to 37±2^{o}C.

6.2.1 Results of Digestion at 55^{o} C

The substrates were loaded to batch anaerobic digesters simulated by 100 ml plastic syringes, kept in an incubator set at 55^{o} C to digest and the biogas production was recorded everyday at a fixed time of the day to keep a constant interval between consecutive records. The digesters were in triplicates i.e. for each type of substrate we had 3 digesters running in parallel for statistical analysis purposes. In this section of the report we first present the results of the digestion of food wastes, fruit wastes and the mixture of the two for comparison purposes. We later present the results of the digestion of the latter and that of the one which contains human urine for a separate comparison. The records of biogas production were recorded at the digestion temperature (55^{o} C) which is higher than the normal temperature, so we had to find the real volumes at normal temperature (25^{o} C).

Cumulative biogas volume was recorded every day in the purpose of analysing the difference in total biogas quantity produced by the different substrates. On the first day, food wastes had produced a small volume equal to 0.2453 ml of biogas, fruit wastes had not produced any significant amount of biogas and the mixture of both had produced quite higher biogas volume equal to 1.817 ml, this difference in biogas production went on until the end of the experiments. Volume recording was stopped on the twelfth day when no more significant production was noticed. For all of the substrates the cumulative biogas production in millilitres over 12 days of retention time is summarised in table 6.3 with standard deviations in parentheses.

Table: 6.3 Biogas produced at 55^{o} C over twelve days

Days	**Food wastes**	**Fruit wastes**	**Food wastes + Fruit wastes**
1	0.245305 (0.216)	0 (0)	1.817073 (0.047)
2	3.270732 (0.163)	0.817683 (0.1)	4.179268 (1.548)
3	4.270122 (0.805)	0.99939 (0.2)	5.269512 (1.883)
4	6.541463 (2.076)	1.226524 (0.35)	16.2628 (9.487)
5	14.99085 (1.416)	4.542683 (1)	20.89634 (8.158)
6	18.625 (2.869)	5.542073 (0.2)	26.43841 (7.330)
7	21.44146 (1.156)	6.223476 (0.15)	31.70793 (7.840)
8	23.07683 (1.575)	6.677744 (0.05)	37.70427 (1.087)
9	27.80122 (0.860)	7.495427 (0.25)	38.52195 (0.826)
10	28.25549 (0.694)	7.604451 (0.2)	39.43049 (0.942)
11	28.52805 (0.776)	7.631707 (0.09)	39.6122 (0.489)
12	28.52805 (0.694)	7.631707 (0.049)	39.70305 (0.589)

After 12 days of retention time food wastes had produced 28.528 ml, fruit wastes 7.631 ml and the mixture had produced 39.703 ml of biogas.

For a simpler analysis and comparison figure 6.3 present the graph of the cumulative biogas production versus time showing the progress of biogas production over twelve days of digestion.

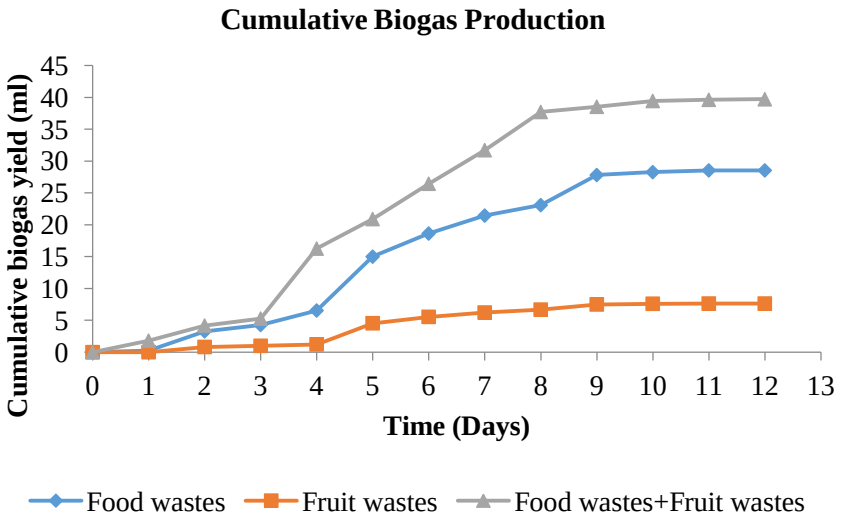


Figure: 6.3 Cumulative biogas yields at 55^{o} C

Biogas production is the highest (39.703 ml) for the mixture of food wastes and fruit wastes. Looking at the graphs we see that biogas production of fruit wastes was far lower than the other two samples and that it took a bit longer for a significant volume of biogas to be produced from fruit wastes. It can also be seen on the figure that a noticeable increase in biogas produced was observed between the third and the fifth day.

