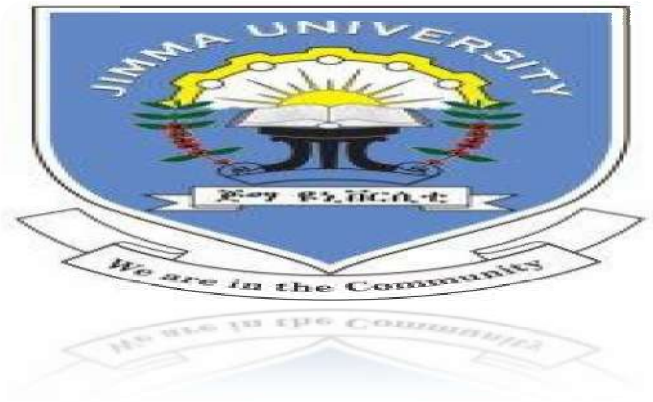

JIMMA UNIVERSITY

INSTITUTE OF HEALTH SCIENCE

FACULTY OF PUBLIC HEALTH

DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY

BIOGAS PRODUCTION POTENTIALS FROM ANAEROBIC CO- DIGESTION OF FOOD WASTE AND HUMAN EXCRETA IN JIMMA UNIVERSITY, SOUTH WEST ETHIOPIA

BY: BIRUK DEMISSIE

A MASTER THESIS SUBMITTED TO DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY, FACULTY OF PUBLIC HEALTH, INSTITUTE OF HEALTH SCIENCE AND JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER'S OF SCIENCE IN ENVIRONMENTAL HEALTH

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JIMMA, ETHIOPIA

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NOVEMBER, 2018

JIMMA, ETHIOPIA

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SCHOOL OF GRADUATE STUDIES

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Biruk Demissie on assessment of biogas production potentials from anaerobic co- digestion of food waste and human excreta in Jimma University**.** We recommended that it be submitted as fulfilling the thesis requirement.

Gudina Terefe (PhD) _____ _____

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Embiale Mengiste (PhD) _____ _____ Co-advisor Signature Date

As member of the board of examiners of the M.Sc. thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Biruk Demissie and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Environmental health.

_____ _____ _____

Chairperson Signature Date

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Internal Examiner Signature Date

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External Examiner Signature Date

Approval sheet

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Biruk Demissie entitled as "Biogas production potentials from anaerobic co digestion of food waste and human excreta in Jimma town, Southwest, Ethiopia". We recommended that it could be submitted as fulfilling the thesis requirement.

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As member of the board of examiners of the MSc. thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Biruk Demissie and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Environmental health Science.

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Chairperson Signature Date

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Internal Examiner Signature Date

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External Examiner Signature Date

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles in the preparation, data collection, analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

This thesis is submitted in partial fulfillment of the requirement for M.Sc. degree at Jimma University. The Thesis is deposited at the University Library to be made available to borrowers under the rules of the Library. I solemnly declare that this thesis is not submitted to any other institution, elsewhere, for the award of any academic degree, diploma, or certificate.

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Name: Biruk Demissie

Signature: _____

Place: Jimma University

Date of submission _____

Abstract

Introduction: *Biogas is produced by the anaerobic digestion of biodegradable materials.* Co-digestion is a simple approach of anaerobic digestion by mixing wastes together in different ratio and proportion.*About 2.4 billion people still do not have access to improved sanitation.* In Ethiopia the prevalence of diarrhoea is slightly higher for children in households with unimproved sanitation than in households with improved sanitation1.3 billion tons of food is wasted annually across the world. From organic solid waste stream available, food waste accounts 45-70 %. Global food waste ranked the third top methane emitter after China and United States of America.

