ADDIS ABABA UNIVERSITY

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SCHOOL OF CHEMICAL AND BIO-ENGINEERING

ANAEROBIC CO-DIGESTION OF SLAUGHTERHOUSE WASTEWATER WITH WATER HYACINTH (*Ecchornia crassipes*) FOR BIOGAS PRODUCTION USING RUMEN FLUID AS INOCULUM: CHARACTERIZATION AND PARAMETRIC OPTIMIZATION

BY

RAKEB KIFLE

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BY

RAKEB KIFLE ALEMU

A thesis submitted as a partial fulfillment to the requirements for the award of the degree of Master of Science in environmental engineering

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Approval Page

This is to certify that the thesis prepared by Ms. Rakeb Kifle Alemu entitled "Anaerobic Co-Digestion Of Slaughterhouse Wastewater With Water Hyacinth (*Eccharnia crassipes*) For Biogas Production Using Rumen Fluid As Inoculum: Characterization And Parametric Optimization" and submitted as a partial fulfillment for the award of the Degree of Master of Science in Environmental Engineering complies with the regulations of the university and meets the accepted standards with respect to originality, content and quality.

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Declaration

I hereby declare that this thesis entitled “Anaerobic Co-Digestion of Slaughterhouse Wastewater with Water Hyacinth (*Ecchornia crassipes*) for Biogas Production Using Rumen Fluid as Innoculum: Characterization and Parametric Optimization” was prepared by me with guidance of my advisor. The work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted, in whole or in part, for any other degree or professional qualification.

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Abstract

The purpose of the present study was to characterize and optimize the co-digestion of Slaughterhouse wastewater with Water Hyacinth (WH) for biogas production using rumen fluid as inoculum. The slaughterhouse wastewater and water hyacinth were first characterized to determine their potential for biogas production. Water Hyacinth reducing sugar content was determined (11.94 g/L) using dinitrosalicylic acid (DNSA). Co-digestions were carried out in batch reactors. The effects of substrate composition (ratio of WH to SWW), hydraulic retention time and pH on methane production and COD removal efficiency were investigated and optimized using RSM-Optimal (custom) design. Accordingly, the optimum methane production and COD removal efficiency were found to be 76.2% and 59.1%, respectively at HRT of 40 days, a substrate composition of 50%SWW: 50%WH and a pH of 7. Furthermore, the bio-digestate was investigated for fertilizer potential. Results show its nutrient values were below the FAO suggested values. FAO suggests a minimum of 5% sum (N+P+S) for an organic fertilizer. Results show that co-digestion of SWW with WH is promising way for producing biogas and simultaneously to control the spread of WH. In addition, it is suggested to blend the bio-digestate with organic manure for enhancement of fertilizer potential.

Keywords: Anaerobic Co-digestion, Slaughterhouse waste water, Water hyacinth, Rumen fluid

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List of Abbreviation and Acronyms

APHA	American Public Health Association
AD	Anaerobic Digestion
BBD	Box-Benkhen Design
BOD	Biochemical Oxygen Demand
BR	Blending Ratio
CCD	Central Composite Design
COD	Chemical Oxygen Demand
FAO	Food and Agriculture Organization
FS	Fixed Solid
FTIR	Fourier Transform Infrared spectroscopy
GC	Gregorian Calendar
HRT	Hydraulic Retention Time
IEA	International Energy Agency
K	Potassium
Mg/L	Milligrams per Liter
N	Nitrogen
OLR	Organic Loading Rate
P	Phosphorus
pH	Power Of Hydrogen
ppm	Parts Per Million
RSM	Response Surface Methodology
S	Sulfur
SWW	Slaughterhouse Waste Water
TS	Total Solid
UASB	Up-flow Anaerobic Sludge Blanket

VP	Substrate Volume Percentage
VS	Volatile Solid
WH	Water Hyacinth
%(w/w)	Weight percent or percent weight of solute by weight of solution
%(v/v)	Volume percent or percent volume of solute by volume of solution
%(w/v)	Percent weight of solute by volume of solution

1. Introduction

1.1 Background

The advance of fast industrialization, global population growth followed by transformation of life style give way for escalating the rate of global energy consumption. It was reported that global energy demand rose by 2.3% in 2018 which was the fastest speed in decades. In order to meet this increasing global energy need, the demand for all fuel sources has risen drastically. Fossil fuel provides the majority of world energy and has risen to its ultimate point than before. This augmented utilization of fossil fuel has in turn amplified the release of CO_2 to the environment. In 2018 alone, global energy-related CO_2 emissions rose by 1.7% amounting to 33 Giga tons (Gt) as compared to 1.6% in the previous year (IEA report, 2019; Industrial Development Report, 2018). Carbon dioxide released from fossil fuel sources is a key constituent of greenhouse gas sources. The escalating level of CO_2 in the environment causes an excess of greenhouse gases that trap extra heat resulting to the speedy increase in surface temperature with exceptionally pronounced warming trend currently. This trapped heat eventually results in melting of ice caps, glaciers, rising of sea and ocean levels that cause flooding, devastating and irrecoverable weather pattern.

Since fossil fuel is the major source of CO_2 emission to the environment, use of alternative renewable energy sources with net zero or reduced carbon dioxide emission is necessary to lower the effect of greenhouse gases. Currently, there is a hopeful interest in the use of renewable energy sources both in developing and developed nations; however, its global energy portion is still the lowest. According to International Energy Agency (IEA) report, renewable energy sources comprise only about 11% of global energy demand. Among renewable energy sources, biofuel covers the greater part of global renewable energy supply which is about 77%. Biomass, obtained by photosynthesis, is a multipurpose feedstock available in different parts of the world. Biomass as biofuel can be used for heating and cooking purposes without further processing in most rural areas of developing nations or it can be transformed into solid, liquid or gaseous biofuel to enhance its fuel efficiency and diversity. Biofuels include bioethanol, biogas, biodiesel and bio-hydrogen (Robak & Balcerek, 2018).

Biofuels are produced from organic materials derived from biological origin called biomass. Biomass feedstocks can be obtained from plants like algae, crops and trees or from organic wastes municipal solid waste, industrial waste, animal manure and aquatic plants. These biomass feedstocks can be converted to three end products like electrical/heat energy, transport fuel and chemical feedstocks. The main compositional components of biomass are cellulose, hemicellulose and lignin (McKendry, 2002). Among biofuel, bioethanol (CH_3CH_2OH) is the most widely used commercial fuel. It is used for complementing petrol as a liquid biofuel either in pure form or blended with petrol at different proportions. In US and Brazil more than 70% of petrol vehicles, including nearly all new passenger cars, can use a petrol blend containing up to 27% of ethanol (E27). Bioethanol can be first, second or third generation bioethanol based on its feedstock sources and technology employed during production (Robak & Balcerek, 2018).

Biogas is another type of biofuel that is obtianed by anaerobic digestion of different biomass like animal waste, municipal wastes, agricultural waste and industrial wastes. Anaerobially digesting feedstocks has been proven to be an eco-friendly or environmetally friendly porcess and cost-effective as well. Unfortunately, the technology has suffered a set back in its development, especially in Sub Saharan African countries where energy is scarce , due to the lack of information on its economic benefit and the incompetence of the governments to set energy policies eventhough promising conditions are available for the technology promotion (Mohammed, Egyir, Donkor, Amoah, & Nyarko, 2016).

Waste management has become one of the most significant problems of our time. Solid waste of over 5.84 million tons is produced annually in urban areas (Rouf et al., 2016). According to Tchobanoglous et al.(2002), solid wastes include wastes that are produced from human activities which are wastes from commercial, industrial, residential, institutional, costruction and demolition, treatrment plant site, municipal sevices and agriculture. One way of managing these wastes can be anaerobically digesting them into biogas. As to other renewable energy sources, this conversion process requires less capital and per unit production cost (Rao, Baral, Dey, & Mutnuri, 2010).

Slaughterhouse or abbattoir waste is another form of waste that needs to be managed. Slaughterhouses generate large volumes of wastewater. The total fresh water consumed by the meat processing industry is about 24% of the food and beverage industry consumption and 29% of the agricultural sector consumption, world wide. If this wastewater is not properly managed it will pollute the surrounding environment and in turn affect the society (Bustillo-lecompte & Mehrvar, 2015).

Untreated slaughterhouse wastewater contains a mixture of fats, proteins and fibers, creating a high content of organic matter and results a polluting impact on the rivers and sewage systems. It also upsurges nitrogen, phosphorus, solids and BOD_5 levels of the recipient water bodies, potentially leading to eutrophication. However, the wastewater from the slaughterhouses are, directly or indirectly, dischared into the environment without treatment (Mulu, 2003). It was studied and proven that this negative impact can be converted into a positive energy by anaerobic digestion of the slaughterhouse wastewater into biogas and a good outcome can be achieved (Temsgen & Ketema, 2020).

Other types of biomass are aquatic plants. Among these, WH an aquatic weed that floats over freshwater, has an attractive blue, lilac to purplish flowers and round to oval leaves. It is native to Brazil and Ecuador region (Bhattacharya & Kumar, 2010). It is an invasive plant that reproduces very fast producing over thousands of seeds each year. It has broad, thick, glossy, ovate leaves that distinguish it from other aquatic weeds. In South East Asia, some were found to grow 2 to 5 meters in a day. Water hyacinth has been listed as one of the 100 most dangerous invasive aquatic species and one of the top 10 worst weeds in the world if not properly managed and converted to use products (Dersseh, et al., 2019). Water hyacinth obtains all of its nutrients directly from the water that it inhabits. It grows, most efficiently, in habitats with tropic climates and nutrient-enriched water. Their growth in fresh waters causes serious problems for water activities. They affect activities like swimming, boating and fishing. It can block water ways interfering with transport and irrigation. This, in turn, affects the economy of the country. So converting it into energy can be one of the many ways of changing this negatively perceived weed into a positive outcome.

Ethiopia is a developing country with a high population, scarcity of energy and poor waste management. So, the need for utilizing every opportunity that presents itself is a crucial move. The anaerobic co-digestion different biomass has been proven to have a good outcome than that of a single biomass. So, the present work attempts to study the anaerobic codigestion of WH and SWW to produce biogas.

1.2 Statement of the problem

The Kera abattoir wastewater (slaughterhouse wastewater) is directly or indirectly discharged into the Kera River. The river water is used for irrigation to grow vegetables like lettuce, spinach, cabbage, zucchini, pumpkin, etc. which are eventually sold to the community. However, untreated slaughterhouse wastewater contains pathogenic organisms like *Clostridium perfringens, Pseudomonas aeruginosa, Vibrio sp, Staphylo coccus sp, Salmonela sp, Proteus sp* which are highly detrimental to the human health (Rouf, Islam, Rabeya, Mondal, Khanam, & Samadder, 2016). The Kera slaughterhouse generates 363.35 m^3 of wastewater per day. This wastewater characterized by high amount of COD and BOD causing severe environmental pollution. Moreover, such high COD and BOD amounts are important indicators for biogas production which can be trapped (Worku & Leta, 2017).

According to Musa et al. (2018), slaughterhouses produce wastewater that has high amount of organic contaminants, such as protein, fats, oil and grease, and nutrients resulting in high COD and BOD contents. Even though this is a good precursor for biogas potential, its removal is also important since untreated discharge of wastewater with high COD creates bad aesthetic and ruins the environment by increasing the nutrient (N,P,K,S…) contents; creating nutrient imbalance in the water body(Mulu & Ayenew, 2015).Therefore, COD removal while producing biogas is a plus.

Water hyacinth, on the other hand, is currently another nuisance to the environment by invading water bodies, making them lose their biodiversity and creating loss of income for people leaving in marginal areas. In Ethiopia, water hyacinth first appeared in 1965 at Koka reservoir and Awash River. Other infested areas in the country were Gambella region, Blue Nile from just below Lake Tana to Sudan and Lake Ellen near Alem Tena (Dersseh, et al., 2019). It has been studied and proven that water hyacinth has a potential for biogas production (Hounwanou, Aina, Tchehouali, Adjahatode, Yao, & Matejka, 2012).

Nugraha et al.(2018) reported on the use of water hyacinth for biogas production and the effect that the food to micro-organism ratio has on the biogas production. It was observed that the highest biogas production was found on the lowest food to micro-organisim ratio due to the existence of smaller total solid. The lower the total solid, the more mass transfer between microbes and material. Eventough the feed to inoculum ratio was investigated, there are also other factors that affect the biogas production process that need to be studied. According to the investigations made by Tsegaye (2016), slaughter house waste (blood, manure and undigested stomach content) was used for the production of biogas. During the study, the effect of pH, temprature and substrate proportion was investigated. The optimum condition for an optimum methane yield of 79.26% was a substrate proportion of 20% blood:20% manure:60% undigested stomach content, a pH of 7.88 and temperature of 3.49^{0}C. The methane yield could infact cover for 70.7% of boiler furnace oil and gas oil consumption but the effect of the retention time is not investigated.

The anaerobic co-digestion of water hyacinth with ruminal slaughterhouse waste for biogas production was investigated by Omondi et al. (2019). Different mix proportions of water hyacinth with ruminal slaughterhouse waste was investigated with varying temperature. during the investigation, that the co-digestion of these substrates led to the improvement in the biogas quality that indicated the complementary properties of the substrates to each other that promoted the biogas yield but optimization of the digestion was not done.

Slaughterhouse wastewater has significant concentration of nutrients that can complement the digestion of other substrates such as water hyacinth. The current dependence on fossil fuel is presenting a threat to the environment particularly climate change (Omondi, Gikuma-Njuru, & Ndiba, 2019). Therefore, alternative renewable energy sources are becoming essential in order to address the energy demand by society while protecting the environment from pollution. Therefore, this proposed research work aims in incorporating biogas production plant that will co-digest SWW with WH. It will help in managing waste while producing renewable energy.

1.3 Objective

1.1.1. General objective

The main objective of the research work was to produce biogas by anaerobic co-digestion of slaughterhouse wastewater with water hyacinth (*Ecchornia crassipes*).

1.1.2. Specific objectives

The specific objectives were:

- Collection of the substrates such as water hyacinth and slaughterhouse wastewater
- Characterization of the feedstocks i.e. slaughterhouse wastewater and water hyacinth
- Investigating the effects of the processing variables such as HRT, substrate composition and pH on biogas production and COD removal efficiency
- To determine the optimum operating condition
- To evaluate the quality of bio-digestate for the potential use as bio-fertilizer

1.4 Significance of the study

The proposed research work will be beneficial in determining the synergy of WH and SWW during the co-digestion and on the biogas output. It will also determine the characteristics and biogas potential of the proposed feed stocks. But the main significances of the study are the reduction of environmental pollution by SWW, the conversion of the problematic and invasive WH into a useful energy source, the production of a renewable alternative energy and to create the understanding that while managing waste properly, environmentally clean energy can be produced and motivate industries like this to go green.

1.5 Scope of the study

The study focused only on the WH collected from Lake Zeway in the month of December, 2020. WH found in different seasons and in different water bodies are not considered. The slaughterhouse wastewater was that collected from the Addis Ababa Slaughterhouse effluent (Kera) in April, 2021 only. The study approach was firstly, to characterize the feedstocks regarding their suitability for biogas production and then to study the co-digestion process under different conditions. Although many factors can affect the responses, only effects of HRT, substrate composition and pH were considered. After biogas production, the bio-slurry was analyzed for possible use as fertilizer.

2 Literature review

The use of fossil fuels has become increasingly expensive over the years. This calls for a cheaper alternative that can give a somewhat similar benefit as to fossil fuels. This is where biofuels come in and among them; biogas tends to be relatively cheaper and cost-effective. This part will focus on what is already known about the feed stocks for biogas, anaerobic digestion of feed stocks, types of digesters and factors affecting biogas production.

2.1 Introduction to biofuels

Biofuels are renewable sources of energy that are produced from biological origin known as biomass. They are energy sources that have the potential to replace non-renewable energy sources like fossil fuels in a way that reduces the emission of greenhouse gases. For developing countries, biofuels offer the opportunity of reduced energy import bills and earning foreign exchange. Biofuels are classified as bioethanol, biodiesel and biogas based on the (Arshad, Zia, Shah, & Ahmad, 2018).

2.1.1 Historical background of biofuels

The oil crises that occurred in 1973 and 1979 G.C led to the use of biofuels as replacement to fossil fuels for transportation. Then again, during the 2000s G.C, the interest in biofuels was rekindled due to climate change issues, non-renewable energy sources depletion and high fluctuation in oil price. During the period of 2008-2011 G.C, there was confusion on whether to support biofuel policies made by different countries or not because the feed stocks used for biofuels generation were food sources which put food security at risk. This decreased the previous enthusiasm towards biofuels but they are still a good option (Arshad, Zia, Shah, & Ahmad, 2018).

In Ethiopia, especially the rural areas, most of the energy demand is fulfilled by using woody biomass i.e. direct firing of woody biomass but the pressure of the need for firewood is causing an environmental and energetic crisis and it is also depleting the forest. This leads to the loss of soil fertility, unavailability of fire wood and loss of preservation of aquifers. Sometimes, Instead of firewood, sun dried cow dung is used as source of heat which has a conversion efficiency of less than 8%. But nowadays, the conversion of organic waste like livestock manure into biogas has made it possible to achieve up to 55% conversion to heat. It also produces a bio-slurry that can be utilized as an enriched fertilizer (Kamp & Forn, 2016).

2.1.2 Classification of biofuels

There are numerous types of biofuels but the most frequent types of biofuels are bioethanol, biodiesel, biogas and bio-hydrogen. Based on the feed stocks used, the production method and the technology, biofuels are classified as 1^{st}, 2^{nd}, 3^{rd} and 4^{th} generation biofuels.

2.1.2.1 First generation biofuels

These are fuels that produced from food crops that contain sugar/starch and vegetable oil. Examples of these crops include corn, sugar cane, sunflower, canola, soybean and wheat. The fact that these crops require land that is fertile enough to be used for agriculture and the pressure it is putting on food security has made first generation fuel mediocre. In order to overcome these disadvantages, second generation fuels are more preferable now (Akbar, Subedi, Rolfe, Ashwath, & Rahman, 2019).

2.1.2.2 Second generation biofuels

These biofuels, on the other hand, are fuels generated from different types of feed stocks that are non-edible like Lignocellulosic biomass, municipal solid waste and agricultural and forest wastes. The feed stocks are either crops that can grow on marginal land i.e. energy crops or organic wastes that can be converted to one of the biofuels easily. The technologies to be used for the generation of these biofuels are either ‘thermo’ or ‘bio’ pathways.

The ‘thermo’ pathway is conversion of biomass to biofuels by applying heat with a minimal amount of oxidizing agent. These conversion processes result in three different products: bio-char which is solid, bio-oil which is liquid and syngas which is a gas composed of carbon monoxide, hydrogen, carbon dioxide and short chain alkanes.

The ‘bio’ pathway is an approach where the cellulose is first isolated from other Lignocellulosic components. This has challenge because the process has to produce very pure cellulose so that most inhibitors are removed without it being too energy intensive or too chemical consuming. Once the cellulose is isolated, it is saccharified into its monomers by using either enzymatic or chemical hydrolysis (Lee & Lavoie, 2013).

2.1.2.3 Third generation biofuels

These are fuels produced algal biomass (Lee & Lavoie, 2013). These are easily degradable biomass so they are they are good solutions for both the drawbacks of first and second generation biofuels (Akbar, Subedi, Rolfe, Ashwath, & Rahman, 2019). The amount of biofuel obtained from these feed stocks depend highly on the lipid content of the micro-organisms i.e. cholera, for example, are targeted due to their high lipid content and their high productivity (Lee & Lavoie, 2013).

2.2 Introduction to biogas

Biogas technology is an alternative energy technology which uses various organic wastes in order to generate Biogas (cooking, heating and lighting), mineralized water and organic fertilizers. Biogas refers to a mixture of various gases produced by the breakdown of organic matter in the absence of oxygen. Biogas can be produced from raw materials such as agricultural waste, manure, municipal waste, plant material, sewage, green waste or food waste. Biogas is an eco-friendly fuel that releases a very small carbon footprint (Lahlou, 2017).Biogas can be produced by the anaerobic digestion of anaerobic organisms, which assimilate material inside a closed system, or fermentation of biodegradable materials. Biogas is primarily methane and carbon dioxide and may have small amounts of hydrogen sulfide moisture and siloxanes (Mosquera, Varela, Santis, Villamizar, & Paola, 2020). This source of renewable alternative energy can be made from different feed stocks. The most common feed stocks are short cycle crops. But due to the fact that these crops are sources of food in third world countries called for the utilization of other types of feed stocks like Lignocellulosic biomass. The use of short cycle crops created the question to choose between food and energy (Omondi, Gikuma-Njuru, & Ndiba, 2019).

2.3 Anaerobic digestion

Anaerobic digestion is a natural biological process of producing biogas by the reduction of organic carbon to methane and oxidation to carbon dioxide in addition to other trace gases. This is one of the mechanism in which waste can be managed by biological treatment while producing biogas (Mosquera, Varela, Santis, Villamizar, & Paola, 2020). As the name indicates, the digestion or degradation takes place in an oxygen free environment while a consortium of microorganisms is present in a controlled environment. The microbes help catalyze successions of complicated microbial reactions (Patila, Raja, BB, Shetty, & Kumar, 2014).

