

**BIOGAS PRODUCTION FROM WATER HYACINTH (*Eichhornia
crassipes*) CO-DIGESTED WITH POULTRY DROPPING**

MSc THESIS

ADUGNA LEMESSA

OCTOBER 2016

HARAMAYA UNIVERSITY, HARAMAYA

**Biogas Production from Water Hyacinth (*Eichhornia crassipes*) Co-digested
with Poultry Dropping**

**A Thesis Submitted to the College of Natural and Computational Sciences
Department of Biology, Postgraduate Program Directorate
HARAMAYA UNIVERSITY**

**In Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE IN BIOLOGY**

Adugna Lemessa

**October 2016
Haramaya University, Haramaya**

DEDICATION

The thesis is dedicated to my parents who always give me unconditional love and encourage me in my work

STATEMENT OF THE AUTHOR

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Name: Adugna Lemessa

Signature_____

Place: Haramaya University

Date of Submission: _____

BIOGRAPHICAL SKETCH

The author was born in January 1980 G.C at Jimma Arjo Woreda, E/Wollega Zone of Oromia Regional State from his father Ato Lemessa Wodajo and his mother W/o Mulu Tujuba. He attended his elementary and secondary school from Hara Gabato and Arjo Secondary School, respectively. After completion of the Secondary School at Arjo Secondary School, he joined Jimma Teachers College in 1998 G.C and Ambo University in 2006 and graduated with B.Sc. degree in Biology in November, 2009 G.C. Soon after graduation, the author joined Haramaya University School of Graduate Studies, to pursue his M.Sc degree in Biology.

ACKNOWLEDGMENTS

Above all, I would like to thank my God who strengthened me as I did this thesis paper and completed the course peacefully by being beside me in every place.

I am very much indebted to Dr. Meseret Chimdessa, My major advisor, who has been very kind and generous to me in sharing his ideas and providing me with illuminating suggestions and constructive comments from the very initial stages of title selection up to detailed analysis of my research work, and up to final write up of the thesis by providing valuable, practical and useful comments. My thanks also go to My Co-advisor Dr. Manikandan Muthuswamy for his valuable comments and support on my research work.

I would like to give special thanks to microbiology laboratory assistants for their help in simple examinations and data collection.

Lastly, but not least, My warmest thanks and appreciation goes to My lovely wife Asnakech Kelbessa and my children Gifti and Gad Adugna for their spiritual encouragement throughout the four years continuous summer programs

LIST OF ABBREVIATIONS/ACRONYMS

AD	Anaerobic Digestion
ANOVA	Analysis of Variance
BSP	Biogas Support Program
CSA	Central Statistical Agency
CO	Carbon Monoxide
FS	Fresh Sample
GHG	Green House Gases
HRT	Hydraulic Retention Time
LPG	Lubricated Petroleum Gas
PM	Poultry manure
SPSS	Statistical Package for Social Sciences
TS	Total Solid
VFA	Volatile Fatty Acid
VS	Volatile Solid
WHO	World Health Organization

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BIOGAS PRODUCTION FROM WATER HYACINTH (*Eichhornia crassipes*) CO-DIGESTED WITH POULTRY DROPPING

ABSTRACT

*Production of biogas through anaerobic digestion of organic waste materials provides an alternative environmentally friendly renewable energy. Water hyacinth is one of the fastest growing aquatic weed known to man. The objective of this study was to determine biogas yield using water hyacinth as a sole substrate and co-digestion with poultry dropping. Anaerobic digestion (AD) was done using the two substrates in five mix ratio was evaluated under mesophilic conditions (38⁰C) using a batch digester in laboratory, but it should be carried out at room temperature (20⁰C) and at thermophilic condition (55⁰C). In all treatments, total solid, volatile solid, organic carbon, and pH were measured before and after AD. The daily biogas production was subsequently measured by water displacement method for 31days. All measured physico-chemical parameters of each substrate were significantly varied between before and after AD, $p < 5\%$. Gas production was noticed in all of the substrate types from the first day of digestion experiment and became zero on 31 days in all substrates. Assessment of cumulative biogas production revealed that substrate in a mix ratio of 75% *Eichhornia crassipes* and 25% poultry dropping showed the highest biogas production, suggesting this mix ratio of the two substrates is in an optimal mix to yield better amount of biogas. Overall results indicate that the increment of biogas yield and VS, and TS reduction can be significantly enhanced when EC and poultry dropping are co-digested.*

Keywords: Biogas, Co-digestion, Poultry dropping, Total solids, Volatile solids

1. INTRODUCTION

Most of the developing countries are suffering from energy crisis, which is attributed to depletion of locally available energy sources and dependence on imported fuel. Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Biogas, a renewable bio-fuel, is becoming increasingly important as a consequence of major concern for depleting oil reserves, rising crude oil prices and greenhouse effect. As more and more trees are cut down in unsustainable manner, the impact on environment like soil erosion, landslide, etc..., becomes significantly visible. Furthermore, use of crop residues and dung cakes, as substitute for wood fuel, reduces the soil fertility and agricultural productivity (Aklilu *et al.*, 2008).

According to the report by CSA, (2007), 83.3 percent of the total 79.1 million population of Ethiopia live in rural areas. From the total population living in urban areas, 86 percent have access to electricity while the access to this technology is only limited to two percent living in rural areas. Traditional fuels contribute the rest of the rural energy consumption, with fire wood being by far the most important source (81.8%), followed by dung (9.4%), crop residues (8.4%) and small amount of charcoal. On the whole, over 95% of the domestic energy needs are met from biomass based fuels thereby contributing to deforestation, loss of soil nutrients, and organic matter. In any case, Ethiopia is one of the countries that rely extremely on biomass for cooking and lighting (Hilawe, 2010; Aklilu *et al.*, 2008). To overcome these problems alternative energy sources have recently become more and alternative due to the increasing demand for energy, the limited resource for buying fossil fuel, the environment concerns ,and the strategy to survive post-fossil fuel economy era (Siltan *et al.*, 1985).

Ethiopia is one of the top ranking countries in Africa and among the first ten in the world in terms of livestock resource (FAO, 2009). The majority of the rural population in Ethiopia is involved in some way in animal husbandry. So the country has the greatest potential to the development of biogas technology (Zenebe *et al.*, 2010). Biogas technology is a modern and eco-friendly technology based on the decomposition of organic materials in anaerobic

environment at suitable and stable temperature by anaerobic bacteria. Anaerobic digestion consists of several interdependent, complex sequential and parallel biological reactions in the absence of oxygen, during which the products from one group of microorganisms serve as the substrates for the next, resulting in transformation of organic matter (biomass) mainly into a mixture of methane and carbon dioxide commonly referred to as biogas (Werner *et al.*, 2000).

Apart from the benefit of biogas for cooking and lighting, the effluent that comes as slurry is rich with various plant nutrients such as nitrogen, phosphorous, and potash, which are essential for plant growth. Well fermented bio-slurry improves the physical, chemical, and biological properties of the soil resulting in enhancing qualitative as well as quantitative yield of food crops. The slurry is free of parasites and pathogens, it is highly recommended for use in farming. The economic value of the slurry is high because it saves the money for importing inorganic fertilizers and it improves the yield more than inorganic fertilizer by adding both micro and macro nutrients to the soil (Shrestha, 2010). Furthermore, biogas is also improving the environment indoors and outdoors. The indoor environment is enhanced by reduction in the incidents of illness from burning of firewood and dung, outdoors by reduction in carbon dioxide and methane emission (Siltan *et al.*, 1985). Biogas is also a proven technology that contributes to the reduction of the deforestation rate and helps to save the trees to sequester more carbon from the atmosphere and the local effects of trees being cut down that otherwise cause soil erosion, desertification, loss of soil fertility, and landslides (Marry *et al.*, 2007).