Objective*: The aim of this study was to assess biogas production potentials from anaerobic co-digestion of food waste and human excreta.* ***Methods****: lab based experimental study design was employed. TS, , VS, MC, C/N ratio, fecal coliform and ova of S mansoni were determined before and after AD. pH, temperature, daily biogas and methane content was measured every other day until the end of the experiment. Biogas and methane was measured using water displacement method and 10 % sodium hydroxide solution respectively. The data was entered in excel 2016 version and subjected to analyze of variance using SPSS version 21.*

Result: *The highest reduction of VS was recorded in digestion of co substrates (14.5-20 %) compared to digestion of mono substrates (7.6-9.9 %). Optimum C/N ratio was obtained from T2, T3 and T4 (21.6-28) compared to T1 and T5 (34.6 and 13.4. The result showed that biogas production was started from second day and reached zero at day 40(T1), 42(T5) and 44 (T2, T3, T4). Two and half times higher biogas yield was obtained from co digestion of FW and HE compared to mono digestion of food waste and human excreta. The highest and lowest percentage of methane was obtained from T3 (72.5%) and T5 (57.7%). 1.07± 0.4 log CFU/ml (45.5%) of fecal coliform were removed after 44 days retention time. S mansoni was completely removed after 44-day retention time anaerobic digestion.*

Conclusion and Recommendation: *The highest biogas and methane was produced from co digestion of FW and HE opposed to mono digestion. In order to have more biogas and methane yield co digestion of 50% FW + 50% HE mixture should be applied.*

Keywords: *Biogas, Co-digestion, Anaerobic digestion, Food waste, Human excreta*

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II. ACKNOWLEDGMENT

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III. ABBREVIATIONS

AD -Anaerobic digestion

ANOVA- Analysis of variance

EDHS- Ethiopia demographic health survey

FC- fecal coliform

FW-Food waste

GHG- Greenhouse gas

HE- human excreta

HRT- Hydraulic retention time

JMP-joint monitoring program

MDG- Millennium Development goal

MC- Moisture content

MPN-most probable number

MSW – Municipal solid waste

OLR- Organic loading rate

SSA- sub Saharan Africa

SW- solid waste

SWM- solid waste management

TKN-total Kjeldahl nitrogen

TOC- total organic carbon

TS-total solid

UFW- urban food waste

UNICEF-united nation international child fund

USA- United States of America

USEPA- united states of environmental protection agency

USD – united states dollar

VFA- volatile fatty acid

VS-volatile solid

WASH- water sanitation and hygiene

WHO- world health organization

1. INTRODUCTION

1.1 Background

Biogas is an organic colorless, flammable gas used as a fuel which is produced through anaerobic digestion(AD) of any biodegradable organic wastes and it is composed of 50 to 70 % methane(CH_4), 20 – 40 % carbon dioxide(CO_2), and traces of others gases. It is estimated that about 40 to 60% of the OM(organic matter) presents in the feedstock is converted to biogas(1).

If the content of CH_4 is 50% and above biogas burns very well and its ignition temperature is found in the range between 650°C to 750 °C(2). Methane is a major component of purified biogas generated through natural process(3). It is also colorless, odorless and flammable gas with a wide distribution in nature (4).

Co-digestion is an approach of AD by mixing of two or more wastes together in different ratio and proportion (5). Anaerobic biodegradation process takes place in the absence of oxygen and the presence of anaerobic microorganisms including to non methanogenic and methanogenic bacteria. AD is the processes of degrading and converting organic materials into methane and carbon dioxide.AD is the consequences of a series of metabolic interactions among various groups of microorganism (6). Bacteria and archaea are responsible for AD process in strict anaerobic conditions (7).

Anaerobic digestion process carried out by four key stages of biochemical processes. These are hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis is the first step in AD, hydrolysis of complex organic compounds like cellulose, proteins and fats converted into simple sugar, amino acid and fatty acid by hydrolase exoenzyme. The degree of hydrolysis and speed of the processes depends on pH, temperature, concentration biomass and particle size(8).

Acidogenesis (formation of organic acid) – acidogenic bacteria further degrade the products of hydrolysis into short-chain organic acids. The main products of this stage are acetate, carbon dioxide and hydrogen(70%) and as well as VFA(volatile fatty acid) and alcohol (30%)(1,9).

Acetogenesis stage, Products which cannot be directly converted to methane by methanogenic bacteria are converted into methanogenic substrate. VFA and alcohol are oxidized into acetate, hydrogen and carbon dioxide. The acetate serves as a substrate for methane – forming bacteria and acetogenic bacteria, which grows in a synergetic relationship with methane forming bacteria(7,10).

Acetogenesis and methanogenesis usually run in parallel with the two groups of bacteria in symbiosis particularly in the maintenance of hydrogen partial pressures that suit both acetogenic bacteria and methanogenic bacteria. This stage can limits the rate of degradation in the methanogenic stage and directly influences the quantity and quality of biogas produced (11).

