JIMMA UNIVERSITY

JIMMA INSTITUTE OF TECHNOLOGY

FACULTY OF CIVIL AND ENVIRONMENTAL ENGINEERING

ENVIRONMENTAL ENGINEERING CHAIR

BIOGAS PRODUCTION FROM THE BLENDS OF WASTEWATER AND MACRO ALGAE

BY: WAGARI MOSISA KITESSA

A THESIS SUBMITTED TO ENVIRONMENTAL ENGINEERING CHAIR, JIMMA INSTITUTE OF TECHNOLOGY, FACULTY OF CIVIL AND ENVIRONMENTAL ENGINEERING, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN ENVIRONMENTAL ENGINEERING

OCTOBER, 2017

JIMMA, ETHIOPIA

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OCTOBER, 2017

JIMMA, ETHIOPIA

APPROVAL SHEET

As a thesis advisor, we hereby approved that we have read and evaluated this thesis prepared, under our guidance, by Wagari Mosisa Kitessa entitled Biogas production from blends of second and third generation prototype feedstock: wastewater and macro algae. We recommend that it can be submitted as fulfilling thesis requirement.

Dr.-Ing. Fekadu Fufa	_____	_____
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Mr. Dida Abera (PhD candidate)	_____	_____
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As a member of board examiner of MSc. thesis final defense examination, we certify that we have read, evaluate thesis prepared by Wagari Mosisa Kitessa and examined the candidate. We recommend that, the thesis could be accepted as fulfilling the thesis requirements for the degree of masters of Science in Environmental Engineering.

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(Internal Examiner)	Signature	Date
_____	_____	_____
(Chair Person)	Signature	Date

DECLARATION

I, Wagari Mosisa, hereby declare that the research work on 'Biogas production from the blends of second and third generation prototype feedstock: Wastewater and Algae biomass' for the MSc degree at the Jimma University is my original work and the research has not presented for award of any degree either in Jimma University or any other university.

Wagari Mosisa Kitessa

(Researcher) _____ Signature _____Date

This thesis has been submitted for examination with our approval as university advisor and program chairperson.

Advisor: Dr.–Ing. Fekadu Fufa

(Main advisor) _____ Signature _____Date

Mr. Dida Abera (PhD Candidate)

(Co-advisor) _____ Signature _____Date

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ACRONYMS

AD	Anaerobic digestion
APHA	American Public Health Association
BMP	Biochemical Methane Potential
BOD	Biochemical Oxygen Demand
BOD_5	Five days Biological Oxygen Demand
MA	Macro algae
CH_4	Methane
C/N	Carbon to Nitrogen Ratio
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
FC	Faecal Coliform
GA	Gas Analyser
GHG	Green House Gas
HRT	Hydraulic Retention Time
H_2S	Hydrogen Sulphide
H_2O	Water
JiT	Jimma Institute of Technology
JU	Jimma University
MC	Moisture Content
MJ	Mega Joules
mL	Milliliter
NH_3	Ammonia
OLR	Organic Loading Rate
OM	Organic Matter
PASDEP	Plan for Accelerated and Sustained Development to End Poverty
pH	Power of Hydrogen Ion Concentration
SD	Standard Deviation

TC	Total Coliform
TK	Total Potassium
TOC	Total Organic Carbon
TOD	Total Oxygen Demand
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solid
VFA	Volatile Fatty Acids
VS	Volatile Solid
WW	Wastewater

ABSTRACT

Energy is the fundamental requirements for the economic development of the world. It can be obtained from renewable and non-renewable sources. Energy from non-renewable sources has been exploited to assure and sustain the need for sustainable development. However, non-renewable energy sources have been depleted and forced researchers to search for alternative cost effective and environmental friendly energy sources. Thus, energy production from biomass has obtained considerable attention. The aim of this study was to investigate the enhancement of biogas production through AD from blends of WW and MA. The MA are functioned as a co-substrate. A single factor experimental design with three level of mix in triplicate of each level was used with the response variable; quantity of biogas varies with the variation of mix ratio. Series of laboratory scale batch anaerobic co-digestion of the WW and algae biomass were carried out under mesophilic condition for 21days. Biogas production rates from WW alone, and different blends of WW and MA were analysed. In addition, the nutrient values and reduction in volume of the WW after digestion were determined. The results show that CH_4 productions of 39.7%, 51.7% and 57.9% were obtained for WW alone and for WW: MA mix ratios of 3:1 and 3:2, the values of TS, VS and COD also reduced by 43.11%, 40.09% and 71.99% at optimum mix ratio respectively. At optimum mix ratio 1732.77, 77.14 and 174.26 kg/year of Urea, diammonium phosphate and potash fertilizer respectively were obtained. The results indicate that biogas production can be improved through co-digestion of WW and MA as a co-substrate; thus warranting further investigation for practical application in the energy production.

Keywords: *biogas; Anaerobic co-digestion; Wastewater; Macro algae*

CHAPTER ONE
INTRODUCTION

1.1 Background

In recent years, there has been increased interest in converting fraction of the WW due to the high decomposition potential and production of CH_4 as a valuable product. AD has been recognized as one of the best options for treating WW since it results in two valuable final products: biogas and bio-fertilizer that may be utilized for electricity production and as soil conditioner respectively. Also, the WW utilities have shown increased interest for identifying an alternative supplemental carbon and nutrient source to the use of methanol for enhancing the process of de-nitrification and meeting regulatory nitrogen standards [1].

Algal biomass is considered as a third generation biomass, which does not require arable land for cultivation. Despite research on the concept of producing energy from blue green algal biomass dating back to the 1960s, there has been limited commercial development and the environmental advantages are still in doubt [2].

Anaerobic conversion of organic materials and pollutants are established technology for environmental protection. The end product is biogas, a mixture of CH_4 and CO_2, oxygen, H_2S, carbon monoxide and other trace gases. AD is a technologically simple process, with a low energy requirement, used to convert organic material from a wide range of WW types, solid wastes and biomass into CH_4 [2]. In the 1980's several projects were initiated in the Netherlands to produce biogas from wastes [3].

Over the recent years the industrialization of biogas production were increasing [3]. Initially, the aim was simply to generate energy in the form of heat and electricity. While electricity and heat are still the main products of biogas utilization, other interests in the use of biogas have steadily grown and now include utilization as a vehicle fuel and all applications that natural gas has found over the last century [4]. In addition to energy, the AD process has a residue, the digestate, which contains valuable nutrients and can therefore be used as a bio fertilizer. This short summary describes the developments in the biogas sector in terms of the drivers for AD deployment, the technologies adopted, and utilization of the products, biogas and digestate [4, 5].

However, locally the demand for biogas is continuously growing and the biogas substrate, such as algae, may soon become limited and it is therefore important for biogas producers to expand the range of substrates. Much attention has been focused on the improvement of CH_4 production in order to prevent the limitation. An interesting option for improving CH_4 yields is co-digestion [4-6]. This is a process where resource recovery can be optimized by improving the nutrient and organic content of substrates to be used in anaerobic digester along co-digester.

Numerous feedstocks can be used in the AD process. Feedstock can include animal and human manure, WW, food waste, garden/yard waste, greases, oils, fats, and some industrial waste/ WW s, such as paper mill and brewery effluent [7]. Biogas composition, especially the CH_4: CO_2 ratio vary greatly depending on the type of feedstock, or feedstock (if co-digesting) [8]. The concept of using WW streams as a source of nutrients to grow microalgae is not new, nor is the possible use of this biomass to produce bio-fuels [9].

So far, the process, although technically feasible, has not proven to be economical. However, considering the extremely high research and development efforts under way, it is possible that an economic process will be developed in the future.

It is, however, very likely that such a process would only be economically viable in very large piggeries because of the economies of scale. Unless the algae biomass has high lipid content the optimum strategy would appear to be fermentation of the biomass to produce CH_4. Alternatively, hydrothermal liquefaction could be used to convert the algae biomass to fuel [10, 11]. Therefore, it is possible to use blends of MA and WW to produce biogas [10].

The present study was explored the possibilities to use MA from Oxidation pond of JiT, as a co-substrate to WW in biogas production under mesophilic conditions.

1.2 Statement of the problem

The deficiency of energy is continuously occurring in the daily activities of the nation [12]. Therefore, the problem turns the researcher to find environmentally acceptable and economically sound energy production techniques to sustain the development. AD

technology has been used throughout the world by using biomasses such as WW as a substrate [13, 14]. However, energy production rate from digestion of WW alone is minimal [15]. Consequently, co-digestion process has acquired a notice. Accordingly, the current study has considered the use of MA as co-substrate in the process of resources (bio energy and bio fertilizer) recovery from batch an anaerobic digestion of WW under mesophilic conditions.

1.3 Significance of the Study

The use of WW for energy production would reduce emissions, leachates and also to recover valuable by-products like biogas for energy conversion. This study helps to produce biogas from WW and indirectly suggests the mechanisms to manage the WW generated from the campus, JiT. It also establishes database for further investigating AD as other option to solve similar problems in other parts of the country. In addition to these significances, the study evaluates the potential value of co-digestion of WW and algae biomass as non-renewable energy source.

1.4 Objectives

1.4.1 General Objective

To investigate biogas production from the blends of wastewater and macro algae

1.4.2 Specific objectives

The specific objectives of the study are:

1. To analyze physico-chemical composition of the WW and MA biomass before and after digestion;
2. To determine the amount of biogas produced from WW alone and from different mix ratios of the WW and MA;
3. To determine the optimum mix ratio for maximum biogas production
4. To analysis reduction percentage of the physicochemical characteristics of WW after digestion and
5. To evaluate bio-fertilizer potential of the digestate.

1.5 Research Questions

1. What does the physicochemical and bacteriological contents of WW look like before and after digestion?
2. Can blending WW with MA biomass enhance the production of biogas?
3. At what optimum mix ratio of WW and MA biomass does highly biogas can be obtained?
4. Can blending WW with MA biomass reduce volume of the WW?
5. Does the digestate can be used as bio fertilizer?

1.6 Scope

The study focused on the WW generated from the cafeteria of JiT, Jimma University. In addition, for co-digestion purpose, the algae biomass was collected from oxidation pond of JiT.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

Biological treatment of WW through AD is a matured technology commonly used in WW treatment facilities for sludge volume reduction, pathogen reduction and stabilization [15]. WW is readily biodegradable and requires less solid retention time in AD processes than that of food waste or other type of organic wastes [16]. AD is an established technology for the production of biogas from Biomass. The final Product is biogas: a mixture of CH_4 and CO_2 that can be used for heating, upgrading to natural gas quality or co-generation of electricity and heat [14].

The socioeconomic changes in the past decades related to increases in urban populations, development of food industries, the intensification of livestock operations, and the increasing tendencies of population consumption have favoured an increase of concentrated organic waste production which has resulted in serious environmental problems [7].

Organic wastes are produced by many activities, for example, livestock and food processing industries. In many years, an array of ideas for the utilisation of these streams have been put forward, but AD of organic waste streams to produce biogas, which is recoverable as energy, is the most likely option to be interesting for the markets, provided that the economics were favourable [18]. In spite of that, waste management strategies have frequently consisted of landfilling (controlled or un-controlled) of these wastes; however recent stricter legislations are imposing limitations to this practice suggesting alternative strategies. As consequence AD of OM with different origin has been presented as an adequate and profitable technology used for the treatment of organic residues and for the production of energy from biogas combustion [6, 18]. In addition, political changes in renewable energies and environmental policy are converging in an AD technological platform, although, unfortunately, not in the same degree in all the countries [19].

The anaerobic co-digestion process, which can be defined as the joint treatment of several organic waste streams by AD [20, 21], offers great potential for the adequate disposal of a range of organic wastes [22]. Additionally, co-digestion overcomes some of the inherent mono-digestion problems [8] and as result, higher digestion efficiencies are achieved [1, 22].

2.2 Theoretical review/Conceptual Framework

Research conducted on the subject of recovering energy from algal biomass has always been most prevalent around times of energy insecurity [3]. This is the case for both freshwater algae and marine algae [23]. Research investigating the potential of energy recovery from freshwater algae was first initiated in the 1950s. The first concept was derived as a result of ideas for the use of algal biomass in WW treatment ponds where the biomass was used as an oxygen source for oxidizing bacteria [1]. Studies conducted investigating the energy recovery possibilities of aquatic biomass became more prominent in the late 1970s as a result of the oil crisis during this decade. Research continued to mainly focus upon the production of CH_4 although attention started to spread to the production of biodiesel [15]. Current research on algal bio energy is varied however the main focus has been on algal biomass with conversion to biodiesel. biogas from algae has increased rapidly over the past 10 years, particularly for microalgae and biodiesel production [13, 24]. It can be observed that previous research had focused on biogas generation but this was over taken by biodiesel generation around 2008 and more recently slightly by bio ethanol production. The reason for the interest in biodiesel production is partly because biodiesel is considered a more valuable fuel than biogas as it can be used as a direct substitute for diesel [15].

Research investigating the potential for biogas recovery has seen a strong resurgence from the late 2000s due to climate change and fossil fuel security and costs. However the concept of converting algal biomass to bio energy remains at an early stage with many obstacles needing to be overcome [25, 26].

The aim for the generation of biogas is to produce a sustainable energy source. There is no definite description of this but in general it denotes a source that produces a positive energy

balance (or net energy ratio above one) with limited environmental impacts or impacts that are at least lower than those of conventional fossil fuels [27].

In order to improve the performances of anaerobic digesters, the co-digestion of waste activated sludge together with other organic wastes is a common practice adopted in WW treatment plants [21]. Co-digestion is the concurrent digestion of a homogenous mixture of substrates in the same unit. Through co-fermentation, that means through the joint treatment of biogenic wastes (co-substrates) in the digesters of the WW treatment plant, the digester gas production can be in-creased considerably [26]. Depending on the type and quantity of the co-substrates added, gas generation can increase so strongly that a self-sufficient energy level for the operation of the WW treatment plant can be realized and excess energy can be passed to the grid [15, 27]. Traditionally, AD was a single substrate, single purpose treatment [15]. The use of co-substrates usually develops the biogas yields from anaerobic digester due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates [18].