A better substrate would be the one which would produce a higher yield at a reasonable biogas production rate. If the overall production of biogas is the only target, the daily biogas production would not be of a great importance as long a total higher biogas quantity is expected to be collected from a given anaerobic digestion process. In contrast, if a daily biogas yield is of interest like in a situation where a continuous use of biogas is needed as biogas is being produced then daily biogas production would be the most important parameter. So, it is up to the operator to balance between the two parameters and decide accordingly. Therefore, it is important to investigate the daily biogas yield from the substrates. Table 6.4 and figure 6.4 illustrate the daily biogas production of the samples.

Table: 6.4 Daily biogas yields at 55^{o}C

Days	Food wastes	Fruit wastes	Food wastes + Fruit wastes
1	0.245305	0	1.817073
2	3.025427	0.817683	2.362195
3	0.99939	0.181707	1.090244
4	2.271341	0.227134	10.99329
5	8.44939	3.316159	4.633537
6	3.634146	0.99939	5.542073
7	2.816463	0.681402	5.269512
8	1.635366	0.454268	5.996341
9	4.72439	0.817683	0.817683
10	0.454268	0.109024	0.908537
11	0.272561	0.027256	0.181707
12	0	0	0.090854

For the very first days, table 6.4 shows that daily biogas production was lower for all substrates, but it later increased but it was not stable at all.

The same observation is clearly indicated by figure 6.4.

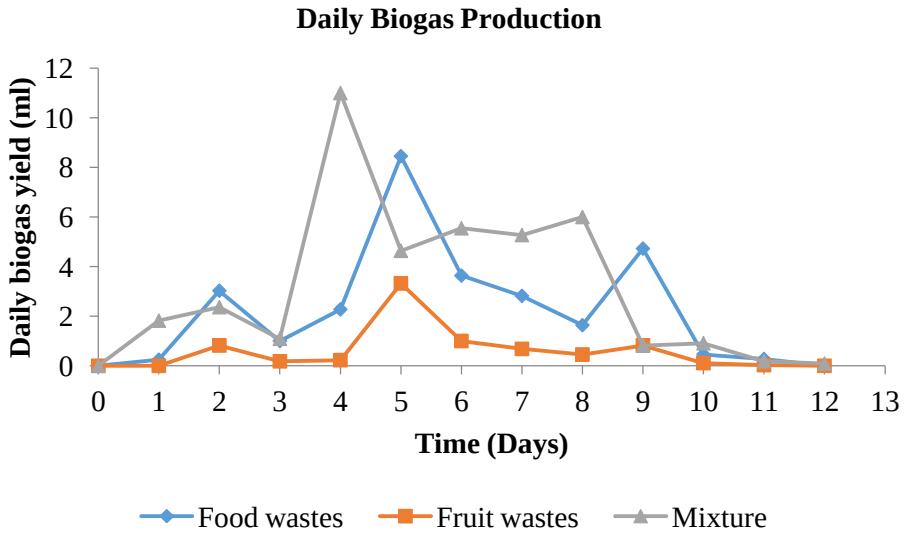


Figure: 6.4 Daily Biogas yield

Figure 6.4 indicates that the mixture of food and fruit wastes attained its peak in daily biogas production earlier; on the fourth day. The other two samples had their peaks a bit late compared to the mixture; on

the fifth day. Moreover, daily biogas yield peaks are far different with the mixture having the highest peak biogas yield followed by food wastes and fruit wastes being the least of all. It is worth noting that even after the fifth day the mixture kept a bit higher daily biogas production until the eighth day then down until the last day. None of the three had a steady daily biogas production.

Looking at the average biogas production in ml/day illustrated by table 6.5 and figure 6.5, the mixture always presents higher values in terms of daily biogas production followed closely by food wastes.

Table: 6.5 Average daily biogas yield

Food wastes	**Fruit wastes**	**Food wastes + Fruit wastes**
2.377337	0.635976	3.308587

There is no big difference in average daily biogas production between food wastes and the mixture but a clear difference is readable between the two and fruit wastes.