Globally biogas fuel helps to reduce greenhouse gas emissions. Domestic biogas installations can reduce greenhouse gas (GHG) emissions in three ways: by changing the manure management modality; by substituting fossil fuels and non-renewable biomass for cooking (and to a smaller extent for lighting) with biogas, and by substituting chemical fertilizer with bioslurry (Shrestha *et al.*, 2010). Utilizing biogas as an alternative to fossil based fuels reduces the net amount of carbon dioxide emitted to the atmosphere. The biogas used in a sustainable basis assures the CO₂ associated with biogas combustion will be reabsorbed in the processes of the growth of fodder and food for animals. Moreover, the emissions of methane gas, which is more aggressive greenhouse gas than carbon dioxide from otherwise naturally digested dung can be prevented (Huong *et al.*, 2010). Generally, domestic biogas benefits over multiple levels (micro, meso and macro) to the Shrestha *et al.*, 2010).

Biogas production in smaller agricultural units can additionally reduce the use of forest resources for household energy purpose and thus slowdown deforestation. This will maintain the water cycle and avoid alternative periods of flood and drought in the affected areas (Dagnachew *et al.*, 2003). Due to massive deforestation, about 50 to 100 species of animals are being lost each day. The outcome of which is the local extinction of animals and plants on a massive scale which are the source of herbal medicines of about 80% of the world population in developing countries (WHO, 1998).

Water hyacinth (*Eichhornia crassipes*) is a perennial, herbaceous, aquatic plant. The stem and leaves contain air filled sacs, which help them to stay afloat in water. Water hyacinth is considered as a noxious weed in many parts of the world as it grows very fast and depletes nutrient and oxygen rapidly from water bodies, adversely affecting flora and fauna. It is a free floating weed known for its production abilities and pollutant removal (Bolzonella *et al.*, 2006). It can double its size in 5 days and a mat of medium sized plants may contain 2 million plants per hectare that weigh 270 to 400 tones. It poses serious socioeconomic and environmental problems on millions of people in riparian communities. The weed obstructs electricity generation, irrigation, navigation, and fishing; increases evapo-transpiration resulting in water loss; increase cost of crop production; provides habitat for vectors of malaria and bilharzias; harbors poisonous snakes; causes skin rashes; and can host agents of amoebic dysentery and typhoid (Mironga *et al.*, 2004). Therefore, there is a need to manage its spread through suitable control measures. However, the fact remains that water hyacinth has successfully resisted all attempts of its eradication by chemical, biological, mechanical, or hybrid means (Abbasi *et al.*, 1998).

In Ethiopia also, Water hyacinth is considered as a constraint on the development of the country (Firehun *et al.*, 2007). Several authors have tried to put an approximate figure to the economic consequences caused due to water hyacinths problem specifically on utilization of water for irrigation. According to (Gopal *et al.*, 1987) the annual water loss through evapo-transpiration due to water hyacinth in Sudan would be enough to irrigate more than 400 ha of land. Similarly Firehun *et al.* (2007) reported that than weed causes 393,660 to 2,945,160 m³ water loss at Wanji-Shoa. Water hyacinth has attracted the attention of scientists to use it as a potential biomass as it is rich in nitrogen, essential nutrients and has a high content of

fermentable matter. Apart from biogas the sludge from biogas process contains almost all of the nutrients and can be used as a good fertilizer with no detrimental effects on the environment. With these points in mind, this project is designed to evaluate biogas production from Water hyacinth (*Eichhornia crassipes*) in sole or co-digestion with poultry manure with the following objectives.

Objectives

The general objective of this study was to determine biogas yield using water hyacinth (*Eichhornia crassipes*) as a sole substrate and co-digestion with poultry manure.

The specific objectives of this study were:-

- ❖ To characterize water hyacinth and poultry manure interms of total solid, volatile solid, organic carbon and pH before and after anaerobic digestion.
- ❖ To evaluate the biogas yield of solo and mixed substrates under mesophilic (38)anaerobic digestion process
- ❖ To evaluate the optimal mix ratio of water hyacinth and poultry manure co-digestion in biogas yield.

2. LITERATURE REVIEW

2.1. Biogas

Biogas is an alternative and renewable energy source produced through the anaerobic digestion process, which is a natural biological process in which an interlaced community of bacteria cooperates to obtain a stable fermentation through assimilation, transformation and decomposition of the organic matter present in waste into biogas. This is a complex multistep process in terms of microbiology, where the organic material is degraded to obtain methane gas under the absence of oxygen. Biogas consists primarily of utilizable methane (CH₄) and inert carbon dioxide (CO₂), which are both colorless and odorless. However, depending on the source of the organic matter and the management of the anaerobic digestion process, small amounts of other gases may be present (Arogo *et al.*, 2009; Madu and Sodeinde, 2001). Methane has 20 times more greenhouse gas potential than carbon dioxide, so the capture and burning of methane significantly reduces the greenhouse gas effect (Atkins *et al.*, 2008).

Table 1. Biogas composition

Constituents	%Composition
Methane	55 - 75
Carbon-dioxide	30 - 45
Hydrogen sulphide	1 - 2
Nitrogen	0 - 1
Hydrogen	0 - 1
Carbon monoxide	Traces
Oxygen	Trace

Source: Madu and Sodeinde (2001)

2.2. Biogas in Ethiopia

Biogas technology was introduced in Ethiopia in 1979. Even if biogas technology has multitude of advantages to rural households society and for forming sustainable environment, the wider dissemination of the technology is limited until the National Biogas Program (NBP) is launched in 2008. To implement the technology widely, it needs encouraging the households because in lacking technical and financial support to rural households who are more or less unaware of the technology difficult to use it consistently (Getachew *et al.*, 2006).As (NBP, 2007) reported, around 1000 biogas plants were constructed in various parts of the country. Approximately 40% of these plants are not operational due to lack of effective management and follow-up, technical problems, loss of interest, evacuation of ownership and water problems. Other reasons for the limited success of the technology in Ethiopia include the adoption of a project-based stand-alone approach without follow-up structure in place, variations in design, and the absence of a standardized biogas technology (NBP, 2007)

2.3. Biogas Production

Biogas digesters are technology that can produce methane gas for cooking and lighting as well as organic nutrient-rich bio-slurry for use as fertilizer. Methane is produced by the anaerobic breakdown of organic materials, particularly cow dung, but other organic wastes including sewage can also be fed into well-manage digesters. The resulting bio-slurry has a reduced load of parasitic diseases and pathogenic bacteria, but further processing, for example through composting, is needed to eliminate these pathogen. Biogas fermenting microorganism in which organic material- splitting bacteria and methane bacteria are included, degrade complex organic materials to produce methane under anaerobic conditions, (Madu and Sodeinde 2001). The process of biogas production includes the stages of hydrolysis, acidogenesis, acetogenesis and methanogenesis (Getachew *et al.*, 2006; DFID *et al.*, 2011) and different bacteria act upon the organic matter resulting in the formation of methane and acids.

2.4. Description of the Feedstock

2.4.1. Water Hyacinth (*Eichhornia crassipes*)

Water hyacinth (*Eichhornia crassipes*) is originally from South America and is one of the world's most widespread invasive aquatic plants. This vascular floating plant is known to cause major ecological and socio-economic alterations (Center, 1994). It normally forms thick,

intertwining mats due to its fast reproductive rate and intricate root structure. Water hyacinth is indigenous to the Amazon Basin of Brazil but has been introduced to tropical and subtropical regions around the globe. Holm *et al.* (1977) noted that 56 countries including the United States had reported water hyacinth as a noxious weed. Holm and others that water hyacinth has invaded freshwater systems in over 50 countries on five continents and that it is especially pervasive throughout Southeast Asia, the southeastern United States, central and western Africa, and Central America (Brendonck *et al.*, 2003).