2.3 Critique of the existing literature relevant to the study

The raw material for the production of biogas can be processed from various feedstock sources [12]. For instance biogas is produced from nearly all types of organic materials including vegetable, animal and WW feedstocks. The origin of the feedstock can vary, ranging from livestock waste, manure, harvest surplus and vegetable oil residues. Recently, WW, municipal solid wastes and organic wastes from households have been introduced as feedstock. Another feedstock source is the collection of biogas from landfill sites [23]. One main advantage of biogas production from WW is the ability to use so-called wet biomass as feedstock source because biomass cannot be used for the production of other biofuels such as biodiesel [15]. Examples for wet biomass are WW, manure from dairy and swine farms as well as residues from food processing are all used in biogas production [12, 17]. Therefore, it is possible to recover energy from WW and possible to increase amount of energy to be recovered by mixing with co-substrate.

2.4 Wastewater

Wastewater has been identified as a major problem that has reached proportions requiring drastic measures [5]. WW management system in Finland has been undergoing major changes during the last few decades. The changes in the national legislation have been the requirement to comply with the European Union's legislation [30]. The framework for waste legislation in European Union is based on three Acts: waste framework directive (Directive 2008/98/EC) providing the general framework of waste management requirements, decision 2000/532/EC establishing the list of wastes and Regulation (EC) No 1013/2006 of the European Parliament and of the Council on shipments of waste [31].

The organic WW requires to be managed in a sustainable way to avoid depletion of natural resources, minimise risk to human health, reduce environmental burdens and maintain an overall balance in the ecosystem [32]. AD could be an appealing option for converting solid organic waste into useful product like biogas, which will play an important role in meeting the world ever increasing energy demand in the future. Finland has implemented a renewable energy policy which aims to increase the use of biogas as a renewable energy source up to 0.7TWh by year 2020 [33].

Thus, use of AD technology for simultaneous treatment of WW and production of renewable energy in the form of biogas would not only facilitate the waste management requirements but also enable achieving the renewable energy target in sustainable manner.

2.4.1 Wastewater Characteristics

Wastewater quality can be defined by physical, chemical, and biological characteristics. Physical parameters include colour, odours, temperature, solids (residues), turbidity, oil, and grease. Solids can be further classified into suspended and dissolved solids (size and settleablity) as well as organic (volatile) and inorganic (fixed) fractions. Chemical parameters associated with the organic content of WW include the BOD, COD, total organic carbon (TOC), and total oxygen demand (TOD). BOD is a measure of the organics present in the H_2O, determined by measuring the oxygen necessary to bio stabilizes the organics (the oxygen equivalent of the biodegradable organics present). Inorganic chemical parameters include salinity, hardness, pH, acidity, alkalinity, iron, manganese, chlorides,

sulfates, sulfides, heavy metals (mercury, lead, chromium, copper, and zinc), and nitrogen (organic, NH_3, nitrite, and nitrate), and phosphorus. Bacteriological parameters include coliforms, fecal coliforms, specific pathogens, and viruses [20, 37].

Pathogenic organisms in WW can be categorized as bacteria, viruses, protozoa, and helminthes. Because of the many types of pathogenic organisms and the associated measurement difficulties, coliform organisms are frequently used as indicators of human pollution. On a daily basis, each person discharges from 100 to 400 billion coliform organisms, in addition to other kinds of bacteria [32]. In terms of the indicator concept, the presence of coliform organisms indicates that pathogenic organisms may also be present, and their absence indicates that the H_2O is free from disease-producing organisms. TC and FC are often used as indicators of WW effluent disinfection [42]. In general, the numbers of pathogenic microorganism in the digestion are too many. But, in the digestion process number pathogen before digestion is greater than the number of pathogen after digestion. The impact of discharging untreated wastewater to the environment may cause odour, different waterborne diseases and can reduce the aesthetic value of environment.

2.4.1.1 Physical characteristics of wastewater

Physical examination of WW is carried out in order to determine its physical characteristics. These include tests for determining turbidity, colour, odour, and temperature [39].

I) Turbidity

WW is normally turbid, resembling dirty dish H_2O or WW from baths having other floating matter like faecal matter, pieces of paper, cigarette-ends, match sticks, greases, vegetable debris, and fruit skins, soaps etc. the degree of turbidity can be measured and tested by turbidity rods or by turbid meter.

II) Colour

The colour of WW can normally be detected by the necked eye, and it indicates the freshness of WW. If its colour is yellowish, gray, or light brown, it indicates fresh WW.

However, if the colour is black or dark brown, it indicates stale and septic WW. Other colours, may also be formed due to the presence of some specific industrial wastes [12].

III) Odour

Fresh WW is practically odourless. But in 3 to 4 hours, it becomes stale with all oxygen present in WW being practically exhausted. It then starts omitting offensive odours, especially that of H_2S gas, which is formed due to decomposition of WW.

IV) Temperature

The temperature has an effect on the biological activity of bacteria present in WW, and it also affects the solubility of gases in WW. In addition, temperature also affects the viscosity of WW, which, in turn, affects the sedimentation process in its treatment.

2.4.1.2 Chemical characteristics of wastewater

Testes conducted for determining the chemical characteristics of WW help in indicating: the stage of WW decomposition, its strength, and extent and type of treatment required for making it safe to the point of disposal. Chemical analysis is, therefore, carried out on WW in order to determine its chemical characteristics [42]. It includes tests for determining: TS , suspended solids, settleable solids, chloride content, pH value, nitrogen content, presence of fats, greases, and oils, sulphides, sulphates and H_2S gases, DO, COD, and BOD.

I) Total solid and volatile solid

TS is defined as the material residual left in the Dish after the continuous evaporation of a well-mixed sample in the oven dry at a temperature of about 105°C until the weight becomes constant. In principle, TS is the weight sum of dissolved and suspended solids[16]. On the other hand, VS is defined as the weight loss at a temperature of 550°C after the combustion of the TS [37]. TS indicates that there is the effect of run off on WW.

II) pH value

The pH value of WW indicates the negative log of hydrogen ion concentration present in WW.$pH = -logH^+$. It is, thus, an indicator of the alkalinity of WW. If the pH value is less than 7, the WW is acidic, and if the pH value is greater than 7, the WW is alkaline.

The determination of pH_value of WW is important, because of the fact that efficiency of certain treatment methods depends upon the availability of a suitable pH value.

III) Nitrogen contents

The presence of nitrogen in WW indicates the presence of OM, fertilizer, and may occur in one or more of the following forms: free NH_3, called NH_3 nitrogen, albuminoid nitrogen, called organic nitrogen, nitrates and nitrites. The sources of nitrogen used in fertilizers are many, including NH_3, diammonium phosphate ($(NH_4)_2HPO_4$), ammonium nitrate (NH_4NO_3), ammonium sulfate ($(NH_4)_2SO_4$), calcium cyanamide ($CaCN_2$), calcium nitrate ($Ca(NO_3)_2$), sodium nitrate ($NaNO_3$), and urea (N_2H_4CO).

IV) Phosphorus contents

Nitrogen and phosphorus is the essential components of a microbial cell [37]. The acid forming bacteria in this stage required for their growth and synthesis of new cells and their metabolite products. According to [37], the highest COD removal and VFA production of 45.0%, and 8.32 g/l, respectively, were found at COD: N: P of 100:1.1:0.5. This ratio was slightly different from theory for the AD (COD: N: P = 100:2:0.3). However, [39] found that a COD: N: P ratio of 100:0.5:1 was an optimum for the acidogenic step for bio hydrogen fermentation of wheat powder solution. Therefore, the effect of N and P on an AD depends on the source of materials and OLR. The phosphorus content of a fertilizer is specified as the amount of P_2O_5, because this is the anhydrous form of phosphoric acid [42].

v) Potassium

Potassium (K), which is said to be potash, is important for general health of plants. It is key in the formation of chlorophyll and other plant compounds. Potassium is also known to help with disease resistance [21]. Potassium deficit is hard to symptomize, however plants are generally sickly, with small fruit, yellowing from the older leaves upwards, and sickly blooms [31]. Sources of organic potassium include greensand and liquid fertilizers such as Earth Juice's Meta-K [35]. No any plants live without P and required for photosynthesis, osmotic regulation and the activation of enzyme systems. Potassium

deficiency in cereal crops consequences in reduced growth, delayed ripeness, lodging cau sed by weak straw, and low bushel weight [42].

VI) Dissolved Oxygen (DO)

The determination of DO present in WW is very important, because: while discharging the treated WW in to river stream, it is necessary to ensure at least 4ppm of DO in it; as otherwise, fish are likely to be killed, creating nuisance near the vicinity of disposal. The DO test performed on WW before treatment, helps in indicating the condition of WW. It is well known by now, that only very fresh WW contains some DO, which is soon depleted by aerobic decomposition.

VII) Chemical oxygen demand (COD)

The OM present in H_2O can be measured in a number of ways; VS determination being crude measure of OM. OM is most often assessed in terms of oxygen required to oxidize completely the OM to CO_2, H_2O and other oxidized species. The DO required to oxidize the OM present in a given WW can be theoretically computed, if the organics present in WW are known. Thus, if the chemical formulas and the concentrations of the chemical compounds present in WW are known to us, we can easily calculate the theoretical oxygen demand of these compounds by writing the balanced reaction for the compound with oxygen to produce CO_2, H_2O and oxidized inorganic components.

VIII) Biochemical Oxygen Demand (BOD)

The OM, in fact, is of two types; i.e. that which is biologically oxidized (i.e. oxidized by bacteria) and is called biologically active or biologically degradable; and that which cannot be oxidized biologically and is called biologically inactive. If sufficient oxygen is available in WW, the useful aerobic bacteria will flourish and cause the aerobic biological decomposition of WW, which will continue until oxidation is completed. The amount of DO consumed in this process is the BOD. The BOD of during 5 days at 20°C is generally taken as standard demand and is written as BOD_5, or simply as BOD. It is determined in the laboratory by mixing or diluting a known volume of a sample of WW with a known volume of aerated pure water, and then calculating the DO of this diluted sample. The aerated water should be mixed with reagents like phosphate buffer, magnesium sulphate,

calcium chloride and ferric chloride. These reagents are used for bacteria as energy source to reduce the amount final DO.

$$BOD_5 = \left(DO_i - DO_f\right) * D_f \quad 2.1$$

$$D_f = \frac{V_{sample}+V_{dlw}}{V_{sample}} \quad 2.2$$

Where: DO_i = DO before incubation

DO_f = DO after incubation

D_f =dilution factor

V_{sample} =volume sample to be measured

V_{dlw} =volume of diluted water

BOD_5 after and before digestion are calculated using equation 2.1 and 2.2 above [32].

2.5 Macro algae

Algae that predominate in ponds are the motile genera such as Chlamydomonas, Pyrobotrys, and Euglena. These algae species are capable of optimizing their vertical position in the pond H_2O column to incident light intensity and temperature. Non-motile algae such as Chlorella are also found in ponds and they rely on mixing initiated by wind and temperature [43].

The mixing mechanisms enable them to access the incident light, allowing them to photosynthesize. The concentration of algae in a healthy pond depends on surface BOD loading and temperature. The majority of the algae occupy a band about 200mm deep that moves up and down the H_2O column, presumably in response to nutrient concentration and incident light during the day [44]. Anaerobic reactions initiated by acid-forming, CH_4-forming bacteria and sulphate-reducing bacteria in the lower layers may feed VFA and sulphides to the upper layer where they may affect the ecology of the pond algae. These products of AD have been observed to inhibit both growth and production of pond algae. It has been argued that high NH_3 and sulphide concentrations in a facultative pond may result in the replacement of Euglena with more tolerant algae species such as Pyrobotrys and Chlorella [45].

The algae biomass and its productivity cause a marked diurnal and vertical variation in the levels of DO, pH, sulphide and NH_3. It has been observed that when CO_2 is taken up faster than bacterial respiration can supply, the concentration of CO_2 drops causing a dissociation of the bicarbonate ion to form CO_2 and alkaline hydroxyl [43]. This results the rise of pH levels in facultative ponds. A rise of pH in facultative ponds exceeding 9.0 and this is important in killing FC. NH_3 and sulphide toxicity have been observed to be pH-dependent. As the pH of a facultative pond increases, the unionized form of NH_3 increases while sulphide production decreases. The effect of this toxicity is to inhibit algae growth and production and these mechanisms are thought to be self-sustaining [46].

From figure 2.1 [17], MA contains high content of nitrogen bonded with different carbon compounds which can be used for bacteria as a source of food to survive. That means in AD bacteria has the main role to degrade complex organic compounds, and algae can be used a source of food for bacteria to function this process.

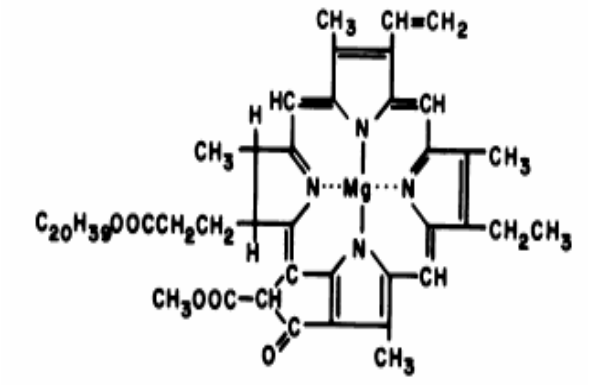


Figure 2.1 Structure of Macro algae

2.6 Anaerobic Digestion

Anaerobic digestion is one of primary WW treatment by which microbiological process that converts the chemically complex OM to CH_4, CO_2, and in offensive humus like material. The reactions occur is devoid of oxygen. The conversion takes place through a series of reactions. First, the solid matter is made soluble by enzymes, and then the substance is fermented by a group of acid-producing bacteria, reducing it to simple organic acids such as acetic acid. The organic acids are then converted to CH_4 and CO_2 by bacteria.

WW is heated as possible to the digester, where it remains for 10 to 30 days and is decomposed. Digestion reduces OM by 45 to 60 percent [2].

2.7 Experiences in anaerobic digestion of wastewater

Wastewater treatment and disposal is becoming an important issue. Traditionally, sludge from WW treatment plants (WTPs) was applied on farmland as fertilizer and soil amendment. Significant inactivation of pathogens also occurs during the AD, depending on the process temperature and technological layout [42].