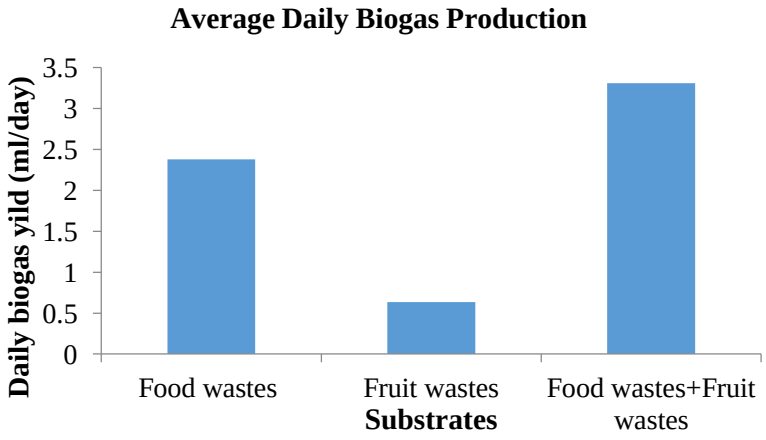


Figure: 6.5 Average daily biogas production

Average daily biogas production of food wastes is 28.14 % lower than that of the mixture but that of fruit is much lower (80.77 %) than that of the mixture.

6.2.2 Digestion at 37^{o} C

The substrates were loaded to batch anaerobic digesters simulated by 100 ml plastic syringes, kept in an incubator set at 37^{o} C to digest and the biogas production was recorded everyday at a fixed time of the day to keep a constant interval between consecutive records. The digesters were in triplicates i.e. for each type of substrate we had three digesters running in parallel for statistical analysis purposes. The data presented in table 6.6 are average cumulative biogas yields with standard deviation in parentheses

Table: 6.6 Cumulative biogas Yields at 37^{o} C

Days	Food wastes	**Fruit wastes**	**Mixture**
1	0 ()	0 (0)	0 (0)
2	0 (0)	0 (0)	0 (0)
3	0 (0)	0 (0)	0.3 (0.047)
4	0.2(0.047)	0.1 (0)	0.7 (0.124)
5	0.6 (0.047)	0.1 (0.047)	1.1(0.124)
6	1 (0.124)	0.2 (0.124)	1.4 (0.047)
7	1.2 (0.081)	0.4 (0.124)	1.7 (0.081)
8	1.5 (0.094)	0.4 (0.169)	2 (0.094)
9	1.7 (0.124)	0.6 (0.216)	2.3 (0.094)
10	2 (0.094)	0.9 (0.046)	2.5 (0.094)
11	2.2 (0.169)	1.2 (0.205)	2.6 (0.047)
12	2.6 (0.244)	1.4 (0.169)	2.8 (0.94)

At mesophilic temperature biogas production is lower compared to thermophilic temperature. We also notice that the biogas yield of food wastes is closer to that of the mixture. Biogas was still being produced when data recording was stopped.

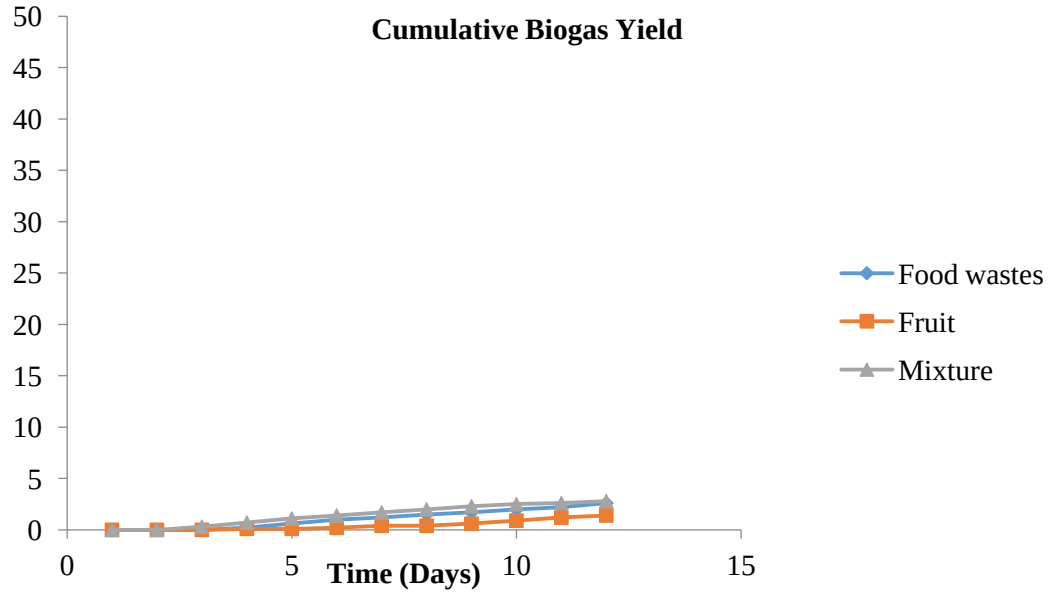


Figure: 6.6 Cumulative Biogas yields at 37^{o}C

Figure 6.6 show that biogas yields at 37^{o} C are very low, but still the biogas yield is higher for the mixture.