2.5. Anaerobic Digestion

Anaerobic digestion is a natural process during which organic matter, in the absence of oxygen is degraded to methane (60-70%), CO₂ (30-40%), water vapor and traces of nitrogen, hydrogen sulphide and ammonia (Berglund, 2006). This mixture of gases is called biogas. Anaerobic digestion is used in waste water treatment as a biological step and plays three main roles: Production of a stabilized bio solids residue that enables further treatment and safe utilization, reduction of pathogens, odor and amount of bio sludges. The main disadvantage of anaerobic fermentation is the long hydraulic retention time that is necessary for the conversion of organic matter to biogas. Therefore, large tanks, pumps, heat exchanges and compressors are needed for running the process. Specialized digesters with mesophilic or thermophilic environment are used in one, or two- phase digestion. Even though the digestion takes about 30 days (mesophilic conditions), it is not 100% degradation of biosolids (Ciborowski, 2004).

2.5.1. Hydrolysis

Hydrolysis is the first step in the anaerobic digestion. During this phase, undissolved compounds, such as polysaccharides, proteins, and fats get degraded in to their monomers, such as sugars amino acids, fatty acids (Parawira *et al.*, 2009). This is performed by extracellular hydrolytic enzymes, which use water to cut the covalent bonds in the polymers. The hydrolytic enzymes include cellulases, hemicellulases, amylases, lipases, and proteases (Parawira *et al.*, 2009). Many cellulase-degrading organisms have their enzymes in exo-enzymes complexes, called cellulosomes. These complexes are attached to the cellular wall and simultaneously they attach to substrate for more effective degradation. The hydrolysis of complicated structures, like lignocelluloses, can require weeks, and the degradation is often

not complete (Gerardi *et al.*, 2003). In hydrolysis complex organic substance are converted to simple ones (Carbohydrates to sugars, fats to fatty acids and proteins to amino acids) by use of hydrolytic bacteria. This step takes long time due to limited accessibility of the extra cellular enzymes to intracellular polymeric materials, which are protected by cell membrane (Rilling 2005).

2.5.2. Acidogenesis

In the acidogenesis phase, the monomers produced in the hydrolysis phase are further degraded by fermentative bacteria into short-chain organic acids, with one to five carbons (valeric acid, butyric acid, propionic acid, acetic acid, and formic acid), alcohols, hydrogen, ammonia, and CO₂. In a stable process, with low partial pressure of hydrogen, the main products formed by the fermentative bacteria is acetate, CO₂, and hydrogen. Acidogenesis products are volatile fatty acids (VFA), alcohols, aldehydes, hydrogen and CO₂. Decomposers are fermentative bacteria or anaerobic oxidizers (Garcia-Heras *et al.*, 2002). When the partial pressure of hydrogen is high, more intermediates such as volatile fatty acids and alcohols are formed (Shink *et al.*, 1997).

2.5.3. Acetogenesis

Products from acidogenesis are converted to acetate, hydrogen and CO₂ by acetogens. High concentration of hydrogen disables acetogens to act that is why that is of importance that methanogenic bacteria use the hydrogen (Zaher *et al.*, 2007). Some of the degradation products from the acidogenesis phase can be directly used by methanogens. However, the fatty acids longer than two carbon atoms, alcohols longer than one carbon atom, and branched chain and aromatic fatty acids are degraded further into acetic acid, hydrogen and carbon dioxide in the acetogenic phase.

The acetogenic microorganisms are obligatory hydrogen producers, and for the degradation to proceed, thermodynamically, the acetogenic microorganisms need low partial hydrogen pressure (Shink *et al.*, 1997). Therefore, the acetogens are found in symbiotic relationship with hydrogen consuming methanogens, keeping the partial hydrogen pressure low enough for the growth of acetogenic microorganisms. When the concentration of hydrogen is too high, butyric, caproic, propionic, and valeric acids and ethanol concentrations are increased which

in turn are toxic for the methanogens. In parallel with the formation of acetic acid from short chain organic acids homoacetogenic microorganisms reduce hydrogen and carbon dioxide into acetic acid (Gerardi *et al.*, 2003).

2.5.4. Methanogenesis

The last step in the anaerobic digestion is the methanogenesis. The methanogenic microorganisms work under strictly anaerobic conditions. The methanogens which belongs to the group Archaea differ from the other organisms in the anaerobic reactor which are bacteria. Archaea are more sensitive compared with the bacteria, regarding environmental stresses in the reactor, such as pH, or toxic compounds such as heavy metals or different toxic organic materials (Liu *et al.*, 2008). The methanogens mainly use acetate, carbon dioxide, and hydrogen, but also methylamines, alcohols, and formate for the production of methane (Liu *et al.*, 2008). About 70% of the methane production arises from the acetate, and about 30% of the methane arises from hydrogen and carbon dioxide (Gerardi *et al.*, 2003). The methanogens have the longest generation times (2-25 days) of the microorganisms in the reactor which makes this step the most time limiting step for easily hydrolyzed materials (Annaschnurer *et al.*, 2009).

2.6. Factors Affecting Anaerobic Process and Biogas Production

Environmental factors which influence biological reaction, such as pH, temperature, nutrients and inhibitors concentrations are amenable to external control in the anaerobic process. Any drastic changes in these factors can adversely the biogas production (Chatterjee *et al.*, 2007; Marchaim *et al.*, 1992)

2.6.1. pH

The optimum biogas production is achieved when the pH value of the input mixture in the digester is between 6 and 7. The pH in a biodigester is also a function of the retention time. In the initial period of fermentation as large amount of organic acids are produced by acid-forming bacteria, the pH inside the digester can decrease to below 5 (Marchaim *et al.*, 1991). Low pH inhibits the growth of the methanogenic bacteria and hence gas generation (Da Silva 1979). Methanogenic bacteria are very sensitive to pH and do not thrive below a value of 6.5.

Later, as the digestion process continues, the concentration of NH_4 increases which in turn increase the pH value to above 8. When the methane production level is stabilizing, the pH ranges remains buffered between 7.2 and 8.2 (FAO, 1996b). A pH higher than 8.5 will start showing toxic effect on methanogens population.

2.6.2. Temperature

The methanogens are inactive in extreme high and low temperatures. The optimum temperature is 35 °C. When the ambient temperature goes down to 10 °C, gas production virtually stops. Satisfactory gas production takes place in the mesophilic range between 25 and 30 °C. Temperature variations can have adverse effects on mesophilic (35 °C) digestion, or thermophilic (55 °C) digestion. Organic material degrades more rapidly at higher temperature because the full ranges of bacteria are at work (Marchaim, 1992).

The temperature effect also depends significantly on the solid concentrations of the fermentation. When high concentrations of organic loading are used (over 10% solids), bacterial activity increases with temperature (Marchaim *et al.*, 1983). Proper insulation of the digester helps to increase gas production in the cold season. When the ambient temperature is 30 °C or less, the average temperature within the digester usually remains about 4 °C above the ambient temperature (Lund *et al.*, 1996).