So far, anaerobic treatment has been applied in Colombia, Brazil, and India, replacing the more costly activated sludge processes or diminishing the required pond areas. In various cities in Brazil, they show an interest in applying anaerobic treatment as a decentralized treatment system for "sub-urban", poor, districts. The beauty of the anaerobic treatment technology is applied to a very small and very big scale which result sustainable option for a growing community [40].

In Sweden, many treatment plants have also been supplemented with biogas extraction where WW is digested in a biogas digester in order to recover energy (for electricity generation and heat production), to minimize sludge volumes and facilitate the reuse of sludge as a soil improver. Re-circulation of the nutritious in WW sludge as an alternative to land filling is a matter of great attention [28].

Co-digestion of WW with other organic waste is prone in Dutch in order to recover energy and phosphorus from WW. It was proved that digestion of the WW with other wastes, like livestock manures, food waste, or other industrial organic wastes. Hence, co-digestion is an efficient way for increasing the yield of biogas and further reduction of CO_2 emission [13]. Anaerobic treatment of WW treatment with long retention times has a very long history in some of the central European countries but has improved considerably: These anaerobic systems can be built and operated on various scales in size with a high degree of technical sophistication and automation, but sometimes are technically quite simple as well.

In Central Europe, AD of WW is presently a routine process implemented in combination with the aerobic activated sludge process, which is the standard technology for municipal WW treatment. WW is the TS material that results from sedimentation and bacterial activity and growth during aerobic WW treatment. The floating and sinking layers formed before, during and after a treatment of the WW are normally all fed to digester. Here, anaerobic fermentation takes place at process temperatures of 35°C (mesophilic) to 55°C (thermophilic) and biogas is generated. To generate appropriate reactor temperatures, a heating system is required. Its energy demand can partly, sometimes fully, be covered by utilizing the produced gas, which can either be burnt directly or in cogeneration units [16].

In developing countries the polyethylene tubular digester was promoted to reduce production cost by using local materials and simplifying installation and operation [14]. The resulting low cost digester has been well received by poor farmers, especially when farmers participate fully in the necessary maintenance and repair work [11]. Within 10 years, more than 20,000 polyethylene digesters were installed and mainly paid by the farmers themselves. However, the digesters are still not fully integrated into the farming system, as there is only limited use of the effluent as fertilizer for fish and crops [13]. In addition, the potential for improving the digester efficiency, ease of maintenance, and durability.

2.8 Mixing wastewater with other substrates

In order to improve the performances of anaerobic digesters, the co-digestion of waste activated sludge together with other organic wastes is a common practice adopted in WW treatment plants. Co-digestion is the simultaneous digestion of a homogenous mixture of two or more substrates in the same unit (digester). Through co-fermentation, which means through the joint treatment of biogenic wastes (co-substrates) in the digesters of the WW treatment plant, the digester gas production can be in-creased considerably. Depending on the type and quantity of the co-substrates added, gas generation can increase so strongly that a self-sufficient energy level for the operation of the WW treatment plant can be realized and excess energy can be passed to the grid [15, 16].

For the joint anaerobic treatment with the raw WW treatment plant a number of biogenic wastes and plants like algal biomass are suitable for co-fermentation [17]. Traditionally, AD was a single substrate, single purpose treatment. The use of co-substrates usually improves the biogas yields from anaerobic digester due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates [13, 18]. Some of the merits of co-digestions are: Improved nutrient balance for an optimal digestion and a good fertilizer quality, homogenization of particulate, floating, or settling wastes through mixing with animal manures or MA, increased, steady biogas production throughout the seasons, higher income thanks to gate fees for waste treatment, aadditional fertilizer (soil conditioner) and renewable biomass production for digestion energy crop as a potential new income of agriculture. In general, the strength of substrate used during anaerobic process must be in some proportional ratio with the necessary nutrients which can enhance the growth of anaerobes.

In addition the cause why the second and third generation prototype is chosen is that second-generation prototype (WW) is locally available waste generated everywhere and MA is high content of nutrient that can be used as a food for microorganism [40, 45]. Nevertheless, first generation prototypes (cereal crops) are not economical, because it can cause crop to be invested [40]. This may charge capital.

2.9 The outputs of anaerobic digestion of wastewater

The challenge for the 21st century in terms of a sound waste management strategy is the transformation of waste into resources for the future [42]. One way of achieving this is through AD, providing avenues to recover energy and compost whilst reducing waste at the same time. Operating and maintaining healthy anaerobic digesters requires understanding of the substrate biodegradability, gas yields, toxicity and other anaerobic problems. The main theme of this project is to assess the enhancement of CH_4 potential, and organic fertilizer potential and the reduction in solid wastes as the result of co-digestion of WW from JiT, Jimma University and MA from oxidation pond of JiT.

2.10 History of Anaerobic Digestion

In 1808, Sir Humphrey Davy determined that CH_4 was present in the gases produced by cattle manure. The first anaerobic digester was built by a leper colony in Bombay, India in 1859. In 1895 the technology was developed in Exeter, England, where a septic tank was used to generate gas for the sewer gas destructor lamp, a type of gas lighting. In 1907, in Germany, a patent was issued for the Imhoff tank, an early form of digester [42].

Through scientific research AD gained academic recognition in the 1930s. This research led to the discovery of anaerobic bacteria, the microorganisms that facilitate the process. Further research was carried out to investigate the conditions under which methanogenic bacteria were able to grow and reproduce. This work was developed during World War II where in both Germany and France there was an increase in the application of AD for the treatment of manure.

With the increased interest in biomass-derived energy, there is a great opportunity for looking at the potential role of AD. In the 1970s numerous studies were carried out in which the BMP of crop species, wastes and other forms of biomass was reported. This biogas can be used as an energy source when its CH_4 content exceeds 30% [42].

2.10.1 Status of Anaerobic Digester in Ethiopia

Woody biomass represents the principal form of cooking and lighting fuel in Ethiopia's rural areas. An increasing fraction of the population is being confronted with the difficult choice between eating its food poorly cooked and travelling long distances to collect fuel for cooking. The scarcity of fuel wood has led to an increased utilization of dung and agro-residues for cooking, which could otherwise have been used to enhance the nutrient status and texture of the soil and contribute positively to agricultural production.

Biogas offers an attractive option to replace unsustainable utilization of wood and charcoal. It complies with the principles put forward in the country's Energy Policy and Environmental Protection Strategy, and closely meets the terms of the PASDEP as well: it is a local, renewable resource that addresses the basic needs of rural households amongst which energy; it supports decentralized access to household energy; its digestate, enhances

agricultural productivity and promotes organic farming, thus offering opportunities for niche markets and export. On the whole, it ensures environmental sustainability and its use as domestic fuel improves development conditions and opportunities for women and girls.

Biogas technology was introduced in Ethiopia as early as 1979, when the first batch type digester was constructed at Ambo Agricultural College with the plan of reducing the critical energy crisis of the 1970s [42]. In the last two and a half decades, around 1000 biogas plants were constructed in various parts of the country of which approximately 40% of these plants are not operational due to a lack of effective management and follow up, technical problems, loss of interest, reduced animal holdings, and evacuation of ownership and H_2O problems. Other reasons for the limited success of the technology in Ethiopia include the adoption of a project-based stand-alone approach without follow-up structure in place, variations in design, and the absence of a standardized biogas technology [12].

2.10.2 Current status and the ongoing projects in Ethiopia

In March 1994, the Transitional Government of Ethiopia released its energy policy, the first of its kind [9]. By way of 2012, this is still in potency as the plan of energy. It targets to address household energy deficiency by promoting energy supply from agro-forestry, increasing the efficiency with which biomass fuels are utilized, and facilitating the shift to greater use of modern fuels. The policy states that the country will rely mainly on hydropower to increase its electricity supply, nevertheless also take advantage of Ethiopia's geothermal, solar, wind and other renewable energy resources from different sources like wastes, where appropriate. Furthermore, it aims to explore and develop oil and gas investments. It encourage energy preservation in industry, transport, institutions and major energy consuming sectors, to ensure that energy development is environmentally sustainable and, to provide appropriate incentives to the private sector [9].

2.11Anaerobic Digestion Processes

Anaerobic digestion, which is also referred to as biomethanization, is a natural process that takes place in absence of air (oxygen). It involves biochemical decomposition of complex organic material by various biochemical processes with release of energy rich biogas and

production of nutrious effluents. CH_4 production pass over three general steps: hydrolysis, acidification and methanogenesis

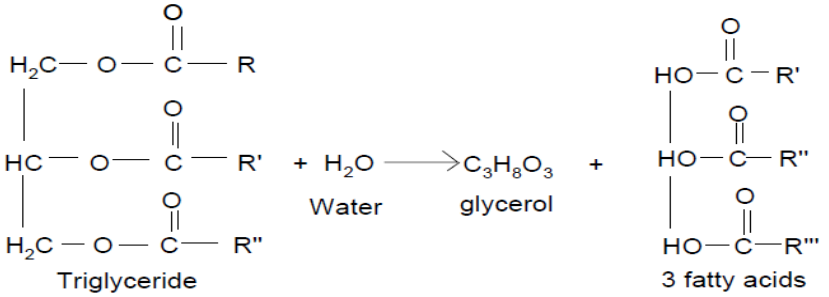
2.11.1 Hydrolysis

Hydrolysis is the first step in which the OM is enzymolysed externally by extracellular enzymes, cellulase, amylase, protease and lipase of microorganisms. Bacteria decompose long chains of complex carbohydrates, proteins, and lipids into small chains. For example, Polysaccharides are converted into monosaccharide. Proteins are split into peptides and amino acids. The important bacteria involved at this stage are: *Clostridium, Vibrio, Bacillus, Micrococcus and Peptococcus* [4].

This stage is also popularly known as the polymer breakdown stage.

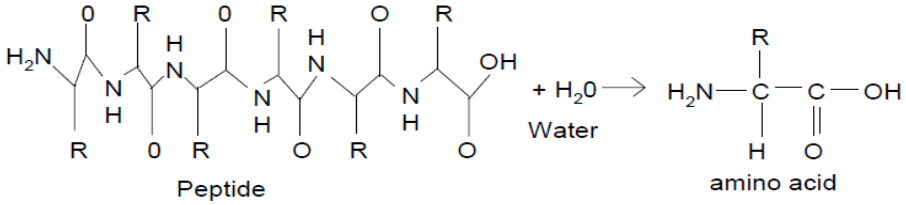
$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6 \qquad 2.3$$

In the case of lipids, usually triglycerides are split into three fatty acids and glycerol by the addition of three H_2O molecules, as illustrated at equation 2.4 [37].



2.4

In the case of proteins, peptide bonds are broken to separate amino acids, equation 2.5 [38].



2.5

2.11.2 Acidifications

Acid-producing bacteria, involved this step, convert the intermediates of fermenting bacteria into acetic acid, hydrogen and CO_2 [38]. These bacteria are anaerobic and can grow under acidic conditions. To produce acetic acid, they need oxygen and carbon. For this, they use DO. Hereby, the acid-producing bacteria create anaerobic condition, which is essential for the CH_4 producing microorganisms. Also, they reduce the compounds with low molecular weights into alcohols, organic acids, amino acids, CO_2, H_2S and traces of

CH_4. From a chemical point, this process is partially endergonic (i.e. only possible with energy input), since bacteria alone are not capable of sustaining that type of reaction [36]. The important bacteria involved in this stage of the process are: Clostridium, Rumino coccus, Propioni bacterium and Desulphobacter streptococcus [39].

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad 2.6$$

2.11.3 Methanogenesis

This is CH_4 formation stage in which CH_4-producing bacteria, which are involved in the third step, decompose compounds having low molecular weight [28]. They utilize hydrogen, CO_2 and acetic acid to form CH_4 and CO_2. Under natural conditions, CH_4 producing microorganisms occur to the extent that anaerobic conditions are provided, e.g. under H_2O (for example in marine sediments), and in marshes. They are basically anaerobic and very sensitive to environmental changes, if any occurs. The methanogenic bacteria belong to the archaebacter genus, i.e. to a group of bacteria with heterogeneous morphology and lot of common biochemical and molecular-biological properties that distinguishes them from other bacteria. The main difference lies in the makeup of the bacteria's cell walls [23].

The important bacteria involves methanogenic stage are: Non sporulating methanobacteri um, Sporulating methano bacterium and Sarcinaea [34].

Hydrogen: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ 2.7

Formic acid: $4HCOOH \rightarrow CH4 + 3CO_2 + 2H_2O$ 2.8

Methanol: $4CH_3OH \rightarrow 3H_4 + CO_2 + H_2O$ 2.9

Acetic acid: $CH_3COOH \rightarrow CH_4 + CO_2$ 2.10

The net of biochemical process is summarized by the Buswell formula. This formula gives the total stoichiometric relation of the complete AD process.

$C_nH_aO_b + (n - a/4 - b/2)\ H2O \rightarrow (n/2 - a/8 + b/4)\ CO2 + (n/2 - a/8 - b/4)\ CH_4$ [40]. 2.11

2.12 Bacteria

CH_4 and acid-producing bacteria act in a symbiotically way. Acid producing bacteria create an atmosphere with ideal parameters for CH_4 producing bacteria (anaerobic conditions, compounds with a low molecular weight) [2].

On the other hand, CH_4-producing microorganisms use the intermediates of the acid producing bacteria. Without consuming them, toxic conditions for the acid-producing microorganisms would develop [24]. In real time, fermentation processes the metabolic actions of various bacteria acts in a design. No single bacteria is able to produce fermentation products alone as it requires others too as shown below [9].