All of the results presented in this document show that the mixture of food and fruit wastes gives better yields in terms of cumulative biogas production, daily production and peak daily biogas production.

The experiments went on to the digestion the mixture of food wastes, fruit wastes and urine at 55^{o} C, where a half of the mixture of food and fruit wastes was replaced by the same volume of urine. So, the mixture formed was 50 % urine and 50 % food and fruit wastes. The gas yield of this mixture was compared to that without urine.

The cumulative gas yield in ml was much higher for the mixture containing urine as it can be seen from the table 6.6 and figure 6.7.

Table: 6.7 Average cumulative gas yield

Days	**Food wastes + Fruit wastes**	**Food wastes + Fruit wastes + urine**
1	0.245305	5.542073
2	3.270732	47.2439
3	4.270122	58.78232
4	6.541463	63.77927
5	14.99085	81.13232
6	18.625	86.76524
7	21.44146	100.9384
8	23.07683	113.9305
9	27.80122	125.7415
10	28.25549	126.65
11	28.52805	125.9232
12	28.52805	126.1049

The mixture containing urine managed to produce 126.1049 ml in 12 days of digestion; 77.37 % higher than that of the mixture without urine.

Looking at the figure 6.6 we figure out that the addition of urine to the substrates increased the gas yield of the digestion process. From the very first day the mixture containing urine had already produced a significant amount of gas and between the first day and the second day there was an abrupt increase of the gas yield far more than the mixture without urine.

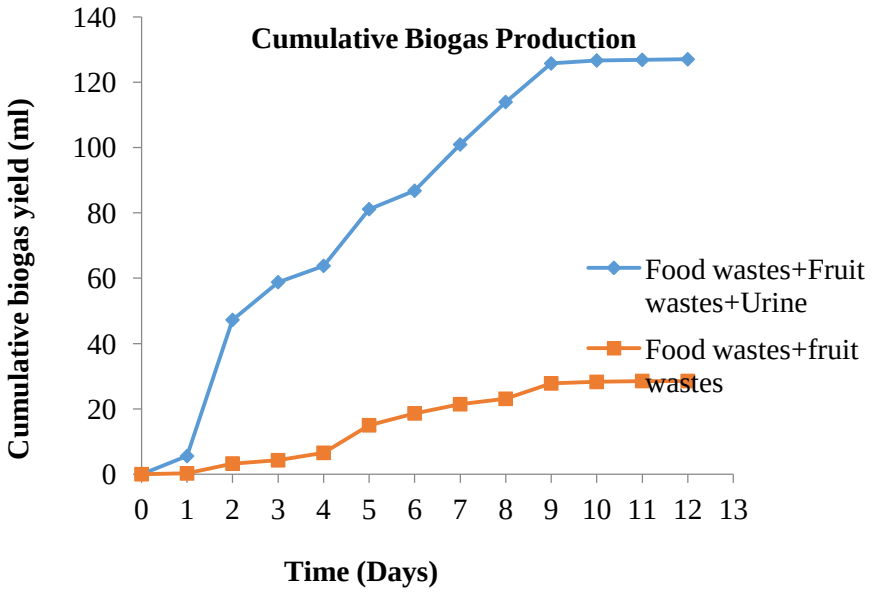


Figure: 6.7 Cumulative gas yields with and without urine

There is a big difference in the gas yield between a mixture without urine and the one with urine. But let us look at the daily biogas production of the two mixtures; we use figure 6.7 to analyse.