In order to increase the microbial degradation of the organic waste or biomass, it has been studied and proven that the synergy of simultaneous digestion or co-digestion of feed stocks has a better performance than the individual feed stock digestion. Other benefits of co-digestion are the reduction of concentration of toxic materials, nutrient concentration increment, substrate loading improvement, provision of buffer capacity and hygienic stabilization of enzymes. As a result of these improvements, the anaerobic process will be stable and efficient. Therefore, co-digestion offers the opportunity to increase the biogas yield at a lower hydraulic retention time (Omondi, Gikuma-Njuru, & Ndiba, 2019).

2.4 Principles of anaerobic digestion

The aim of anaerobic digestion is to either treat biodegradable wastes or to produce saleable products i.e. heat, electricity and soil amendment. If the anaerobic digestion is aimed at producing biogas, energy crops can be grown and used. The bio-slurry produced after digestion will be used as soil amendment as well. Nonetheless, anaerobic digestion is mainly beneficial in managing waste while producing biogas and byproducts that are useable. The process of anaerobic digestion is classified into 4 stages that are namely: pretreatment, digestion, gas upgrading and digestate treatment.

The science behind anaerobic digestion is better understood by classifying it into three i.e. **hydrolysis, acidogenesis, acetogenesis and methanogenesis**. **Hydrolysis** is the degradation of recalcitrant, complex organic matter into their monomers, such as fatty acids, amino acids and sugars, by the aid of fermentative bacteria. The hydrolytic step can be rate limiting in wastes with high organic matter. **Acidogenesis** is the second step where the hydrolyzed product is converted to simple organic acids, carbon dioxide and hydrogen with the help of acidogenic bacteria. The organic acids primarily formed are acetic acid, butyric acid, propionic acid and ethanol. **Acetogenesis** is where acetate is formed by the acetogenic bacteria. Lastly, in **methanogenesis** methanogenic bacteria will produce methane in two ways i.e. either by reduction of carbon dioxide with hydrogen or by cleaving two acetic acid molecules to produce carbon dioxide and methane. Due to the limited amount of hydrogen, the primary producer of the methane is the acetate reaction (Rao, Baral, Dey, & Mutnuri, 2010).

2.5 Types of anaerobic digesters

Anaerobic digesters work either in batch, Single-stage or multi-stage system (Table 2.1). These systems are comprised in different types of digesters. Anaerobic digesters can be broadly classified as to their ability to digest liquid or solid wastes. There are several digesters in action; the most common one is the fixed dome digester, while the floating dome digester and bag digester are found in many developing countries.

Table 2. 1. Anaerobic digesters types and description; adopted from (pew center on global climate change, 2011)

Types of waste	Types of digesters	description
Liquid waste	Covered lagoon digester/Up flow anaerobic sludge blanket(UASB)/Fixed Film	Covered lagoon or sludge blanket type digesters are used with wastes discharged into water. The decomposition of waste in water creates a naturally anaerobic environment.
Slurry waste	Complete mix digester	Complete mix digesters work best with slurry manure or wastes that are semi-liquid (generally, when the waste's solids composition is less than 10 percent). These wastes are deposited in a heated tank and periodically mixed. Biogas that is produced remains in the tank until use or flaring.
Semi-solid waste	Plug flow digester	Plug flow digesters are used for solid manure or waste (generally, when the waste's solids composition is 11 percent or greater). Wastes are deposited in a long, heated tank that is typically situated below ground. Biogas remains in the tank until use or flaring.

2.6 Factors affecting anaerobic digestion

The efficiency of an anaerobic digester increases when the microbial activity increases and this is achieved by controlling the operating parameters of the digester (Monnet, 2003).

2.6.1 Total solid content (TS)

Total solids are the material residue left in a vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Solid contents are classified into three ranges: high solid systems have 22-40% TS, medium solid systems contain from 15-20% TS while low solid systems contain less than 10%TS. When total solid content increases, the digester volume decreases because of low water requirement (Monnet, 2003).

2.6.2 Temperature

Temperature affects anaerobic digestion in two ranges: mesophillic conditions exist between the temperature of 20 and 45^{o}C and thermophillic conditions exist between the temperature of 50 and 65^{o}C(Bakraoui et al., 2020). The optimum condition for each temperature range is 35 and 55^{o}C, respectively. The growth rate and activity of the bacteria, in the mesophillic range, decreases by 50% for every 10^{o}C drop. If the temperature decreases to 20^{o}C , biogas production starts to fall. Increasing the temperature above 37^{o}C will lead to the decrease the rate of biogas generation (Cioabla, Ionel, Dumitrel, & Popescu, 2012).

2.6.3 pH

The pH has a significant effect on AD because the change in the pH affects the microbes. Optimum pH of AD lies in the range of 6.8-7.2 but a pH of 6.5-8.0 can be tolerated (Cioabla, Ionel, Dumitrel, & Popescu, 2012). The optimum pH conditions for acidogenesis and methanogenesis are different. Since acidogenesis produces organic acids like acetic acid, lactic acid and propionic acid, the pH falls. pH below 6.4 is unsuitable for methane-forming bacteria because the optimal range for methanogenesis is between 6.6 and 7 (Monnet, 2003).

2.6.4 Retention time

The retention time is the time required for the complete degradation of the organic matter. The retention time is determined based on parameters like process temperature and waste composition. Therefore, the retention time for mesophillic digester is from 15 to 30 days while for thermophillic digesters it is from 12 to 14 days (Monnet, 2003).

2.6.5 Carbon to nitrogen ratio (C: N)

The carbon to nitrogen ratio affects methanogens i.e. the methane forming bacteria. High C: N ratio is an indication of fast consumption of nitrogen by the methanogens and low gas production will be obtained. Low C: N ratio, on the other hand, indicates accumulation of ammonia. The optimum C: N ration for a good anaerobic digestion lies in the range between 20 and 30 (Monnet, 2003).

2.6.6 Organic loading rate (OLR)

This is the measure of biological conversion capacity of the anaerobic digestion system. If there is high OLR, then the biogas yield of the digester will be low due to the accumulation of inhibiting substances. This happens because the microorganisms will be overfed so they will not be able to consume leading to accumulation (Monnet, 2003). On the other hand, low OLR means that the microorganisms will be starved. Therefore, OLR is determined by the feeding material and reactor temperature (Lohani & Havukainen, 2018).

2.6.7 Particle size and moisture content of substrate

The particle size of a substrate affects the kinetics of the digestion while the moisture content affects the AD process and methane yield. A small particle size insures a good substrate utilization which improves the kinetic process; this in turn improves the methane yield. The substrate should be dried before use because a study done previously stated that the increase in moisture content resulted in the decease of methane yield because of the decrease in efficiency of carbohydrate removal (Lohani & Havukainen, 2018).

2.7 Feed stocks for biogas production

Biogas is an ecofriendly fuel produced from the anaerobic digestion of organic waste like sewage sludge, organic farm waste (Monnet, 2003), Waste from dairy production, Food industries, Agricultural remains, Kitchen waste, Animal waste, Slaughter house waste as well as Lignocellulosic biomass. Various works are being done in order to produce this clean fuel by digesting organic material (Shivani & Misbah, 2018). Among

Lignocellulosic biomass, water weeds can be used for the production of biogas and of these water weeds, water hyacinth shows promise for biogas production.

2.7.1 Water hyacinth

Water hyacinth is an invasive, aquatic weed that floats over fresh water. It reproduces very fast producing over thousands of seeds each year. It has broad, thick, glossy, ovate leaves that distinguish it from other aquatic weeds. Above water surface, it can grow up to 1 meter and below water surface, the roots can grow up to 80 centimeters. The leaves are 10 to 20 centimeters wide. It has long and spongy stalks. The seeds can stay intact for more than 28 years. In south East Asia, some were found to grow 2 to 5 meters in a day. Water hyacinth has been listed as one of the 100 most dangerous invasive aquatic species and one of the top 10 worst weeds in the world. Water hyacinth has the potential to be used as a biofuel feed stock. Among these biofuels, biogas can be produced from these particular invasive weed (Bhattacharya & Kumar, 2010). Water hyacinth is composed of different materials as stated in Table 2.2.

Table 2. 2.Chemical and structural composition of water hyacinth *(Rathoda, et al., 2018)*

Composition	Amount (%)
Water content	90
Cellulose	24
Hemicellulose	30
Lignin	16
Ash	20
Nitrogen	2.9
Carbon	38.4
Hydrogen	5.85
Oxygen	28.1
Potassium	2.78
Sulfur	0.47
Phosphorus	0.77
Calcium	1.32
Sodium	1.44

2.7.2 Slaughterhouse wastewater (SWW)

Slaughterhouse wastewater consists of blood, urine, washings of intestinal content as well as pathogenic microbes (Rouf, Islam, Rabeya, Mondal, Khanam, & Samadder, 2016). It also has rumen content, inedible fats, womb, large intestine and udder. The wastes with the exception of rumen contents are characterized by large organic content composed mainly of animal proteins and fats (Omondi, Ndiba, & Njuru, 2019). The foremost features of slaughterhouse wastes are high organic strength, sufficient organic biological nutrients (Table 2.3), adequate alkalinity, relatively high temperature (20 to 30°C) and free of toxic material. Slaughterhouse wastewaters with the preceding properties are appropriate for anaerobic treatment and the efficiency in reducing the BOD5 ranged between 60 and 90% (Pilgonde, 2017).

Table 2. 3. Slaughterhouse wastewater characteristics *(Omondi, Ndiba, & Njuru, 2019)*

Parameters	Amount
Carbon (C) (mg/L)	26,984 ± 600
Nitrogen (N) (mg/L)	1430 ± 260
Potassium (K) (mg/L)	7694 ± 19.65
Phosphorus (P) (mg/L)	41 ± 1
C/N ratio	18.86:1
C/P ratio	656:1

Different digestions and co-digestions of slaughterhouse wastewater and water hyacinth are stated in Table 2.4.

Table 2. 4.summary of digestion of water hyacinth and slaughterhouse wastewater separately and co-digestion with other substrates

References	Raw material used	Digestion technology	remark
(Patil et al., 2012)	• Water hyacinth • Inoculum: poultry litter	• Mesophillic condition • Batch anaerobic digestion using 0.25L digesters	• Kinetics of the anaerobic digestion process was carried out by modified gompertz equation • The biogas production increased as the substrate to inoculum ratio but decreased when only poultry litter was digested. • **Gap**: co-digestion as well as its optimization might lead to a better result.
(Worku & Leta, 2017)	• Slaughterhouse waste water	• Mesophillic environment • Pilot scale semi-continuous anaerobic digester • Digestion process was optimized	• Optimum methane yield: 72.75% • Optimum COD removal: 84.54% • **Gap**: there might be improvement of methane yield if co-digested with other substrates
(Bharati & Kalamdhad, 2018)	• Water hyacinth • Inoculum: cow dung • Microbial pretreatment of water hyacinth	• Batch anaerobic digestion in 1L digesters	• Feed to microbe ratio was analyzed. • Methane yield from untreated water hyacinth: 57% • Methane yield from treated water hyacinth: 59.9% • **Gap**: co-digestion and other pretreatment processes can be tested out.

(Borja et al., 1998)	• Slaughterhouse waste water	• Laboratory scale anaerobic reactor with a combination of sludge blanket and a filter arrangement (submerged small cubes of polyurethane foam) • Mesophillic operation	• Maximum methane generation: 74% • Maximum COD removal of 96.2% was achieved. • The packed –bed portion of the reactor helped in the further retention of active biomass which promoted organic matter removal • **Gap**: investigation of the possibility of producing similar methane amount with a less complicated reactor
(Aberra & Fufa, 2016)	• abattoir wastes (AW) with sewage sludge (SS)	• Laboratory-scale batch anaerobic co-digestion • Under mesophillic conditions	• Different mix ratios of sewage sludge and abattoir waste were analyzed. • Maximum methane yield of 56.9% and COD removal of 71.8% was obtained from a mix-ratio of 3SS: 2AW. • **Gap**: investigation of lignocellulosic biomass and a specific inoculum for biogas production
(Otero & Mor, 2008)	• Slaughterhouse solid waste with the organic fraction of municipal solid waste • Inoculum: previous digestate from wastewater treatment plant	• Mesophillic semi-continuously fed digester	• A maximum methane yield of 64.5% was achieved on the 25th day. • Methane production decreased with increase in retention time • **Gap**: investigation of the effect of parameters other than HRT.

2.8 Advantages of co-digestion

Co-digestion offers a better nutrient balance and digestion than a single substrate digestion. It also offers Equalization of particulate, floating, settling, acidifying, etc. wastes, through dilution by manure or sewage sludge, collection of additional biogas, additional fertilizer recovery and renewable energy crops disposable for digestion in agriculture (Wu, 2020). Table 2.5 shows the different effects of different co-digestions.

Table 2. 5. Different co-digestions, with the digesters used with their respective remarks

References	Co-digestion	Digester	Remark
(Berhe, 2017)	• Co-digestion of tannery and dairy industry wastes	• Two-phase anaerobic sequencing batch reactor	• Mesophillic condition (38±2^{0}C) • OLR, HRT and mixing ratio were optimized. • **Gap**: complex process of digestion
(Rahmansyah et al., 2017)	• Co-digestion of water hyacinth and cow manure	• Anaerobic batch digester	• Varying substrate mix ratio • Chemical Pretreatment using 5% sulfuric acid • Methane gas produced: 15.56% • **Gap**: the methane produced is very low considering the materials used
(Andri & Corre, 2017)	• Co-digestion of water hyacinth and earthworm bedding wastewater	• Laboratory scale anaerobic batch digester	• RSM-CCD optimization of TS of substrates, particle size of WH and initial pH • Maximum bio-methane potential

			was 35.50%. • COD removal efficiency obtained was 65.26% • **Gap:** methane yield is very low eventhough the COD removal was high.
(Pan-in & Sukasem, 2017)	• Seeds, corn cobs and corn husk (sweet corn wastes) co-digested with pig dung, cow dung and goat dung	• A 1-Liter glass anaerobic digester.	• Pretreatment of the sweet corn wastes using 2% (w/v) NaOH • 1:1 mixture of the sweet corn wastes and animal dung resulted in maximum methane yield of 46.13% and COD removal efficiency of 74.37%. • **Gap**: other types of pretreatments should be considered to improve the methane yield
(Nam et al., 2016)	• Co-digestion of water hyacinth and pig manure	• Anaerobic batch digesters	• Different (7) mix ratios of the substrates were investigated in 5 replications for 60 days. • Methane produced ranged from 29.6% to 53%. • **Gap**: low methane yield and

			other parameters should be considered for process optimization.
(Ogunwande et al., 2018)	• Co-digestion of water hyacinth (*eichhornia crassipes*) and duckweed (*lemna minor*)	• Anaerobic digesters	• Inoculum: cow dung slurry • Duckweed produced more biogas than water hyacinth • Duckweed to Water hyacinth ratio of 7:3 resulted higher biogas production • **Gap**: other parameters should be considered since one parameter can explain a process
(Patil et al., 2014)	• Co-digestion of water hyacinth and sheep waste	• Bio-methanation unit with connected with gas collectors through tubes and biogas produced measurement by downward water displacement	• Inoculum: predigested material from previous experiments • Water hyacinth was alkali pretreated. • Maximum methane yield of 60.84% was obtained. • **Gap**: process optimization and other pretreatments can be considered for improved methane yield.

(Ehiri et al., 2014)	• Co-digestion of water hyacinth and fresh rumen residue	• 7500ml capacity batch process bio-digester	• Biogas production started on the 9^{th} day and the digestion was stopped on the 39^{th} day. • Optimum biogas yield was 16.4 ml on the 17th day • Kinetics of the digestion process: second-order type • **Gap**: other parameter effects should be considered.
(Adanikin et al., 2017)	• Co-digestion of water hyacinth with morning glory	• Anaerobic digesters made of cube-shaped $25cm^3$ plastic kegs	• Inoculum: cow dung slurry • 5 mix ratios of the substrates were digested for 17 weeks. • Kinetics of digestion: first order type • The morning glory to water hyacinth ratio of 70%:30% resulted in the highest biogas yield • **Gap**: other parameters should be considered since one parameter cannot explain a whole process.
(Tallou et al., 2020)	• Co-digestion of olive mill wastewater, municipal waste	• Anaerobic batch digesters	• Inoculum: cow dung is used as both substrate and

	water and cow dung		inoculum. • 1:1:1 combination of the substrates produced the largest volume of methane per gram of volatile solid • **Gap**: parameters like HRT should also be considered since time is essential for biogas production.

3 Materials and methods

The anaerobic co-digestion of multiple raw materials for the production of biogas is currently being practiced extensively, mostly co-digestion of organic waste. This section clearly discusses the materials used and the methods applied for the collection and characterization of raw materials, the biogas production process and the characterization of the product.

3.1 Materials Used

The major equipment and chemicals utilized will be presented here forth.

3.1.1 Equipment used

The major apparatus used during the present study were COD and multi-parameter photometer (HI83099, HANNA instruments), muffle furnace (MV 106, NUVE), UV-Visible Spectrometer (UV-1800, SHIMADZU), fourier transform infrared radiation (FTIR) (Thermo Scientific is50 ABX), Gas analyzer (Biogas 5000, geotech), soxhlet extraction unit (BST/SXM-6, BIONICS), dry oven (TD-1315, GENLABPRIME), COD reactor (HI839800-02, HANNA instruments), autoclave, analytical Balance (AD300-3), sieve shaker (IC-205/EV), grinder (NIMA, Japan /220v/50-60Hz), magnetic stirrer with hot plate (MS-H280-PRO), pH meter, incubator, DO meter, vacuum pump (MZ 2C NT), water bath (HH-6) and other glass ware like beaker, flask etc.

3.1.2 Chemicals used

The chemicals used during the present study were acetone (≥ 99.5 %v/v, RANCHEM), 3,5-dinitrosalicylic acid (reagent grade), sodium bisulfate, sodium-potassium tartarate (NEOLAB), d-glucose (LOBACHEMIE), COD reagent (HR(0-15,000 mg/L)), sodium acetate anhydrous (≥ 99 %w/v, CENTRAL DRUG HOUSE), sodium hydroxide (≥ 99 %w/v, NEOLAB), sulfuric acid (≥ 98 %v/v, CARELABMEB) etc.

3.2 Overview of the methodology

Schematic presentation of the experimental steps is given in Fig. 3.1.

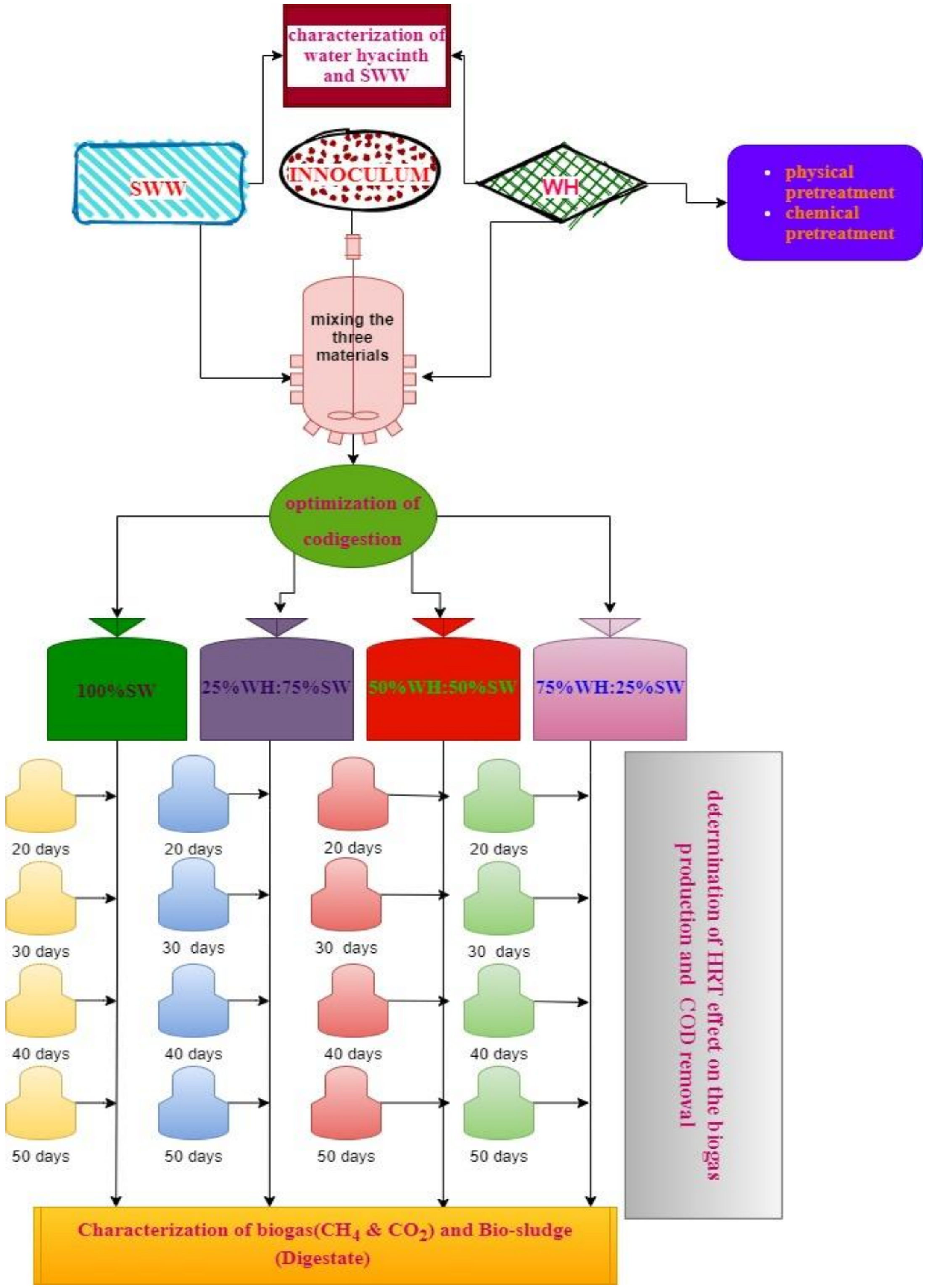


Figure 3. 1. Experimental steps for the production of biogas

3.3 Raw materials

The raw materials used during this work were WH and SWW as substrate and rumen fluid as inoculum.