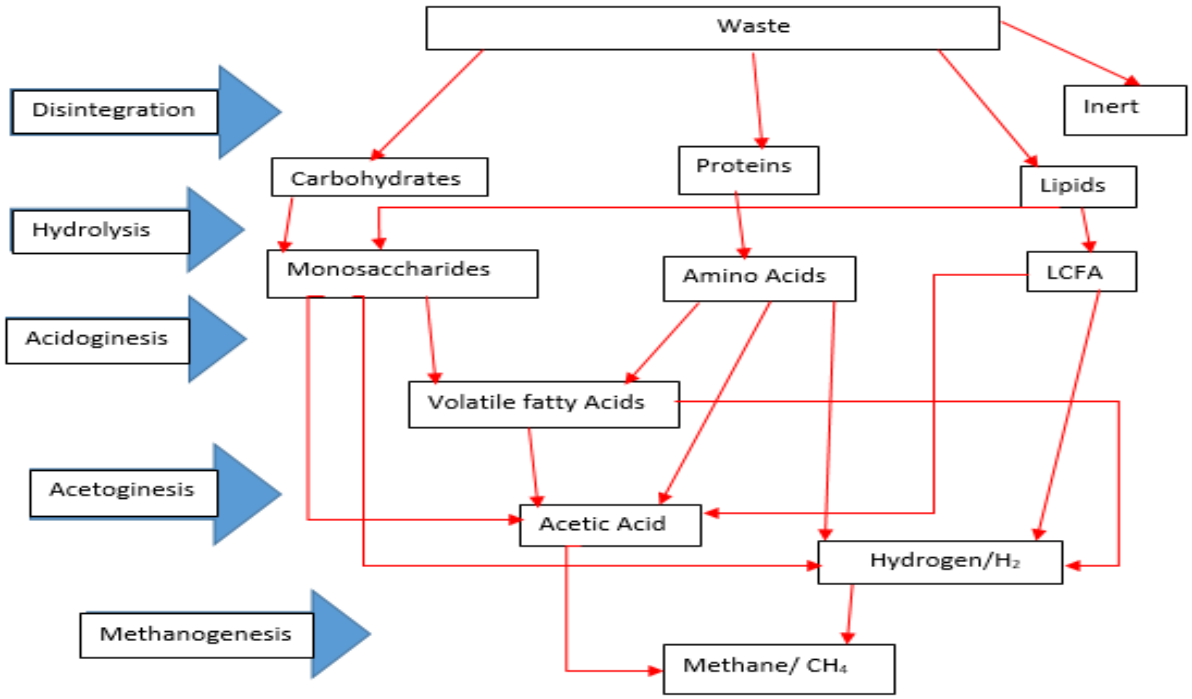


Figure 2.1 Schematic of anaerobic digestion process

2.13 Characteristics of Biogas

Biogas and natural gas have something in common; for example, they are both produced in an anaerobic environment, and CH_4 (CH_4) is their major component. Natural gas is formed after million years of high pressure and temperature acting on dead biomass, converting it to CH_4, ethane, propane, condensates, etc. However, it takes only about 15 days to convert biomass to biogas [34].

Biogas has CH_4 and CO_2 as its main constituent that is produced by the anaerobic biodegradation of the organic material of the wastes by microorganisms under anaerobic conditions. It also consist other gases like hydrogen sulfide, hydrogen, carbon monoxide

and others, but in small amount [1]. It results in residual wastes which are of superior nutrient quality as a fertilizer [35].

Composition of biogas depends upon feed materials or substrate. Biogas is about 20% lighter than air has an ignition temperature in range of 650 to 750^{0}C [20]. Its caloric value is 20 MJ /m3 and it usually burns with 60 % efficiency in a conventional biogas stove [2]. This gas is useful as fuel to substitute firewood, cow-dung, petrol, diesel, and electricity, depending on the nature of the task, and local supply conditions and constraints.

2.14 Post treatment

After the completion of the AD, the remaining biodegradable organic material, digestate or effluent, is subjected further to post treatment processes. This includes dewatering, aeration and leachate treatment. The importance of aeration process in post treatment is to remove the left over biodegradable organics by aerobically reducing the organic compounds to valuable material, which is used as soil conditioner [42].

2.15 Feedstock

2.15.1 Substrate

All biomass resources, such as sewage sludge, agricultural, and industrial or municipal organic wastes including WW, can act as the substrate in biogas generation [18]. It is wise to select appropriate food waste as the substrate under the consideration of energy supply and waste disposal. The output of an AD process much depends on the composition of the adopted substrate. The composition of WW may vary from place to place and from country to country. The chemical and physical characteristics of the WW selected for biogas production can influence the yield and process stability [16].

Therefore, they should be considered while designing and operating the anaerobic digester. MC, ratio of carbon to nitrogen (C/N), volatile solids content (VS), nutrient contents, particle size, and biodegradability are useful parameters describing food waste undergoing AD [9].

2.15.2 Inoculums

Inoculum is a necessary substance that contains rich seeding microorganisms to initiate anaerobic reactions; therefore, it is often added together with the substrate at the beginning. It is often from the digested sludge of running biogas plants, wastewater treatment plants, or rotted manure. Difficulty and long start-up time are some barriers to AD. When inoculum is used, more biogas with higher CH_4 composition is produced, and a shorter period is needed for start-up [41]. In this study, cow dung was used as inoculum.

2.16 Operational parameters of anaerobic digestion process

The complete process of AD requires a complex interaction of several varieties of bacteria that must be in equilibrium in order for the digester to remain stable. Changes in environmental conditions can disturb the equilibrium and result in the build-up of intermediaries that may inhibit the overall process or shut it down altogether.

It is crucial to manage and use design control technologies to continually monitor and adjust the environment to prevent this.

Anaerobic digestion is a complicated process, which involves varieties of microorganisms to convert a substrate to biogas. The growth of microorganisms requires a favourable environment [41]. The composition of the end-product highly depends on the types of microorganism species. If the microorganisms live in an optimal environment, a balanced procedure can be achieved. However, the optimal environment for microorganism species in each step is so diverse that it requires appropriate monitor and control of several operational conditions, such as pH and temperature [28]. Typical parameters that affect AD include ratio of carbon and nitrogen (C/N), volatile solid (VS), pH, temperature, retention time, particle size, toxic materials [39].

2.16.1 Temperature

The biogas production differs greatly due to change in temperature. In case of thermophilic temperature, operation of reactor biogas production might be higher and the retention time of the substrate is low due to faster digestion by thermophilic bacteria, but the CH_4 content of the biogas may be low because the optimum temperature of the methanogenic bacteria lies in mesophilic range. In case of mesophilic temperature, operation of the

digester the process is slow and the retention time of the substrate material is also high. But the biogas production is stable when the digester is operated at optimum condition [33].

2.16.2 pH Value

The pH value of WW indicates the negative log of hydrogen ion concentration present in WW. pH = -log H+ .It is, thus, an indicator of the alkalinity of WW. If the pH value is less than 7, the WW is acidic, and if the pH value is greater than 7, the WW is alkaline. The determination of pH value of WW is important, because of the fact that efficiency of certain treatment methods depends upon the availability of a suitable pH value. The pH value can be measured quickly and automatically with the help of pH meter, which measure the electrical potential exerted by the hydrogen ions, and thus indicating their concentration [42].

The pH of the digester is an important indicator of the performance and the stability of an anaerobic digester. The pH level changes in response to biological conversions during the different processes of AD. A stable pH indicates system equilibrium and digester stability. Many aspects of the complex microbial metabolism are greatly influenced by pH variations in the digester. Although acceptable enzymatic activity of acid forming bacteria can occur at pH 5.0, Methanogenesis proceeds at a high rate only when the pH is maintained in the neutral range. Most anaerobic bacteria, including CH_4-forming bacteria, perform well within a pH range of 6.8 to 7.2 reported that AD of kitchen wastes with controlled pH value at 7.0 resulted in a relatively high rate of hydrolysis and acetogenesis with about 86% of TOC and 82% of COD were solubilised [16].

The pH value of the digester decreases with the days increases. Thus, pH of the digester needs to be maintained around 6.5 during the acidification step. The pH during the methanogenic step may go up to 8.5 or more due to higher NH_3 production but it is not favourable thus pH needs to be maintain around 7.0 for the optimal functioning of the methanogenic bacteria [9].

2.16.3 Toxic Substances

Methanogens are considered the most sensitive to toxicity in AD [42]. Inhibition can be caused by substances either entering with influent substrate or being produced by the

anaerobic process itself. Products in the chain of simultaneous biochemical reactions, such as NH_3, H_2S and VFA are pH dependent since only the non-ionized forms exhibit microbial toxicity.

2.16.4 Carbon to nitrogen ratio (C/N)

The percentages of carbon and nitrogen in the sample are other limiting factors in an AD process. For bacteria, carbon represents the energy source, and nitrogen serves as their growth [2]. However, if the percentage of nitrogen is low, the population of bacteria increases slowly and more time is required to degrade the substrate. That means nitrogen present in the substrate will be consumed too rapidly as compared to carbon, by the methanogenic bacteria to meet their protein requirements and thus the function of the bacteria will be affected as they will no longer be able to act on the remaining carbon content, resulting in low gas production. On the other hand, the high percentage of nitrogen leads to the generation of too much NH_3 gas that inhibits the growth of bacteria [1]. In anaerobic processes, the digestion rate of carbon is 30 to 35 times faster than the conversion rate of nitrogen. Therefore, in principle, the optimal ratio of carbon to nitrogen (C/N) is from 20:1 to 35:1 [41].

2.16.5 Organic Loading Rate (OLR)

Organic loading rate is a measure of the biological conversion capacity of the AD system and is defined as the mass of VS added each day per reactor volume. [42]. The potential danger of a rapid increase in the OLR would be that the hydrolysis and acidogenic bacteria would produce intermediary products rapidly. The accumulation of fatty acids will lead to a pH drop and affect the activity of methanogenic bacteria, causing the digester failure [6]. The recommended OLR for high-rate AD is 1.6- 4.8 kg VS/ (m^3*d) in mesophilic, and the recommended OLR for low-rate AD (digestion with no heat and no mixing) is 0.5-1.6 kg VS/(m^3*d). The recommended OLR for thermophilic 5-10 kg VS/(m^3*d) [5]. The recommended OLR for standard rate anaerobic digester should be 1.0-3.5 kg VS/(m^3*d) [18].

2.16.6 Hydraulic Retention Time (HRT)

Hydraulic retention time is the average time spent by the input slurry inside the digester before it comes out. The retention time is determined by the average time it takes for organic material to digest, as measured by the COD and BOD of exiting effluent. In tropical countries HRT varies from 30-50 days while in countries with colder climate it may go up to 100 days. Shorter retention time is likely to face the risk of washout of active bacterial population while longer retention time requires a large volume of the digester and hence more capital cost. Hence there is a need to reduce HRT for domestic biogas plants based on solid substrates [42].

It is determined by the average time needed for decomposition of the organic material, as measured by the COD of the influent and the effluent material. The longer the substrate is kept under proper reaction conditions, the more complete will be its degradation. However, the rate of the reaction decreases with longer residence time, indicating that there is an optimal retention time that will achieve the benefits of digestion in a cost effective way [10]. The required retention time for completion of the AD reactions varies with differing technologies, process temperature, and waste composition. The retention time for wastes treated in mesophilic anaerobic digester ranges from 3 to 55 days, depending on the type of waste, operational temperature, process stages and configuration of the digesters [4].

2.16.7 Percentage of Solids

AD of organics will proceed best if the input material consists of roughly 8% solids. In the case of fresh cow manure, this is the equivalent of dilution with roughly an equal quantity of H_2O. Digestion is practiced in two broad categories of solid content: "dry digestion," with typical dry solids content of 25-30% and "wet digestion," with dry solids content of less than 15% [17].

A higher TS contents leads to smaller and thus less costly, reactors. This price savings may be offset, however, by the more expensive pumps needed to move denser material. Higher TS values cause excessive resistance to flow in pipes as well [24]. Systems with lower TS tend to have much better mixing and thus increasing the degree of digestion because the bacteria can more easily access liquid substrate and because the relevant reactions require H_2O. An additional benefit to lower solids content is that mixing is more complete when

the solid content is lower. It also more amenable to co-digestion with more dilute feed stocks, such as wastewater or manure.

2.17 Organic fertilizer potential of the digestate

Plants must obtain the elements essential for their growth, other than carbon, oxygen, and hydrogen, from the soil. These essential elements are called nutrients; those needed in the greatest amount are called macronutrients whereas those needed in lesser amounts are called micronutrients. Among the macronutrients are Fertilizers. The term complete fertilizer often refers to any mixture containing all three important elements; such fertilizers are described by a set of three numbers. They are usually sold in packages, on which the percentage by weight of the macronutrients nitrogen (N), phosphorus (P), and potassium (K) are listed on the label, always in the order N-P-K. For example, a fertilizer that is labelled 10-5-3 is 10 percent nitrogen, 5 percent phosphorus, and 3 percent potassium [42].

2.18 Biogas production process design

The general biogas production system consists of the following stages:

1. **Macro algae and Wastewater collection measure:** The wastewater and macro algae were collected and mixed together for analysis.
2. **Pre-treatment:** In this stage, the WW and MA were separated from materials like those that plastics that cannot be digested by the microbes are removed before the wastes are added to the digester so that they do not affect the activity of digester.
3. **Homogenization:** In this stage the WW and algae were mixed to homogenize the feedstock. This was done laboratory shaker.
4. **Feeding:** The substrate materials were fed to the digester tanks to start the digestion.
5. **Anaerobic digestion:** The wastes were digested by the various microbes involved in the process. The maintenance of pH, temperature and other factors influencing the digestion of the wastes for optimum digestion of the substrate and the production of biogas [28].
6. **Production and utilization:** The biogas produced due to the AD of the feedstock is by cleaning and removing contaminant gases. This biogas can be directly used by combustion. The sludge that is produced as by product is dried to remove H_2O.

This sludge can be utilized as fertilizer as it is rich in nutrients like phosphorus and nitrogen [35, 37].

2.19 Benefits of Bio-digester Technology

There are a number of benefits resulting from the use of AD technology. Some benefits of biogas include: production of energy (heat, light, electricity). And also transformation of organic wastes into high quality fertilizer, improvement of hygienic conditions through reduction of pathogens, worm eggs and flies. In addition, reduction of workload, mainly for women, in fire wood collection and cooking. Environmental advantages through protection of forests, soil, H_2O and air, reduce global warming by reducing CH_4 emission from waste disposal site. Thus, biogas technology can substantially contribute to conservation and development, if the concrete conditions are favourable. However, the required high investment capital and other limitations of biogas technology should be thoroughly considered.

2.19.1 Economic Benefits

Considering the whole life cycle, biogas is more cost-effective than other treatment options. In addition, it can represent kerosene, diesel fuel and, possibly, wood or charcoal. Energy supply for commercial activities of biogas is about 6kWh/m^3. This correspondent to half a litter of diesel oil. It can enhance soil productivity because of the use of bio-slurry. Thus, savings on chemical fertilizers and/or additional income from higher agricultural yields, effective workload reduction on women in searching for fire wood [42].