2.6.3. Carbon to Nitrogen (C/N) ratio

The relationship between the amount of carbon and nitrogen present in organic materials expressed in terms of the carbon/nitrogen (C/N) ratio. A C/N ratio ranging from 20 to 30 is considered optimum for anaerobic digestion (FAO, 1996b). If the C/ N ratio is very high the nitrogen will be consumed rapidly by methanogens for meeting their protein requirements and will no longer react on the left over carbon content of the material. As a result gas production will be low. On the other hand, if the C/N ratio is very low, nitrogen will be liberated and accumulated in the form of ammonia. According to TERI (1994), solid concentration in the feed material is also crucial to ensure sufficient gas production, as well as easy mixing and handling 8-10% of total solids is the normal value required.

The concentration of total solids in the input suspension can be varied within the range of 20 to 100g/liter. In practice it is recommended to limit the total solids concentration to the range of 20 to 30g/liter (Preston *et al.*, 1999).

2.6.4. Organic loading rate (OLR)

The rate at which substrate is supplied to the digester is referred to as organic loading rate and is usually expressed in terms of Kg volatile solids per m³ and day. The gas production rate in the digester is highly dependent on the organic loading rate (Yadvika *et al.*, 2004). The organic loading rate (OLR) determines the volatile solids input to the digester. The OLR parameter has a significant performance. It is expressed as the amount of organic matter (COD or volatile solids) per reactor volume. If the digester is overfed, acids will accumulate and methane production will be inhibited since micro-bacteria cannot survive in acidic situation. Similarly, if the digester is underfed, the gas production will also be low because of alkaline solution, which is also not a favorable condition for anaerobic bacteria. Organic loading rate (OLR) is a measure of the biological conversion capacity of the AD system (Vandevivere *et al.*, 1999).

2.6.5. Dilution and Consistency of Inputs

Before feeding the digester, the excreta, especially fresh poultry dropping, has to be mixed with water at the ratio of 1:1 on a unit volume basis (i.e. same volume of water for a given volume poultry dropping). However, if the poultry dropping is dry form, the quantity of water has to be increased accordingly to arrive at the desired consistency of the substrate (e.g. ratio could vary from 1:1.25 to even 1:2). The dilution should be made to maintain a total solid content from 7 to 10 percent (Shrestha *et al.*, 2010).

2.6.6. Inhibitory substances

An organic waste from livestock farms and plants contains a variety of poisonous substances, such as disinfectants, pesticides, and heavy metals, which will inhibit the growth, metabolism, and propagation of the anaerobes (Chen *et al.*, 2008). A variety of substances have been reported to be inhibitory to the AD process. A material may be considered inhibitory when it causes an adverse alteration in the microbial community or inhibition of the bacterial growth. Inhibition is usually revealed by a decrease in methane production and an accumulation of

volatile fatty acids. Considerable variations in the inhibition/toxicity levels of some substances (e.g, ammonia, certain light metal ions such as Na, K, Mg, Ca and Al, heavy metals at high concentrations, chlorophenols, halogenated aliphatic, N-substituted aromatics including nitrobenzene's, nitro phenols, aromatics amines, long-chain fatty acids including lauric and oleic acid, and lignin related compound such as hydroxyl methyl furfural and others) have been reported in the literature (Chen *et al.*, 2008).

2.6.7. Retention Time

Retention time is the average period that a given quantity of the substrate takes to move from the inlet to the outlet pipes (the average time the substrate remains in the biodigester). The retention time is calculated by dividing the total volume of the digester by the volume of input added daily. In general, the faster the rate of digestion, the shorter can be the retention time. When the temperature in the digester increase, the retention time can be shorter. A normal period for the digestion of poultry dropping would be 2 to 4 weeks (Da Silva *et al.*, 1979).

2.6.8. Ammonia

Ammonia is an important inhibitor in anaerobic digestion when treating nitrogen-rich substrates, such as livestock wastes. As previously explained, nitrogen is one of the essential elements, with a concentration in the range of 50-200mg N L⁻¹ being reported as necessary for anaerobic bacterial growth (McCarty, 1964). Proteins are broken down during anaerobic digestion; increasing ammonium concentration inside the reactor. Free ammonia is the nitrogen species which in fact causes toxicity and it is in equilibrium with ammonium. The free ammonia concentration can be calculated according to Hansen *et al.* (1998).

Poultry manure has a higher fraction of biodegradable organic matter than other livestock wastes. Yet this substrate, rich in organic nitrogen, when anaerobically digested at its original solids content of 20-25%, can cause a reduction of process performance caused by ammonia accumulation. A common approach to this problem relies on dilution of the manure to 0.5-3.0% total solids (TS) thereby eliminating ammonia inhibition of the digestion (Hill, 1983).

2.6.9. Seeding

To start up a new anaerobic process, it is critical to use inoculums of microorganisms to commence the fermentation process. The common seeding materials include digested sludge from a running biogas plant or material from well-rotted manure pit or cow manure slurry. (Yadvika *et al.*,2004) states that rumen fluid inoculums caused biogas production rate and efficiency increase more than two times in compare to manure slurry to the batch reactor as part of the starter improved the biogas production. Normally, the volume of the inoculums should be above 10% of the total working volume of the digester, and 20-30% supplementation of inoculums would favor the smooth start-up of AD digesters (Neves *et al.*, 2006).

2.6.10. Particle size

The production of biogas is also affected by particle size of the substrate. Too big particle size is problematic for microbes to digest and it can also result in blockage in the digester, where as small particle size gives a large surface area for substrate adsorption and thus allows the increased microbial activity followed by increase in the production of biogas. Physical pretreatment such as grinding could significantly reduce the volume of the digester required, without decreasing biogas production (Yadvika *et al.*, 2004).

2.6.11. Mixing condition

The close contact between microorganisms and the substrate material is important for an efficient digestion process. The agitation of the digester contents has a number of benefits, one of the most obvious being that it helps to mix up material, evening out any localized concentrations, thus helping to stop the formation of dead zone or scum. In addition, it increases the wastes availability to bacteria, helps remove and disperse metabolic products and also acts to ensure a more uniform temperature within the digester (Yadvika *et al.*, 2004)

2.7 .Co-digestions

A combination of two or more substrates in anaerobic digestion is attractive technique by which the substrates digestion process improvement is reported; the anaerobic co-digestion of various agricultural residues, various municipal solid wastes and various other specific organic

wastes, is becoming attractive for producing energy in the form of biogas (Ahring *et al.*, 1992). The use of a co-substrate in most cases improves the biogas yields due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates. In addition, economic advantages derived from the fact of sharing equipment are quite significant. Sometimes the use of a co-substrate can also help to establish the required moisture contents of the digester feed. Other advantages are the easier handling of mixed wastes and the use of common access facilities (Tafdrup, 1995)

The work of Sosnowski *et al.* (2003) showed that optimization of the carbon to nitrogen ratio was found when municipal wastes and sewage sludge are co-digested. The improvement of the buffer capacity is also reported as a positive effect in the co-digestion process by Mshandete *et al.* (2004). Akuzuo *et al.* (2010) shows that blending the paper waste with cow dung or any other animal waste will give sustained gas flammability throughout the digestion period of the waste since animal waste are good starters for poor producing wastes. Mata-Alvarez *et al.* (2006) showed that co-digestions of cattle slurry with poultry litter (7.5% and 15%TS) gave higher cumulative productions of methane, and the system with the lower concentration of poultry litter gave a higher specific methane yield. However, there was some evidence of ammonia inhibition. Comparing the single waste digestions with co-digestion of combined wastes, it was shown that co-digestion resulted in higher methane gas yields. Poultry litter has been reported as good substrates for co-digestion studies and the literature has encouraging reports regarding the use of chicken manure for co-digestion purpose.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The experiment was conducted in the laboratory of Biology Department at Haramaya University. The university is located at latitude of 9°26' N, longitude of 42°03'E and an altitude of 1980 meter above sea level (Google Earth, 2013). The mean annual temperature is 17°C with mean minimum and maximum temperatures of 3.8 and 25°C, respectively.