Water hyacinth was collected from Ziway Lake located in the south east Shewa zone in Oromia region 168 km away from Addis Ababa (Figure 3.2). It was collected with a poly bag having internal lining in the month of December 2020. After collection it was brought straight to the Addis Ababa Science and Technology University, Chemical Engineering Department laboratory for drying and size reduction.

Figure 3. 2. Water hyacinth that took residence on Ziway Lake

The slaughterhouse wastewater and cows' rumen content was collected in April 2021 from Addis Ababa abattoirs enterprise located around Kera, Addis Ababa at $8^0$59´12´´N and $38^0$44´49´´E coordinates. It was collected using 7 L plastic bottles washed with a sodium hydroxide solution and rinsed with distilled water. The samples were immediately placed in cold boxes and brought to the Addis Ababa institute of technology environmental laboratory for refrigeration. Samples were kept in a refrigerator at 4^{o}C until use.

3.4 Raw material characterization

The water hyacinth and slaughterhouse wastewater were characterized proximate, physicochemical and elemental analyses before any type of processing.

3.4.1 Proximate analysis of water hyacinth

The proximate analysis of water hyacinth was investigated to determine the potential of the hyacinth to be used as a co-digestion material for biogas production. The investigation included bulk density, moisture content, volatile matter content, ash content and fixed carbon content which were performed as per ASTM standards (D 3173- D 3175). The characterizations were carried out separately for the roots, leaves and shoot samples of the water hyacinth as shown in Figure 3.3.

3.4.1.1 Moisture content investigation

The moisture content was determined on the as-received samples after separating the leaves, stem and roots and chopping the parts into smaller sizes. The sample trays were dried separately in order to remove any moisture adhering on the trays so that only the moisture from the water hyacinth parts can be determined. 5 g of each chopped part of the water hyacinth sample was measured and placed on pre-dried trays. The samples were then dried in an oven at a temperature of 105±3^{o}C for 24 h. The moisture content was then determined as per equation 3.1 (ASTM standard D 3173 – 03, 2013).

$$MC = \frac{M_w - M_d}{M_w} \quad 3.1$$

Where: MC: moisture content

M_w : mass of wet sample.

M_d : mass of dried sample .

Figure 3. 3. Parts of water hyacinth (A) Leaf, (B) shoot, (C) root of water hyacinth

3.4.1.2 Volatile matter content

The volatile matter content was determined as per ASTM D 3175. Two g each of the separated water hyacinth samples were first put in pre-dried ceramic crucibles. The crucible plus sample was then placed into a muffle furnace which was set at a temperature of 950^{o}C. The crucible-plus sample were withdrawn from the furnace after 7 minutes and placed inside a desiccator in order to avoid moisture reabsorption during cooling. This analysis was done in triplicates. The samples were then weighed and recorded. The volatile matter was determined using equation (3.2) (ASTM standard D 3175 – 89a, 2013).

$$VMC\% = \frac{W_{ods} - W_{mfs}}{W_{ods}} \times 100\% \tag{3. 2}$$

Where: VMC%: percentage volatile matter content

W_{ods}: weight of oven dried sample

$W_{mfs:}$ weight of the sample after it is burnt in the muffle furnace

3.4.1.3 Ash content determination

Determination of ash content was conducted by taking 2 g each of the separated water hyacinth sample. The samples were put into crucibles and placed in the muffle furnace. The muffle furnace was set at a temperature of 550±50^{0}C and left for 3 h. After the 3 h, the crucibles were taken out of the furnace and placed in the desiccator for cooling and avoiding moisture contamination. After the samples were cooled to room temperature, the samples were weighed and recorded. This was also done in triplicates as well. The ash content was determined using equation (3.3)(ASTM standard D 3174 – 02, 2013)

$$AC\% = \frac{W_{AR}}{W_{ods}} \times 100\% \tag{3. 3}$$

Where AC%: percentage ash content

W_{AR}: weight of ash residue

3.4.1.4 Fixed carbon content

The fixed carbon composition was determined by subtracting the percentage of moisture content, volatile matter and ash content of the sample from the total mass as per ASTM standard D 3172 – 89 (Reapproved 2002)(ASTM standard D 3172 – 89 R02, 2002).

3.4.2 WH Bulk density

According to ASTM 7481, bulk density was measured by using a glass measuring cylinder of 50 milliliters. The empty measuring cylinder was weighed first. Then, the water hyacinth powder was packed into the measuring cylinder by pushing it with a piston-like material assuming there is no space for air and weighed again. Finally, the bulk density was determined by using the formula shown in equation (3.4).

$$\rho(\frac{kg}{m^3}) = \frac{M_{pc} - M_{ec}}{V_c} \tag{3. 4}$$

Whereρ: is the bulk density

M_{pc}: is the weighed mass of the packed cylinder (Kg)

M_{ec}: is the weighed mass of the empty cylinder (Kg)

V_f: is the volume of the volumetric flask (m^3)

3.4.3 Determination of the lignocellulosic components WH

The determination of the lignocellulosic components of the root, leaf and shoot of the water hyacinth is useful in determining which part is relatively more suitable for the biogas production process. The decision of which part to use relies on the amount of cellulose, hemicellulose, lignin as well as extractives (fats, proteins, waxes, chlorophyll and so on) and ash present in the biomass. Therefore, it is important to investigate the composition of each component in the water hyacinth (Malveaux, 2013).

3.4.3.1 Extractives determination

Determining the amount of extractives in the specific biomass helps in the determination of other constituents of lignocellulosic biomass. Extractives were determined by using a Soxhlet apparatus in two consecutive stages. 5 g of each part of prepared sample of water hyacinth were placed into a filter paper. The samples were put into the Soxhlet chamber, consecutively, equipped with a condenser on top of it and a three necked round bottom flask on the bottom as presented in Figure 3.4. The extraction was conducted using ultra-purified water for 5 hours. The heating mantle was used to supply heat for the extraction and the temperature inside was maintained near the boiling point of water (Toribio-cuaya et al., 2014). The remaining un-extracted sample was recovered from the filter paper, dried and measured to record the amount of extracted matters using water as a solvent. The dried samples were then further extracted using acetone as a solvent in Soxhlet apparatus. For the extraction process using acetone as a solvent, the temperature in the solution is adjusted around the boiling point of acetone (70^oC). The extraction was conducted for 4 h(Ayeni et al., 2015). After the extraction, the remaining biomass was sun dried to let acetone evaporate from the samples for about 1 h. The samples were further dried in an oven at a temperature of 105^0C until constant weight. The amount of extractives was determined by subtracting the mass of remaining biomass after both water and acetone solvent extraction from the original sample mass (Ayeni et al., 2015).

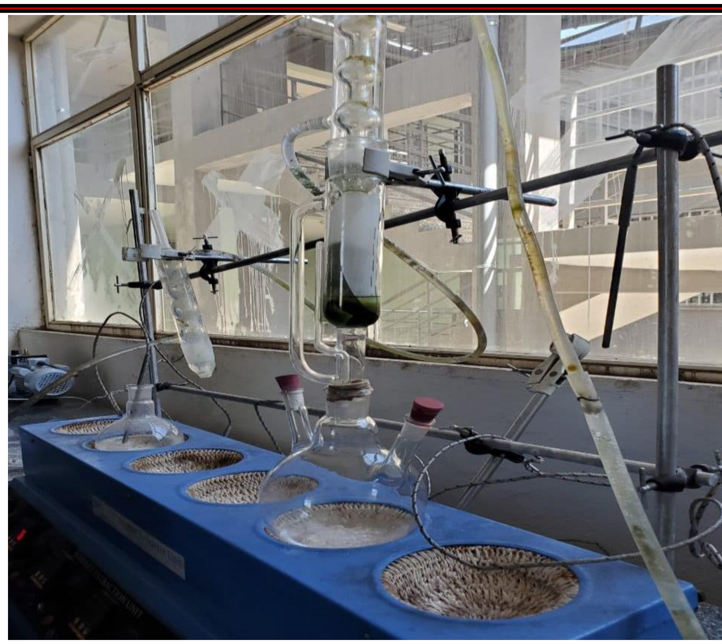


Figure 3. 4. Extractives investigation using a soxhlet extraction unit

3.4.3.2 Hemicellulose

For the determination of hemicellulose content, 2 g of each part of the biomass recovered after extraction was placed inside a 250 mL Erlenmeyer flask at separate times. Then 200 mL of sodium hydroxide solution at a concentration of 0.5 M was added to the flask and boiled for 3.5 h. After boiling, it was cooled to room temperature and filtered using a vacuum filter. The biomass solid recovered after filtration was dried in an oven adjusted to a temperature of 105^{0}C until constant weight. Finally, the hemicellulose amount was determined by subtracting the final mass of the solid from the original extractive-free biomass (Ayeni et al., 2015). These investigations were done for the root, leaf and shoot parts of the water hyacinth.

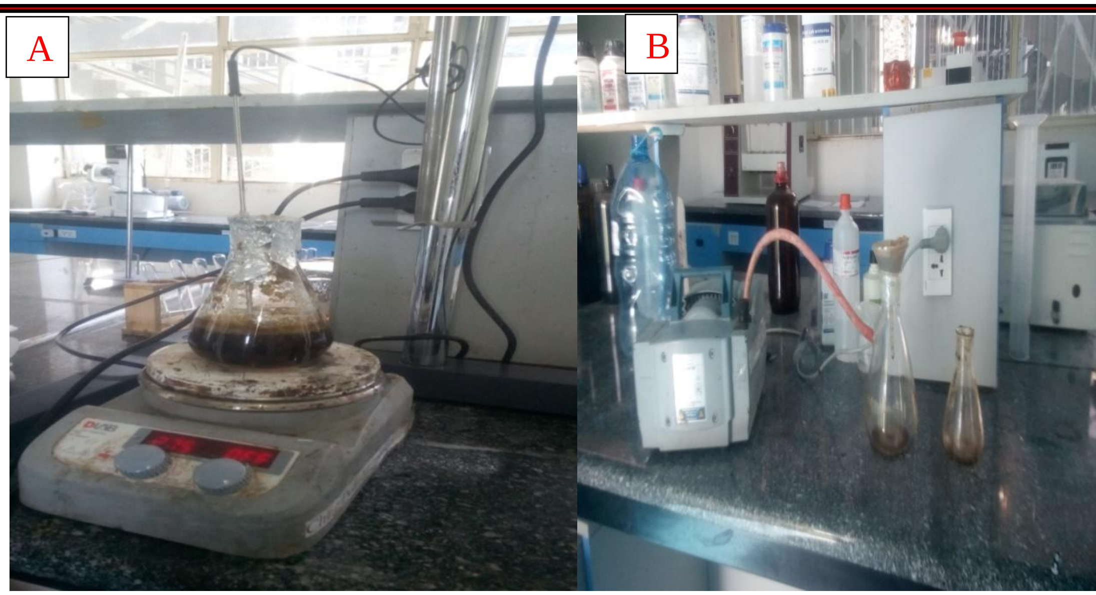


Figure 3. 5. Hemicellulose determination process (A) cooking the water hyacinth parts (B) vacuum filtration of the cooked slurry

3.4.3.3 Lignin

The amount of lignin was determined by using a 2 step hydrolysis process. One gram of the extractive-free biomass parts were transferred into a 250 mL Erlenmeyer flask. Then, 10 mL of 72 %(v/v) sulfuric acid was added into each flask, at separate times, to produce slurry. The slurries were kept at room temperature for 2 h while shaking at 30 min interval for complete hydrolysis. In the second step, dilute acid hydrolysis was started by adding 280 mL of distilled water into the slurry in order to reduce the concentration of acid solution to 4 %(v/v). The solution was then autoclaved at a temperature of 120^0C for 1 h. The liquid hydrolysate was cooled to room temperature and filtered by using an ashless filter paper. After filtering out, the solid residue was dried until constant mass in an oven at 105^0C. The final mass was measured and recorded. The ash was then determined by incinerating the solid residue in a muffle furnace at a temperature of 575^0C for 3 h. Lignin was determined as acid soluble lignin and acid insoluble lignin.

The acid insoluble lignin, determined by equation 3.5, is the residue recovered as a solid. Acid insoluble lignin is further incinerated for the investigation of ash in the insoluble lignin which, in turn, is used to analyze the inorganic components of lignocellulosic biomass. The liquid hydrolysate was analyzed by the UV-spectrophotometry. The absorbance was measured at a 320 nm wave length in order to determine the acid soluble lignin, as given in equation (3.6). The total lignin amount was determined from the sum of the acid soluble lignin and the acid insoluble lignin, as shown in equation (3.7), according to the national renewable energy laboratories (NREL) laboratory analytical procedure (LAP) (Sluiter et al., 2012). This investigation is done for the root, leaf and stalk of the water hyacinth as well.

$$\%AIL = \frac{W_{C+AIR} - W_C \;-\; W_{C+ASH} - W_C}{ODW_{sample}} \times 100 \qquad 3.5$$

Where: % AIL: percent acid insoluble lignin

W_C: weight of the crucible

W_{C+AIR}: weight of the crucible plus the acid insoluble residue

W_{C+ASH}: weight of the crucible plus the ash

ODW_{sample}: oven dried weight of the sample

$$\%ASL = \frac{UV_{abs} \times volume_{filtrate} \times Dilution}{(\varepsilon \times ODW_{sample} \times pathlength)} \times 100 \qquad 3.6$$

Where: %ASL: percent acid soluble lignin

UV_{abs}: absorbance read from the UV spectrophotometer

ε: absorptivity of biomass at a specific wavelength

Pathlength: cuvette diameter in cm

$$Dilution = \left(\frac{V_s + V_{ds}}{V_s} \right) \qquad 3.7$$

Where V_s: volume of the sample

V_{ds}: volume of the diluting sample

$$\%Lignin_{exf} = \%AIL + \%ASL \tag{3.8}$$

Where $\%Lignin_{exf}$: percent extractive free lignin

3.4.3.4 Cellulose

The amount of cellulose was estimated by simply subtracting the amount of extractives, hemicellulose, lignin and ash obtained from the original mass of each part (root, leaf and stalk). The quantities of other constituents were assumed to be insignificant(Ayeni et al., 2015).

3.4.4 Slaughterhouse wastewater (SWW) characterization

Slaughterhouse waste water was investigated for properties like total solids, fixed solids and volatile solids according to the standard methods set by American public health association (APHA) section 2540 B and E (APHA, 1999). The pH and temperature of the slaughterhouse waste water were also measured using a pH meter and thermometer, respectively.

3.4.4.1 Total, volatile and fixed solids in the SWW

The total solid was determined as per the method of APHA 2540B. Ceramic crucibles were washed and dried in an oven set at 105°C for 30 min to remove any adhering moisture. After drying, the crucibles were transferred into a desiccator, cooled and weighed. 5 g of the homogenized slaughterhouse waste sample and rumen content were placed in an oven set at 105°C for an hour. After an hour, the samples were placed in a desiccator to avoid moisture contamination, cooled and weighed. After weighing, the samples were ignited in a muffle furnace at 550°C for an hour. The samples were cooled in a desiccator and weighed. The ignition, cooling and weighing was repeated until constant weight or until below 4% weight difference was obtained. These investigations were carried out in triplicate. Finally, the parameters were determined by using equations (3.9-3.11).

$$\%TS = \frac{A - B}{C - B} \times 100 \tag{3.9}$$

$$\%VS = \frac{A-D}{A-B} \times 100 \quad 3.10$$

$$\%FS = \frac{D-B}{A-B} \times 100 \quad 3.11$$

Where, TS: is the total solid

VS: is the volatile solid

FS: is the fixed solid

A: weight of the dried residue plus the dish, mg

B: weight of the dish, mg

C: weight of the wet sample plus the dish, mg

D: weight of residue plus the dish after ignition, mg

3.4.4.2 Biochemical and chemical oxygen demand analysis

The 5-day biochemical oxygen demand (BOD_5) test was carried out for the raw waste water using the standard method set by APHA. Section 5210B was used in particular. As stated in the standard method, the major apparatus used were a DO (dissolved oxygen) meter and an incubator set at 20 ± 1^0C. The initial dissolved oxygen and the final dissolved oxygen after 5-day incubation were measured and recorded. The BOD_5 was then determined using the equation (3.12)(APHA, 1999).

$$BOD_5, \frac{mg}{L} = \frac{D_1 - D_2}{P} \quad 3.12$$

Where, D_1 is the initial dissolved oxygen of the raw wastewater (mg/L)

D_2 is the final dissolved oxygen after 5-day incubation of the waste water (mg/L)

P is the decimal volumetric fraction of the sample used

The chemical oxygen demand (COD), on the other hand, was carried out using COD reagent, a digester and a multi-photometer. A closed reflux, colorimetric method was used. But instead of preparing a standard curve, a direct reading by the multi-photometer was executed. A modification of section 5220D was employed for COD determination at low cost and less time consumption. The COD amount of the mixture of the water hyacinth and waste water was first determined before the digestion process started (before inoculation). Similarly, the COD was also determined after the digestion period ended. During the analysis, 1mL of the sample to be analyzed was diluted by using 5 mL distilled water. After dilution, 2 mL of the diluted sample was transferred into the COD reagent which was then digested using a digester set at 150°C for 2 h. After digestion, the mixture was cooled and transferred into a vial for a multi-photometer reading. The COD of both the raw sample and the bio-digestate was investigated. This was required to identify the COD removal efficiency of the anaerobic digestion using equation (3. 13)(APHA, 1999).

$$COD_{removalefficency} = \frac{COD_{BD} - COD_{AD}}{COD_{BD}} \times 100\% \quad 3.\ 13$$

Where COD_{BD} = chemical oxygen demand before the anaerobic digestion

COD_{AD}=chemical oxygen demand after anaerobic digestion

3.5 Water hyacinth preparation and pretreatment

The water hyacinth was washed using tap water to remove any adhering dirt or material. The leaf and shoot or stem were then chopped to about 2 cm length and dried using an oven set at 105°C for 24 hours. Dried samples were then ground (NIMA; japan /220v/50-60Hz) and sieved. Sieve analysis resulted in particle sizes ranging from ≤0.04 mm to ≥1.5 mm. Since the gap of the range is very large and in order to minimize wastage, the particle size between 0.25 mm and 1.5 mm was used for the rest of the experiments.

For acid hydrolysis, 50 g of the pretreated WH was put into a 2 L flask containing dilute sulfuric acid solution (1.5 % (v/v)) at a solid to liquid ratio of 1:35. The size reduced water hyacinth was mixed with 1750 mL of dilute sulfuric acid with a concentration of 1.5 % (v/v) i.e. 1:35 solid to liquid ratio since water hyacinth is water loving and does not form an adequate slurry until it is mixed with 35 mL acid. It was then autoclaved at 140°C for 85 min for an optimum hydrolysis (Kifle, 2021). The liquid hydrolysate was then analyzed for reducing sugar content using the dinitrosalicylic acid method(Miller, 1959). After acid pretreatment, the slurry was neutralized (pH=7) by using a 30% sodium acetate solution in order to avoid further hydrolysis and for further processing since the successive process involves micro-organisms that require a neutral pH .

3.5.1 Reducing sugar content determination using the DNSA method for the liquid hydrolysate

The DNSA reagent was composed of 2 g of 3, 5-dinitrosalicylic acid, 2 g of sodium hydroxide, 0.05 g of sodium sulfite and 4 g of sodium potassium tartarate (also known as Rochelle's salt) and a 100 mL of the reagent was prepared (Miller, 1959).

One liter of 1 mg/mL glucose stock solution was prepared by mixing 1 g of glucose with 1 L of distilled water. In the preparation of standard solution, 40, 80, 120, 140 and 200 μL of glucose stock solution were transferred to test tubes. Distilled water was then added to bring the liquid volumes in each test tube to 200 μL. After the preparation of the standard solution, similarly the main hydrolysate sample was prepared. The preparation was conducted by transferring 200 μL hydrolysate sample into labeled test tube. 0.5 mL of the DNSA reagent was added to all standard solution and hydrolysate samples in the tubes. The samples were well mixed to achieve homogeneous mixture. The mixture is then placed in water boiling in a beaker for 5 min. After cooling the samples, UV-spectrophotometer (UV-1800) was used to measure the absorbance of the sample in each test tubes at 540 nm (Zhang et al., 2016). From the absorbance of each test tube, the reducing sugar was estimated by using the "trend" function in Microsoft excel.