2.19.3 Health Benefits

Reduction in smoke borne diseases [3]: eye-irritation, Lung problem, asthma, dizziness/ head ache and respiratory tract infection. The followings are among the principal organisms killed in biogas plats such as: Typhoid, Paratyphoid, Cholera and dysentery bacteria (in one or two weeks), Hook worm and bilharzias (in three weeks), Tapeworm and roundworm die completely when the fermented slurry is dried in the sun. In addition to these, biogas improves household sanitation when latrines are attached to bio-digesters and also easier, cleaner cooking and create better hygiene [39].

2.19.4 Environmental Benefits

Biogas technology has immense benefits in regulating ecosystems: Significantly reduces greenhouse gas (GHG) emissions, eliminates odour, produces a sanitized compost and nutrient-rich liquid fertilizers, and maximizes recycling benefits, prevention of land fertility degradation due to the excessive use of chemical fertilizers. Researches shows that organic fertilizers which comes from biogas plant contains three times more nitrogen than the best compost made through open or air digestion, lessen local deforestation, enhance climate change monitoring strategy [42].

2.20 Types of Biogas Plant

There are different designs of bio-digesters in use today. They can be classified based on feeding and plant types. In AD process technology, two general models are used: the batch process and the continuous process [35].

1. Continuous Feeding: Continuous plants are filled and emptied regularly, normally daily. Each design is suitable for continuous operation, but the feed material must be flow able and uniform. In this process, fresh material continuously enters the tank and an equal amount of digested material is removed. Continuous plants are more suitable for rural households. The necessary work fits better into the daily round. Gas production is constant, and somewhat higher than in batch plants. The disadvantage of the continuous process is the removed effluent is a combination of completely digested and partially digested material. To minimize the removal of partially digested material, some designs dictate the path of the digestate inside the chamber, for example through the use of interior walls [8, 41].

2. Batch Feeding: In the batch process, the substrate was put in the reactor at the beginning of the degradation period and sealed for the complete retention time, after which it is opened and the effluent removed. The disadvantage of this type of system is the large tank volume required due to only about 1/3 of the tank volume is used for active digestion, making this a poor option in crowded urban settings the long retention time, the low OLR and the formation of a scum layer [42]. Therefore, in this study the batch feeding is selected due to the only required is the prototype biogas production of JiT and availability of the digester used for this purpose.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling Area

The study focused on the WW generated from the cafeteria of JiT, Jimma University, Jimma. For co-digestion purpose, MA was collected from oxidation pond of JiT. Jimma is far from the capital city of Ethiopia, Addis Ababa, 335 km having an altitude of 1717 m, 7.66 m latitude and 36.833 m longitude. It has average yearly temperature of 22.8 ^{O}C and 125 mm yearly rain fall.

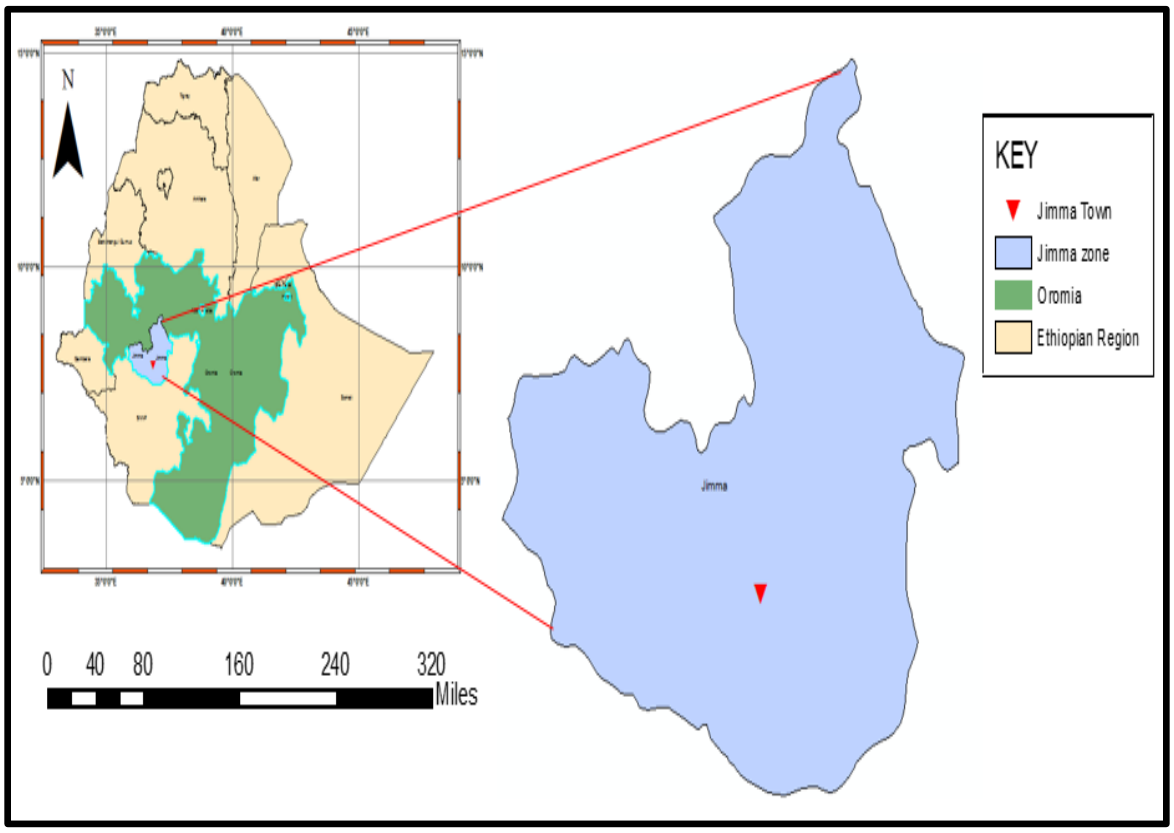


Figure 3.1 Map of study area

3.2 Sampling procedure

Well-mixed representative samples of WW s was collected from of JiT, Jimma University, Ethiopia. The WW was collected for three consecutive days in the morning, mid-day and evening to reduce sample variation. Then, the volume of WW collected during morning, mid-day and evening was mixed together to get one common sample of WW of that day. With the same procedure for the next two days the samples were collected. The collected

samples were preserved in the refrigerator working at 4^0c temperature to prevent result variation during experiment. For blue green algal representative, the sample was collected for three consecutive days and was prepared one day representative and with the same procedure for the next two days. Then it is filtered and preserved in the refrigerator to prevent result variation.

Figure 3.2 Oxidation pond of JiT from where macro algae was collected

3.3 Materials

Glass bottle (for sample measurement), crucibles (for solid analysis), folks, pH meter (for pH measurement), dissector (for taking sample from oven dry), anaerobic digester batch reactor (for gas production), magnetic stirrer (for mixing of inoculum and WW uniformly), incubator (used in measurement of coliforms), furnace (to measure VS), measuring cylinder (also for sample volume measurement), sterile plastic Petri dish (used during coliform investigation process), GA (to measure components of biogas as percentage), gas sampling bag (to collect gas from AD), sodium hydroxide (to adjust acidity of feedstock in the AD). Digital weighing scale was used throughout the experiment.

Analytical instruments (GA, spectrophotometer, DO meter and pH meter) were used for the analysis of WW and MA composition and analysis were conducted for samples from JiT oxidation pond.

3.4 Methods

3.4.1 Study Variables

The study parameters are classified into independent and dependent. Independent parameters include the amount of WW and MA, C/N ratio, dilution rate, temperature, pH, retention time, TN, TP, TK and coliforms and dependent parameter is the amount of biogas produced.

3.4.2 Experimental design

A single factor experimental design with three level of mix in triplicate of each level was used with the response variable; quantity of biogas varies with the variation of mix ratio.

3.4.3 Sample Analysis

Series of batch anaerobic reactor under mesophilic condition (35^{o}C) for a digestion period of 21 days were used in the laboratory. Biogas production was determined by water displacement. OM content was estimated from weight loss upon ignition at 550^{o}C for 3 hours in the furnace at laboratory of Environmental health science and technology department, JU. Coliforms were measured using membrane filtration method. TK, TN and TP are measured using kit method. pH was measured using digital pH meter at Environmental Engineering laboratory of JiT, Jimma University. Parameters such as total solids (TS), volatile solids (VS), biochemical oxygen demand (BOD_5), Chemical oxygen demand (COD), pathogen (total and FC), Ash content, Total Nitrogen (TN), Total phosphorus (TP) and Total potassium (TK) were analysed in the laboratory of Environmental health science and technology department of JU. pH of feedstock under digestion was measured in Environmental Engineering laboratory, JiT, Jimma University. Finally, biogas was analysed at Addis Ababa Institute of Technology, Addis Ababa University.

3.4.4 Experimental Procedure

Digester, necessary fittings, and different measuring device were prepared prior to collect the samples from each site. Sample preparation, testing for different parameters and recording (pH, BOD_5, TS, VS, COD), preparation of different mix and homogenizing were done during experiment and finally experimental results were collected. The pH of solution

(slurry) was adjusted through the production time at standard pH (5-8), at the temperature of mesophilic range (29 – 40 ℃). The biogas produced during digestion process was collected by gas collector and analyzed by employing gas analyzer.

3.5 Experimental set up

EDBON anaerobic digester operation set up

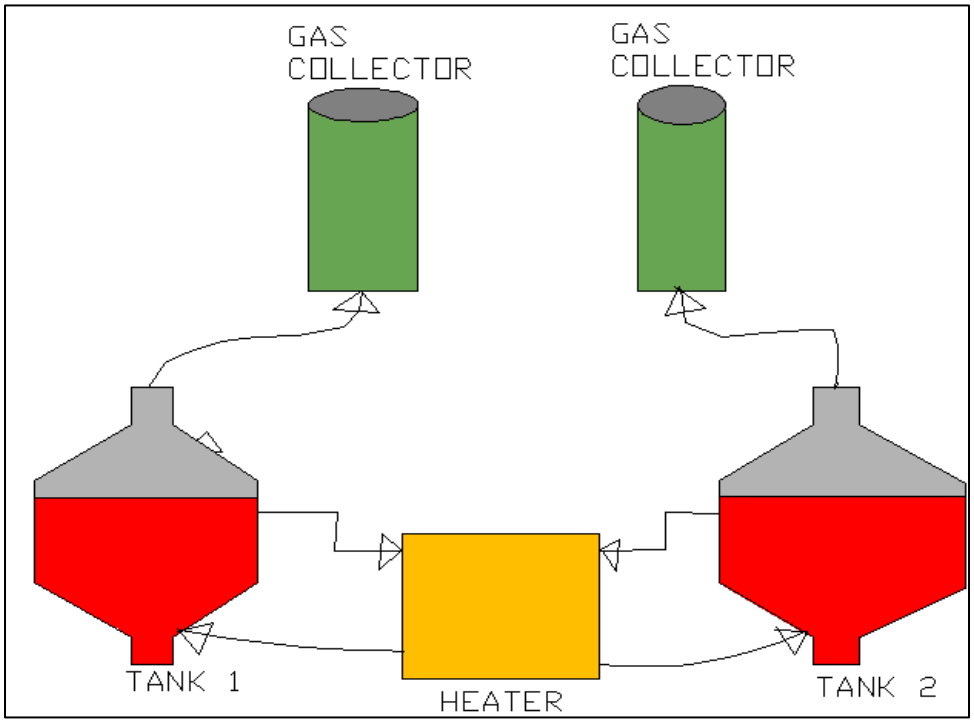


Figure 3.3 Experimental set up

Figure 3.3 shows that two packed column anaerobic digester tanks of 10 litres volume were used. Hot H_2O recycle in shell cover of digester was also used to maintain temperature. From 10 litres of digester 3 litres occupied by packed column, 1 litter free gas generation and only 6 litres of each used for sample volume. Two H_2O displacers or gas collector cylinders of 3 litre capacity of plastic type were used. Gas transport through plastic pipe which is connected top free space of digester to H_2O displacer. The amount of gas produced is equal to H_2O displaced. H_2O bath is temperature adjustment of the digester.

Figure 3.4 Anaerobic digester used in laboratory

3.6 Data Quality Assurance

The quality of the data was assured through triplicate analysis of samples and replication (the average plus or minus was reported) of the samples in operating procedures for quality purpose and software's (excel software, origin pro 8) were used for data report.

3.7 Dissemination Plan

The results of this study will be presented before dissemination for check-up if there is any problem in the study. Then after the efforts will be made to open to concerning bodies for dissemination. Also publication in national and international reputable journals will be considered.

CHAPTER FOUR

RESULTS AND DISCUSSION

Physico-chemical evaluations were carried out to evaluate the potential of co-digestion of WW and MA for energy recovery using AD.

Figure 4.1 Feedstock for the digester

4.1 Experimental Results

4.1.1 Characterization of raw wastewater before digestion

The physiochemical and bacteriological characteristics of the WW used in the study has been determined and the experimental results are displayed in Table 4.1. Accordingly, the TS of the WW was 2271.87± 3.97 mg/l and the VS of the WW was 703.95 ± 1.40 mg/l. The mean value of COD of the WW was 1549.79 ± 2.14 mg/l.

Table 4.1 Composition of Wastewater before digestion

Parameters	Unit	values (Mean ± SD)
pH	-	7.80 ± 0.16
TS	mg/l	2271.87± 3.97
VS	mg/l	703.95± 1.40
BOD_5	mg/l	777.57 ± 4.58
COD	mg/l	1549.79 ± 2.14
DO	mg/l	1.49 ± 0.29
TP	mg/l	3.47 ± 0.10
TK	mg/l	13.40 ± 1.84
TN	mg/l	16.97 ± 0.88
TC	col/100ml	$307*10^4$
FC	col/100ml	$181*10^4$

col = *colonies, SD=standard deviation*

4.1.2 Characterization of the macro algae

The physiochemical characteristics of MA used in the study were determined and the experimental results are tabulated in Table 4.2. TS of the MA was 1979.48 ± 6.48 mg/l and its VS was 720.13 ± 3.48 mg/l. The value of COD of the MA was 61.73 ± 0.21 mg/l.