In methanogenesis stage the methaneogenic bacteria convert acetic acid, hydrogen and carbon dioxide into methane gas and carbon dioxide(1,8,9,12,13).

$CH_3CO_2H \xrightarrow{\text{methanogenic bacteria}} CH_4 + CO_2$ (70% methane originates from acetate) and

$4H_2 + CO_2 \xrightarrow{\text{methanogenic bacteria}} CH_4 + 2H_2O$ (30% methane originates from hydrogen and carbon dioxide)(11)

1.2 Statement of the problem

Tons of human excreta are generated each day and its management is still remains a great challenge worldwide (14). Human excreta are the source of many infectious disease agents (15). Human excreta and fecal sludge contains 10^8 of pathogenic E coli, 10^5 Giardia lamblia, 40 S .mansoni, 10^4Taenia saginata and 10^3 Trichuris trichiura,10^5 CFU of fecal coliform per gram of fresh wet feces (16). The 2015 Millennium development goals (MDG) report indicated that 2.4 billion peoples still do not have access to improved sanitation (17). Sub-Saharan Africa(SSA), Oceania and Southern Asia, were the most vulnerable regions characterized by the lowest sanitation coverage (30, 35 and 42 percent respectively) (14). According to the UNICEF 2015 report improved sanitation coverage in Ethiopia stands at 24% (18).

88 percents all deaths from of Diarrheal disease in developing countries is related to lack of access to WASH (19) and it is the second leading cause of child death globally and the leading cause in SSA (20)**.** WHO estimates, diarrhoea contributes to more than one in every ten child deaths in Ethiopia. According to EDHS 2016 report the prevalence of diarrhoea is slightly higher for children in households with unimproved sanitation than in households with improved sanitation (21). It is estimated that improved sanitation can reduce diarrhoeal diseases by 32 percent worldwide (22).

Rapid growth of population and uncontrolled urbanization has created serious problems of energy requirements and solid waste disposal (23). 1.3 billion tons of solid waste generated (SW) per year, a volume expected to rise to 2.2 billion tones by 2025. In SSA approximately 62 million tones of SW generated per year (24). In developing country more than 50% of the collected waste is often disposed of through uncontrolled landfill (24). Among the organic SW streams available, food waste (FW) accounts 45-70 percent(25). USA is leading food waster in the world. The country generates 36 million tons of FW annually (26). In Africa South Africa is the largest food waster and FW accounts $1/4^{th}$ (177 kg/c/d) of all avoidable waste (27).

In Ethiopia FW is the single largest category of MSW(1). According to studies conducted in different towns of the country, 86.6% (28), 37% (29), 61.5% (30) and 36.03% (31) percents of FW generated in Bahir Dar , Jigjiga, Laga Tafo Laga Dadi and Jimma towns respectively from all avoidable wastes.

In the world it is estimated that 8% of anthropogenic emission of greenhouse gases(GHG) are generated from mainly from landfilled UFW (urban food waste) (32). In average 2.8 to 4.14 tons

of carbon dioxide is produced per tons of food waste (33). Global food wastage ranks as the third top methane emitter next to China and USA by causing 3.3 Gigatonnes of methane emissions (34).

Utilization of fossil fuels converts carbon and stored for millions of years in the earth crust, and releases it as carbon dioxide into the atmosphere. And it increase carbon dioxide concentration in the atmosphere causing global warming (13). Global warming, ozone layer depletion, acidic rain and bad smelling of the city can be considered as the results of poor WM (35). Improving global access to clean drinking water and safe sanitation is one of the least expensive and most effective means to improve and safeguard public health (36). Generating biogas through AD potentially leads to improved handling of human and food waste also provides treatment (37).Many studies have been done for treating various types of organic solid wastes using anaerobic digestion process. But the previous biogas studies were focused on animal waste and food waste without emphasis on human excreta (HE).

A few studies have been conducted on co digestion of food waste with human excreta. Of this anaerobic co digestion of cattle manure with FW and sludge in Spain (38) and anaerobic co digestion of FW and HE in 3:1 ratio in Nigeria (39)are some of related studies done previously. The first study was aimed to evaluate the effect of temperature on biogas production and the second study was done only in 3:1 ratio of FW and HE, and the study has not been clearly shown the optimum mixing ratios of FW and HE because the investigation was done in a single ratio (3:1).