3.5.2 Fourier transform infrared spectroscopy (FT-IR) analysis for the solid hydrolysate

The raw water hyacinth and pretreated water hyacinth were analyzed by using FTIR (Thermo Scientific iS50 ABX) in order to determine the changes that are occurring on the functional groups. The detection was carried out in the wave number range between 400 and 4000 cm^{-1} with a detector resolution of 4 cm^{-1}.

3.6 Rumen fluid preparation

The rumen fluid was prepared by using distilled water and cow's rumen content brought from the abattoirs. 500 g of the rumen content was measured into a 2000 mL beaker. Then 1500 mL of distilled water was added into the beaker in order to produce slurry.

Then the slurry was mixed using a magnetic stirrer for complete mixing. Then the slurry was filtered using a vacuum filter and a filter paper. The separated liquid was put into 6 250 mL Erlenmeyer flasks and covered using aluminum foil. The liquid was then incubated for 7 days at 38^{0}C for starvation prior to use.

3.7 Experimental operation of anaerobic co-digestion and optimization

After collecting and preparing the raw materials, a laboratory scale anaerobic batch digester was established in the Addis Ababa Institute of Technology bio-engineering laboratory. Plastic bottles of 500 mL capacity fitted with screw caps were used as batch anaerobic digesters. The working volume of the digesters was 400 mL and 100 mL was left as head space for gas. A newly bought urine bag was used for gas collection which was connected to the screw cap that was pierced on the top. The tube end was secured to the screw cap by using a silicone gasket maker to prevent any leakage of gas. The reactors were fed with different ratios of water hyacinth and slaughterhouse wastewater and were kept at a mesophilic range temperature of 38 ± 2^{0}C by using a thermostated water bath in order to match the cattle core temperature to create an optimum environment for the microbes in the rumen fluid to grow. Each digester was fed with 100 mL rumen fluid that is used as inoculum for investigation of its digesting abilities as stated in Table 3.1(Erick Auma Omondi et al., 2019; Rabiu et al., 2014).

Table 3. 1. Substrate preparation for anaerobic co-digestion in a batch bio-digester

	Substrate composition (%)		Substrate volume (mL)		Rumen fluid added (mL)	Working volume (mL)
Sr. no	SWW	WH	SWW	WH		
1	25	75	75	225	100	400
2	50	50	150	150	100	400
3	75	25	225	75	100	400
4	100	0	300	0	100	400

To scrutinize the methane yields and COD removal of the anaerobic co-digestion at different operating conditions, one variable and interaction effects have been studied. The one-variable-at-a time (OVAT) study was used to determine the ranges of the affecting parameters despite its long retention time and the interaction effect to examine how the parameters affect the anaerobic co-digestion altogether. In this regard, the OVAT results were the inputs for the interaction effect. The interaction effects that variables have on the anaerobic digestion were studied using RSM (response surface methodology) followed by optimal (custom) method.

After surveying different articles out there in the literature, four parameters that are considered to affect the anaerobic digestion mostly, among others, were selected. These are substrate volume percentage (VP), hydraulic retention time (HRT) and pH. The volume percentage was the mix proportion of the volume of water hyacinth to the volume of slaughterhouse wastewater (%SWW: %WH) while the hydraulic retention time is the time that takes the digestion process to produce biogas. The responses studied were methane production and COD removal efficiency. The optimal (custom) design was chosen because some modifications were needed to be done which were not possible to do so if central composite design or Box Benkhen design were used.

3.7.1 Effect of parameters one at a time

The effect of each parameter on the methane production and COD removal efficiency was studied based on the one variable at a time approach. Therefore, Volume percentage (VP) ranging from 20–100%, hydraulic retention time (HRT) from 10–70 days and pH from 5–9, have been considered. In this approach, only the effect of one variable was examined while the others were assumed to be constant. Hence, varying the volume percentage to 20, 40, 60, 80, & 100% and fixing hydraulic retention time at 40 days and pH at 7, the effect of volume percentage on the methane production and COD removal of anaerobic digestion was studied (Auma, 2020). In the same fashion, the effect of individual parameters at given operating conditions was scrutinized in the range shown in Table 3.2.

Table 3.2. Factors and levels of for OVAT analyses

Factors	Unit	level				
Volume percentage	%	20	40	60	80	100
pH	–	5.0	6.0	7.0	8.0	9.0
Hydraulic retention time	day	10	25	40	55	70

The pH of the solution was adjusted using 0.1M NaOH and HCl solutions. This study was conducted to determine the best possible operating conditions for enhanced biogas production and performance of the anaerobic digester during the anaerobic co-digestion of water hyacinth and slaughterhouse wastewater using a batch setup. Each of the reactors was inoculated with 0.1 L of fresh rumen fluid. Then the two substrates i.e. pretreated water hyacinth and slaughterhouse wastewater i.e. washed blood from slaughter area and rumen fluid from ruminal waste disposal area were added by using different mixing ratio. Mix ratios were chosen based on the investigation made by Omondi et al.(2019) on the co-digestion of water hyacinth and ruminal slaughterhouse waste. The responses namely methane yield and COD removal were determined using gas analyzer (Biogas 5000, Geotech) and a COD and multi-parameter photometer (HI83099, HANNA instruments), respectively.

3.7.2 Interaction effects of parameters and model evaluation using RSM

Now that screening experiments have been conducted to determine the effects of the operational parameters (volume percentage, hydraulic retention time and pH) individually, it is imperative to investigate the interaction effect of the parameters all at once. So, the data from the screening experiments were used to evaluate the applicable range of these parameters, and they were used to deduce an experimental design using Response Surface Methodology (RSM). It is a technique often used to describe the behavior of experimental data by generating a different order models to represent the data. Thereafter, statistical analyses have been made to determine the accuracy of the model. The model further utilized to optimize and tune the system output to achieve the best possible outcome with that specific set of operating parameters. It was designed to give insight into the potential interactions between the operating parameters. This provides a key advantage for Design of Experiment (DOE) over other approaches, as the variables are not varied one at a time while the others are held constant.

Based on the preliminary experiments conducted, the levels of each parameter which this research focused on were set as shown in Table 3.3.

Table 3.3. Factors and corresponding levels for optimal (custom)experiments

Factors	Factor symbol	Unit	Level			
			Level 1	Level 2	Level 3	Level 4
Volume percentage	A	min	25	50	75	100
pH	B	–	6.0	6.5	7.0	7.5
Hydraulic retention time	C	day	20	30	40	50

A total of 20 experiments were conducted separately for experimental response of both responses (Table 3.4). Mathematical model was selected to relate the methane production and COD removal with all parameters. Response surface regression for the design response was analyzed by quadratic model equation (3.14**)** generated by design expert in the following format.

$$Y = \beta + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_4 X_4 + \mathrm{b}_1 \mathrm{X}_1 X_2 + \mathrm{b}_2 \mathrm{X}_1 X_3 + \mathrm{b}_3 \mathrm{X}_1 X_4 + \mathrm{b}_4 \mathrm{X}_2 X_3 + \mathrm{b}_5 \mathrm{X}_2 X_4 + \mathrm{b}_6 \mathrm{X}_3 X_4 + \mathrm{c}_1 {X_1}^2 + \mathrm{c}_2 {X_2}^2 + \mathrm{c}_3 {X_3}^2 + \mathrm{c}_4 {X_4}^2 + \xi \quad 3.\ 14$$

Where, Y is response variable, β is intercept constant, $\alpha_1 - \alpha_4$ are main linear effects constant, $\mathrm{b}_1 - \mathrm{b}_4$ are linear-linear coefficients, and $\mathrm{c}_1 - \mathrm{c}_4$ are main quadratic effect coefficients, ξ is error and X_1–X_4 are the independent variables (Behboudi-Jobbehdar et al., 2013).

For a three-factor system, a total of 20 experiments were generated to determine the optimum conditions for optimum methane production and COD removal, taking into consideration the interaction among the factors.

Table 3.4. Experimental design matrix of factors for optimal (custom) design

	Factor 1	Factor 2	Factor 3
Run	A:Volume percentage (%)	B: HRT (days)	C: pH -
1	100	20	7
2	75	20	6
3	25	50	7.5
4	75	40	6
5	100	50	6
6	75	30	7.5
7	25	30	6.5
8	100	30	6
9	100	20	7
10	50	30	6
11	75	30	7.5
12	25	50	6
13	25	30	6.5
14	50	20	6.5
15	50	40	7
16	25	20	7.5
17	100	50	7.5
18	50	40	7
19	50	40	7
20	75	50	6.5

3.7.3 Optimization of process variables and model performance evaluation

The performance and significance of the model equations were statistically evaluated using analysis of variance (ANOVA) and multiple correlation coefficients (R2). The parameters considered in this study namely, volume percentage, hydraulic retention time and pH were optimized using numerical optimization to obtain optimal methane production and COD removal. The validity of optimum condition was confirmed with triplicate experimental data and the error was calculated.

3.8 Biogas and bio-digestate characterization

A portable gas analyzer (Biogas 5000, Geotech) was used in order to determine the amount of methane, carbon dioxide, hydrogen sulfide, oxygen and other residual gases in the biogas.

The bio-digestate, on the other hand, was analyzed for its potential to be used as a bio-fertilizer. The contents of total nitrogen (TN), total phosphorus (TP), nitrate (NO_3^-), ammonia (NH_4), phosphate (PO_4^-) and sulfate (SO_4^-) were determined using a colorimetric method by a COD and multi-parameter photometer (HI83099, HANNA instruments) after the necessary treatments and reagent additions were made.

Nitrate was examined by measuring 20 mL of filtered bio-digestate into a vial with a screw cap. Reagents like nitratest powder (1 spoonful) and nitratest tablet (1 tablet) were added and the vial was inverted for one minute after screwing on the cap. After shaking, the vial was let to rest for 2 min settlement of some suspended material. Then, 10 mL of the liquid was slowly transferred into the cuvette for multi-parameter photometer reading. Another reagent tablet, known as nitricol tablet, was finally added, crushed and left for dissolving for 10 min. The nitrate method was chosen for photometer and a blank solution, which is distilled water, was used to set it at zero. After the 10 min was done, the nitrate was measured directly using the multi-parameter photometer.

Ammonia was inspected using reagents known as Palintest Ammonia No 1 Tablets and Palintest Ammonia No 2 Tablets. Here as well, 20 mL of filtered bio-digestate was put into a vial with a screw cap. The tablets were then added consecutively, crushed and mixed. The solution was then left for 10 min to develop a reddish color. 10 mL of the solution was slowly transferred in to the photometer cuvette. Ammonia measurement was chosen for the photometer and was set at zero using a blank solution. After the 10 min, the cuvette was put into the photometer to record the ammonia reading.

Phosphate was determined using reagents known as Palintest phosphate No. 1 and 2 tablets. After measuring 20 mL of filtered bio-digestate, add the tablets consecutively, crush and mix them. 10 mL of the solution was slowly transferred in to the photometer cuvette. The solution was left for 10 min to develop a bluish color. Phosphate measurement method was selected for the photometer and was set at zero using a blank solution. After the 10 min, the cuvette was put into the photometer to record the phosphate reading.

Sulfate was inspected using a reagent known as Palintest sulfate turb tablets. 20 mL of filtered bio-digestate was measured and one tablet, crush and mix it. 10 mL of the solution was slowly transferred into the photometer cuvette. The solution was left for 5 min to develop a cloudy texture. Sulfate measurement method was selected for the photometer and was set at zero using a blank solution. The cuvette was put into the photometer to record the sulfate reading.

Total nitrogen (TN) and total phosphorous (TP) were also determined by using the standard methods for examination of water and waste water set by APHA(Auma, 2020).

4 Results and discussion

This chapter presents the results obtained during the course of the work with their respective discussion. Different justifications are stated with the results discussed.

4.1 Results of proximate analysis of water hyacinth

The moisture content, volatile matter, ash content and fixed carbon of each part (root, leaf and stalk) of the water hyacinth were investigated (Appendix A) for choosing the part that would be suitable for biogas production.

The moisture content was investigated as received and was found to be 84.76 ± 0.53 wt.% for the root, 91.57 ± 0.23 wt.% for the leaf and 92.47± 0.25 wt.% for the stalk. The values indicate that the stalk retains more water than the other parts which is attributed to its spongy nature. The volatile matter for the root, leaf and stalk was also investigated and average values of 64.56 ± 1.5 wt.%, 81.57 ± 0.27 wt.% and 55 ± 1.31 wt.% were obtained, respectively. Higher volatile matter indicates the need for further processing of the biomass for energy use since direct use will create a lot of smoke (Jimoh et al., 2016). The ash content of the Water hyacinth parts was investigated and average values of 22.17 ± 4.42 wt.% for the root, 15.17 ± 0.623 wt. % for the leaf and 10.33 ± 1.43 wt.% for the stalk were obtained. The root has more ash content than the other parts which indicates the higher presence of inorganic matter (Sukarni et al., 2019).

The fixed carbon content of the root was found to be 13.27 wt.% while the leaf and stalk were found to have 3.26 wt.% and 34.67 wt.%, respectively. The fixed carbon content is an indication of the amount of carbon present in the biomass precursor that can be utilized as energy source. The stalk has the highest fixed carbon content which makes it suitable for energy production(Wauton & William-Ebi, 2019). According to the proximate analysis carried out, the leaf and stalk were found to be suitable precursors for biogas production due to the fact that they contain higher amount The above stated results, which are also annexed in appendix A, indicate that the leaf and stalk are found to be complementary to each other with their properties.

After choosing the leaf and stalk for biogas production, further proximate analysis was carried for the mixture in order to see the complementary effects of the parts on each other. The investigation resulted in average values of 92.78 wt.% moisture content, 79.44±3.14 wt.% volatile matter, 10.81±0.17 wt.% ash content and 9.75±3.178 wt.% fixed carbon content as can be seen in Table 4.1. According to Cavalaglio et al. (2020), high volatile matter increases the energy consumption as combustion need to be conducted for extended time to achieve maximum weight loss. The maximum temperature is the parameter used in measuring the reactivity of the sample as it helps to evaluate the anaerobic digestion rate of biomass in the process of biogas production.

The ash content is required to be as low as possible since it is the part of the biomass that will be a residual due to its inorganic nature. Higher amount of ash present in a biomass is an indication of low energy content of the biomass. Other types of lignocellulosic biomass such as agricultural residues have ash contents of up to 15% (Jönsson & Martín, 2016). This indicated that water hyacinth is a good candidate to be used as energy sources such as for the production of biogas.

Kang et al. (2014) have conducted the investigation of various biomass fixed carbon including water hyacinth and reported that the fixed carbon contents of biomass ranges from 5-25 wt.%. The authors pointed out that the variation in fixed carbon might be mainly attributed to environmental impact such as climatic and weather condition, nutrient values in the water body at different location, harvesting time and practice and the type of species. This exhibits that the fixed carbon content of the present study is within the limit to be used as energy sources after maximizing its energy value such as bioethanol.

Bulk density was also investigated and found to be 0.435±0.0519 g/cm^3 on a dry basis. Due to the removal of a substantial amount of water content, the bulk density was found to be low. According to Melane et al. (2017), it was reported that the lower the bulk density of the biomass is, the lower (cheaper) the material handling cost.

Table 4. 1. Proximate analysis results of water hyacinth in comparison with references

Reference	Moisture content (wt.%)	Volatile matter (wt.%) (dry basis)	Ash content (wt.%) (dry basis)	Fixed carbon (wt.%) (dry basis)
This study	92.78	79.44	10.81	9.75
(Melane et al., 2017)	94.7	85.5	17.1	-
(Sukarni et al., 2019)	4.9 (dry basis)	61.2	20	13.8
(Wauton & William-Ebi, 2019)	-	71.27	14.56	14.56
(Hu et al., 2015)	-	70.35	15.32	14.33

4.2 Lignocellulosic components determination

Water hyacinth, as a lignocellulosic biomass, is expected to have components such as extractives, hemicellulose, lignin and cellulose. The composition of these components is highly affected by the environmental condition such as harvesting time, climatic conditions and nutrient values of the water body that the water hyacinth grows on. Investigating the amounts of extractives, hemicellulose, lignin and cellulose is essential in the biogas production process as these components are degraded during the anaerobic digestion. During the course of the present work, the amount of lignocellulosic components of water hyacinth was investigated for the root, leaf and stalk separately as illustrated in Figure 4.1. Extractives were found to be 15.92 wt.% for the root, 8.43 wt.% for the stalk and 7.44 wt.% for the leaf while the amounts of hemicellulose were 39.6 wt.% in the root, 44.8 wt.% in the stalk and 46.4 wt.% in the leaf. The root, stalk and leaf were found to have lignin amounts of 25.58 wt.% , 12.22 wt.% and 14.12 wt.%, respectively and cellulose amounts of 18.9 wt.%, 33.7 wt.% and 32.04 wt.%, respectively. The results show a higher content of hemicellulose and cellulose in the leaf and stalk of the water hyacinth which indicates suitability for biodegradation due to their organic nature during anaerobic digestion(Auma, 2020) and represent the organic matter in the lignocellulosic biomass that are degradable to reducing sugar and in turn to methane, than that of the root (Cater et al., 2014). Lignin also acts as a shield for the hemicellulose and cellulose consequently inhibiting the hydrolysis process of the anaerobic digestion(Ma et al., 2019). Lignin is also an organic component but is not composed of sugar instead it is made of aromatic compounds like phenyl-propane units such as syringyl, guaiacyl and p-hydroxyphenyl(Cater et al., 2014).

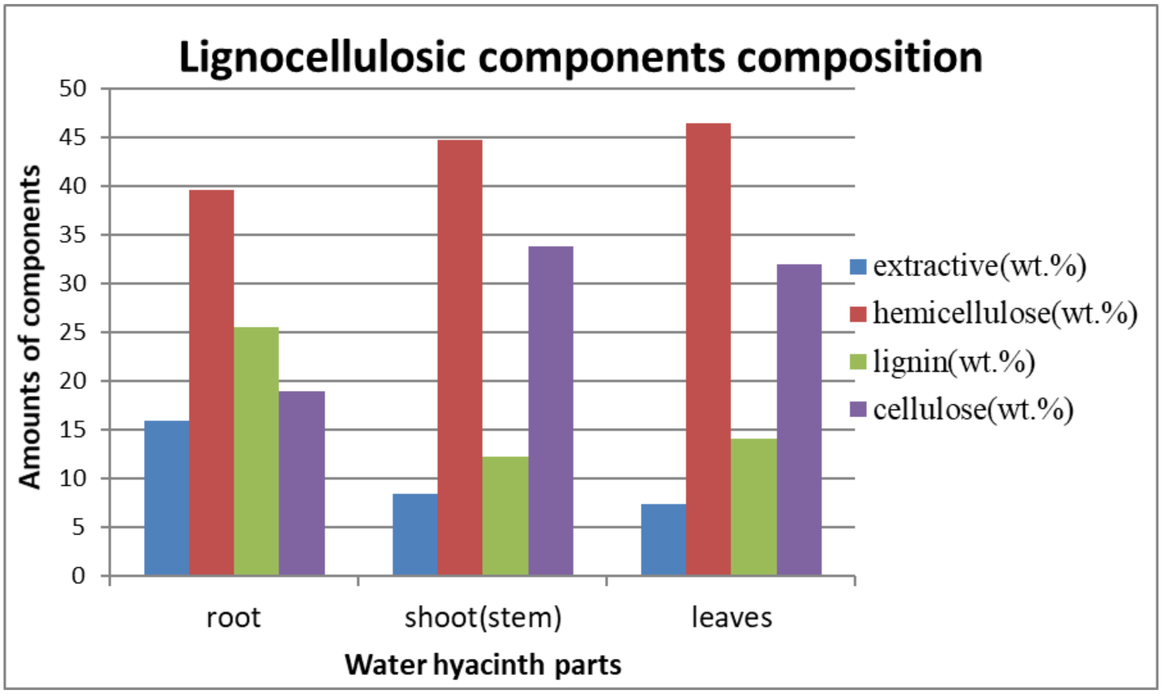


Figure 4. 1. The amounts of lignocellulosic components in the different water hyacinth parts

The mixture of the leaf and stalk was further investigated to justify the fact that they will have high biodegradable and low inorganic components. The extractives content in the mixture was found to 8.92 wt.% while those of hemicellulose, cellulose and lignin were found to be 45.13 wt.%, 33.51 wt.% and 12.43 wt.%, respectively, as illustrated in Figure 4.2.