Table 4.2 Composition of raw macro algae

Parameters	Unit	values (mean ± SD)
pH	-	8.20 ± 0.08
TS	mg/l	1979.48 ± 6.48
VS	mg/l	720.13 ± 3.48
BOD_5	mg/l	40.67 ± 0.85
COD	mg/l	61.73 ± 0.21
DO	mg/l	10.28 ± 0.42
TP	mg/l	4.62 ± 0.49
TN	mg/l	59.63 ± 0.70

The characteristics of WW as main substrate and the MA as co-substrate mixed in different mix ratio were analysed and the results are displayed in table 4.3. When the mixed substrates were characterized, the mean value of the TS of 3:1 mix by volume was 2032.68 ± 4.72 mg/l and that of 3:2 mix of WW to MA was 1711.93 ± 4.38 mg/l; the VS of the two mixes were 634.43 ± 2.68 mg/l and 564.75 ± 5.83 mg/l, respectively. The COD of the mixture were 1390.38 ± 3.35 mg/l and 1292.37 ± 4.12 mg/l for 3:1 and 3:2 mix ratio respectively. From table 4.3, the C/N of WW before digestion is about 41:1 which shows the deficiency of nutrients for efficient AD. From table 4.2, the C/N ratio of MA is about 13:1, which is more than the nutrient values needed for anaerobes to carryout AD. Hence, mixing WW and MA, C/N ratio has improved as the mix ratio increases.

Table 4.3 Characteristics of blends of WW and MA at different mix ratio before digestion

Parameters	Unit	WW alone	WW: MA (3:1)	WW: MA (3:2)
pH	-	7.80 ± 0.16	7.77 ± 0.09	7.93 ± 0.12
TS	mg/l	2271.87 ± 3.97	2032.68 ± 4.72	1711.93 ± 4.38
VS	mg/l	703.95 ± 1.40	634.43 ± 2.68	564.75 ± 5.83
BOD_5	mg/l	777.57 ± 4.58	702.94 ± 3.95	652.03 ± 2.15
COD	mg/l	1549.79 ± 2.14	1390.38 ± 3.35	1292.37 ± 4.12
DO	mg/l	1.49 ± 0.29	2.03 ± 0.08	2.30 ± 0.14
TP	mg/l	3.47 ± 0.10	3.99 ± 0.02	3.96 ± 0.06
TK	mg/l	13.40 ± 1.84	12.61 ± 1.16	11.28 ± 0.65
TN	mg/l	16.97 ± 0.88	25.44 ± 0.78	28.21 ± 1.69
TC	col/100ml	$307*10^4$	$301*10^4$	$298*10^4$
FC	col/100ml	$181*10^4$	$178*10^4$	$171*10^4$

Col = colonies, SD= standard deviation

4.1.3 Characterization of the feedstocks after digestion

The physicochemical and bacteriological characteristics of the feedstocks after the digestion processes are expressed in table 4.4. TS s of 1342.33 ± 1.93 mg/l, 1161.41 ± 2.01 mg/l and 973.91 ± 2.52 mg/l for WW, 3:1, and 3:2 mix ratio were reported respectively. The values of VS after digestion processes were 503.97± 2.74 mg/l for WW alone, 414.13 ± 2.94 mg/l and 338.33 ± 3.31 mg/l for 3:1 and 3:2 mix ratio respectively were recorded.

Table 4.4 Characteristics of feedstock after digestion

Parameters	Unit	WW alone	WW: MA (3:1)	WW: MA (3:2)
pH	-	5.80 ± 0.08	6.03 ± 0.09	6.00 ± 0.08
TS	mg/l	1342.33 ± 1.93	1161.41 ± 2.01	973.91 ± 2.52
VS	mg/l	503.97 ± 2.74	414.13 ± 2.94	338.33 ± 3.31
BOD_5	mg/l	291.70 ± 4.26	216.46 ± 4.30	186.43 ± 3.90
COD	mg/l	583.70 ± 2.60	433.25 ± 2.28	361.99 ± 4.18
DO	mg/l	5.57 ± 0.30	6.30 ± 0.42	6.47 ± 0.38
TP	mg/l	3.07 ± 0.12	3.58 ± 0.10	3.61 ± 0.13
TK	mg/l	10.43 ± 0.69	7.82 ± 0.46	6.85 ± 0.06
TN	mg/l	50.99 ± 0.79	55.10 ± 1.44	60.66 ± 0.61
TC	col/100 ml	$113*10^4$	$68 * 10^4$	$23 * 10^4$
FC	col/100 ml	$64*10^4$	$40 * 10^4$	$12 * 10^4$

Finally, as presented in the table 4.2, 4.3, 4.4, 4.5 and 4.6 the physiochemical and bacteriological characteristics of WW exhibit extreme variations. These variations have also been observed in a number of prior studies and are attributed to several factors such as origin of the waste, type of on-site sanitation system, amount of ageing that has taken place, extent of storm H_2O, temperature and infiltration, and user habit[42]

4.1.4 Temperature, p^H and amount of gas produced measured in volume (L)

The relationship between parameters such as temperature, pH and the amount of gas produced during the processes are expressed on Figure 4.2, 4.3 and 4.4.

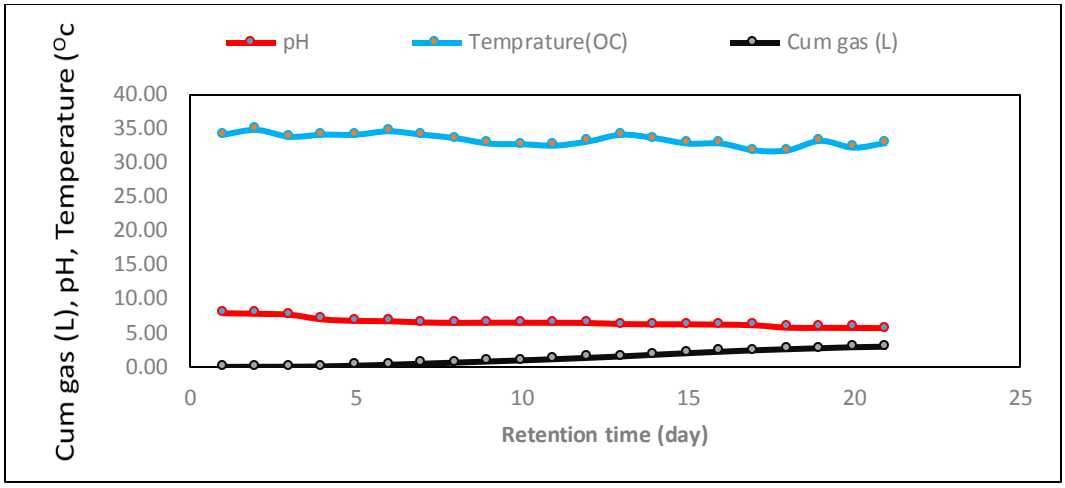


Fig 4.2 Relation between temperature, p^H and amount of gas produced from WW only

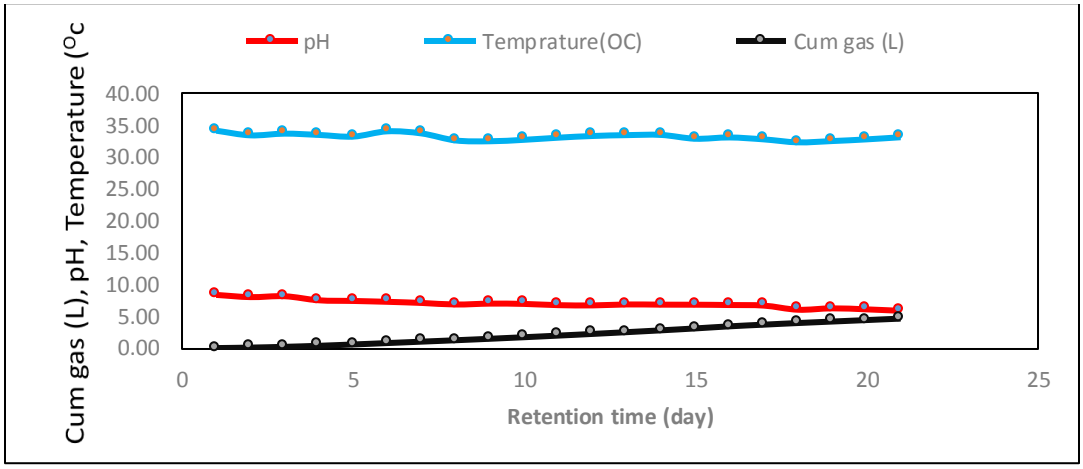


Figure 4.3: Relation between temperature, p^H and amount of gas produced at 3:1 ratio (WW: MA)

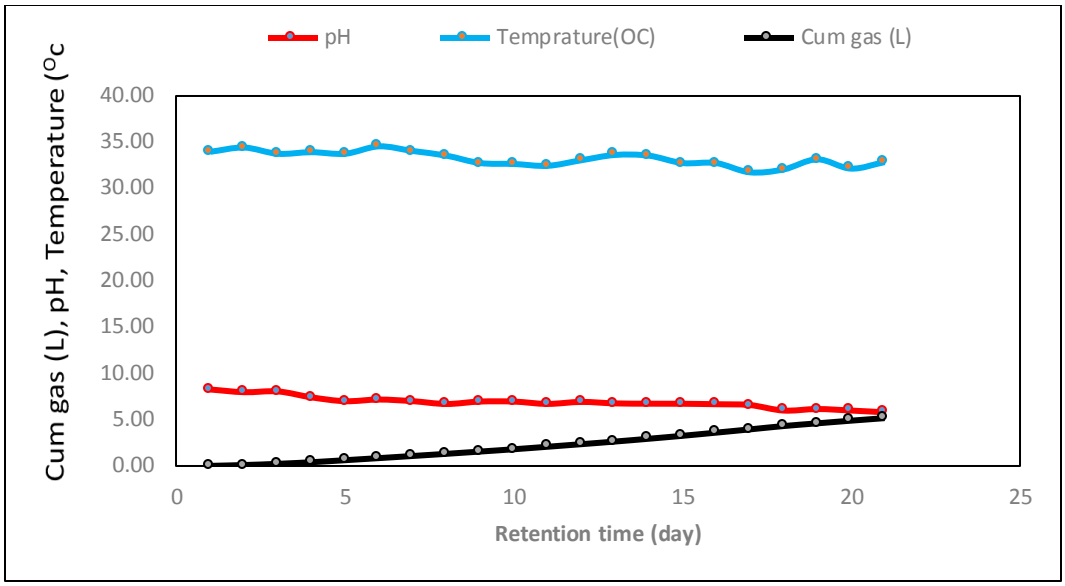


Figure 4.4 Relation between temperature, p^H and amount of gas produced at 3:2 ratio (WW: MA)

The temperature of H_2O bath for the anaerobic digester was set to 35℃. This temperature was continuously maintained until the retention time was completed. However, from the above three figures (Figures 4.2, 4.3 and 4.4), when the pH of the feedstock being digested was checked along the internal temperature of the digester, this temperature is changed with insignificance. This change is due to H_2O bath is open to atmospheric temperature, which influence temperature uniformity. And also due to different structure of digester system there is temperature loss when it circulates between the digester tank and H_2O bath. Gas measured in volume is increased as mix ratio is increased i.e. from WW only to 3:2 (WW: MA). The values were 2.955, 4.631 and 5.150 L for WW only, 3:1 (WW: MA) and 3:2 (WW: MA) respectively measured at the end of 21 days.

4.2 Determination of biogas production for each mix ratios

For the determination of maximum CH_4 in the study from digestion and co-digestion of WW and MA: WW only, 3:1 and 3:2 mixes of WW and MA were used. The cumulative biogases produced during the experimental period are displayed in table 4.5. From the digestion of WW alone: : 0.028 $m^3/d/m^3$ biogas with 39.7% CH_4 was produced; 0.044 $m^3/d/m^3$and 0.049 $m^3/d/m^3$ biogas with 51.7% and 57.9% CH_4 were produced from 3:1 and 3:2 mix ratio of the substrates respectively. More detail is shown on appendix II.

Table 4.5 Percentage composition of biogas produced

Average Volume of gas produced (L)	2.955L	4.631L	5.150L
Percentage mix of MA	0	20	40
CH_4	37.1	51.1	57.4
CO_2	55.4	42.9	38.8
H_2S	16ppm	11ppm	10ppm
O_2	1.8	1.2	0.8
Others	5.7	4.8	3.0

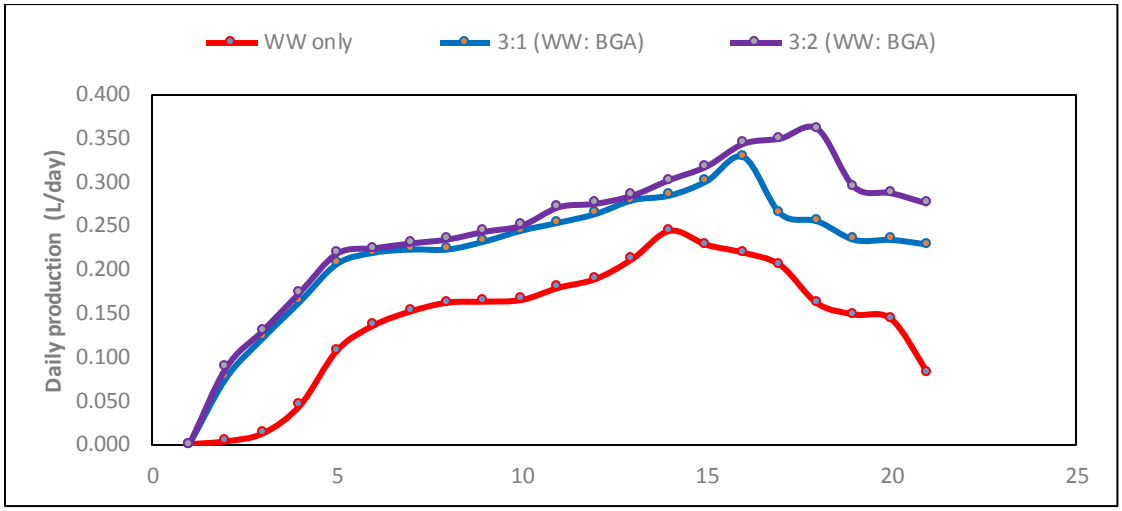


Figure 4.5 Daily production of each mix ratio

From figure 4.5 y-axis is labelled by amount of biogas produced daily in L/day and x-axis is labelled retention time in day. As in indicted the amount of biogas produced is increased more up to 3:2 (WW: MA) mix ratios. This was because more food was available for bacteria to degrade organic compound.