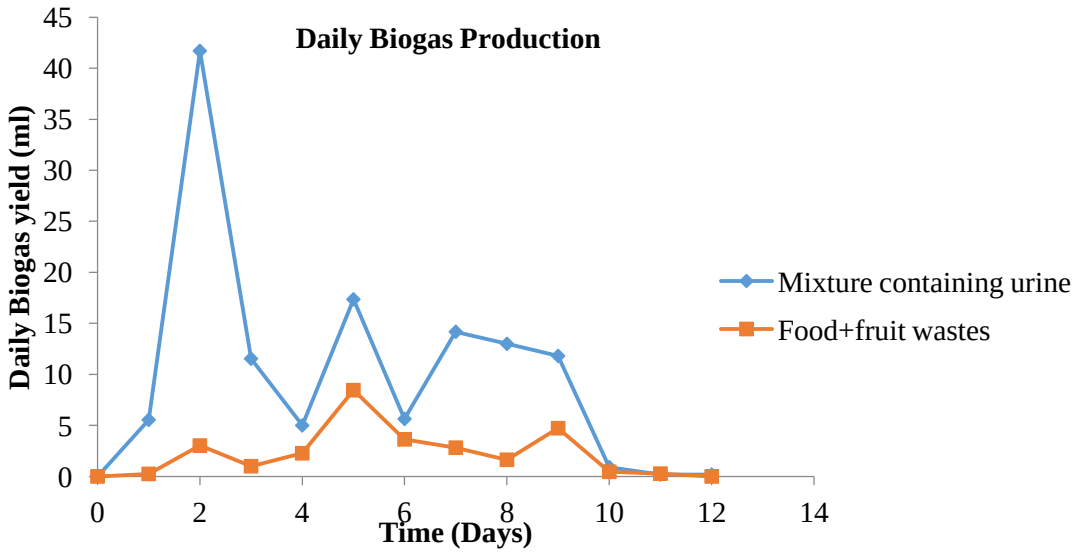


Figure: 6.8 Daily gas yields

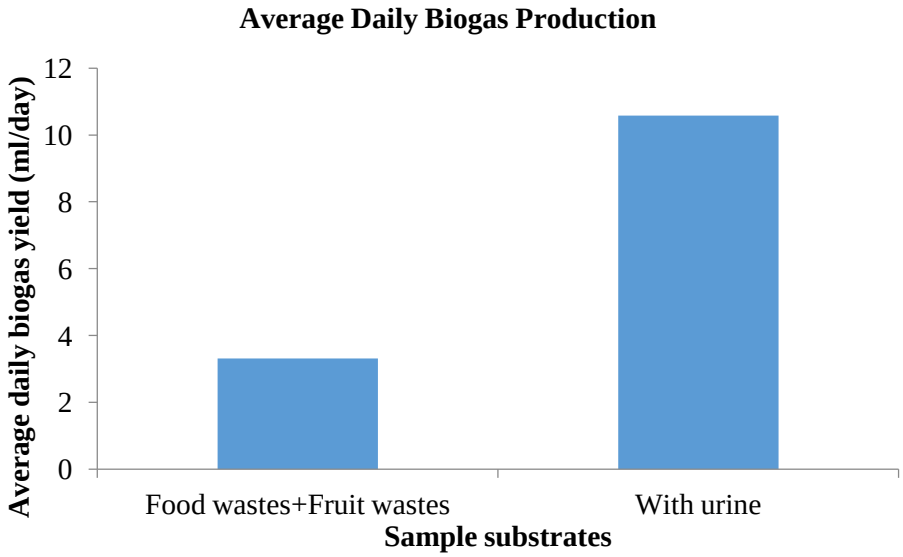
The mixture containing urine reached its peak daily gas production right away at the second day whereas the mixture without urine waited until the fifth day to rich its peak. The variability of the daily gas production between the two mixtures is the same though the mixture with urine has higher values.

Let us now look at the average daily biogas production in ml/day of the two mixtures

Table: 6.8 Average daily gas production

Mixture without urine	Mixture with urine
3.308587	10.58445

The average daily biogas production of the mixture containing urine is 68.74 % higher than that the mixture without urine.



The average daily gas production of the mixture containing urine is far higher than that without urine.

6.3 Effluent Characterisation Results

After twelve days of digestion the effluent from digesters at 55^{o} C were characterised for their TS, VS and VS/TS ratio and the results of the characterisation process are tabulated in table 6.9.

Table: 6.9 Effluent characteristics

Effluents	TS (%)	VS (%)	VS/TS (%)	VS reduction (%)	pH
Food wastes	8.78802	93.36515	90.58747	2.769956	4.69
Fruit wastes	7.282653	96.4771	92.45142	0.586217	4.34
Mixture	12.24044	95.93445	87.24083	0.828525	4.37

The results of the effluent characterisation show that the volatile solids reduction was very poor during the experiments, the same for total solids reduction level but this one is a bit higher. But it shows in contrast that the volatile solids reduction of food wastes is higher than that of both fruit wastes and the mixture.