3.2. Feedstock and Inoculums

Two types of substrates namely *Eichhornia crassipes* (EC) and poultry manure were used as feedstock for anaerobic digestion. *Eichhornia crassipes* was collected from east Wallaga zone particularly from Jima Arjo Didessa Lake. The fresh poultry manure was obtained from Haramaya University poultry farms. After collection; these substrates were kept in a refrigerator at 4°C until use. The ruminant fluid were used as a starter of AD were obtained from slaughter house of Haramaya University and filtered through a cloth of 0.5mm sieve diameter to separate solid content from slurry. Prior to use, the inoculum was starved for one week by incubating at 38°C to remove the easily degradable VS present in inoculum (LoNiece-Liew, 2011)

3.3. Design of the Experiment

This study consisted of anaerobic digestion of two substrates. The two substrates were mixed in five different combinations and used as five treatments (treatment A-E) before AD). Treatments were randomly arranged in the lab and done in three replicates.

Table 1. Treatment description

Treatment	Description	Ratio
A	EC	100:0
B	PM	100:0
C	A+B	50:50
D	A+B	75:25
E	A+B	25:75

EC=*Eichhornia crassipes*, PM=poultry manure

3.4. Determination of the Physico-Chemical Properties of the Substrates

Both EC and poultry manure and their combinations in different ratios were analyzed for total solids (TS), volatile solids (VS), fixed solids, Organic carbon and pH before and after AD process based on the standard methods for the Examination of water and wastewater (APHA, 1999) as follows.

3.4.1. Total Solids

To measure TS, first a clean evaporating dish was oven-dried (at 105°C for one hour), cooled in desiccators and weighed immediately before use. Feedstock (10g) was placed on the evaporating dish and put in an oven (105°C) using a crucible to evaporate for 24 hours. After 24 hours the crucible was taken out from the oven, cooled in desiccators and weighed. Then, the percentage of TS calculated using the following formula (APHA 2540 B, 1999).

$$\% \text{TS} = \frac{m_{DS}}{m_{FS}} \times 100$$

Where: %TS= percentage of total solids, mDS = mass of dry sample (final weight) in gram
mFS = mass of fresh sample in gram

The percentage TS removal was calculated using the formula below.

$$\text{TS removal (\%)} = \frac{TS_i - TS_f}{TS_i} \times 100$$

Where: TS_i= initial total solids (%). TS determined before AD, TS_f= final total solid (%).TS determined after AD

3.4.2. Determination of volatile solid and Fixed Solids

Once the TS was determined, the oven dried feedstock was ignited at 550°C in a muffle furnace for 3 hours to determine the volatile and fixed solid content of the feedstock. The following formula was employed to calculate the percentage of volatile solid content of the TS (APHA 2540 E, 1999)

$$\% \text{VS} = \frac{m_{DS} - m_{(ash)}}{m_{DS}} \times 100$$

Where: %VS=percentage of volatile solids, mDS= mass of dry solids in gram

m (ash) =remaining mass after ignition = fixed solid in gram.

i.e. TS=VS+FS

Then percentage VS removal was calculated using the equation below.

$$\text{Vs removal (\%)} = \frac{vsi - vsf}{vsi} \times 100$$

Where: vsi =initial volatile solids (%), Vsf =final volatile solids (%) after AD

3.4.3. pH Value Determination

The pH value was determined using digital pH meter before and after AD. In the case of before AD, an electrode was inserted into samples of feedstock that was diluted using distilled water before inoculation with poultry dropping fluid. The pH measurement after AD was done using pH electrode which was inserted into samples of the feedstock that have undergone digestion for about 31 days in AD process.

3.4.4. Organic Carbon

The carbon content of the feedstock was obtained from volatile solids data using an empirical equation as reported by Badger *et al.* (1979)

$$\% \text{ carbon} = \frac{\% \text{ VS}}{1.8}$$

Where, VS =Volatile solid

3.4.5. Anaerobic Digestion of the Substrates

The co-digestion experiments were conducted in batch mode in 0.5L digester with 2 feed stocks prepared in five mixtures at different proportion (water hyacinth and poultry manure) labeled as A to E (Table 2). Once the TS was determined, feedstock were mixed with appropriate amount of distilled water and inoculums to achieve the recommended (8% w/w) total solid content in the fermentation slurry (Tchobanoglous *et al.*, 1993). The total amount of liquid (distilled water and rumen fluid) need to be added to the digester was determined by the following formula.

$$Y = \frac{mTS - 8\%x}{8\%}$$

Where,

Mts=mass of total solids, X=mass of fresh substrate

Y=mass of fluid (distilled water and rumen fluid) to be added to get 8% total solids in the digester. Then, by fixing the amount of inoculums (100ml) that was added finally to facilitate digestion, the amount of distilled water that has to be added was then determined using the following formula.

$$Z=y-100$$

Where, Z=amount of distilled water, Y=total amount of liquid (distilled water and rumen fluid)

Treatments were randomly arranged in the lab and done in three replicates. The temperature of biodigester was maintained at 38°C by keeping in incubator, which represents mesophilic condition (Knottier, 2003). Initial pH values were also maintained within the pH range for optimal biogas production up to 31 days with biogas yield measured every other day starting from the first day of treatment (Yadvika *et al.*, 2004; Thy *et al.*, 2003).

Table 2. Proportion of different substrates.

	Mix ratio		TS of		Amount of		Volume of water (ml)	Volume of inoculums (ml)	Total volume (ml)
	%EC	%PM	%EC	%PM	fresh sample	EC			
			(g)	(g)	EC	PM	(ml)	(ml)	(ml)
A	100	0	30	0	135	0	315	100	550
B	75	25	22.5	7.5	100	70	280	100	550
C	50	50	15	15	75	87	288	100	550
D	25	75	7.5	22.5	35	115	300	100	550
E	0	100	0	30	0	155	295	100	550

EC= *Eichhornia crassipes*, PM=poultry manure, TS=Total solid

Anaerobic digesters were generally constructed in bench-scale experiments where biogas is produced out of the degradation of organic matter in 0.5L digester with appropriate working volume (Table 3). The three plastic bottles were arranged in such a way that the first bottle contained substrate, the middle contained acidified brine solution and the last for collecting the brine solution that was expelled out from the second container. The acidified brine solution was prepared by adding NaCl to distilled water until a supersaturated solution was formed to prevent the dissolution of biogas in the water. Then 3 drops of sulphuric acid were added using a dropper to acidify the brine solution. All the three containers were interconnected with a plastic tube having a diameter of 1cm. The tube connecting the first bottle to the second was fitted just above the slurry in the first bottle to help gas collection. Thus, the biogas produced by fermentation of the slurry was driven from the first bottle to the second bottle that contained a brine solution so as to displace a volume of the brine solution equivalent to the volume of biogas produced. The lids of all digester were sealed tightly using super glue in order to control the entry of oxygen and loss of biogas. After measuring the pH values of all the digesters, their pH values were arranged between 6.8 and 7.4 by adding buffer solution. The temperatures of all the digesters were maintained at 38⁰C by keeping them in incubator, which represents mesophilic condition. The amount of gas produced was measured by water displacement method using 90% NaCl solution (brine) (Yetilmezsoy and Sakar, 2008). The daily gas production was recorded for different treatments until the gas production ceases.