However, no study has been conducted on anaerobic co digestion of FW and HE in five different mixing ratios operated at ambient temperature. Therefore the present study was aimed to assess the potential of biogas production from anaerobic co digestion of food waste and human excreta in five different ratios operated at ambient temperature.

1.3 Significance of the study

Developing nations especially Ethiopia both energy demands and waste production rate is increased from day to day. In Ethiopia it is oblivious that communicable diseases are prevalent and majority of them are related to poor sanitation and hygiene problem, beside this solid waste in Ethiopia caused for a hundreds of death in Addis Ababa particularly Koshe village due to the incident of land slide since 2009 EC. Food waste and human excreta are the most commonly generated waste in every home. Therefore this study will contribute on setting a clear direction on sustainable waste management options and will find an alternative sustainable energy source through converting waste to energy.

Additionally the study will contribute to policy development on sustainable waste management practice and energy sources at national and international level. This study will also be added to existing literature on energy particularly biogas production to help academic and other discourses. The last not the least the study will also help to increase the knowledge of the researcher in the field of anaerobic co digestion technology.

2. LITERATURE REVIEW

2.1 Biogas

Biogas is produced naturally in the absence of oxygen usually in swamps, bogs, rice paddies, sediment(40), digestive tract of ruminant mammals(12). Before 17th C biogas was not known by peoples and it was considered as a waste(41), but after 17th C the concept and understanding of biogas was raised(42) and the first actual biogas plant was installed in 19th C (9). The first biogas plant construction was done in 1890s by Donald Cameron, and he was constructed a septic tank and gas was collected and he used for street lighting. Biogas industry was grown after Second World War, but the interest of this technology was not reawakened until 1970s(9). However after 1973 the interest and awareness of biogas was increased due to the incident of oil crisis (41). In Ethiopia biogas generation was introduced in 1957/58 in Ambo Agricultural College. The data shows from 1980 – 2000 more than 1000 biogas plants were constructed in government and private institutions(1).

2.1.1 Advantages of biogas technologies

The benefits of AD include renewable energy generation, GHG emission reduction, and reduced water pollution, a reduced dependency on fossil fuels, creates job opportunity, closing of the nutrient cycle, convert waste to valuable resource, it contributes on waste volume and waste disposal cost reduction. Biogas is an environmental friendly technology used as a renewable energy source and it helps to preserve natural resource by reducing, deforestation and pollution through converting waste to useful product like biogas (43,44). Carbon dioxide released from biogas plant directly up taken by photosynthesis of plant, while carbon dioxide from non renewable resource like fossil fuel stored in earth for long period of time in the form of carbon and finally it released to the atmosphere in the form of carbon dioxide and causes green house effect (13). According to USEPA, methane is over 20 times more effective at trapping heat in the atmosphere than carbon dioxide (45). Previous studies shows that using human waste as a substrate for biogas production can reduce water borne disease and about 90% of parasitic egg and ova inactivated in AD process (2).

2.1.2 Challenges of biogas technology

Initial investment cost, social and cultural barriers are the common challenge that can hinders the growth of biogas technologies. Social and cultural barriers may also hinder harnessing fully the

benefits of the technology. Some cultures against using human excreta as a source of fuel and they believed that biogas from human excreta is 'unclean' for cooking(2).

2.2 Factors Affecting Anaerobic Digestion

2.2.1 PH

At low pH non-methanogenic microorganisms responsible for hydrolysis and digestion, while the methanogenic microorganisms unable to function at low pH (46). Methanogens are difficult to survive when pH is in acidic or alkaline medium. The optimal pH values for acidogenesis and methanogenesis stages are different. In acidogenesis stage acidogenic bacteria produce organic acids: acetic, lactic, and propanoic acids that tend to lower the pH of the bioreactor. During the initial time of experiment pH is declined because of high production of carbonic acid and it increases when methanogenic bacteria enriched (47). Methanogenic bacteria is very sensitive if the pH-value below 6,0 or above 8,3(41,48).

Methanogenic archaea function in the pH range between 6.5-8 and fermentative bacteria in a wider pH range of 4-8.5 (49). The acidogens prefer a pH 5.5- 6.5 (40). Studies shows that more biogas yield was recorded in neutral pH than others (50). A study done in India on anaerobic digestion of FW shows highest biogas yield was obtained from pH 7 compared to 5, 8 and 9 (51).