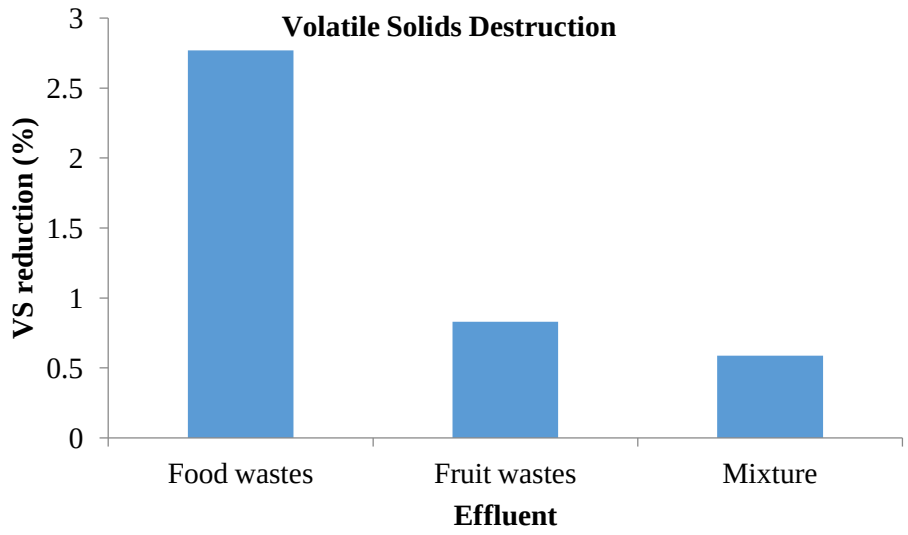


Figure: 6.9 VS reduction level

The figure 6.9 illustrates the VS reduction level is the highest in the food wastes that in other effluents, this would then imply that the addition of fruit wastes to food wastes as co-digestion substrate would reduce the volatile solids destruction level of food wastes. For the purpose of wastes disposal of fertilizer improvement, based on these results mixing these two substrates will not be a good option. But this was not the purpose of this study. Therefore, it will not be explained in details in the next sections.

Looking at the level of the total solids reduction of the effluent the results stipulated that the total solids reduction level was higher in the food wastes digestion followed by fruits wastes. But these values are higher that the reduction of volatile solids.

7 Discussion

Goals and objectives were set at the beginning of this project; guided by these objectives work has been done to achieve the overall goal which was to assess the effect of the improvement of substrate nutritional composition on the yield of the process.

The first objective was set to be the detailed description of the anaerobic digestion. To achieve this objective, anaerobic digestion process was described as a process which is completed in three main complementary stages and that the optimal biogas yield is produced when each stage is completed at its maximum. To achieve the second objective which was the study of the factors affecting the anaerobic digestion process, factors affecting the anaerobic digestion were studied. It was found that they are parameters that need to be controlled carefully to be able to get the desired yield from the anaerobic process. Those include substrates nutritional composition and temperature. Existing anaerobic digesters were reviewed and it was noticed that multi-stage anaerobic digestion systems have many advantages over single stage anaerobic digestion systems, but the use of the multi-stage systems is mostly used for research purposes with little penetration in industrial practices. This is due to the complexity and higher operational cost that are attributed to this type of digesters.

The fourth objective was the study of the mostly used types of substrates and their optimal biogas yield it was achieved by studying different substrates that are mostly used for biogas production focusing on their respective biogas production potentials. Cattle manure, pig manure, agricultural residues, waste activated sludge, organic fraction of municipal wastes, fruit and vegetable wastes as well as food wastes were found to be the mostly used substrates and having comparatively better biogas potentials. We also found that mixing different substrates is also a practice that is advanced in the field of anaerobic digestion for energy production. There were no examples where fruit wastes are separately digested, but they are in most of the cases mixed with vegetables to make one substrate referred to as fruit and vegetable wastes (FVW). Even when fruit and vegetable wastes are co-digested with other substrates they are considered as one complete substrate and therefore it is characterised as a single substrate. This might be due to two facts; one is that in most of the cases research samples for these products are collected from markets and municipal areas where these wastes are disposed together. Another but technical cause of this would be the composition of fruits which is dominated by sugars and carbohydrates lacking proteins and lipids making it inappropriate for single substrate digestion.