Figure 1. Configuration and setup of the digester

3.4.6. Data Analysis

Data were subjected to analysis of variance (one-way ANOVA) using SPSS for windows 16.0 (SPSS; Chicago, IL, USA). Fishers Least Significant Difference (LSD) was used to investigate statistical significance between the different treatments, whereas Paired samples T-test was used to investigate statistical significance within a treatment. Difference between means was considered statistically significant at $P < 0.05$.

4. RESULTS AND DISCUSSION

4.1. Physico-Chemical characteristics of the substrates (*Eichhornia crassipes* and poultry manure)

The physico-chemical characteristics of EC and PM in sole and mixed in different proportions were determined before and after anaerobic digestion, and results are shown in Table 4

4.1.1. Total solid content and Volatile Solid

The total solid content of all mix ratios before AD was between $17.90 \pm 0.05\%$ (1.79 gram of TS from 10gm sample) and $22.90 \pm 0.05\%$ (2.29 gram of TS from 10gram sample). EC had the highest TS value before AD while PM had the least, suggesting the high content of organic matter for degradation in EC than PM. TS was found to decrease in all substrates after AD. Similarly, VS also reduced after AD and extent of reduction was higher in 75%EC+25%PM than other substrates used (Table 4) and reduction in TS and VS might be due to the degradation of organic matter by microbes in the process of biogas production similar suggestions were presented (Bagudo *et al.*, 2008). Total solids and Volatile solids destruction is a good parameter for evaluating the efficiency of AD (Abubaker and Ismail, 2012).

Table 3. Comparison of %TS and %VS before and after AD for various treatments (values are mean \pm SE, n=3)

Treatment	Parameter			
	%TS		%VS	
	Initial	Final	Initial	Final
100%EC	22.90 ± 0.05^{Aa}	12.40 ± 0.05^{Ab}	20.04 ± 0.04^{Aa}	18.21 ± 0.09^{Ab}
75%EC&25%PM	21.80 ± 0.05^{Ba}	10.00 ± 0.05^{Cb}	19.03 ± 0.05^{Ba}	14.01 ± 0.00^{Cb}
50%EC&50%PM	20.50 ± 0.25^{Ca}	10.80 ± 0.05^{Bb}	18.83 ± 0.06^{Ba}	15.62 ± 0.00^{Bb}
25%EC&75%PM	19.50 ± 0.01^{Da}	10.30 ± 0.05^{Bb}	18.82 ± 0.05^{Ba}	14.5 ± 0.00^{Cb}
100%PM	17.90 ± 0.05^{Ea}	10.20 ± 0.50^{Cb}	16.80 ± 0.01^{Da}	14.02 ± 0.06^{Db}

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between treatments.

4.1.2. The pH value and Carbon content of the digester

Table 4. Comparison of pH and % organic carbon between before and after AD of the various substrates (values are mean \pm SE, n=3)

Treatment	parameter			
	pH		%C	
	Initial	Final	Initial	Final
100%EC	6.19 \pm 0.10 ^{Ea}	8.53 \pm 0.00 ^{Ab}	11.65 \pm 0.02 ^{Aa}	9.56 \pm 0.03 ^{Ab}
75%EC&25%PM	6.41 \pm 0.00 ^{Da}	8.04 \pm 0.03 ^{Bb}	10.55 \pm 0.03 ^{Ba}	7.33 \pm 0.00 ^{Bb}
50%EC&50%PM	6.18 \pm 0.01 ^{Ca}	8.45 \pm 0.02 ^{Cb}	9.89 \pm 0.04 ^{Ca}	7.56 \pm 0.00 ^{Cb}
25EC% %75PM	6.84 \pm 0.01 ^{Ba}	8.64 \pm 0.05 ^{Cb}	9.32 \pm 0.03 ^{Da}	7.76 \pm 0.03 ^{Eb}
100%PM	6.92 \pm 0.00 ^{ABa}	8.73 \pm 0.00 ^{AB}	8.74 \pm 0.01 ^{Ea}	7.24 \pm 0.05 ^{Db}

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between treatments.

The pH gives an indication of chemical factors in the digester. After AD, the pH values of all treatments were found to be alkaline which might also be due to the build up of ammonium compounds. The pH of 100% PM slurry before anaerobic digestion was about 6.92, but 100%EC was 6.19 (Table 5). The pH value of 100% PM was optimum for biogas production, whereas 100%EC was less optimal (Yadvika *et al.*, 2004; Thy *et al.*, 2003). Mixing the substrates resulted in the rise of pH compared to that of EC alone, but decreased pH from that of PM alone.

The pH of substrates varied significantly between treatments both in before and after AD ($p < 0.05$). PM alone had the highest pH value in both cases. Mixing of PM with EC raised the pH to near optimal for biogas production than EC alone, suggesting mixing of substrates improves pH for AD (Hills and Roberts, 1981). Optimum biogas production is achieved when the pH value in the digester is between 6 and 7 (Garba, 1996). Low pH value inhibits methanogenic bacteria and methanogenesis. The high pH value recorded in this study could be attributed to large ammonia losses resulting from carbon to nitrogen ratio of poultry waste (Gray *et al.*, 1971). The pH value increases by ammonia accumulation of volatile fatty acid,

resulting from degradation of organic matter decreases the pH value.

There was significant difference (paired sample-T-test, $p < 0.05$) in %C content between treatments both in before and after AD. Before AD, highest was recorded in EC alone and that of PM alone was least. After AD %C was significantly reduced compared to that of before AD and relatively extent of reduction appeared to happen in 75%EC+25%PM (Table 5). Decrement in organic carbon shows effective degradation process during AD. That is increased consumption of organic carbon at higher temperature is ascribed to its utilization by bacteria for various metabolic requirements including biogas production (Geradi, 2003).

4.1.3. Analysis of Average daily and Cumulative Biogas production of each treatment

Biogas measurement was done daily for 31 days after incubation. Gas production began right on the first day of incubation in all treatments, but the amount was varied from treatment to treatment (Fig 2). Over all gas production was halted at about 31 day after incubation, suggesting the depletion of substrate from the system

It was observed that biogas production was slightly slow at the beginning and the end period of observation. This is predicated because biogas production rate in digester condition is directly proportional to specific growth rate of methanogenic bacteria in the biodigester from rumen fluid inoculums added (Nordberg and Edstrom, 2005). Compared to the pure samples, mixing of EC and PM generally increased biogas yield. The maximum biogas yield was attained with mixtures of 75%EC+25%PM produced more gas as compared to 100%PM. According to Hobson's (1981) findings that attributed the lower production to low biodegradable material in the poultry manure.

So the presence of more biodegradable material in EC than PM used as a source of energy for microbes (Yeole and Ranande, 1992). Thus, biogas production is more of the presence of the feed stocks organic content and its biodegradability (Macias-Corral *et al.*, 2008).