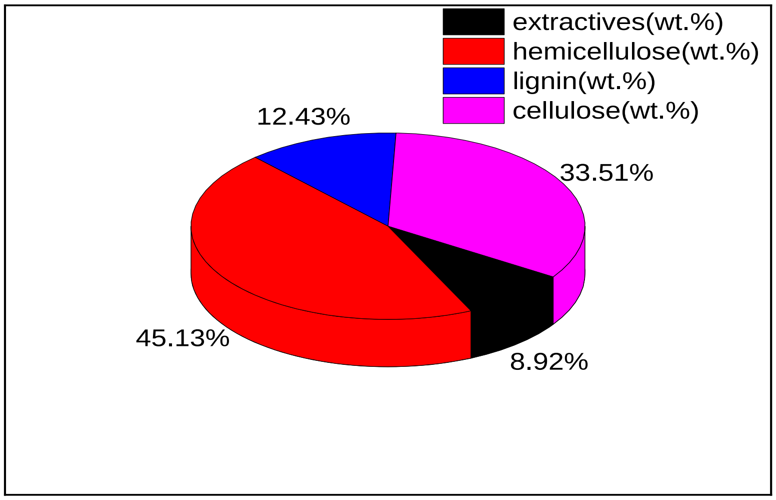


Figure 4. 2. Lignocellulosic components amount for the mixture of water hyacinth leaf and stalk

4.3 Slaughterhouse wastewater characterization

The slaughterhouse wastewater was investigated for different parameters to determine its potential for biogas production and to analyze the COD removal efficiency of the successive anaerobic digestion process. The parameters investigated were moisture content, total solid, volatile solid, fixed solid, COD, BOD_5 and pH as shown in Table 4.2. The investigation revealed that slaughterhouse wastewater had a total solid of 5.4 wt.% indicating that additional water is not required and high volatile solid of 85.2 wt.% which can be attributed to the solid wastes that are washed out during the meat and slaughter area cleaning(Worku, 2018). A BOD_5 of 4372.22 mg/L and COD of 6699 mg/L were also obtained which are in agreement with values reported by (Worku & Leta, 2017). These values were found to be high due to the presence of blood in the wastewater which in turn indicates high organic matter presence in the wastewater.

The pH was also investigated and found to be 7.17±0.036. As reported by Worku & Leta (2017), the pH was seen to oscillate between values of 7.14 ± 0.16 and 7.23±0.27 which suggests that the effluent is more or less neutral. According to Mulu & Ayenew (2015), the pH of the Kera abattoirs waste water was reported to be 6.8 to 7.6. It was stated that the pH was within the range of the EPA standard values (6.0-9.0) that were set for abattoirs discharge into surface water.

The pH and percentage total solid, volatile solid and fixed solid were also investigated for the rumen content. On average, the pH of the rumen content was found to be 7.82±0.12 which is slightly basic. According to Petrovski (2017), a pH value between 7.5-8.5 with light brown color is a result of simple indigestion or starvation. The total solid, on the other hand, was found to be 37.2 wt.% while the volatile solid was obtained to be 88.4 wt.% and fixed solid was found to be 11.6 wt.%. Since the total solid is more than 10%, additional water is required. Therefore, the rumen content was mixed with water and filtered. The values obtained by Tsegaye (2016) for volatile and fixed solid was comparable even though the total solid values were different which might be due to the heterogeneity of the feed for the cattle that the rumen content was obtained from. The characterization of the rumen fluid was necessary in order to determine the amount of water required in producing the rumen fluid and the pH indicates what the effect is on the digestion process.

Table 4. 2. Slaughterhouse waste water and rumen content characteristics

Characteristics	SWW	Rumen content
Moisture content (wt.%)	94.6	62.8
Total solid (wt.%)(wet basis)	5.4	37.2
Volatile solid (wt.%) (dry basis)	85.2	88.4
Fixed solid (wt.%) (dry basis)	14.8	11.6
BOD_5 (mg/L)	4372.22	-
COD (mg/L)	6699	-
pH	7.17±0.03	7.82±0.12

4.4 Effect of water hyacinth pretreatment

The dilute acid hydrolysis pretreatment of the water hyacinth resulted in the reduction of the polysaccharide structure into monosaccharide. The reducing sugar obtained in the liquid hydrolysate was 11.94 g/L. In this pretreatment, the hemicellulose and lignin are expected to be reduced. According to Barua & Kalamdhad (2016), the pretreatment of water hyacinth led to the softening of the lignin which is an indication of the destruction of the sturdy structure as well as the bio-accessibility of the cellulose.

4.4.1 FTIR analysis of the raw and pretreated water hyacinth

The raw and dilute acid pretreated water hyacinth was analyzed using the Fourier transform infrared spectroscopy (FTIR). During FTIR analysis interpretation, it should be known that there are two regional designations that ease the interpretation. These are the 'functional group' region which is found in the range of 1500-4000 cm^{-1} and the 'fingerprint' region which is found in the range of 400-1500 cm^{-1} as illustrated on Figure 4.3.

In the functional group region, it can be seen that there are broad peaks that occur between 3200 and 3500 cm^{-1}. These peaks are reported to represent the OH stretch which is an indication for the presence of primary alcohols and water molecules (Anjos et al., 2016; Sataloff et al., n.d.). As can be seen from Figure 4.3, the intensity of the peak for the pretreated water hyacinth is lower than that of the raw water hyacinth which is an indication of the removal of some of the OH containing, presumably, water molecules.

In the raw water hyacinth spectrum, there are two peaks occurring in the functional group region. One of the peaks is occurring at 2914.5 cm^{-1} indicating that there is a presence of alkanes or a C-H stretch. These components occur in the range of 2800-3000 cm^{-1} wavenumber. As can be seen from the graph, this peak disappears in the pretreated water hyacinth which is an indication of removal or degradation of components. The other peak appears at a wavenumber of 1615 cm^{-1} which a representation of a C-C stretch or alkenes. This peak is seen to be dissolved into two peaks, appearing at 1644.9 and 1555.1 cm^{-1}, with lower intensity in the pretreated water hyacinth spectrum. This shows that the pretreatment is indeed successful in changing the structure of the water hyacinth(Fallis, 2013).

In the fingerprint region, on the other hand, there are two peaks appearing at 1305.6 cm^{-1} and 1007.1 cm^{-1} wavenumbers. These peaks represent the existence of C-N stretch (amines) and aromatic C-H bends, respectively(Fallis, 2013; Sataloff et al., n.d.). The amine stretch is seen to decrease significantly or even disappear in the pretreated water hyacinth spectrum. This indicates the removal of amines from the water hyacinth during pretreatment. But a peak with low intensity appears at a wavenumber of 1017.1 cm^{-1} indicating the existence of a C-O stretch of esters that occurs in the range of 1300-

1000(Sataloff et al., n.d.). The overall analysis indicates that the pretreatment was successful in the removal of unwanted materials and conversion of some.

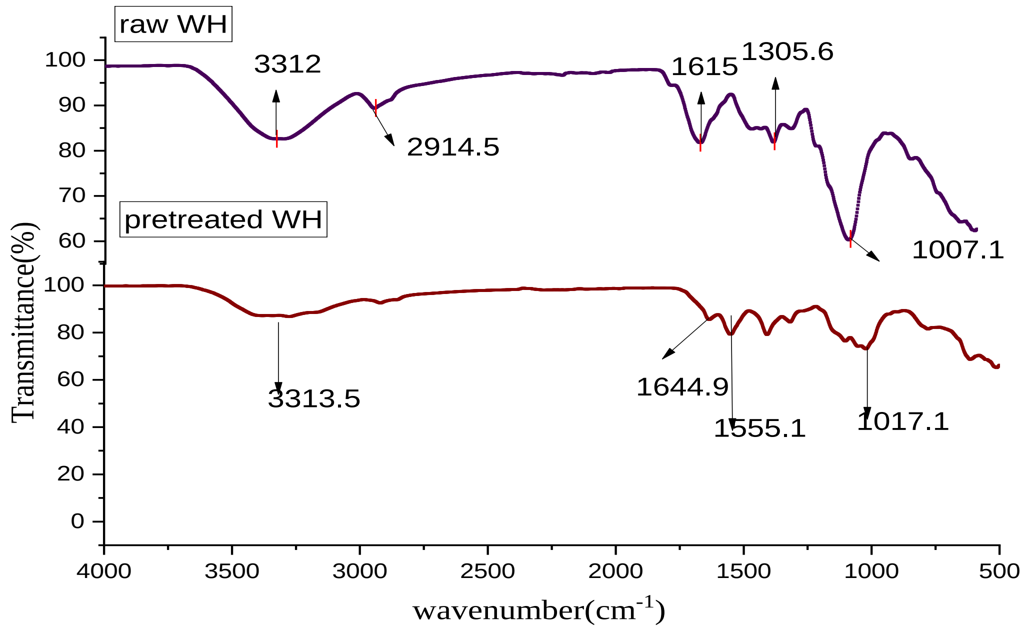


Figure 4. 3. FTIR analysis of water hyacinth before and after pretreatment

4.5 Experimental design for determination of optimum anaerobic co-digestion process

A preliminary study based on the one variable at a time approach and the interactive effect of process parameters which are substrate volume mix ratio, hydraulic retention time and pH on the anaerobic digestion process was made. The preliminary study was used to scope the domain of this research with regard of the parameters' levels. The levels of each variable were determined based on the effect they have on the methane production and COD removal efficiency.

In a given process, be it digestion or another, Mother Nature does not function on single variable basis. Rather, the parameters that characterize the process are more likely interactive; one variable affects the other and the process. Therefore, it is important to investigate the effects of the interactions of the pertinent parameters .

4.5.1 Effect of individual parameters

In this preliminary study, the effect of individual parameters on the anaerobic digestion process was examined by varying one parameter and keeping the others constant. Thus, volume percentage of substrates in the range of 20–100%, hydraulic retention time from 10–70 and pH 5–9 have been considered.

4.5.1.1 The effect of volume percentage on the methane production and COD removal efficiency

The effect of volume percentage on methane production and COD removal of the anaerobic digestion process at 20, 40, 60, 80 and 100% SWW was studied at a fixed hydraulic retention time of 40 days and pH of 7. As can be seen from Figure 4.4 (A) and (B), both methane production and COD removal efficiency exhibit an increasing-and-then-decreasing patterns. The methane production increases from 25.7% to 76.4% as the substrate volume percentage increases form 20% SWW to 60% SWW. However, when the substrate volume further increases to 100% SWW, the methane production declines to 50.2%. The increase of methane production with substrate volume percentage is owing to the favorable conditions created due the increase in the reducing sugar concentration added. Water hyacinth produced reducing sugar after pretreatment which is added to the digestion bottle after neutralization of the acid in the hydrolysate. The increase in the presence of water content owing to the increase in SWW volume percentage is also beneficial in reducing the restriction of mass transfer of particulate substrates (Nugraha et al., 2018). The decrease in the methane production observed at substrate compositions higher than 60 % (v/v) might be due to the decrease in the organic matter which resulted from the decrease in the water hyacinth amount. Furthermore, high amount of water hyacinth (corresponding to low values of SWW) is seen to be not beneficial towards methane production. This might be due to the high volatile matter content of water hyacinth which leads to the accumulation of volatile fatty acids (Erick Auma Omondi et al., 2019). The COD removal efficiency also increased when the volume percentage increased which might be due to the appropriate amount of organic matter and reducing sugar available for microbial action but decreased as the substrate volume percentage further increased. This might be attributed to the microbial action inhibition due to shock from high organic matter presence and the deprivation of reducing sugar.

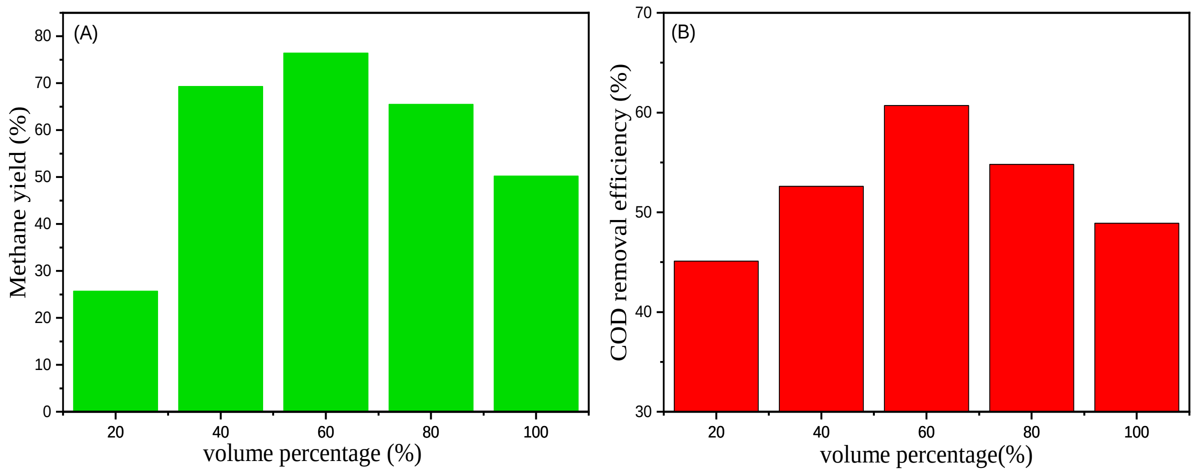


Figure 4.4. Effect of volume percentage on methane production and COD removal from anaerobic digestion at constant HRT and pH

4.5.1.2 The effect of pH on the methane production and COD removal efficiency

The effect of pH on the production of methane and COD removal of anaerobic digestion at 5.0, 6.0, 7.0, 8.0 and 9.0 was studied to examine the optimum pH for rumen fluid since cows' core pH ranges from 6.4 to 7.2at a fixed volume percentage of 60% and hydraulic retention time of 40 days. It is evident from Figure 4.5 (A) that the methane production increases from 55% to 75.2% as the pH increases from 5 to 7. Thereafter, the production of methane decreases down to 57.1%. Similarly, the COD removal efficiency increases from 38.8% to 61.5% when pH varies from 5 to 7 and then falls to 36.1% when the pH increases to 9. As can be seen from 4.5 (A) and (B), conversion of substrates to methane and COD removal efficiency of the digestion process is favored near neutral pH. This may be due the favorable operating conditions for the microbes when the environment is 6.5–7.0 pH. According to Hoda (2020), the pH affects the microbes for both methane production and COD removal. The increase in methane production is an indication to the growth of methanogens while the decline indicates their inhibition due to acidic or basic pH. The COD removal is affected due to the fact that microbes are being used for its removal. Therefore, their growth increases removal while their inhibition decreases removal.

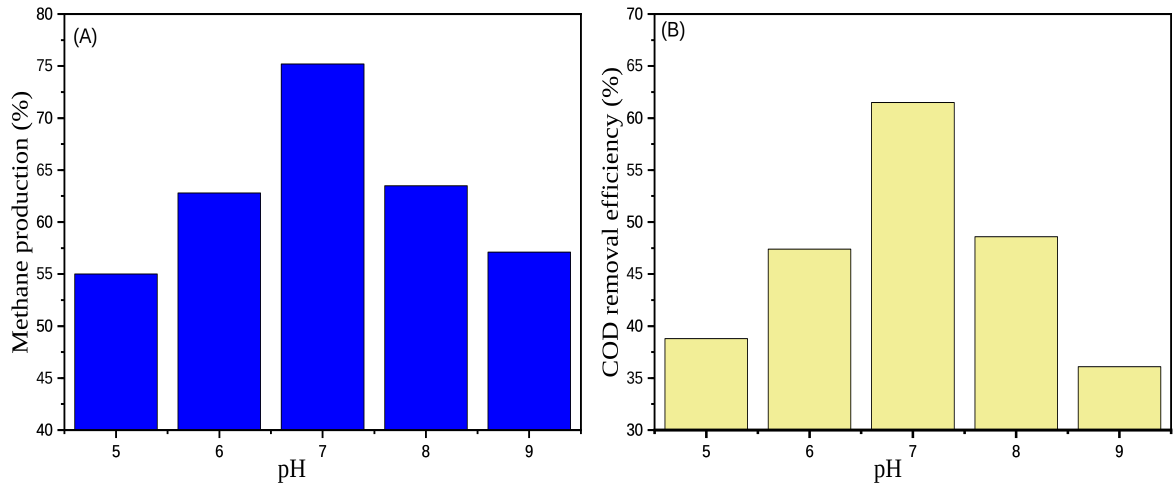


Figure 4.5. Effect of pH on methane production and COD removal from anaerobic digestion with constant HRT and volume percentage

4.5.1.3 The effect of hydraulic retention time (HRT) on the methane production and COD removal efficiency

The effect of hydraulic retention time on the production of methane and COD removal of anaerobic digestion at 10, 25, 40, 55 and 70 days (Omondi et al., 2020)was studied at a fixed volume percentage of 60% and pH of 7. The methane production as function of hydraulic retention time is demonstrated in Figure 4.6(A). The amount of methane production with respect to digestion days was found to increase from 5% to 70.5% as the HRT increased from 10 to 40 days beyond which the methane production tends to be almost constant. This is due to the favorable condition for growth of methanogens as they get accustomed to the conditions created in the digester. But as time increases they are inhibited due to the accumulation of volatile fatty acids (VFAs), such as acetic acid, which create acidic conditions which are not favorable for the growth of methanogens. Otero & Mor (2008) reported that the anaerobic digestion of solid slaughterhouse waste started producing biogas right after loading. But the methane production was seen to decrease as the hydraulic retention time increased which was attributed to the accumulation of VFAs in the reactor inhibiting acetate consuming methanogens.

A similar trend was also observed with the COD removal efficiency, as is seen in Figure 4.6 (B). The efficiency of COD removal was found to vary from 15% to 60.2% initially as the hydraulic retention time increases to 40 days due to the consumption of the easily available organic material which creates high microbial population. The COD removal efficiency becomes almost constant even though the time increases to 70 days. The COD removal is carried out using microbial action. So the inhibition of the microbes due to the changing conditions inhibits the COD removal as well. According to an investigation done by Musa & Idrus (2020) on the effect of hydraulic retention time on the treatment of real cattle slaughterhouse wastewater and biogas production, the COD removal was seen to increase with increase in time which was attributed to the increase in the microbial population. But as the HRT increased the COD removal efficiency was seen to remain constant.

The anaerobic digestion of these substrates is such a slow process that took more than 40 days to produce 70% methane and to remove 60% of the COD. When the substrate depleted, the methane production and COD removal efficiency exhibit constant trends.

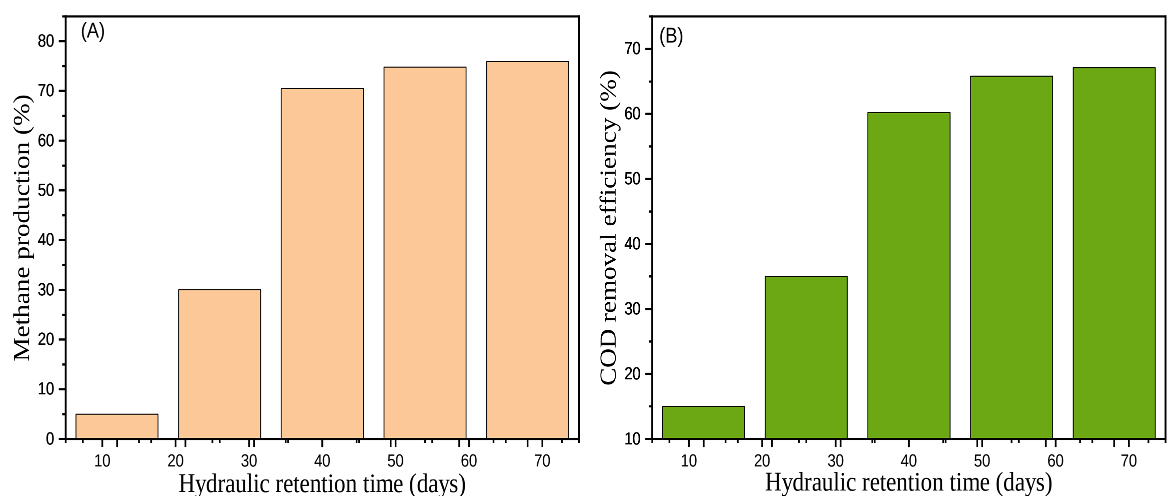


Figure 4.6. Effect of hydraulic retention time on methane production and COD removal from anaerobic digestion

4.5.2 Interactive effect of parameters and process optimization using RSM

Response surface methodology (RSM) is an advanced statistical analysis tool used to develop empirical models and optimize multivariate processes (Panjiara & Pramanik, 2020; Wenyan et al., 2020; Yahya et al., 2020). It analyzes the effect of parameters and interaction of parameters on the response, generates model mathematical equation, evaluates the significance of parameters, interactive terms and model equations, and plots 3D surfaces that create ease to interpret.

Based on the individual parameters effect results, the levels of the variables used for the RSM study were determined. The range of parameters; volume percentage (25–100%), hydraulic retention time (20–50 days) and pH (6–7.5) were set. In these given ranges, 20 runs were conducted and the effects inspected by examining the significance of the model, parameters, terms and the process were optimized and the result was validated.

It is also necessary to determine the initial COD concentration of the different volume ratio of the substrates to be co-digested i.e. mixture of slaughterhouse waste water and water hyacinth. As can be seen in Table 4.3, the COD value decreases with increase in the water hyacinth which is attributed to the relatively lower organic component present in the Water hyacinth(Auma, 2020).

Table 4. 3. Initial COD concentration of the mixture (SWW and WH) to be co-digested

Substrate volume percentage	25%SWW:75%WH	50%SWW:50%WH	75%SWW:25%WH	100%SWW
COD concentration	5055	5481	5821	6699

4.5.2.1 Model fitting and analysis of variance (ANOVA)

The software design expert uses the experimental data and performs non-linear regression to make a mathematical model that represents the response as function of the variables in question. This model, thereafter, is used to draw the 3D surface plots and optimize the process variables. Given the experimental data, the developed mathematical model should, however, be statistically evaluated as to whether it represents the process mathematically based on the variables considered. The parameters that characterize the significance of the model fit are P-value and R-square (determination of coefficient) value. A model with P-values less 0.05 and R-square close to unity implies significance and that the response predicted by the model and the experimental data are close to each other. Among the models proposed by the software, as stated in Table 4.4 and 4.5, a quadratic model was found to be the best fit with P-value of <0.0001 and R-square of 0.96 and 0.987 for methane production and COD removal efficiency.