2.2.2 Temperature

At high temperatures the rate of chemical and biological reactions became faster, but if the temperature is low the reverse is true. If the temperature is beyond the optimum level proteins and cellular components of the microbes may be irreversibly damaged (52). Highest biogas yield is obtained in mesophilic and thermophilic bacteria.

Mesophilic bacteria are most active in 35-40°C temperature range (49) and thermophilic bacteria in the range 50-60°C. The methanogens are not active in extreme high and low temperatures (48).

A thermophilic temperature reduces the required digestion time. The microbial growth, digestion capacity and biogas production could be enhanced by thermophilic digestion, since the specific growth rate of thermophilic bacteria is higher compared to mesophilic bacteria (40). A study done in Hawassa shows biogas and methane production potentials from coffee husk reviled that more biogas and methane yield was recorded in thermophilic temperature (52±2 in 20 day retention time) than mesophilic temperature (37±2 in 40 day retention time) (53).

2.2.3 Air

Methanogenic bacteria are unable to survive in the presence of oxygen. Strict anaerobes and facultative aerobes are found in the AD process. Strict anaerobes only grow in the absence of oxygen (54). Breakdown of organic materials in the presence of oxygen produce CO_2 while in the absence of oxygen produces methane gas (49).

2.2.4 Carbon to nitrogen (C/N) ratio

Carbon and nitrogen in the basic elements feedstock are the chief foods of anaerobic bacteria(40). Anaerobic microorganisms used carbon in carbohydrates for energy source and nitrogen in proteins is required for building of bacteria cell structures (42). The ideal and optimum C/N ratios in AD are 20:1 – 30:1. Which means micro-organisms consume carbon 20 to 30 times more faster than nitrogen(1,9). When C/N ratio is very high nitrogen will be used quickly by methanogens for meeting their protein demand and carbon remain in the form of leftover. As a result gas production will be low due to assemblage of organic acid. Or when the C/N ratio is very low, nitrogen will be liberated and accumulated in the form of NH3 (2)which is toxic to methanogens (40). The previous study done in Abuja, Nigeria indicated that highest biogas yield was obtained in 24 C/N ratio compared to 10 and 47(8). 25 C/N ratio was described optimum for biogas production (55).

2.2.5 Nutrients

Methanogens need a number of macro- and micronutrients in order to grow. N and P are needed in relatively large quantities by all bacteria cell. However K, Mg, Ca, Fe, Na, Cl, Zn, Mn. are required in relatively small quantities. Most nutrients can be inhibitory for methanogens if it is present beyond the recommended values(1,9). The content of biogas yield varied with the content of carbohydrates, proteins, and lipids. Lipids (fats, oils and greases they are commonly present in food wastes) provide the highest biogas yield, but it takes more digestion time because of its slow biodegradability, whereas carbohydrates and proteins show quicker conversion rates but lower gas yields (56).

2.2.6 Particle size

The production of biogas is also affected by particle size of the substrate. Large particle size can affect the digestion processes of substrate, whereas small particle size gives a large surface area for substrate adsorption and it increased microbial activity, resulted higher biogas production (40). This is more important for digesting slowly biodegradable materials (57). A study

conducted in Dar-es- Salaam-Tanzania reviled that highest biogas production was observed in lower(0.001mm) particle size than large particle size(0.5mm), which was 0.9 and 0.34 L respectively(57). Another study done in South Africa shows the amount of biogas recovered from 25µm was almost double as compared to that of 100µm, 250µm and 500µm particle size in mesophilic batch digestion tests (58).

2.2.7 Hydraulic Retention time

Hydraulic retention time (HRT) isthe average time of the substrate remains in the bio-digester. It depends on amount and type of effluent and incubation temperature. When the temperatures increase in the digester, the retention time can be shorter (2). The expected retention times of mesophilic and thermophilic digesters range between 25 and 35days (40). The volume of biogas increases when residence time increased and substrate concentration decreased. Biogas production rate is directly proportional to specific growth rate of methanogenic bacteria in the digester (59).