The above findings lead us to be interested in fruits as single substrate and co-digesting it with kitchen food wastes. These two substrates helped us to achieve the fifth research objective; carry out anaerobic digestion experiments using selected substrates. Food and fruit wastes were selected and digested to

identify their biogas yield. The characterisation of substrates right after their collection showed that fruit wastes have TS of 17.007 % lower than that of food wastes of 21.623 as shown in table 6.1. This is in accordance with literature which suggests that fruits have higher moisture content compared to that food wastes [36]. VS/TS ratio of fruits is higher than that of food implying that a big portion of the solids in fruits are volatile solids. The results of the characterisation of prepared samples are given in table 6.2.

Fruits are reported to be rich in carbohydrates at 90 % of their dry matter, sugars and vitamins and are poor in lipids and proteins. Carbohydrates are highly degradable but their biogas yield is lower, sugars are energy source for anaerobic microorganisms and vitamins are source of nitrogen. Lipids have higher potential to produce biogas but their retention time is longer and low biodegradability, proteins are also good candidate for biogas production. Kitchen food wastes have all it takes for the production of biogas; those are carbohydrates, lipids and proteins. Higher contents of animal derived foods and cooking oil make food wastes richer in lipids may reduces the biodegradability of food wastes.

From the figure 6.3 the general view is that for all samples there is a slow increase in biogas production at the beginning of the process and later on the biogas production rate increases and ends by stabilising before the production stops. This results from the fact that microorganisms need time to stabilise and be able to actively decompose the substrates at a steady rate. On the other hand, biogas yield from fruit wastes is lower than that of food wastes; this is due to what is explained in the preceding paragraph that fruits are poor in lipids and proteins which could boost the production of biogas. Presence of sugars though good source of energy for microorganisms, might lead to high production of acids at the beginning of the reactions.

Mixing fruit wastes to food wastes as seen on figures 6.3 and 6.4 increases cumulative and daily biogas yields. This is due to the fact that carbohydrates and moisture content of the fruits boost the biodegradability of the substrates, vitamins from fruits increase nitrogen content of the substrates to balance with carbon content to come out with desired C/N ratio. In addition, the lipids concentration of the food wastes is reduced by the addition of sugars and carbohydrates from fruit wastes which increases biodegradability and reduces the risk of foaming. But proper mixing should be considered not to add higher quantities of fruit wastes which could produce acids that would compromise the methanogenesis stage leading to poor biogas yield and reduced methane content. From figure 6.6 we see that biogas yield is lower for the anaerobic digestion at mesophilic temperature due to reduced biodegradability of substrates at lower temperatures. But it is still true that mixing fruit wastes to food wastes increases the biogas yield.

Addition of 50% urine as co-substrate showed a higher gas yield compared to the mixture without urine, considering that urine has a very low C/N ratio and that its total solids content is low the biogas produced was dominated by the ammonia, this was confirmed by the fact that when this gas was mixed to distilled water, the pH of the mixture shifted to 8.2 from neutral pH of 7 characteristics for distilled water. Coming to the effluent analysis presented in table 6.9, the VS reduction was very poor for all of the substrates and pH did not change so much, this is a result of initial acidic pH that did not favour a complete decomposition of the substrates. The literature suggests that a better biogas yield is achieved with a pH range between 6.8 and 7.2. pH change was not in the scope of our study that why we did not manipulate the substrates to adjust their pHs, substrates were used in their pure nature to deal with their nutritional composition. The results show that by mixing fruit wastes to food wastes biogas yield is increased to an optimal value that could not be achieved by digesting food wastes as a single substrate.

8 Conclusions

Anaerobic digestion process is used for different purposes including wastewater treatment, municipal solid waste treatment and energy production. For any purpose of the process, operators have interest in maximising the yield. For energy production purposes, one will need to maximise the biogas yield and methane content which defines the energy content of the yield. Many parameters have effect on the yield of the biogas that is collected from the anaerobic digester; those include temperature, carbon to nitrogen ratio, retention time, organic loading rate, pH and alkalinity of the substrates as well as substrates nutritional composition.

Improving the substrates nutritional composition would greatly affect the yield of the process by increasing the biodegradability, boosting microorganisms' activity, minimising inhibitory effects, and reducing the retention time. There are organic materials that cannot produce biogas when digested as single substrates but which can be co-digested with other feedstock and boost the biogas yield. But some operators, especially in developing countries rely on separate substrates to produce biogas which in some cases will not be good enough in terms of biogas yield.