Table 4. 4. The Model fit summary for methane production

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.3873	0.0002	0.0118	-0.4254	
2FI	0.3229	0.0002	0.0609	-4.4372	
Quadratic	**< 0.0001**	**0.0537**	**0.9225**	**0.5794**	**Suggested**
Cubic	0.0537		0.9736		Aliased

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	14.21	0.1678	0.0118	-0.4254	5532.36	
2FI	13.85	0.3574	0.0609	-4.4372	21102.80	
Quadratic	**3.98**	**0.9592**	**0.9225**	**0.5794**	**1632.44**	**Suggested**
Cubic	2.32	0.9930	0.9736		*	Aliased

Table 4. 5. The Model fit summary for COD removal efficiency

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.4751	0.0001	-0.0203	-0.4418	
2FI	0.6513	< 0.0001	-0.1122	-5.2082	
Quadratic	**< 0.0001**	**0.5094**	**0.9753**	**0.9400**	**Suggested**
Cubic	0.5094		0.9750		Aliased

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	12.54	0.1408	-0.0203	-0.4418	4219.98	
2FI	13.09	0.2390	-0.1122	-5.2082	18170.42	
Quadratic	**1.95**	**0.9870**	**0.9753**	**0.9400**	**175.60**	**Suggested**
Cubic	1.96	0.9934	0.9750		*	Aliased

The quadratic equations that predict the methane production and COD removal efficiency as function of volume percentage, hydraulic retention time and pH are given as equation (4.1) and (4.2)

$$\begin{aligned} MP &= -757.04 - 0.2105 * VP + 1.59 HRT + 235.68 * pH \\ &+0.0053 * VP * HRT + 0.1665 * VP * pH + 0.356 * HRT * pH \\ &-0.0095 * VP^2 - 0.0566 HRT^2 - 18.87 pH^2 \end{aligned} \quad 4.1$$

$$\begin{aligned} CODRE &= -1349.8 + 0.311 * VP + 3.18 * HRT + 396.86 * pH \\ &+0.008 * VP * HRT - 0.004 * VP * pH + 0.168 * HRT * pH \\ &-0.004 * VP^2 - 0.06 * HRT^2 - 29.94 * pH^2 \end{aligned} \quad 4.2$$

Where;

MP is methane production, %

VP is substrate volume percentage, %

HRT is the hydraulic retention time, days

COD RE is the COD removal efficiency, %

Similarly, the significance of the main variables and the other interactive terms were statistically analyzed using P-value and F-value. As is shown in Table 4.6, all the parameters were significant with VP<0.0269, HRT <0.0013 and pH <0.0339. For the COD removal efficiency response, p-values are <0.0002, <0.0001, <0.0452, respectively, as stated in Table 4.7.

Table 4. 6. ANOVA analysis of model, main parameters and terms methane production response

Source	Sum of Squares	df	Squares Mean	F value	P-value Prob>F	
Model	3722.90	9	413.66	26.13	<0.0001	Significant
A	106.21	1	106.21	6.71	0.0269	
B	307.80	1	307.80	19.45	0.0013	
C	95.55	1	95.55	6.04	0.0339	
AB	64.75	1	64.75	64.75	0.0707	
AC	142.78	1	142.78	9.02	0.2105	
BC	108.20	1	108.20	6.84	0.0133	
A^2	569.03	1	569.03	35.95	0.0258	
B^2	513.07	1	513.07	32.41	<0.0001	
C^2	388.74	1	388.74	24.56	0.0002	
Residual	158.29	10	15.83			
Lack of Fit	131.31	5	26.26	4.87	0.0537	Not significant
Pure Error	26.98	5	5.40			

Table 4. 7. ANOVA analysis of model, main parameters and terms COD removal efficiency response

Source	Sum of Squares	df	Squares Mean	F value	P-value Prob>F	
Model	2888.75	9	320.97	84.23	<0.0001	Significant
A	130.57	1	130.57	34.26	0.0002	
B	668.09	1	668.09	175.32	0.0001	
C	19.94	1	19.94	5.23	0.0452	
AB	146.81	1	146.81	38.52	0.0001	
AC	0.0968	1	0.0968	0.0254	0.8766	
BC	24.33	1	24.33	6.38	0.0300	
A^2	88.28	1	88.28	23.17	0.0007	
B^2	601.53	1	601.53	157.85	0.0001	
C^2	979.04	1	979.04	256.92	0.0001	
Residual	38.11	10	3.81			
Lack of Fit	18.84	5	3.77	0.9780	0.05094	Not significant
Pure Error	19.26	5	3.85			

Now that the models are statistically significant as measured with P- and R-square values, it is expected that responses (methane production and COD removal efficiency) values predicted by the mathematical models and actual values found form experimental data are close to one other. For this particular study both values are more or less alike since R-squares are close to unity (0.96 and 0.987) and P-value is less 0.0001. The predicted and the actual values are shown in Table 4.8.

Table 4. 8. Experimental design matrix used in RSM

	Factor 1	Factor 2	Factor 3	Response 1		Response 2	
Run	A:Volume percentage	B:HRT	C: pH	methane production		COD removal efficiency	
	(%)	(days)	-	Actual (%)	Predicted	Actual (%)	Predicted
1	100	20	7	40.8	38.58	27.8	30.49
2	75	20	6	41.2	43.50	24	25.18
3	25	50	7.5	50.1	47.41	26.5	26.26
4	75	40	6	60.8	58.05	48.9	47.57
5	100	50	6	37.3	33.23	41.9	41.16
6	75	30	7.5	61.5	62.63	38.4	38.54
7	25	30	6.5	67.2	63.76	49.5	48.70
8	100	30	6	33.2	38.67	39.1	39.29
9	100	20	7	40.3	38.58	33.3	30.49
10	50	30	6	67.2	62.35	40.5	41.06
11	75	30	7.5	62	62.63	38	38.54
12	25	50	6	40.5	43.03	25.2	24.84
13	25	30	6.5	59.9	63.76	46.7	48.70
14	50	20	6.5	59.4	58.38	41	38.59
15	50	40	7	75.7	75.60	59	58.25
16	25	50	7.5	32.8	34.50	15	15.44
17	100	50	7.5	55.8	56.35	42.5	42.09
18	50	40	7	75.4	75.60	58.6	58.25
19	50	40	7	75.8	75.60	58.5	58.25
20	75	50	6.5	58.8	63.40	53.1	55.72

The model predicted and experimental values are also presented in Figures 4.7 and 4.8 for methane production and COD removal efficiency, respectively.

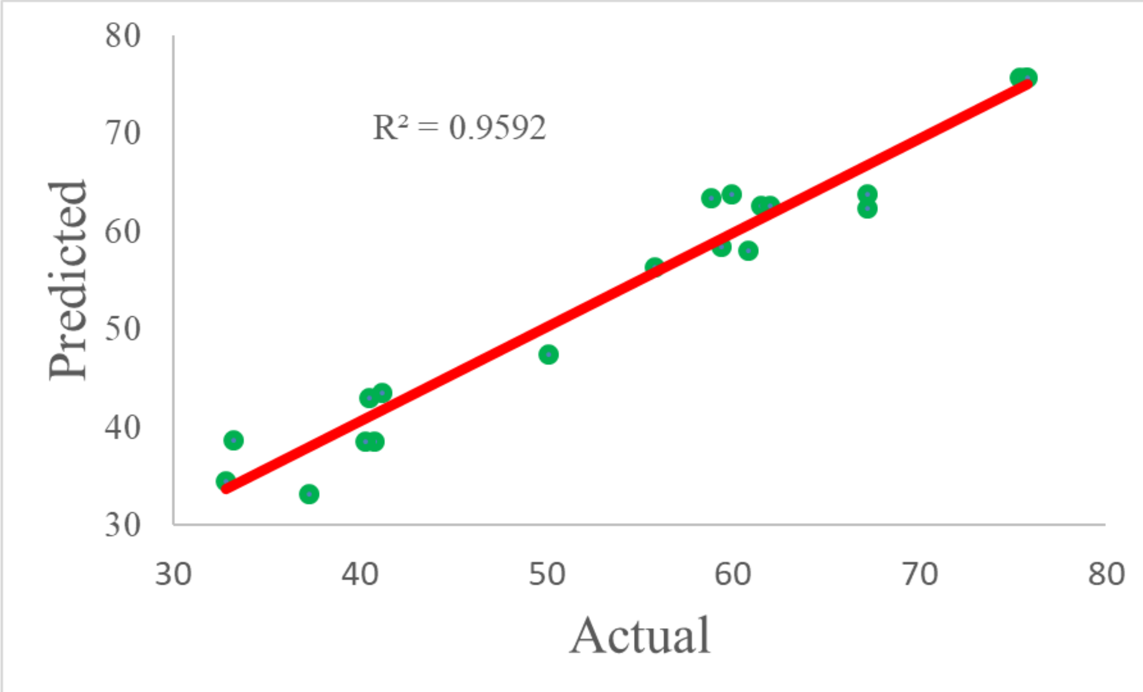


Figure 4. 7. Model predicted versus experimental values of methane production

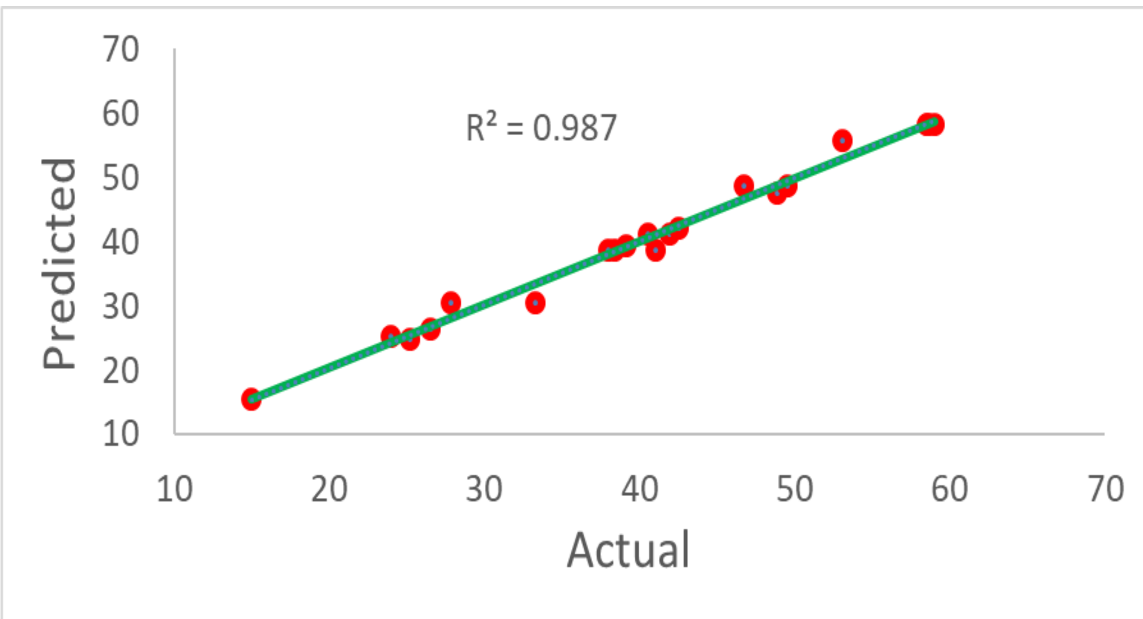


Figure 4. 8. Model predicted versus experimental values of COD removal efficiency

4.5.2.2 Interactive effect of variables

The interaction effect of substrate volume percentage and hydraulic retention time on the methane production of the anaerobic digestion process is shown in Figure 4.9. Figure 4.9 reveals that the interaction effect of substrate volume percentage and hydraulic retention time at a pH of 6.75. It can be observed that the methane production increases from 51.4% to 76.9% as the volume percentage increases from 20%SWW to 60%SWW and hydraulic retention time increases from 20 days to 39 days. This is due to the fact that the methanogens are given enough time and increased reducing sugar to grow which in turn produce more methane. However, the methane production decreases from 76.9% to 56.4% as volume percentage increases to 100% SWW and hydraulic retention time further increases to 50 days. This might be due to the fact that the microbial community was inhibited due to the accumulation of volatile fatty acid and high organic loading rate (Poh et al., 2016). Investigations were carried out by Worku & Leta (2017) on the anaerobic digestion of slaughterhouse wastewater and it was reported that the microbes might be overwhelmed with the organic matter present in the substrate when the organic loading rate is increased. It is evident from the Figures 4.9 that the methane production seems to have a favorable operating condition of substrate volume percentage of 50%SWW and higher hydraulic retention time.

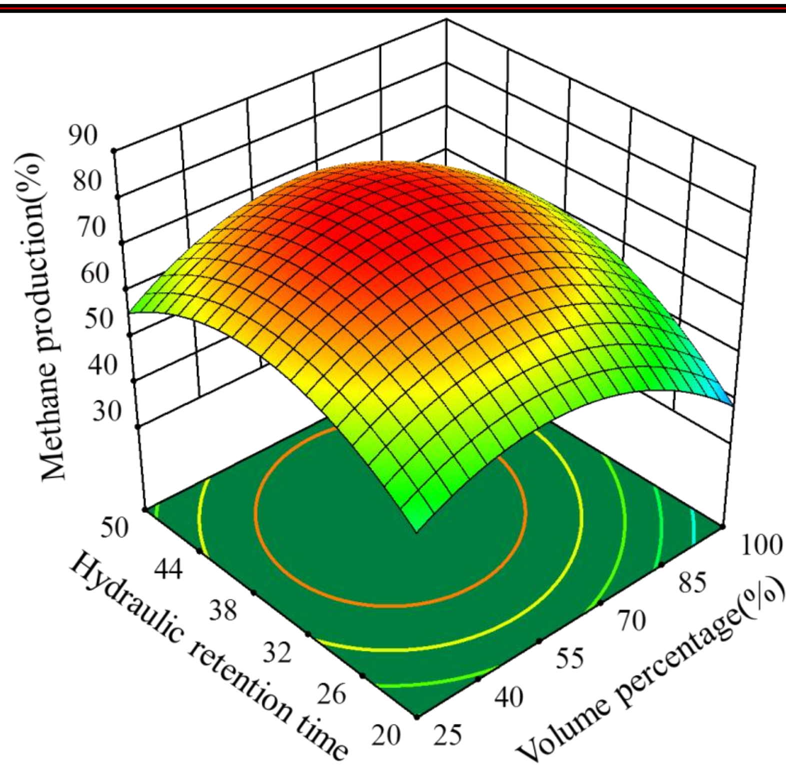


Figure 4.9. Interaction effect of substrate volume percentage and hydraulic retention time

Figure 4.10 depicts the methane production as function of substrate volume percentage and pH. Figure 4.10 shows the interaction between variables substrate volume percentage, pH and the response methane production at hydraulic retention time of 35 days. It can clearly be seen from the graph that the methane production increases from 57.6% to 76.5% as the substrate volume increases from 25%SWW to 60.5%SWW and pH increases form 6 to 6.8 which might due to the favorable condition (good amount of substrate and good pH) created for the growth of methanogens. However, when both substrate volume ratio and pH further increase to 100%SWW and 7.5, respectively, the production of methane form anaerobic digestion process decreases to 58.2% which might be that the methanogens were inhibited by pH increase. An investigation done on the optimization of anaerobic co-digestion of oily-biological sludge and sugarcane baggase suggested that the optimum condition for anaerobic digestion was between 6.2 and 8(Abdulhakim et al., 2020).

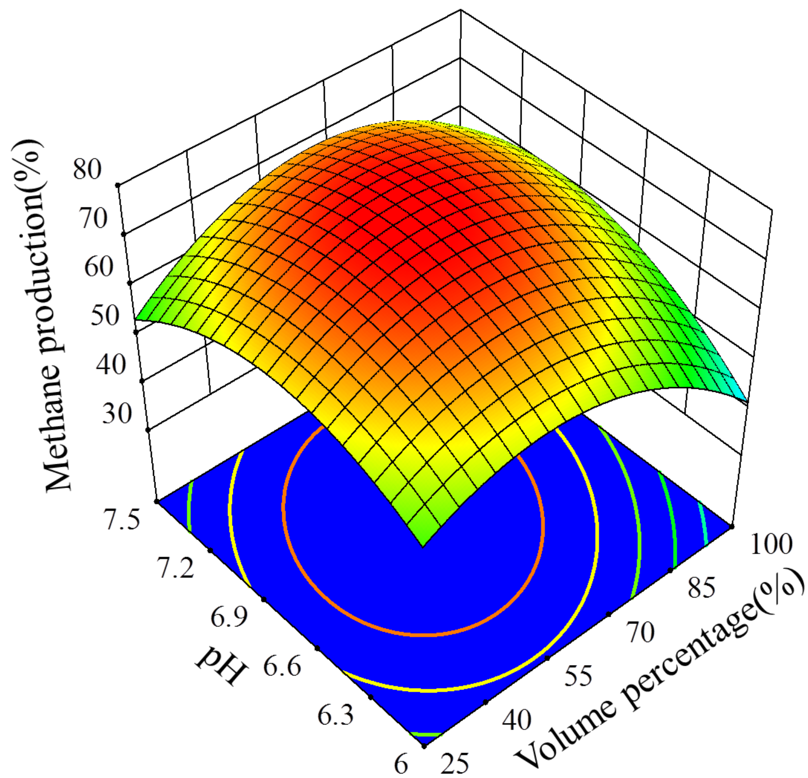


Figure 4.10. Interaction effect of substrate volume percentage and pH

The interaction effect of hydraulic retention time and pH on methane production is demonstrated in Figure 4.11. At a fixed substrate volume percentage, the effect that hydraulic retention time and pH have on the methane production of the anaerobic digestion process is manifested in Figure 4.11. The Figure unfolds the methane production increases from 47% to77.1% as the hydraulic retention time increases from 20 days to 40 days and pH decreases from 7.5 to 6.8 which are attributed to the fact the methanogens were adapting well to the environment because of its suitability for their growth and the digestion process was producing methane. Further increment in hydraulic retention time to 50 days and decrement in pH to 6, however, affect the methane production to decrease to 52.2%. This might be due to the slightly acidic condition which is not favorable for methanogens. The longer time the methanogens spend in the acidic condition, the more they are inhibited. The effect of pH and retention time on the treatment of slaughterhouse wastewater using an up-flow anaerobic sludge blanket was investigated and it was observed that methane production increases when the pH was between 6.5 and 7.2 but decreased when the pH was below 6.5 due to the accumulation of volatile fatty acids at lower pH.

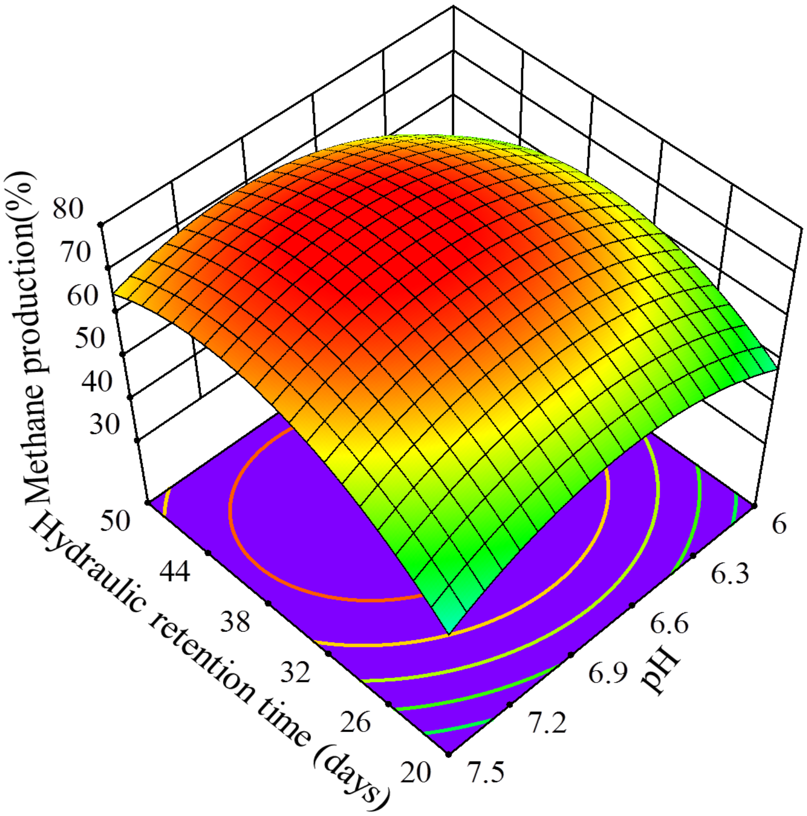


Figure 4.11 Effect of hydraulic retention time and pH

More or less, the interactive effects of the process variables on the COD removal efficiency of anaerobic digestor has similar patterns as that of methane production because methane production and COD removal effeciency are directly proportional(Worku & Leta, 2017). Figure 4.12 illustrates the interaction effect of substrate volume percentage and hydraulic retention time at a 6.75 pH. As is observed in Figure 4.12, the COD removal efficiency of the anaerobic digestion process increases from 34% to 61% as hydraulic retention time increases from 20 days to 39 days and substrate volume percentage decreases from 100%SWW to 53%SWW. Here, the microbes are given enough time as well as reducing sugar as food source for COD removal from the mixture at a decreasing volume percentage. The COD removal efficiency tends to decrease when the substrate volume percentage falls to 25%SWW and the hydraulic retention time goes up to 50 days. This might be due to the fact that decreasing the substrate volume percentage means increasing the volume of water hyacinth. water hyacinth is known to have high volatile solids present which will lead to the production of volatile organic acids such as acetic acid. These organic acids decrease the pH in the digester leading to the inhibition of methanogens and in turn inhibits COD removal by microbes. According to investigations made by Omondi et al. (2019) on the anaerobic co-digestion of water hyacinth with ruminal slaughterhouse waste, it was observed that the pH decreased with increase in the water hyacinth amount. This led to the conclusion that the water hyacinth concentration was responsible for the decrease in pH and inturn microbe inhibition.