High concentrations of substrate added in the digester it required more time for digestion (50). If the retention time is too short, the bacteria in the digester are washed out faster than they can reproduce. If substrates stay more in the digester, complete degradation will occur and more biogas yield can (**1**)(47)

2.2.8 Total and volatile solid

Volatile solids (VSs) are the portions of the total solids (TSs) content of the substrate that can be converted into biogas. 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 in the range between 20 to 30 g/liter (2). Substrates with high VS content produce more biogas yield than low VS if digested properly (40). The previous studies showed that biogas and methane volume increased when TS contents increased. Of this A study done in China shows the average cumulative biogas of 5%TS, 15% TS and 20% TS accounted to 700, 760 and 870 ml and 370, 410 and 480 ml methane content, respectively (60).

Another study conducted in Indonesia indicated that 184.09 ml/ gVS^{-1},186.28 ml/ gVS^{-1} biogas was recorded from7.4% TS and 9.2%TS respectively (61). A study done in Sweden also shows the TS and VS of food waste and human feces were 24.5 and 92.5 % and 21.9 and 87.1 % respectively (62).

Additional study conducted in Indonesia reviled that the TS of human excreta was 23% (63). According to the report of India (64) and Sweden (62) research work the TS of food waste was 28% and 24.5% respectively. A study done in Kochi, South India (65) and Sweden (62) shows the initial VS of food waste was 90.1% and 92.5% respectively. 20% TS of food waste reported an optimum value for biogas production (64)

2.2.9 Water Content

With too much and too low water content not good for AD process and If the water content is too low, acetic acid will accumulate, inhibiting the fermentation process (42,49). The highest methane production rates occur at 60–80% of humidity (23).

2.2.10 Seeding

To start up a new anaerobic process, it is critical to use an inoculum of microorganisms to facilitate the fermentation process. The commonly used seeding materials are digested sludge from a running biogas plant or cow dung slurry (40). Investigation shows that the volume of biogas produced in seeded setup reactor was twice larger than the volume of biogas produced in unseeded setup reactor. This shows that seeding increases the rate of biogas production (66).

2.2.11 Salt

Salts contain essential building blocks for the microorganisms, such as sodium, potassium, and chlorine. Too much salt (or sugar) causes the cells to pump out water and lose both form and function. Salts and sugars generally have a preservative effect; that is, they inhibit bacterial growth. Materials like waste from the food and fisheries production industry may increase salt concentrations in biogas processes. Typically, methanogens are usually the most affected groups of organism which can affect by high salt concentrations in a biogas process (54). Studies shown biogas yield decreased when the content of salt increased. Accordingly more biogas was obtained from 0 g of NaCl l^{-1} (193 ml / g^{-1} VSs) compared with 60 g of NaCl l^{-1} 61 ml/ g^{-1} VSS day^{-1} (67).

2.2.12 Mixing condition

The close contact between micro-organisms and the substrate material is can determine the digestion process. Mixing helps to mix up material, evening out any localized concentrations, thus also helping to stop the formation of ‘dead zones’ or scum (40). It helps to homogenize the incoming feed with the active microbial community of the digester content (68). In addition to increases the waste’s availability to the bacteria, helps remove and disperse metabolic products

and to ensure a more uniform temperature within the digester. Mixing also promotes heat transfer, particle size reduction as digestion progresses and release of produced gas from the digester contents (40). Study was found mixing had improved methane production (5.32 L/day) than unmixed reactor (5.16 L/day) (68).

2.3 Anaerobic digestion and inactivation of pathogen

Fecal related pathogens entering to environment from untreated human and animal faeces can lead to disease (37). AD for biogas generation has the potential to reduce pathogen loadings to the environment (37)

Using human excreta as a substrate for biogas production has potential to reduce the risks of thus pathogens (39). Indicator organisms like E. Coli and fecal coliform (FC) have been the most widely used to assess the efficiency of AD on pathogen removal (69). The previous studies show that thermophilic temperature destroyed more pathogens than mesophilic and moderate temperature. One study done in Canada indicated that more pathogens were removed at 54.9 ^{o} C than 37 ^{o} C from anaerobic digestion of swine slurry (70). A study done in Indonesia found that *E. coli* inactivation in thermophilic temperature were faster than moderate and mesophilic temperatures (63). Another study conducted in Mexico and New York tells that 3 log MPN/g TS at 55 ^{o}C and 1 log MPN/g TS at 35 ^{o} C) (71) and 3 log reduction of fecal coliform was observed after conducting anaerobic digestion process at 100 ^{o} F (72) respectively. According to different study findings Schistosoma parasite was completely removed within 7-22 retention time at moderate, mesophilic and thermophilic incubation temperature (73).