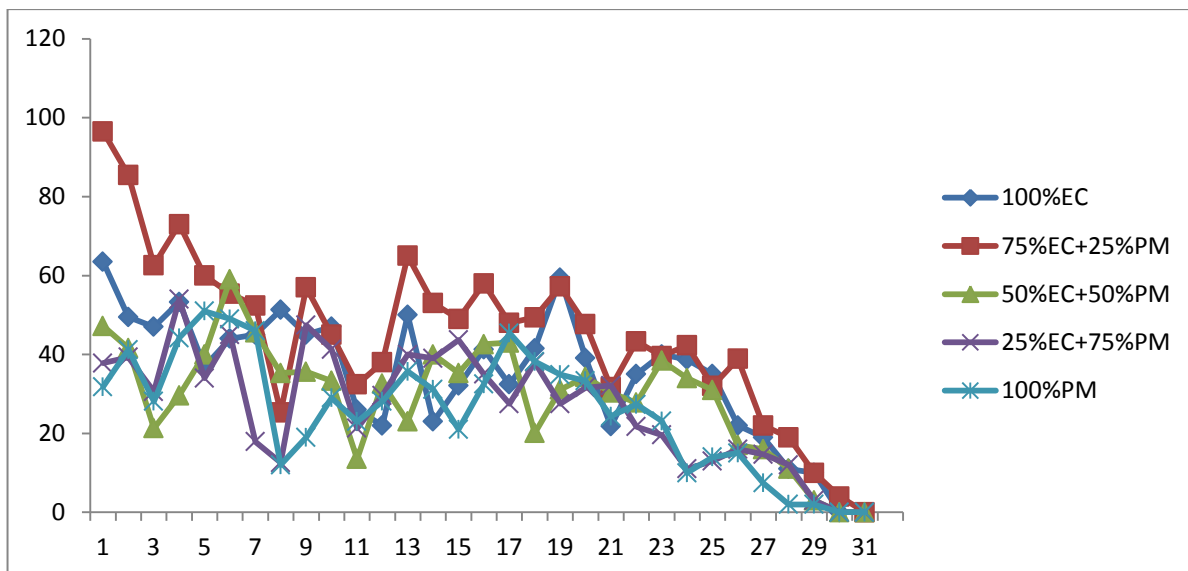


Figure 2. Daily mean biogas of the different substrate combinations

Production of biogas started to fluctuate and reached to zero ml. This might be due to over use of nutrient, accumulation of ammonium, increased pH values (Hansen *et al.*, 1998). It may also be due to accumulation of toxic wastes due to over production of microbes or reaching on carrying capacity of microbes that inhibited gas production. For stable performance of the microbes, the pH must kept in the range of 6.8-7.4. In this experiment, the final pH values of all the digesters were above 8.04. This shows that the presence of more ammonia. The pH value changes below or above the optimal range may stop the process in the reactor since micro-organisms and their enzymes are changed their properties and change the pH activities (Yadvik *et al.*, 2004).

As the ratio of PM in the substrate increased from 25% to 75% the total biogas yield became decreased, as a result of less favourable condition with increasing PM except for 100%EC pure substrates. So from this study the result can be showed that co-digesting improves nutrient balance and enhances pH buffer capacity (Fulford, 1988) and more yield in biogas productions.

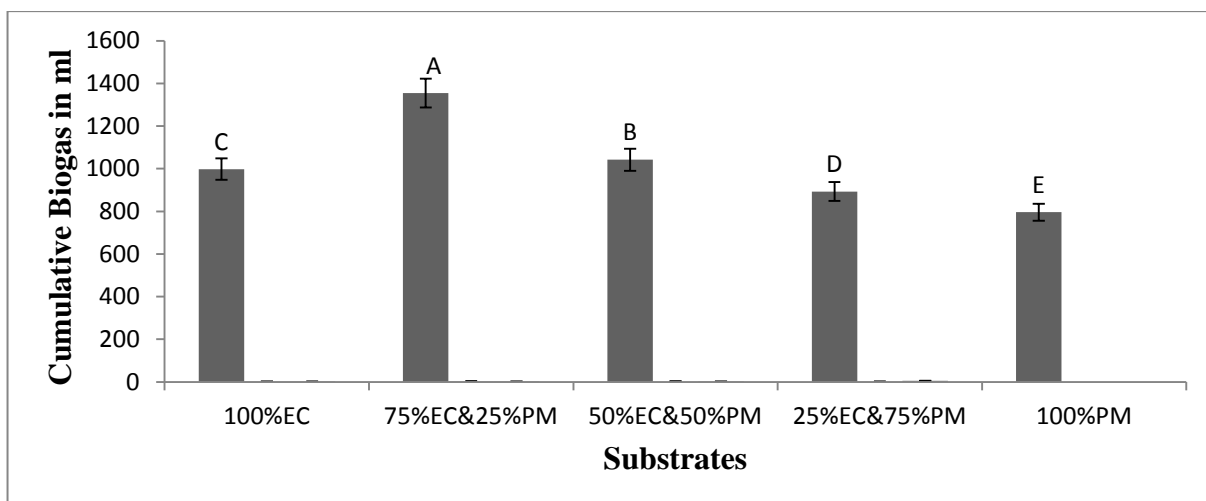


Figure 3. Cumulative biogas yield of the different combinations (values are mean \pm SE), Bars with different Capital letters indicate significant differences between mean.

The cumulative biogas productions for 100%EC, 75%EC+25%PM, 50%EC+50%PM, 25%EC+75%PM, and 100%PM were 998, 1355, 1042, 893, and 796 in ml respectively. The maximum total biogas production was observed with 75%EC+25%PM (1355ml), followed by 50%EC+50%PM (1040ml) and the least in 100%PM (796ml) (figure 3). Though the %VS of 100%EC was higher, it did not result more biogas than the combination with 75%EC+25%PM. This might be due to the less favourable situation of 100%EC to microorganisms as compared to 75%EC+25%PM. As the proportion of PM in the mix ratio increased from 25% to 75%, the cumulative biogas yield decreased, suggesting less favourable situation with increasing PM except for 100%EC which is without the combination of PM. Thus it can be concluded that co-digestion of EC and PM is more productive with PM proportion not exceeding 25%. The higher production from the mixtures could be due to a proper nutrient balance, increased buffering capacity, and decreased effect of toxic compounds resulting from mixing of the substrates (Fulford, 1988; Macias-Corral *et al.*, 2008; Li *et al.*, 2009).

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 .Summary

Water hyacinth fulfils criteria deemed necessary for bio energy production. It is perennial, abundantly available, non-crop plant, biodegradable and has high cellulose content; however its strong disadvantage is that it has over 90% water content which complicates harvesting and processing. Enhancing the use of biogas by using water hyacinth can control the spread of this invasive plant and the biogas production to generate energy for household uses in rural areas. The result of this study contributes to the controlling method of water hyacinth in environmentally safe method which reduces environmental costs and used as sources of energy through biogas. Anaerobic digestion carried out to obtain suitable mix ratio for maximum biogas production from solo and co-digestion of EC with PM at five different treatments in different ratios. The experiment was carried in 0.5L test batch digester under mesophilic condition (38⁰c) at hydraulic retention time. Cumulative biogas production from 100%EC, 75%EC: 25%PM, 50%EC: 50%PM, 25%EC: 75 %PM and 100%PM were statically significant (p<0.05 level).

5.2. Conclusion

As it was shown from the finding of this study 1355 optimum total biogas production was observed in 31 days from 75%EC+25%PM, where as 796 the lowest value was observed in 31 days from 100%PM; from this it is possible to conclude that *Eichhornia crassipes* co-digested with poultry manure in the combination of 75%EC+25%PM improved biogas production than *Eichhornia crassipes* or poultry manure alone.

5.3. Recommendations

- The study revealed that it is possible to produce biogas from a mixture of dried Water hyacinth and poultry manure. Further studies should be investigated on wet Water hyacinth co-digestion with other substrates such as pig manure, **poultry litter** for their application in energy production and determining nitrogen content and the biogas quality of the different combinations by a Gas Chromatography too.

- This investigation was also done at mesophilic temperature (38⁰C) but it should carried out at room temperature (20⁰C) and at thermopilic condition (55⁰C).
- Further research work on this topic particularly with the view of improving digester performance, and mixing ratio of the substrate in order to enhance gas yield is recommended.
- Efforts should also be made to measure the methane quality of the different combinations by a Gas Chromatography too.