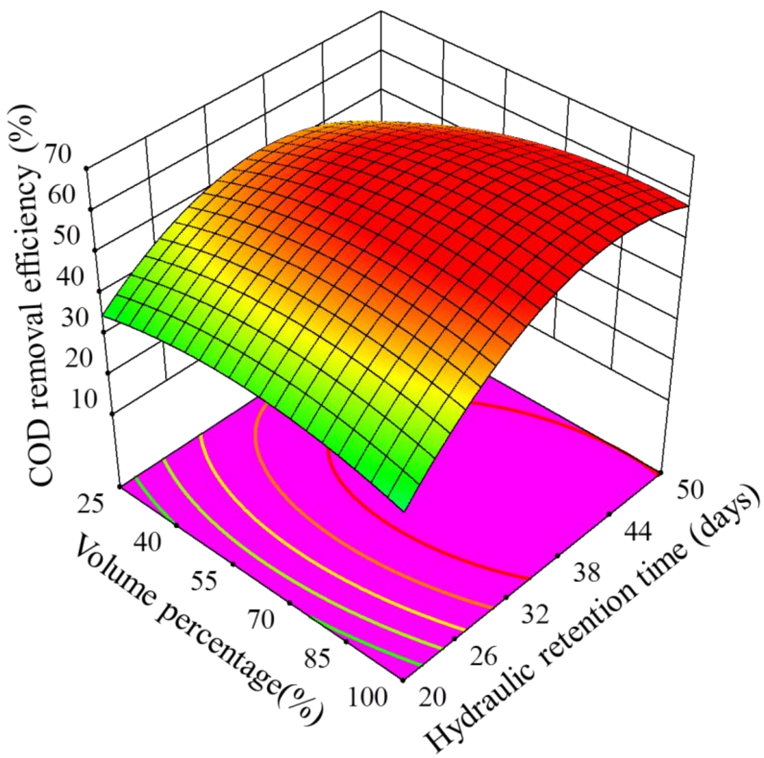


Figure 4.12. Effect of substrate volume percentage and hydraulic retention time

The COD removal efficiency of the anaerobic digestion process is shown in Figure 4.13. It is given as function of pH and substrate volume percentage at constant hydraulic retention time of 35 days. It was found that the COD removal efficiency increases from 37.2% to 62% as both pH and substrate volume percentage increase from 6 to 6.7 and 25 to 65%SWW, respectively which indicates that there is a growth of methanogens that are also responsible for COD removal(Hu et al., 2018). However, when both the pH and substrate volume percentage increase to 7.5 and 100%SWW, respectively, the COD removal efficiency is reduced to 41.8% which might be due to the increase in the slaughterhouse wastewater amount which has higher COD concentration. The higher COD concentration indirectly represents organic loading rate. Therefore, higher COD concentration means higher organic loading rate which causes inhibition of microbes due to scum accumulation(Veroneze et al., 2018). According to Pramanik et al. (2019), the performance of a single stage anaerobic digestion system was investigated using food waste as substrate. During the investigation it was observed that the COD removal efficiency due to the increase in the organic loading which causes scum formation and in turn inhibiting the microbial action. Figures 4.13 is evident to the operating conditions for maximal COD removal are near neutral pH and 50%SWW substrate volume percentage.

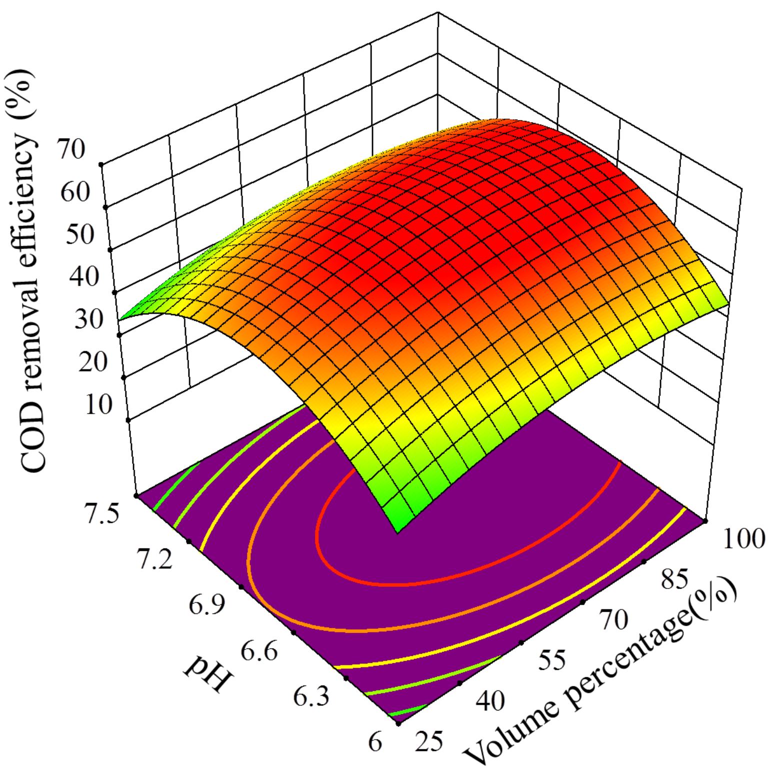


Figure 4.13. Effect of pH and substrate volume percentage

The interaction effect that pH and hydraulic retention time have on the COD removal efficiency of the anaerobic digestion process at fixed substrate volume percentage of 62.5 is given in Figure 4.14. As shown in the graph, the COD removal efficiency increases from 26.2% to 62.6% when the pH varied from 6 to 6.7 and the hydraulic retention time changed from 20 days to 39 days which is due to the favorable condition for microbial growth and their consumption of the readily available organic matter. As both pH and hydraulic retention time further increase to 7.5 and 50 days, the COD removal tends to decline to 40% which might be attributed to the decreased methanogenic activity due to the accumulation of inhibiting agents such as volatile fatty acids. According to investigations made by Chollom et al. (2020) for the optimization of an up-flow anaerobic sludge blanket reactor for treatment of slaughterhouse waste water, different designs were compared (BBD and CCD). But it was concluded that the acidity and alkalinity of a system inhibits the COD removal efficiency of the system. Therefore, the optimum pH condition for biogas production and COD removal was suggested to be 6.5 to 7.2. If the pH is above and below these values, it inhibits methanogenic activity and in turn COD removal efficiency of a system.

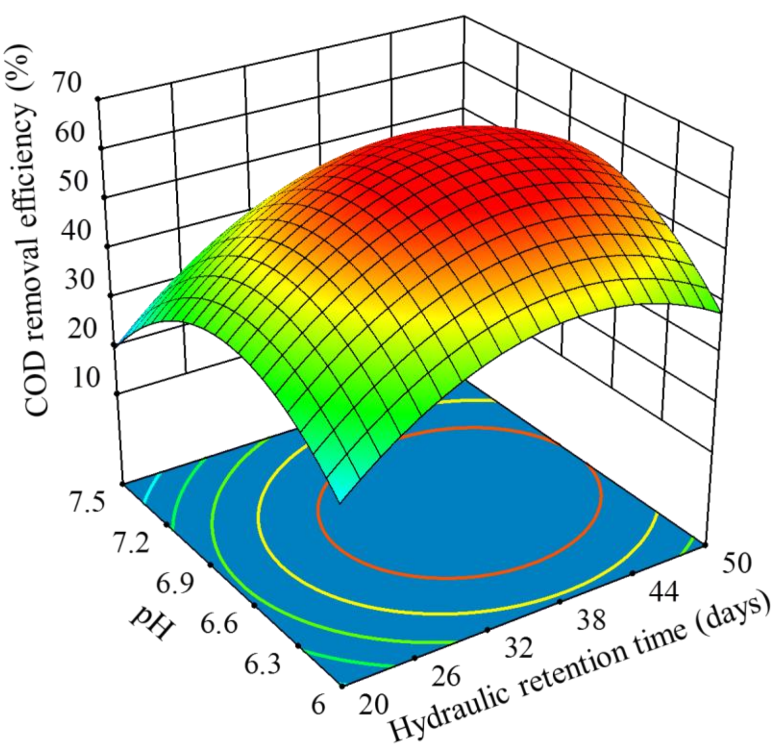


Figure 4.14. Effect of pH and hydraulic retention time

4.5.2.3 Process optimization and process variables validation

The variables, as were discussed at length in section 4.5.2.2, have different effects on responses, methane production and COD removal efficiency. With hydraulic retention time, both increase then tend to a constant value. With substrate volume percentage and pH, there is a trend of increasing and decreasing pattern. This kind of pattern urges the need of optimization which compromises both ways effects by quid pro quo. In order to optimize the adsorption process, the variables set in to the range of the study and based on the goal set, the optimum operating conditions were come out to be substrate volume percentage of 50%, pH of 7, and hydraulic retention time of 40 days to methane production of 75.6% and COD removal efficiency of 58.3%. The summary of the optimization process is given in Table 4.9.

Table 4. 9. Summary of optimization process criteria

Parameters	Goals	Lower limits	Upper limits	Importance
Volume percentage	In a range	25	100	3
pH	In a range	6	7.5	3
Hydraulic retention time	In a range	20	50	3
Methane production	In a range	32.8	75.8	3
COD removal efficiency	In a range	15	59	3

The optimum results predicted by the model have been validated against triplicate experiments conducted at the optimum conditions found from the numerical optimization (Table 4.10). The deviation between experimental and model predicted value were 0.7% for methane production response and 1.3% for the COD removal efficiency. Therefore, it can be established that the fitted models are reliable and significant to predicate the responses since the difference between the values is needed to be below 4%.

Table 4. 10. Model predicted and experimental response at optimum conditions

	Volume percentage (%)	pH	Hydraulic retention time (days)	Methane production (%)	COD Removal efficiency (%)
Predicted	50	7	40	75.6	58.3
Actual	50	7	40	76.2	59.1

According to the investigations made by Borja et al.(1998), slaughterhouse wastewater was digested in a combination sludge blanket and filter arrangement in a single reactor for COD removal. COD removal effciency ranging between 90.2-93.4% (v/v) was reported for different organic loading rates. The result obtained in the current study is lower which may be attributed to the addition of another substrate (water hyacinth).

On the other hand, the anerobic digestion of slaughterhouse wastewater using a pilot scale anaerobic digester was reported to yield an optimum of 72.75% (v/v) methane(Worku & Leta, 2017). This is a lower value than the present study. The present study used co-digestion and rumen fluid as inoculum which might be why the value was higher.

Biogas production from water hyacinth using cow dung and a 6 m^3 tubular biogas plant yielded biogas with methane content ranging from 49-53% (v/v)(Njogu et al., 2015). So the values in the present study is attributed to the co-digestion with slaughterhouse wastewater and the use rumen fluid as inoculum.

4.5.3 Biogas composition and the effect of rumen fluid

The biogas produced during the anaerobic digestion process was analyzed by using a gas analyzer. After optimization and validation, it was found that biogas with a methane composition of 76.2%, carbon dioxide composition of 6.5%, oxygen content of 6.3%, hydrogen sulfide content of 0% and 11% of other trace gases like nitrogen, ammonia and others. In addition to the optimization of methane produced, the amount of hydrogen sulfide in the biogas being zero indicates that the time and operating conditions are suitable for biogas production, as well. According to a research conducted by Herout et al. (2011) on the composition of biogas depending on the type of plant biomass being used, the composition of biogas mainly depends on the type of material being decomposed. It was stated that the biogas composition will be expected to have 50-85% methane, 20-35% carbon dioxide and the rest will be accommodated by trace gases such as nitrogen, hydrogen sulfide, hydrogen as well as others. From the data obtained during current work, the Oxygen content was seen to increase with increase in the water hyacinth volume percentage. So this shows that the water hyacinth feedstock has a role in the presence of oxygen in the biogas since biogas composition depends on the type of biomass used. It was also obtained that 11% of trace gases such as nitrogen, ammonia, hydrogen and others existed in the biogas. Therefore, biogas upgrading is very important in order to remove the other materials and improve methane content.

The microbes found in cows' rumen are mainly bacteria, fungi and protozoa(*The Viral Role of Rumen Microbes _ Department of Agriculture and Fisheries, Queensland*, n.d.). This being the case, during the current work, the anaerobic co-digestion of water hyacinth and slaughterhouse wastewater has been carried out by using a cows' rumen content. The rumen archea was beneficial in the complete degradation of the materials and for the better methane yield. According to an investigation rumen micro-organisms were used to produce enhanced biogas from corn stover and cattle manure in a pilot scale anaerobic digestion plant. It was then found that the microbes in rumen fluid were indeed beneficial in enhancing the process since they were very adoptive to the pH and temperature of the digester(Jin et al., 2018).

4.6 Assessment of bio-digestate for potential use as organic fertilizer

According to the united nations food and agriculture organization (FAO), 'organic fertilizers' are categories of fertilizer, that are carbon-rich, derived from organic materials, including treated or untreated livestock manures, compost, vermicompost, sewage sludge and other organic materials or mixed materials that contain nutrients for plants and organic materials for the improvement of the soils' chemical, biological and physical characteristics(Carapuço, 2016). According to a study done on the Ethiopian soil fertility in 2013, over 150 districts were mapped and it was found that the Ethiopian soil lacks 7 major nutrients such as nitrogen (N), phosphorus (P), sulfur (S), potassium (K), boron (B), copper (Cu) and zinc (Zn)(Tesfahun, 2018). During the present study, the bio-digestate i.e. the anaerobically co-digested slaughterhouse waste water and water hyacinth was considered for assessment as a potential organic fertilizer. The nutrient materials investigated were nitrogen, phosphorus, ammonia, phosphate, nitrate, and sulfate and ammonium ion. During the investigation, the nitrogen was found to be 1.85% (w/v) while the phosphorus was 0.148% (w/v). These two are the major macronutrients useful in plant growth(Lukehurst et al., 2010). Sulfur in the form of sulfate (SO_4-S) was also investigated and found to be 1.75% (w/v). Other nutrients like phosphate, ammonia, ammonium and phosphorus pentoxide were also investigated as shown in Table 4.11. The United Nations FAO sets a minimum of 5% total NPS content and currently in Ethiopia; the government is following the EthioSIS (Ethiopian Soil Information System) recommendation which has introduced new compound (e.g., NPS (19N-46P2O5-7S)) and blended fertilizers containing micronutrients(Elka & Laekemariam, 2020). Therefore, as can be seen from the result, the values of nutrients in the bio-digestate are very low which leads to the suggestion of blending it with cow and other slaughter animal dung for use as fertilizer. According to Bonten et al. (2014), due to the fact that all organic manures contain nutrients(N,P,K,S …), they tend to behave as fertilizers. So the blending of the bio-digestate with organic manure will help in the enhancement of the bio-digestate fertilizer potential.

Table 4. 11. Data obtained from bio-digestate analysis

Nutrient analyzed	Amount in mg/l	Amount in % (w/w)
Nitrogen	18.5	1.85
Phosphorus	1.48	0.148
Sulfur (SO_4-S)	17.5	1.75
Phosphate	2.541	0.254
Phosphorus pentoxide	5.775	0.578
Ammonia (NH_3)	2.424	0.2424
Nitrate	9.83	0.983
Ammonium	2.02	0.202

5 CONCLUSIONS AND RECOMMENDATINS

5.1 Conclusions

The anaerobic co-digestion of slaughterhouse wastewater and water hyacinth was carried out using rumen fluid as inoculum. It was obtained that the leaf and stalk were appropriate for biogas production due to their higher holocellulose (hemicellulose + cellulose) content. The holocellulose can be converted into reducing sugar but not lignin and extractives. The water hyacinth was pretreated physically (size reduction) and chemically (dilute acid pretreatment). The effect of the chemical pretreatment was analyzed using the Fourier transform infrared spectroscopy that showed the removed or degraded and adhering functional groups in the water hyacinth. It was seen that the peaks of the functional groups found in the functional group region of the raw water hyacinth were significantly removed or degraded in the pretreated water hyacinth.

The slaughterhouse wastewater was found to have high BOD_5 and COD, which are good pre-cursors for biogas production. The co-digestion was optimized using response surface methodology and the optimum condition for optimum biogas production and COD removal efficiency was found to be with a substrate volume percentage of 50%SWW: 50%WH, HRT of 40 days and pH of 7. The optimum biogas production and COD removal efficiency were found to be 76.2% and 59.1%, respectively. These values were found to be very good when compared to production with slaughterhouse wastewater and water hyacinth separately. The bio-digestate was, finally, assessed for its suitability for organic fertilizer use. Even though lower nutrient concentrations were found in the bio-digestate, the fact that slaughterhouses produce organic manures as waste and the fact that organic manures have fertilizer potential led to the conclusion that blending the bio-digestate with organic (cattle) manure could enhance the fertilizer potential of the bio-slurry or digestate.

5.2 Recommendations

In order to further refine the current study, the following recommendations are suggested:

- Microbial analysis of the rumen fluid
- Biogas upgrading to improve the quality of methane
- Assessment of bio-digestate and organic manure blend for fertilizer production.

REFERENCES

Abdulhakim, A., Ghaleb, S., Rahman, S., Kutty, M., Ho, Y., Mohammed, N., & Almahbashi, Y. (2020). Response Surface Methodology to Optimize Methane Production from Mesophilic Anaerobic Co-Digestion of Oily-Biological Sludge and Sugarcane Bagasse. *Sustainability*. https://doi.org/10.3390/su12052116

Aberra, D., & Fufa, F. (2016). Bioenergy Production from Anaerobic Co-Digestion of Sewage Sludge and Abattoir Wastes. *Scientifics Research Publishing*, *July*, 281–287. https://doi.org/http://dx.doi.org/10.4236/aces.2016.63028

Adanikin, B. A., Ogunwande, G. A., & Adesanwo, O. O. (2017). Evaluation and kinetics of biogas yield from morning glory (Ipomoea aquatica) co-digested with water hyacinth (Eichhornia crassipes). *Ecological Engineering*, *98*, 98–104. https://doi.org/10.1016/j.ecoleng.2016.10.067

Andri, I., & Corre, O. Le. (2017). Operating condition optimization water hyacinth earthworm bedding wastewater for biogas production. *Energy Procedia*, *138*, 253–259. https://doi.org/10.1016/j.egypro.2017.10.049

Anjos, O., Santos, A. J. A., Estevinho, L. M., & Caldeira, I. (2016). *FTIR – ATR spectroscopy applied to quality control of grape-derived spirits*. *205*, 28–35. https://doi.org/10.1016/j.foodchem.2016.02.128

APHA. (1999). *Standard Methods for the Examination of Water and Wastewater*. *20th ed.*

ASTM standard D 3172 – 89 R02. (2002). *Standard Practice for Proximate Analysis of Coal and Coke 1*. *89*(Reapproved), 1–2.

ASTM standard D 3173 – 03. (2013). *Standard Test Method for Moisture in the Analysis Sample of Coal and Coke 1*. 7–9.

ASTM standard D 3174 – 02. (2013). *Standard Test Method for Ash in the Analysis Sample of Coal and Coke from Coal 1*. *14*.

ASTM standard D 3175 – 89a. (2013). *Standard Test Method for Volatile Matter in the Analysis Sample of Coal and Coke 1*. *Reapproved 1997*, 1–3.

Auma, E. O. (2020). *Department of Civil and Construction Engineering Anaerobic Co-Digestion of Water Hyacinth (Eichhornia crassipes) with Ruminal Slaughterhouse Waste under Mesophilic Conditions By*.

Ayeni et al. (2015). *Compositional analysis of lignocellulosic materials : Evaluation of an economically viable method suitable for woody and non-woody biomass American Journal of Engineering Research (AJER)*. *4*, 14–19. https://doi.org/p-ISSN : 2320-0936

Bakraoui, M., Karouach, F., Ouhammou, B., Aggour, M., Essamri, A., & El, H. (2020). Biogas production from recycled paper mill wastewater by UASB digester : Optimal and mesophilic conditions. *Biotechnology Reports*, *25*, e00402. https://doi.org/10.1016/j.btre.2019.e00402

Barua, V. B., & Kalamdhad, A. S. (2016). Effect of Various Types of Thermal Pretreatment Techniques on the Hydrolysis , Compositional Analysis and Characterization of Water. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2016.12.036

Behboudi-Jobbehdar, S., Soukoulis, C., Yonekura, L., & Fisk, I. (2013). Optimization of Spray-Drying Process Conditions for the Production of Maximally Viable Microencapsulated L. acidophilus NCIMB 701748. *Drying Technology*, *31*(11), 1274–1283. https://doi.org/10.1080/07373937.2013.788509

Berhe, S. (2017). *Anaerobic Co-Digestion of Tannery and Dairy Industry Wastes for Bio Energy Production: An Approach to Convert Waste to Energy in Agro industrial Sector*.