In this study, after a review of potential substrates for the anaerobic digestion, we investigated the effect of improving substrate composition on the biogas yield from the anaerobic digestion. By keeping all the other parameters unaffected we separately digested fruit wastes, food wastes and the mixture of the two. The results show that fruits on their own produced very little biogas equal to 30mL/g VS, food wastes produced 95mL/g VS at 55^{o} C. When fruits were mixed with food wastes the biogas yield was equal to 110mL/g VS at the same temperature, representing an increase of 13.67 %. Therefore, the improvement of the composition of substrates by mixing different organic wastes results in optimal biogas yield.

However, the biogas yield achieved during this study was less than the normal biogas yields. Example is the case of food wastes which are reported to produce 445-540mL/g VS. The lower biogas produced is attributed to the lower pH at which the experiments were carried out leading to incomplete decomposition of the substrates. Future work should focus on reducing the effect of pH on the digestion process without involving additional products which appear to increase the cost of the process. It would also be of interest to study the improvement of the biogas yield by using urine as a co-substrate at well calculated proportions.

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Appendix A

Equipments and methods used in the experiements

Parameter	**Method/Equipment**
Temperature	HI 9214 stick pH meter
pH	HI 9214 stick pH meter
TS	Standard methods
VS	Standard methods
Biogas volume	Plastic syringes

Appendix B

Samples identification for digestion at 55±2^{o} C

Digesters	Samples
A1	Food wastes
A2	Food wastes
A3	Food wastes
B1	Fruit wastes
B2	Fruit wastes
B3	Fruit wastes
C1	Food and fruit wastes
C2	Food and fruit wastes
C3	Food and fruit wastes
D1	Mixture with urine

Cumulative biogas (mL) yields from digesters

Days	A1	A2	A3	B1	B2	C1	C2	C3	D1
1	0.1	0.5	0	0	0	2	2	2.1	6.1
2	3.4	3.8	3.6	0.8	1	6.2	5.1	2.4	52
3	4.2	5.8	4	0.9	1.3	8	6	3.4	64.7
4	5.8	10.1	5.6	1	1.7	25.2	24	4.5	70.2
5	16.1	18.4	15	6	4	29.1	28.5	11.5	89.3
6	20.1	22.7	18.6	6.3	5.9	34.2	34.3	18.7	95.5
7	22.8	25.2	22.7	7	6.7	41.4	39.5	23.9	111.1
8	23.9	27.6	24.8	7.4	7.3	42.6	41.8	40	125.4
9	29.5	31.6	30.7	8.5	8	43	42.9	41.2	138.4
10	30.2	31.9	31	8.6	8.2	43.4	43.9	41.7	139.4
11	30.4	32.3	31.4	8.5	8.3	43.6	44.2	43	139.6
12	30.6	32.3	31.5	8.5	8.4	43.6	44.6	43.2	139.8

Appendix C

Samples identification for digestion at 37±2^{o} C

Digesters	Samples
E1	Food wastes
E2	Food wastes
E3	Food wastes
F1	Fruit wastes
F2	Fruit wastes
F3	Fruit wastes
G1	Food and fruit wastes
G2	Food and fruit wastes
G3	Food and fruit wastes

Cumulative biogas (mL) yields from digesters

Days	E1	E2	E3	G1	G2	G3	F1	F2	F3
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0.1	0	0	0	0.3	0.3	0.2
4	0.2	0.1	0.2	0.1	0.1	0.1	0.8	0.7	0.5
5	0.7	0.4	0.6	0.2	0.1	0.1	1.2	1.1	0.9
6	1	0.9	1.1	0.4	0.2	0.1	1.4	1.4	1.3
7	1.3	1.1	1.3	0.6	0.3	0.4	1.8	1.7	1.6
8	1.5	1.3	1.6	0.7	0.3	0.4	2	2	1.8
9	1.8	1.6	1.8	0.9	0.5	0.4	2.3	2.3	2.1
10	1.9	1.8	2.2	1.2	0.7	0.8	2.5	2.5	2.3
11	2.2	2	2.5	1.4	0.9	1.2	2.6	2.6	2.5
12	2.6	2.3	2.9	1.6	1.2	1.4	2.9	2.9	2.7

Appendix D

Some Used formulas

VS reduction

$$VS_{reduction} = \frac{VS_{in} - VSout}{VS_{in}} * 100$$

VSin: volatile solids content of influents

VSout: volatile solids content of effluents

Standard Deviation

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}$$

N: total number of records

Xi: value of ith record

μ: the Mean

Daily Biogas volume

$$V_{Daily_N} = V_{Cum_N} - V_{Cum_{N-1}}$$

V_{cumN}: cumulative volume of biogas on day N

V_{cumN-1}: Cumulative volume of biogas on the preceding day