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7. APPENDIX

Appendix Table 1: Daily biogas production (mean \pm SE, n=3)

Day	100%EC	75%EC&25% PM	50%EC&50% PM	25%EC&75% PM	100%PM
1	63.5 \pm 0.23	96.5 \pm 0.75	47.2 \pm 0.88	37.8 \pm 0.35	31.8 \pm 0.35
2	49.5 \pm 0.43	85.6 \pm 0.73	41.6 \pm 1.52	39.3 \pm 0.75	41.22 \pm 0.57
3	47 \pm 0.55	62.6 \pm 0.57	21.3 \pm 0.57	30.6 \pm 0.76	28.12 \pm 0.77
4	47 \pm 1.22	73 \pm 0.47	29.6 \pm 0.88	54 \pm 0.87	44.2 \pm 0.73
5	53.3 \pm 1.3	60 \pm 0.73	40 \pm 0.33	34 \pm 0.77	51 \pm 0.57
6	36.9 \pm 0.54	65.4 \pm 0.73	59 \pm 0.97	75 \pm 0.75	49 \pm 0.77
7	44 \pm 0.88	52.4 \pm 0.47	45.6 \pm 0.07	17.9 \pm 0.63	46 \pm 0.45
8	45.3 \pm 1.20	55.3 \pm 0.90	35.3 \pm 0.43	12.6 \pm 0.59	12 \pm 0.59
9	51.3 \pm 0.57	57 \pm 0.65	65.6 \pm 0.05	47.4 \pm 0.58	19 \pm 0.57
10	45 \pm 0.57	45 \pm 0.05	33.3 \pm 0.56	41.3 \pm 0.57	29 \pm 0.77
11	47 \pm 0.87	32.4 \pm 0.04	13.5 \pm 0.76	21.3 \pm 0.77	23 \pm 0.88
12	26 \pm 0.57	38 \pm 0.68	42.6 \pm 0.67	29.6 \pm 0.77	28.1 \pm 1.20
13	22 \pm 0.88	41 \pm 0.05	53 \pm 0.83	40 \pm 0.73	35.7 \pm 0.57
14	50 \pm 1.23	43 \pm 0.56	60.6 \pm 0.32	59 \pm 0.57	31.11 \pm 1.33
15	23 \pm 1.73	39 \pm 0.88	65.3 \pm 0.50	43.7 \pm 0.57	21 \pm 0.57
16	32.1 \pm 1.15	28 \pm 0.57	52.6 \pm 0.05	35 \pm 0.62	32.49 \pm 0.88
17	41.3 \pm 1.45	35 \pm 1.20	43 \pm 0.05	27.5 \pm 0.77	45.41 \pm 0.66
18	32.4 \pm 0.23	49.4 \pm 0.33	20.1 \pm 0.06	38 \pm 0.35	38.2 \pm 0.33
19	41.5 \pm 1.76	59.23 \pm 0.57	31.1 \pm 0.05	27.5 \pm 0.61	34.9 \pm 0.88
20	31.4 \pm 1.20	47.66 \pm 0.57	34.3 \pm 0.76	31.6 \pm 0.55	33.32 \pm 0.88
21	29.1 \pm 0.88	31.7 \pm 1.22	30.3 \pm 0.43	32.11 \pm 0.77	24.34 \pm 1.20
22	21.8 \pm 0.57	43.33 \pm 0.54	27.8 \pm 0.87	21.7 \pm 0.57	27.34 \pm 2.18
23	15 \pm 1.20	29.6 \pm 0.06	38.4 \pm 0.90	19.6 \pm 0.63	23.11 \pm 0.88
24	17 \pm 0.50	42.3 \pm 0.09	34 \pm 0.07	11 \pm 0.61	10.67 \pm 0.88
25	18.7 \pm 0.60	52 \pm 0.05	31 \pm 0.78	13 \pm 0.75	17.33 \pm 0.33
26	15 \pm 0.59	38.9 \pm 0.07	17 \pm 0.09	16 \pm 0.59	13.66 \pm 0.33
27	22 \pm 0.57	32 \pm 0.57	19 \pm 0.88	21.7 \pm 0.57	7.43 \pm 1.85
28	9 \pm 0.59	9 \pm 0.57	11 \pm 0.57	12 \pm 0.57	2 \pm 0.57
29	1 \pm 0.57	1 \pm 0.05	0 \pm 00	3 \pm 0.57	0 \pm 00
30	00 \pm 00	0 \pm 00	0 \pm 00	0 \pm 00	0 \pm 00
31	00 \pm 00	0 \pm 00	0 \pm 00	0 \pm 00	0 \pm 00
Total	998	1355	1042	893	796

Appendix Table 2: Triplicate average value of the parameters of the treatments

Treatment	Repl	Initial(TS)	Final (TS)	Initial(TS)%	Final(TS)%
100%EC	1	2.29	1.25	22.9	12.5
100%EC	2	2.29	1.24	22.9	12.4
100%EC	3	2.29	1.23	22.9	12.3
	AVE	2.29	1.24	22.9	12.4
75%EC&25%PM	1	2.19	1	21.9	10
75%EC&25%PM	2	2.19	1	21.9	10
75%EC&25%PM	3	2.16	1	21.6	10
	AVE	2.18	1	21.8	10
25%EC&75%PM	1	1.97	1.03	19.7	10.3
25%EC&75%PM	2	1.95	1.02	19.5	10.2
25%EC&75%PM	3	1.93	1.04	19.3	10.4
	AVE	1.95	1.03	19.5	10.3
50%EC&50%PM	1	2.07	1.08	20.7	10.8
50%EC&50%PM	2	2.05	1.08	20.5	10.8
50%EC&50%PM	3	2.03	1.08	20.3	10.8
	AVE	2.05	1.08	20.5	10.8
100%PM	1	1.79	1.01	17.9	10.1
100%PM	2	1.79	1.03	17.9	10.3
100%PM	3	1.79	1.02	17	10.2
	AVE	1.79	1.02	17.9	10.2

Treatment	Repl	Initial(pH)	Final(pH)	Initial C(%)	Final C (%)
100%EC	1	6.19	8.53	11.62	9.56
100%EC	2	6.19	8.53	11.62	9.55
100%EC	3	6.18	8.53	11.61	9.56
	AVE	6.19	8.53	11.65	9.56
75%EC+25%PM	1	6.41	8.04	10.55	7.33
75%EC+25%PM	2	6.41	8.04	10.56	7.34
75%EC+25%PM	3	6.41	8.04	10.57	7.33
	AVE	6.14	8.04	10.55	7.33
25%EC+75PM	1	6.83	8.64	9.33	6.71
25%EC+75PM	2	6.84	8.62	9.33	6.72
25%EC+75PM	3	6.85	8.63	9.32	6.71
	AVE	6.84	8.64	9.32	6.71
50%EC+50%PM	1	6.18	8.4	9.89	7.56
50%EC+50%PM	2	6.17	8.4	9.88	7.57
50%EC+50%PM	3	6.18	8.4	9.89	7.55
	AVE	6.18	8.45	9.89	7.56
100%PM	1	6.92	8.73	8.74	7.24
100%PM	2	6.92	8.73	8.75	7.23
100%PM	3	6.93	8.72	8.75	7.25
	AVE	6.92	8.73	8.74	7.24

8. ANNEX

Annex 1: Substrates for anaerobic co-digestions



Fresh Water hyacinth



Poultry manure



Dried water hyacinth



Annex. 2. Rumen fluid used as inoculums