4.3 Identification of optimum mix ratio for maximum biogas production

The rate of production of biogas was measured by H_2O displacement and the volumes of the biogas collected were recorded during the experiment period. The production of biogas was used mainly as an indication of the progress of the digestion process. The cumulative biogas produced for the digestion of WW and its Codigestion (MA) was indicated in Table 4.5 and is elaborated as gas composition in Figure 4.7. The quantity and quality of biogas

produced with different mix ratio of WW to MA (WW only, 3:1 and 3:2) are shown in table 4.7. Starting from WW only to 3:2 (WW: MA), there is slightly increment of biogas. This might be because of the replacement of nutrients lost from WW from MA. The values of daily production are shown on appendix IV.

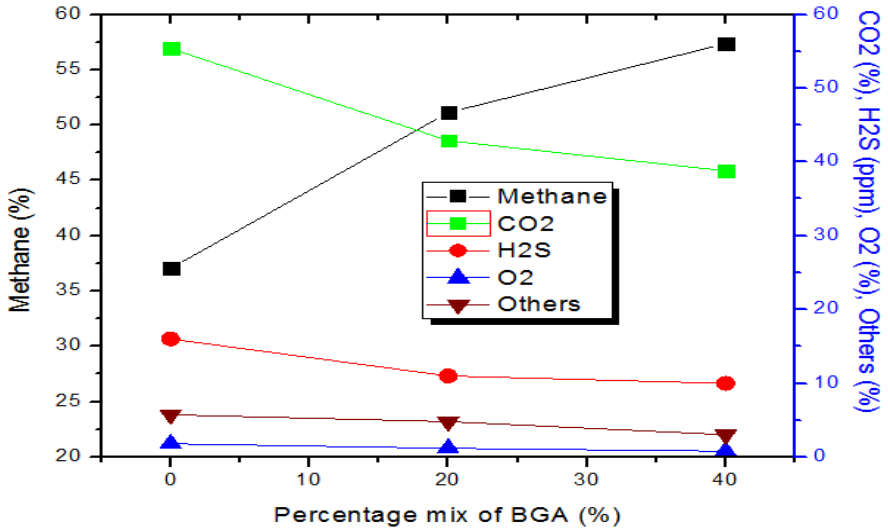


Figure 4.6 Composition of gas produced per mix ratios of MA

4.4 Reduction percentage of the physicochemical characteristics of WW after digestion

Physiochemical and bacteriological properties after digestion of feedstock analysed in this study was discussed in detail as follows:

4.4.1 Total solids (TS)

Reduction of TS by 40.91%, 42.86% and 43.11% of the feed stock for WW, 3:1 and 3:2 mix of WW to MA, were observed respectively. This shows that there is a slightly increase in the removal efficiency of TS as the mix ratio of WW and MA increases.

4.4.2 Volatile solids (VS)

As seen from table 4.4, reductions in VS are also observed with the following percentages: 28.41%, 34.72% and 40.09% for WW alone, 3:1 and 3:2mix of WW to MA respectively. From the reduction percentage of TS and VS, it can be concluded that co-digestion can

reduce the area, which is covered by dry cake in the oxidation pond of JiT, Jimma University.

4.4.3 Chemical Oxygen Demand (COD)

Considerable removal efficiencies of COD were generally observed on WW and MA digestion with the average efficiency of 62.34%, 68.84%, and 71.99% for WW, 3:1 mix of WW to MA and 3:2 mix WW to MA respectively. The COD removal efficiencies over the duration of the experiment were comparable to those reported in the literature ranging from 60-75%. Overall, the high removal efficiencies for COD are a good indication of the fact that the AD under proper operating conditions can be used for the pre-treatment of WW before the conventional WW treatment plant.

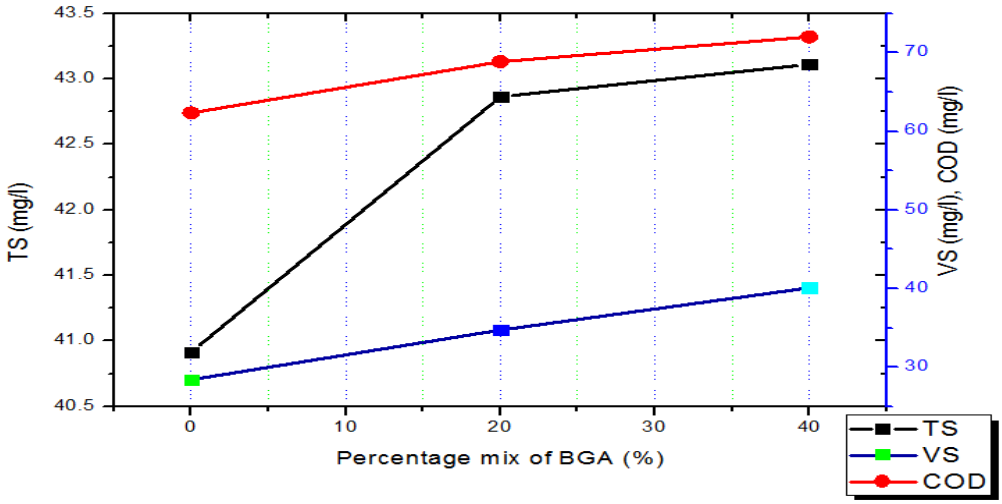


Figure 4.7 Removal efficiency of TS, VS and COD at different mix ratios of MA

4.4.4 Biological Oxygen Demand (BOD_5)

The percentage reduction of BOD_5 were generally observed on WW and MA digestion with the average efficiency of 62.5%, 69.2%, and 71.5% for WW only, 3:1 mix of WW to MA and 3:2 mix WW to MA respectively. The BOD_5 removal efficiencies over the duration of the experiment were almost justify the formula from literature $\frac{BOD_5}{COD} = 0.5$ [38].

4.5 Pathogen

The digestion significantly reduce the coliform bacteria: 92.28% for TC and 92.98% for FC at 3:2 mix ratio; this indicates that the original number of bacteria are died during digestion process for gas production and elaboration is indicated on appendix III.

4.6 Biogas Quality Analysis

The biogas quality was analysed by biogas analyser of model 5000. The biogas quality depends on the gas components. If the composition of CH_4 is maximum biogas considered as high quality because of flammability and combustibility of biogas depends on CH_4 composition. Increasing quantity of CH_4 increases heat value of biogas. In other ways with increasing quantities of CO_2 in biogas decrees heat values of biogas. Therefore, as indicated from the figure 4.5 the quality of biogas increase as mix ratio increase.

4.7 Estimation of fertilizers values

The term fertilizer often refers to any mixture containing all three important elements listed as N (nitrogen), P_2O_5 (phosphate equivalent) and K_2O (potash equivalent). Urea contains 46% N and grade as (46-0-0) and its current price is 740 ETB per 100 kg. Diammonium phosphate (DAP) grade as (18-46-0) contains 18% N and 46% P_2O_5 [42] and its current price is 800 ETB per 100 kg. Potash grade is (0-0-60) contains 60% K_2O and its price is 620 ETB per 100kg.

Nitrogen Fertilizer

Equivalent urea for nitrogen content in WW after digestion is given by:

$$Mass\ of\ Urea = \frac{mass\ of\ nitrogen}{0.46}\ [42] \qquad 4.1$$

$$Mass\ of\ nitrogen\ in\ wastewater = conc. * rate\ of\ flow \qquad 4.2$$

$$= 60.66\ mg/l\ \times 36\ m^3/d.$$

Assuming 365 days in a year, the mass of nitrogen is 797.07 kg/year. Mass of urea is therefore divide the value by 0.46 and equals 1732.77 kg/year. This save the capital to pay urea that cost 12,822.50 ETB be obtained from urea produced during the process.

Phosphorus fertilizer as P_2O_5

Equivalents DAP for phosphorus as P_2O_5 content in WW after digestion is given by:

$$\text{Mass of DAP} = \frac{\text{mass of phosphorus as } P_2O_5}{0.46} \text{ [42]} \quad 4.3$$

Mass of phosphorus in the WW = 3.61mg/l x 36 m^3/d x 365day/year = 47.44 kg/year. Mass of P_2O_5 after digestion is given by using conversion factor 0.748 which is equal to 35.49kg/year; then mass of equivalent DAP is 77.14kg/year and the price is equal to 617.15 ETB. Mass of nitrogen in the DAP is 13.89kg/year which is 102.75 ETB; therefore, the price of phosphorus fertilizer as P_2O_5 is 617.15 ETB-102.75 ETB= 514.40 ETB

Potash fertilizer

Potash fertilizers are quantified by their K_2O equivalent. Mass of potassium in the WW after digestion as K_2O using the conversion factor 1.21 from potassium to K_2O [42] =6.85 mg/l x1.21x 36 m^3/d x 365 days/year = 108.91kg/year. The K_2O equivalent of the mass of potash fertilizer is 174.26kg/year and which is equal to 1080.40 ETB.

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

WW are loaded by organic portion which contains the most valuable elements, carbon, for the formation of CH_4 whereas abattoir effluents contain excess valuable nutrients for anaerobes which lead the co-digestion of the two wastes to high degree of methanization process.

As discussed in the chapter four, the mix ratio (60% by volume of WW to 40% by volume of MA) was observed to produce the maximum quantity of biogas with the maximum percentage of CH_4. This shows that co-digestion of WW, MA enhance the quality, and quantities of CH_4 yield.

The average percentage removal of TS, VS, and COD increases with the mix ratio of the WW and its co-substrate (MA). From the average percentage reduction of those parameters, it can be concluded that there is a reduction in the volume of the waste if anaerobic digester is executed at the oxidation pond of the JiT.

The experimental results showed that, using AD of WW considerable amount of CH_4 can be captured from being emitted into atmosphere so as to prevent greenhouse effect. Into the bargain, valuable energy and high quality organic fertilizers can also be obtained from AD of WW and MA.

5.2 Recommendations

From the findings of this research study, AD technology is recommended for the efficient handlings of ever increasing WW oxidation pond site. MA are suggested to be used as co-substrate for the efficient digestion of WW. However, yet better appropriate co-substrate should be searched and tested to increase the feasibility of the biogas production from WW.

Pilot biogas plant is recommended to be constructed on site with the same operating scheme done at laboratory scale and its effectiveness under different conditions and

seasons should be tested before the execution of this project. In contrast to batch process used in this study, further study is recommended to be done and its effectiveness has to be checked by using continuous process.

Repeatedly researches are also recommended to be carried out in order to assure the feasibility of the process.

5.3 Further Investigations

As observed from this study, biogas generation rate was increase with the mix ratio of MA with WW. This can be as results of increase in dilution of the mixture, which can facilitate sympathetic environment for anaerobes or because of the addition of nutrients to the WW. Hence, therefore, further study is needed in order to justify the reason behinds the increments of biogas generation with mix ratio of MA with WW. In addition, to use the produced CH_4 for different purpose as automobile further qualification is required in order to remove other gases.

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APPENDICES

Appendix I: The average values of temperature, p^H and amount of gas produced during experiment

Days	Mix ratios								
	WW only			3:1(WW:MA)			3:2 (WW:MA)		
	p^H	T (^{0}C)	Gas, L	p^H	T (^{0}C)	Gas, L	p^H	T (^{0}C)	Gas, L
1	7.87	34.07	0.000	8.36	34.17	0.000	8.24	33.90	0.000
2	7.78	34.77	0.003	8.01	33.43	0.076	7.95	34.36	0.088
3	7.64	33.77	0.016	8.15	33.70	0.198	8.03	33.69	0.218
4	6.95	34.07	0.061	7.51	33.50	0.361	7.40	33.86	0.392
5	6.74	34.07	0.168	7.40	33.23	0.567	6.98	33.69	0.610
6	6.69	34.57	0.304	7.27	34.07	0.786	7.16	34.49	0.834
7	6.52	34.07	0.456	7.10	33.73	1.009	6.98	33.99	1.064
8	6.45	33.57	0.618	6.85	32.63	1.232	6.68	33.49	1.298
9	6.47	32.77	0.781	6.99	32.50	1.464	6.94	32.69	1.541
10	6.47	32.67	0.946	6.94	32.73	1.708	6.94	32.59	1.791
11	6.45	32.47	1.125	6.74	33.07	1.961	6.69	32.39	2.062
12	6.43	33.07	1.314	6.71	33.33	2.225	6.90	32.99	2.337
13	6.28	34.07	1.526	6.86	33.47	2.504	6.74	33.56	2.621
14	6.24	33.57	1.770	6.82	33.50	2.788	6.71	33.49	2.923
15	6.23	32.77	1.998	6.81	32.90	3.089	6.70	32.69	3.241
16	6.18	32.77	2.217	6.76	33.10	3.417	6.65	32.69	3.584
17	6.10	31.77	2.422	6.68	32.80	3.681	6.56	31.69	3.934
18	5.74	31.77	2.583	6.03	32.33	3.936	5.97	31.99	4.294
19	5.72	33.17	2.731	6.25	32.53	4.169	6.13	33.09	4.588
20	5.71	32.17	2.874	6.08	32.80	4.403	5.97	32.09	4.875
21	5.67	32.87	**2.955**	5.85	33.13	**4.631**	5.78	32.79	**5.150**

Appendix II: Calculation of daily biogas production

Six litter sample was feed into the digester out of which one litter is starter (inoculums).

$$gas\ volume\left(\frac{\frac{m^3}{d}}{m^3}\right)=\frac{\left(\frac{gas\ produced(L)}{retention\ time(day)}\right)}{feedstock\ volume\ (L)} \quad \text{A1}$$

2.955L/21d biogas was produced from 5L (6L- 1 L) WW feed only.