Bharati, V., & Kalamdhad, A. S. (2018). Anaerobic biodegradability test of water hyacinth after microbial pretreatment to optimise the ideal F / M ratio. *Fuel*, *217*(October 2017), 91–97. https://doi.org/10.1016/j.fuel.2017.12.074

Bonten, L. T. C., Zwart, K. B., Rietra, R. P. J. J., & Haas, R. P. (2014). *Is bio-slurry from household digesters a better fertilizer than manure?* 50.

Borja, R., Bank, C. J., & Mancha, A. (1998). *ANAEROBIC DIGESTION OF SLAUGHTERHOUSE WASTEWATER USING A COMBINATION SLUDGE*

BLANKET AND FILTER ARRANGEMENT IN A SINGLE REACTOR. *65*, 125–133.

Carapuço, M. M. (2016). Sustainable use. In *Encyclopedia of Earth Sciences Series*. https://doi.org/10.1007/978-94-017-8801-4_211

Cater, M., Zorec, M., & Logar, R. M. (2014). *Methods for Improving Anaerobic Lignocellulosic Substrates Degradation for Enhanced Biogas Production*. Springer International Publishing. https://doi.org/DOI 10.1007/s40362-014-0019-x

Cavalaglio, G., Cotana, F., Nicolini, A., Coccia, V., Petrozzi, A., Formica, A., & Bertini, A. (2020). Characterization of Various Biomass Feedstock Suitable for Small-Scale Energy Plants as Preliminary Activity of Biocheaper Project. *Sustainability*, 1–10. https://doi.org/10.3390/su12166678

Chollom, M. N., Rathilal, S., Swalaha, F. M., Bakare, B. F., & Tetteh, E. K. (2020). Comparison of response surface methods for the optimization of an upflow anaerobic sludge blanket for the treatment of slaughterhouse wastewater. *Environmental Engineering Research*, *25*(1), 114–122. https://doi.org/https://doi.org/10.4491/eer.2018.366 pISSN 1226-1025 eISSN 2005-968X Comparison

Ehiri, R. C., Mgbabor, C., & Ogbuanu, C. C. (2014). Kinetics of Biogas Production from a Mixture of Water Hyacinth (Eichornia Crassipes) and Fresh Rumen Residue . *IOSR Journal of Applied Chemistry (IOSR-JAC)*, *7*(7), 36–39. https://doi.org/e-ISSN: 2278-5736

Elka, E., & Laekemariam, F. (2020). Effects of Organic Nutrient Sources and NPS Fertilizer on the Agronomic and Economic Performance of Haricot Bean (Phaseolus vulgaris L.) in Southern Ethiopia. *Applied and Environmental Soil Science*, *2020*. https://doi.org/10.1155/2020/8853552

Fallis, A. . (2013). FTIR spectrum analysis. Lecture notes. *Journal of Chemical Information and Modeling*, *53*(9), 1689–1699.

Herout, M., Malaťák, J., Kučera, L., & Dlabaja, T. (2011). *Biogas composition depending on the type of plant biomass used*. *57*(4), 137–143.

Hoda, A. A. (2020). CHARACTERISTICS OF BIOGAS FROM SAGU WASTE (

Metroxylon sp .) WITH INOCULATION OF RUMENT COW MICROORGANISM AS A SOURCE RENEWABLE INOCULATION OF RUMENT COW MICROORGANISM AS A SOURCE RENEWABLE ENERGY. *Researchgate*, *August*.

Hu, Yan, B., Wang, K. J., & Xiao, X. M. (2018). Modeling the performance of anaerobic digestion reactor by the anaerobic digestion system model (ADSM). *Journal of Environmental Chemical Engineering*, *6*(2), 2095–2104. https://doi.org/10.1016/j.jece.2018.03.018

Hu, Z., Ma, X., & Li, L. (2015). Optimal conditions for the catalytic and non-catalytic pyrolysis of water hyacinth. *Energy Conversion and Management*, *94*, 337–344. https://doi.org/10.1016/j.enconman.2015.01.087

Jimoh, A. O., Namadi, M. M., Ado, K., & Muktar, B. (2016). *Proximate and Ultimate Analysis of Eichornia natans (Water Hyacinth), Pistia stratiotes (Water Lettuce) and Nymphaea lotus (Water Lily) in the Production of Biofuel*. *7*(4), 243–249.

Jin, W., Xu, X., & Yang, F. (2018). Application of rumen microorganisms for enhancing biogas production of corn straw and livestock manure in a pilot-scale anaerobic digestion system: Performance and microbial community analysis. *Energies*, *11*(4), 1–17. https://doi.org/10.3390/en11040920

Kifle, R. (n.d.). *Reducing sugar recovery from water hyacinth for bioethanol production and parametric optimization*.

Lukehurst, C. T., Frost, P., & Al, T. (2010). *Digestate_Brochure_Revised_12-2010*.

Ma, S., Wang, H., Li, J., Fu, Y., & Zhu, W. (2019). *Methane production performances of different compositions in lignocellulosic biomass through anaerobic digestion*. *189*. https://doi.org/10.1016/j.energy.2019.116190

Malveaux, C. C. (2013). *Coastal plants for biofuel production and coastal preservation*.

Melane, M., Ham, C., & Meincken, M. (2017). Characteristics of selected non-woody invasive alien plants in South Africa and an evaluation of their potential for electricity generation. *Journal of Energy in Southern Africa*, *28*(3), 92–98. https://doi.org/10.17159/2413-3051/2017/v28i3a1896

Miller, L. G. (1959). Use of DinitrosaIicyIic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, *lll*, 426–428. http://download.bioon.com.cn/upload/month_1002/20100202_79e2638a4a8db64734c5QyCZjgBzadbY.attach.pdf

Mulu, A., & Ayenew, T. (2015). *Characterization-of-Abattoir-Wastewater-and-Evaluation-of-the-Effectiveness-of-the-Wastewater-Treatment.doc*. *November*.

Musa, M. A., & Idrus, S. (2020). Effect of hydraulic retention time on the treatment of real cattle slaughter house wastewater and biogas production from HUASB reactor. *Water (Switzerland)*, *12*(2). https://doi.org/10.3390/w12020490

Musa, M. A., Idrus, S., Hasfalina, C. M., Norsyahariati, N., & Daud, N. (2018). *Effect of Organic Loading Rate on Anaerobic Digestion Performance of Mesophilic (UASB) Reactor Using Cattle Slaughterhouse Wastewater as Substrate*. https://doi.org/10.3390/ijerph15102220

Nam, T. S., Ngoc, L., Hong, D., Thao, H. Van, Chiem, N. H., Hoang, L., Ingvorsen, K., Vo, N., & Ngan, C. (2016). *Enhancing biogas production by anaerobic co-digestion of water hyacinth and pig manure*. *8*(3), 195–199. https://doi.org/10.13141/jve.vol8.no3.pp195-199

Njogu, P., Kinyua, R., Muthoni, P., & Nemoto, Y. (2015). *Biogas Production Using Water Hyacinth (Eicchornia crassipes) for Electricity Generation in Kenya*. *May*, 209–216.

Nugraha et al. (2018). *Biogas Production from Water Hyacinth (Eichhornia Crassipes): The Effect of F / M Ratio Biogas Production from Water Hyacinth (Eichhornia crassipes): The Effect of F / M Ratio*.

Ogunwande, G. A., Adanikin, B. A., & Adesanwo, O. O. (2018). *comparative evaluation and kinetics of biogas yield from duckweed (lemna minor) co-digested with water hyacinth (Eichhornia crassipes)*. *20*(3), 649–661.

Omondi, E A, Ndiba, P. K., & Abuga, D. (2020). *Dynamics of microbial communities in co-digestion of water hyacinth (Eichhornia crassipes) with ruminal slaughterhouse waste under mesophilic conditions*. *12*(4), 81–89.

https://doi.org/10.5897/IJWREE2020.0946

Omondi, Erick Auma, Gikuma-njuru, P., & Ndiba, P. K. (2019). *Anaerobic Co-Digestion of Water Hyacinth (E . crassipes) With Ruminal Slaughterhouse Waste for Biogas Production*. *8*(3), 253–259.

Otero, M., & Mor, A. (2008). *Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale : Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW)*. *40*, 99–106. https://doi.org/10.1016/j.bej.2007.11.019

Pan-in, S., & Sukasem, N. (2017). Methane production potential from anaerobic different animal dungs and sweet corn residuals. *Energy Procedia*, *138*, 943–948. https://doi.org/10.1016/j.egypro.2017.10.062

Patil, J. H., Antonyraj, M. A. L., Bb, S., & Kumar, M. (2014). Anaerobic Co-Digestion of Water Hyacinth and Sheep Waste. *Energy Procedia*, *52*, 572–578. https://doi.org/10.1016/j.egypro.2014.07.112

Patil, J. H., Raj, M. A., Muralidhara, P. L., Desai, S. M., Raju, G. K. M., & Collection, A. S. (2012). *Kinetics of Anaerobic Digestion of Water Hyacinth Using Poultry Litter as Inoculum*. *3*(2), 3–7.

Petrovski, K. R. (2017). *Assessment of the Rumen Fluid of a Bovine Patient*. *2*(3). https://doi.org/10.19080/JDVS.2017.02.555588

Poh et al. (2016). Optimization of Wastewater Anaerobic Digestion Using Mechanistic and Meta-heuristic Methods : Current Limitations and Future Opportunities. *Water Conservation Science and Engineering*, 1–20. https://doi.org/10.1007/s41101-016-0001-3

Pramanik, S. K., Suja, F. B., Porhemmat, M., & Pramanik, B. K. (2019). *Performance and Kinetic Model of a Single-Stage Anaerobic Digestion System Operated at Di ff erent*.

Rabiu, A., Yaakub, H., Liang, J. B., & Samsudin, A. A. (2014). *Enhancing biogas production rate of cattle manure using rumen fluid of ruminants*. *7*(3), 25–28.

Rahmansyah, M. S., Wilujeng, S. A., & Pandebesie, E. (2017). *Co-digestion of Water Hyacinth (Eichhornia crassipes) mixed with Cow Manureto enhance Biogas Production*. *10*(9), 988–993.

Sataloff, R. T., Johns, M. M., & Kost, K. M. (n.d.). *infrared spectroscopy*.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Nrel, D. C. (2012). *Determination of Structural Carbohydrates and Lignin in Biomass Determination of Structural Carbohydrates and Lignin in Biomass*. *April 2008*.

Sukarni, S., Zakaria, Y., Sumarli, S., & Wulandari, R. (2019). *Physical and Chemical Properties of Water Hyacinth (Eichhornia crassipes) as a Sustainable Biofuel Feedstock Physical and Chemical Properties of Water Hyacinth (Eichhornia crassipes) as a Sustainable Biofuel Feedstock*. https://doi.org/10.1088/1757-899X/515/1/012070

Tallou, A., Pedrero, F., & Haouas, A. (2020). Environmental Technology & Innovation Assessment of biogas and biofertilizer produced from anaerobic co-digestion of olive mill wastewater with municipal wastewater and cow dung. *Environmental Technology & Innovation*, *20*, 101152. https://doi.org/10.1016/j.eti.2020.101152

Tesfahun, W. (2018). *Tef Yield Response to NPS Fertilizer and Methods of Sowing in East Shewa, Ethiopia*. *8*(1), 35–42.

The viral role of rumen microbes _ Department of Agriculture and Fisheries, Queensland. (n.d.).

Toribio-cuaya, H., Pedraza-segura, L., & Macías-bravo, S. (2014). Journal of Chemical , Biological and Physical Sciences Characterization of Lignocellulosic Biomass Using Five Simple Steps. *Journal of Chemical, Biological and Physical Sciences*, *4*(5), 28–49.

Tsegaye, G. (2016). *optimization of biogas production from slaughterhouse waste and digester sizing*. *July*.

Veroneze, M. L., Schwantes, D., Gonçalves, A. C., Manfrin, J., Schiller, P., & Schuba, T. B. (2018). *producion of biogas and biofertilizer using anaeorbic reactors with swine manure and glycerin doses*. https://doi.org/10.1016/j.jclepro.2018.12.181

Wauton, I., & William-Ebi, D. (2019). Characterization of water hyacinth (Eichhornia Crassipes) for the production of thermochemical fuels. *Journal of Multidisciplinary Engineering Science Studies (JMESS)*, *5*(7), 2458–2925. www.jmess.org

Worku, Z. (2018). *Treatability and Biogas Production Potential of Slaughterhouse Wastewater Using Anaerobic Digester Combined with Constructed Wetland at City Abattoir , Addis Ababa Zemene Worku Negie A Thesis Submitted to The Center for Environmental Science Presented in*.

Worku, Z., & Leta, S. (2017). *Anaerobic Digestion of Slaughterhouse Wastewater for Methane Recovery and Treatability*. *6*(5), 84–92. https://doi.org/10.11648/j.ijrse.20170605.13

Appendices

Appendix A

Results for moisture content of water hyacinth parts

Water hyacinth parts	Original mass (in g)	Final mass(in g)	Percentage moisture content (in wt.%)
root	10	1.6	84
	10	1.45	85.5
	10	1.52	84.8
		Average value	84.76667
		Standard deviation	0.530723
leaf	10	0.83	91.7
	10	0.88	91.2
	10	0.82	91.8
		Average value	91.56667
		Standard deviation	0.227303
stalk	10	0.75	92.5
	10	0.79	92.1
	10	0.72	92.8
		Average value	92.46667
		Standard deviation	0.248328

Results for volatile matter of water hyacinth parts

Water hyacinth parts	Original mass (in g)	Final mass(in g)	Percentage volatile matter (in wt.%)
root	3	1.09	63.66667
	3	1	66.66667
	3	1.1	63.33333
		Average value	64.55556
		Standard deviation	1.498971
leaf	3	0.54	82
	3	0.56	81.33333
	3	0.559	81.36667

		Average value	81.56667
		Standard deviation	0.265623
stalk	3	1.3	56.66667
	3	1.34	55.33333
	3	1.41	53
		Average value	55
		Standard deviation	1.312335

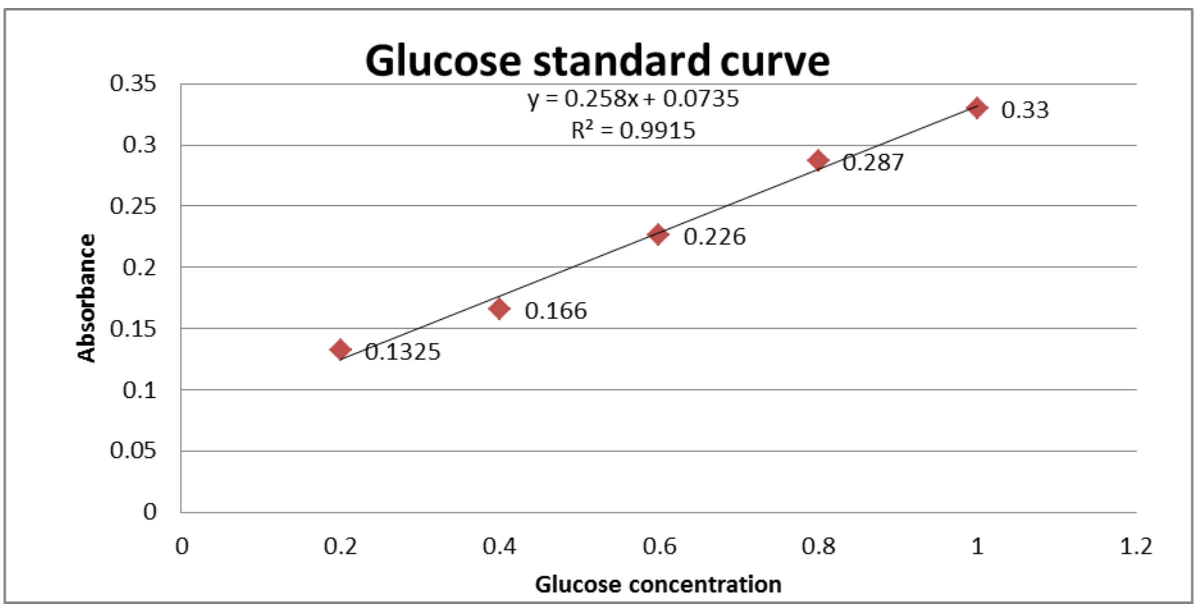
Results for ash content of water hyacinth parts

Water hyacinth parts	Original mass (in g)	Final mass(in g)	Percentage ash content (in wt.%)
root	2	0.5	25
	2	0.53	26.5
	2	0.3	15
		Average value	22.16667
		Standard deviation	4.420596
leaf	2	0.32	16
	2	0.29	14.5
	2	0.3	15
		Average value	15.16667
		Standard deviation	0.62361
stalk	2	0.2	10
	2	0.25	12.5
	2	0.17	8.5
		Average value	10.33333
		Standard deviation	1.428869

Results for fixed carbon content of water hyacinth parts

Water hyacinth parts	Percentage fixed carbon (in wt.%)
root	11.33333
	6.833333
	21.66667
Average value	13.27778
Standard deviation	5.377852
leaf	2
	4.166667
	3.633333
Average value	3.266667
Standard deviation	6.817909
stalk	33.33333
	32.16667
	38.5
Average value	34.66667
Standard deviation	2.383392

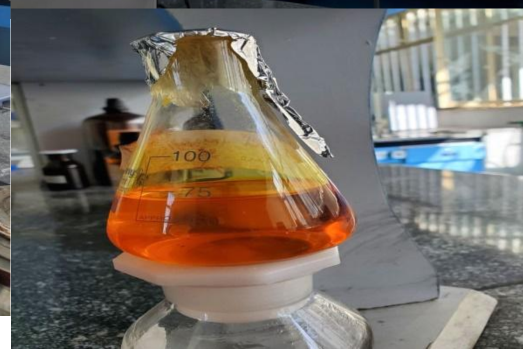
Standard curve for DNSA method



Data from Gas analyzer

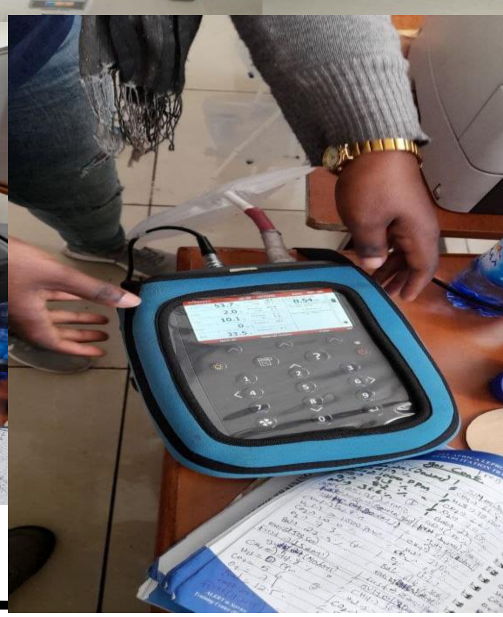
Sr.No.	Methane (% v/v)	Carbon dioxide (% v/v)	Oxygen (% v/v)	Hydrogen sulfide (ppm)	Balance (% v/v)
1	40.8	10.4	9.2	≥1000	39.6
2	41.2	5.3	11.5	≥1000	42
3	50.1	4.0	12.1	12	33.8
4	60.8	6.6	8.4	621	24.2
5	37.3	11.1	21.3	256	30.3
6	61.5	0.4	13.4	32	24.7
7	67.2	3.3	8.8	≥1000	20.7
8	33.2	2.8	9.1	≥1000	54.9
9	40.3	9.4	10.5	≥1000	39.8
10	67.2	7.9	8.6	154	16.3
11	62	0.5	13	36	24.5
12	40.5	5.8	10.9	424	42.8
13	65.9	5	8.7	≥1000	20.4
14	59.4	5.4	7.3	0	27.9
15	75.7	2	10.1	48	12.2
16	52.8	2.1	11.4	15	33.7
17	55.8	7.1	14.9	0	22.2
18	75.4	5.6	6.5	0	12.5
19	75.8	5.4	6.8	0	12
20	58.8	7.1	11.4	0	22.7

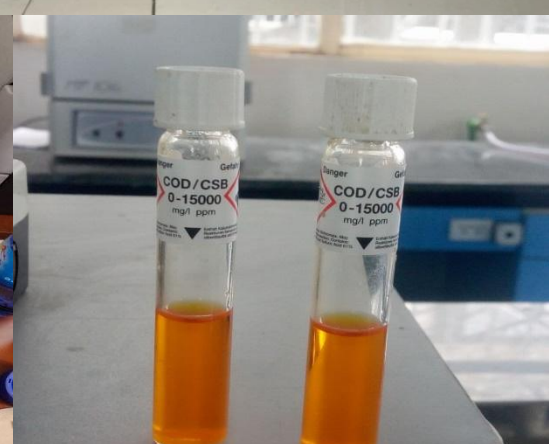
Appendix B




WATER BATH
HH-6


WATER BATH
HH-6




COD/CSB
0-15000
mg/l ppm
COD/CSB
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