2.955L x $10^{-3}m^3$/21d/5 x $10^{-3}m^3$ = **$0.028 m^3/d/m^3$** biogas was produced from WW only

4.631x $10^{-3}m^3$/21d/5 x $10^{-3}m^3$ = **$0.044\ m^3/d/m^3$** biogas was produced from 3:1

5.150x $10^{-3}m^3$/21d/5 x $10^{-3}m^3$ = **$0.049\ m^3/d/m^3$** biogas was produced from 3:2

Note: ($1m^3 = 10^3$ L)

Appendix III: Record of pathogen reduction

Pathogen	Reduction of pathogens (col/100ml)					
	0% MA		20% MA		40% MA	
	BD	AD	BD	AD	BD	AD
TC	$307*10^4$	$113*10^4$	$301*10^4$	$68*10^4$	$298*10^4$	$23*10^4$
FC	$181*10^4$	$64*10^4$	$178*10^4$	$40*10^4$	$171*10^4$	$12*10^4$

Appendix IV: Table that show daily production of biogas

R.T (day)	Average daily production (L)					
	WW only		3:1 (WW: MA)		3:2 (WW: MA)	
	Cum	Daily	Cum	daily	Cum	Daily
1	0.000	0.000	0.000	0.000	0.000	0.000
2	0.003	0.003	0.076	0.076	0.088	0.088
3	0.016	0.013	0.198	0.122	0.218	0.130
4	0.061	0.045	0.361	0.163	0.392	0.174
5	0.168	0.107	0.567	0.206	0.610	0.218
6	0.304	0.136	0.786	0.219	0.834	0.224
7	0.456	0.152	1.009	0.223	1.064	0.230
8	0.618	0.162	1.232	0.223	1.298	0.234
9	0.781	0.163	1.464	0.232	1.541	0.243

10	0.946	0.165	1.708	0.244	1.791	0.250
11	1.125	0.179	1.961	0.253	2.062	0.271
12	1.314	0.189	2.225	0.263	2.337	0.275
13	1.526	0.212	2.504	0.279	2.621	0.284
14	1.770	0.244	2.788	0.284	2.923	0.302
15	1.998	0.228	3.089	0.301	3.241	0.318
16	2.217	0.219	3.417	0.328	3.584	0.344
17	2.422	0.205	3.681	0.264	3.934	0.350
18	2.583	0.161	3.936	0.255	4.294	0.361
19	2.731	0.148	4.169	0.234	4.588	0.294
20	2.874	0.143	4.403	0.234	4.875	0.287
21	2.955	0.081	4.631	0.228	5.150	0.275

Appendix V: Laboratory procedures

a) Total and Faecal coliform (TC and FC)

1) Red Alcohol (C_2H_5OH) is used for hand to hold any equipment in this method, because bacteria may transfer from hand to equipment.
2) Sterilize all the equipment used (forceps, measuring cylinder, pipette, membrane filtration petri-dish, and membrane filtration apparatus) in the Autoclave sterilizer by steam at 120^0C for 15min to remove all bacteria exist, and even the H_2O to be used must be sterilized.
3) Dilute the sample with sterilized H_2O dilution factor of 100,000x (0.1ml sample with 9999.9ml of sterilized cooled H_2O).
4) Mix the powder form of membrane filtration media with appropriate volume of sterilized H_2O (72.9g =1000ml ratio) to prepare the food for bacteria.
5) Prepare filter paper and filter pad.
6) Insert filter paper in the membrane filtration apparatus and pour step 3 in to it. Then open vacuum pump to filtrate it down.
7) Insert filter pad in to membrane filtration petri-dish and pour step 4 using pipette.
8) Pick out filter paper from step 6 and put on step 7 in the petri-dish.

9) Incubate for 37^0C (for 24hr-48hr) to count spore formed for TC and 44.5^0C (for 24hr) for FC and write the colonies/100ml and record in the chapter four.

*Col/100ml = No. of count * dilution factor (D.f = 10^4 for calculation)*

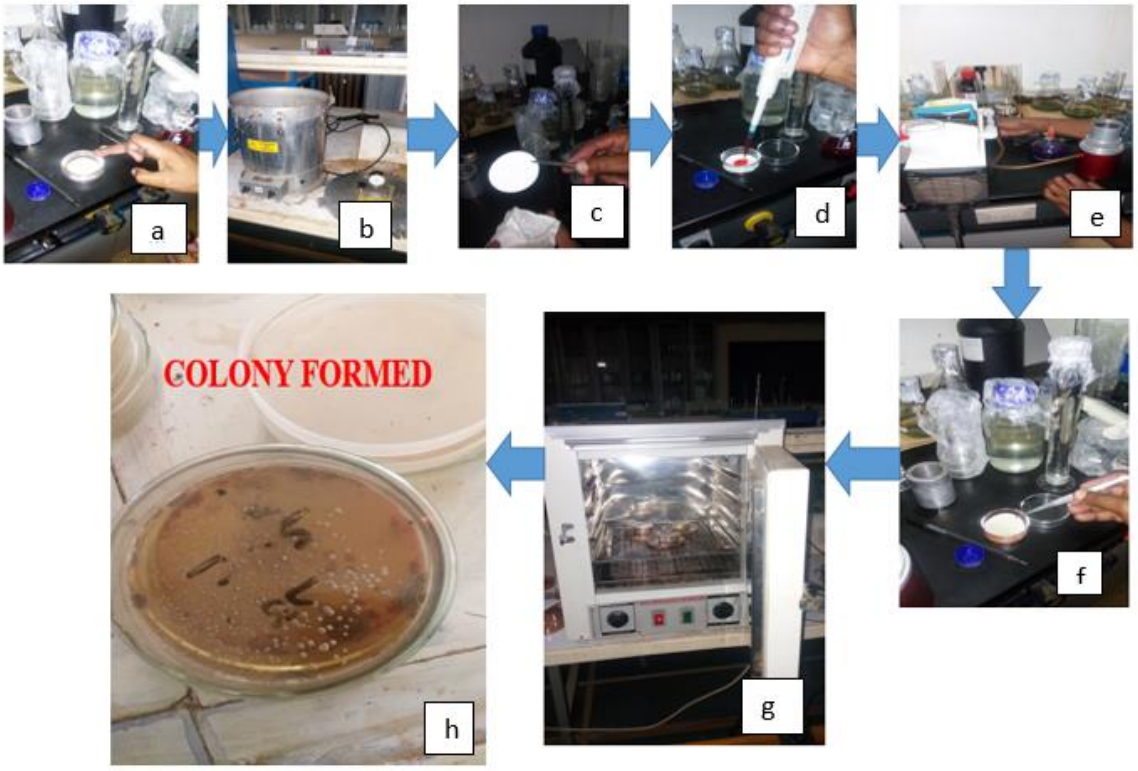

Figure A1 Membrane filtration to determine coliforms set up

a) petri-dish preparation b) Autoclave c) filtration paper d) addition of media to petri-dish e) vacuum pumping f) additional paper into dish g) Incubator h) colony formed

b) Total Nitrogen (TN)

1. Take 0.2ml of sample, 2.3ml of total nitrogen reagent A and 1 piece of total nitrogen reagent B to the empty LCK 338 (kit) and then digest in the HATH LANGE LT 200 digester for 1hr at 100°c.
2. After digestion completed add 1 Microcap from reagent C and shake the kit until only plastic part of Microcap is left.
3. Take another LCK 338 contain chemical produced by manufacturer and add 0.5ml of step 2 above.
4. Add 0.2ml of total nitrogen reagent D to step 3 above and shake quick for a short period, and stay for 15minutes for cooling purpose.

5. Then finally, read TN automatically using spectrophotometer DR 5000 by inserting LCK 338 of step 4.

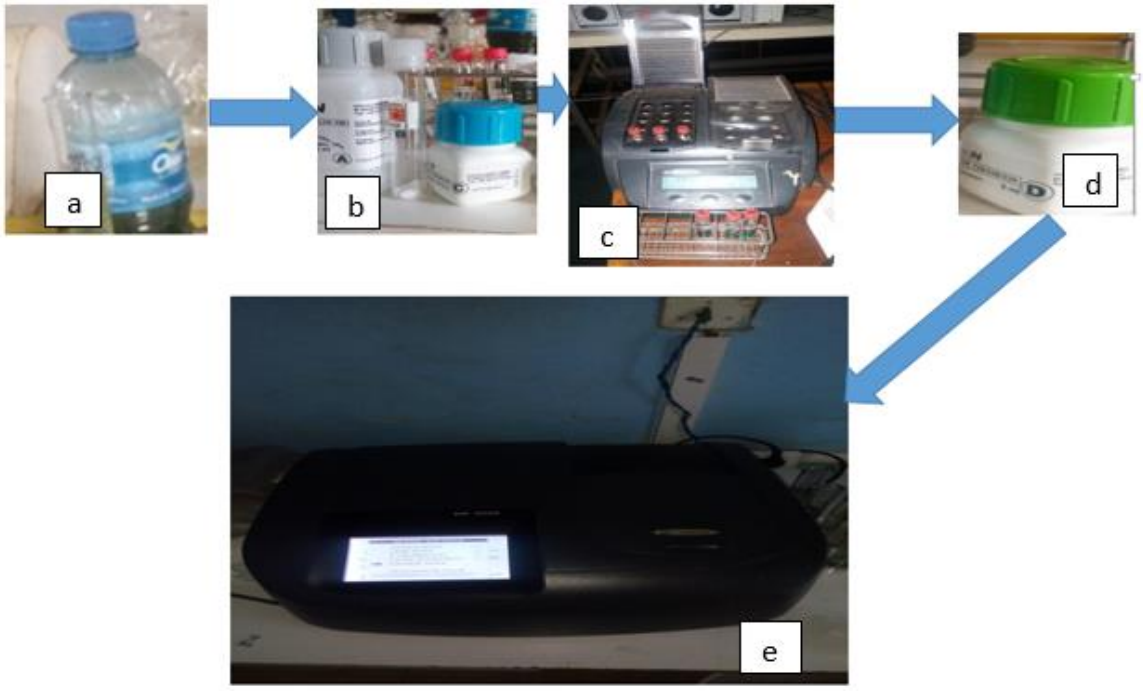

Figure A2 Spectrophotometer DR 5000

a) Sample b) reagent A and C c) TN digester d) reagent D

e) Spectrophotometer

c) Chemical oxygen demand (COD)

1. Bring the sediment into suspension by inverting a few times
2. Carefully pipette 2.0 mL sample.
3. Close cuvette, thoroughly clean the outside.
4. Heat in the thermostat HT 200 S in standard program HT for 15 min.
5. Remove the hot cuvette from HT 200 S after the lock opens, carefully invert twice.
6. Allow to cool to room temperature in the HT 200 S, in the thermostat
7. Sediment must be completely settled before evaluation is carried out. Clean the outside of the cuvette and evaluate using spectrophotometer.

Figure A3 COD measuring equipment

d) Total phosphorus (TP)

1. Carefully remove the foil from the screwed on the Dosicap Zip.
2. Unscrew the Dosicap Zip
3. Pipette 0.4ml sample.
4. Screw the Dosicap Zip back; fluting at the top.
5. Shake firmly.
6. Heat in the thermostat at 100^0C for 60min.
7. Pipette into the cooled cuvette: 0.5ml reagent B (LCK 350 B) and close reagent B immediately after use.
8. Screw a grey Dosicap C (LCK 350 C) onto the cuvette.
9. Invert a few times. After 10min invert a few times more, thoroughly clean the outside of the cuvette and evaluate the total phosphorus available using spectrophotometer model DR 500.

Figure A4 Thermostat digester and spectrometer used in phosphorus determination

e) Total potassium (TK)

1. Take a 100-ml sample and concentrate its potassium content by evaporation until about only 5 ml remain. Transfer this concentrated sample to a 25-ml centrifuge tube and make up to 10.0 ml with deionised distilled H_2O.
2. The reaction is dependent on time and temperature, so both of these should be kept reasonably constant for all samples and standards in a series of tests: 15 minutes and 5°C.
3. At room temperature add, with mixing, 1 ml of the 1 mol L^{-1} nitric acid and 5 ml of the trisodium cobalt nitrite solution. Let stand for 2 hours.
4. Centrifuge for 10 minutes. Carefully pour off the liquid and wash the precipitate with 15 ml of the 0.01 mol L^{-1} nitric acid. Mix with a small glass stirring rod to ensure contact between the precipitate and the wash solution.
5. Centrifuge again for 10 minutes. Pour off the liquid and add, with mixing, 10.00 ml of standard potassium dichromate solution and 5 ml concentrated sulphuric acid.
6. Cool to room temperature. Make up to 100 ml with deionised distilled H_2O. If the solution is turbid, filter it into a Nessler tube and make up to 100 ml.
7. Preparation of standards. Pipette portions of 1, 2, 3, 4, 5, 6 and 7 ml of the standard potassium solution into a series of 25-ml centrifuge tubes, and make up to 10 ml with deionised distilled H_2O. Treat all tubes in the manner described for the sample in steps 3 to 6 above to obtain colour standards containing 1.00 to 7.00 mg K.
8. Calibration curve with absorbance plotted against mg K and the absorbance of the sample is measured and spectrophotometer determine the concentration of potassium from the calibration curve.

f) TS and VS

1. Ignite clean evaporating dish at 550 ^{0}C for 1hr in a furnace for VS
2. Heat clean dish to 103 to 105 ^{0}C for 1hr
3. Store and cool dish in desiccator until needed
4. Weigh immediately before use
5. Pipet a measured volume of well mixed sample to a pre-weighed dish
6. Evaporate to dryness on drying oven

7. If necessary add successive sample portions to the same dish after evaporation
8. Cool dish in desiccator to balance temperature, and weigh
9. Ignite the residue produced by method 2540B in a muffle furnace
10. Transfer to a desiccator for final cooling in a dry atmosphere

Calculation:

$$\text{mg TS /L} = \frac{(A-B)}{\text{sample volume,ml}} * 100 \qquad \text{A2}$$

where: A = weight of dried residue + dish, mg

B = weight of dish, mg

$$\text{mg VS /L} = \frac{(C-D)}{\text{sample volume,ml}} * 100 \qquad \text{A3}$$

where: C = weight of dried residue + dish before ignition, mg

D = weight of residue + dish after ignition, mg

Figure A5: Thermo Scientific Furnace of model FB1410M-33

g) BOD

1. Measure the sample precisely using appropriate over flow and if necessary add nitrification inhibitor (ATH)
2. Insert magnetic stirring rod
3. Place 3-4 drop of KOH solution into the seal gasket and insert gasket in the neck of the bottle
4. Screw the BOD sensors to the sample bottle
5. Place the bottle in the bottle rack

6. Start the measurement
7. Incubate the sample in accordance with the instructions BOD_5 for 5 days at 20^0C
8. Measure after 5 days in spectrophotometer

Calculation is by equation 2.1 and 2.2