2.4 Characteristics of feedstock

2.4.1 Food waste

Food waste (FW) is composed of raw or cooked food materials and includes food loss before, during or after meal preparation in the household, as well as food discarded in the process of manufacturing, distribution, retail and food service activities"(74). FW is rich in nutrients and organic material and it is also easily biodegradable (75). It is rich in protein and lipid if compared to other waste, like sewage sludge. Lipid is the highest contributors of biogas with longer HRT due to retarded biodegradability, whereas proteins and carbohydrates exhibit quicker conversion rates with lower biogas yield. FW is one of the best substrate for AD because of its CH4 producing potentiality (64).

FW has 74–90% of MC, 80 - 97 % VS to TS ratio (VS/TS) and 14.7 – 36.4 carbon to nitrogen ratio (C/N) (64). A C/ N ratio of 20 - 30 is deemed suitable for the AD process (76). Different study findings shows that AD of food leftovers have potential to produce 60 (77) to 64 % (62) and 65 % methane and it contains 2.5 percent nitrogen and 49 -51.4% carbon(62,78).

The degradation of food waste is 20-30 % higher than that of bio waste**.** Leftover foods consisting of cooked foods, such as meat, fish, rice, bread and vegetable are mainly composed of protein, starch, sugar and fat. These food wastes contain highly biodegradable organic matter and thus result in higher methane production (1). A study done by R. K. Somashekar shows 68.8L of biogas was produced from 20 L of food waste within 60 days lab experiment (79). There is high potential biogas yield from food waste because of its high energy content and the presence of easily degradable carbohydrates and proteins (80). If all UFW could be an aerobically treated, the total global energy recovery for 2025 is estimated at 2688 x 1012 KJ. UFW from the Americas, Europe and Africa can produce 616 x 10^{12} KJ, 392 x 10^{12} KJ and 280 x 1012 KJ of energy respectively, in 2025(32).

2.4.2 Human excreta

Human excreta have similar potential in biogas generation compared to the cattle manure. Those advantages might due to the human faeces and cattle manure are derived from the anaerobic degradation in the gastrointestinal tract, so it is possible to contain high fecal anaerobic bacteria. There is a possibility of potential biogas per kg human faeces becomes equal or higher than the manure, and the levels of methane in the biogas can reach 70% (63).

In most cases biogas from human excreta contains 65-66% methane and 32-34% carbon dioxide and 1% hydrogen sulphide and other trace amount of nitrogen and ammonia (81). Additional water is not normally required when the biogas digester is connected to a flush toilet that provides excreta, urine and flush water (23). The main problem of human excreta is the low carbon content (6-10.) This resulted ammonia formation and the system pH becomes alkaline. So to overcome this problem, high carbon content substrates required (63).

Different literatures shows that the pH of human excreta is found between 7.3 to 7.7(63)(82). Human excreta consists of 75% (83), 77% (63) and 79 % (82) of moisture content. Carbon and nitrogen contents of human excreta is between 44 -55 percent and 5% respectively(84,85). Human urine and faeces are rich in plant nutrients. Faeces contain mainly water, bacteria,

nutrients and food residues. It is also contain large concentrations of pathogenic viruses, cysts of protozoa and eggs of helminthes (22).

2.5 Mono digestion

The anaerobic digestion (AD) of single substrates (mono-digestion) presents some drawbacks linked to substrate properties (86,87). High amount of labile organic matter (OM) present in FW, as a result mono-digestion of FW often leads to process instability caused by rapid acid accumulation from the hydrolysis of labile OM. Macromolecules such as carbohydrates, proteins and lipids are hydrolysed by anaerobes, VFAs are produced. VFAs include acetate, propionate, butyrate and valerate, can be consumed by acetogens and methanogens.

The proliferation of acid formers is usually faster than methane gas formers. If the acids production is greater than its consumption, the accumulated acids will lower the pH and lead to process failure. The chance of VFAs inhibition increased following increased proportion of FW in the AD system. So substrates rich in N contents is often used to adjust the nutrient content during AD of FW (76).

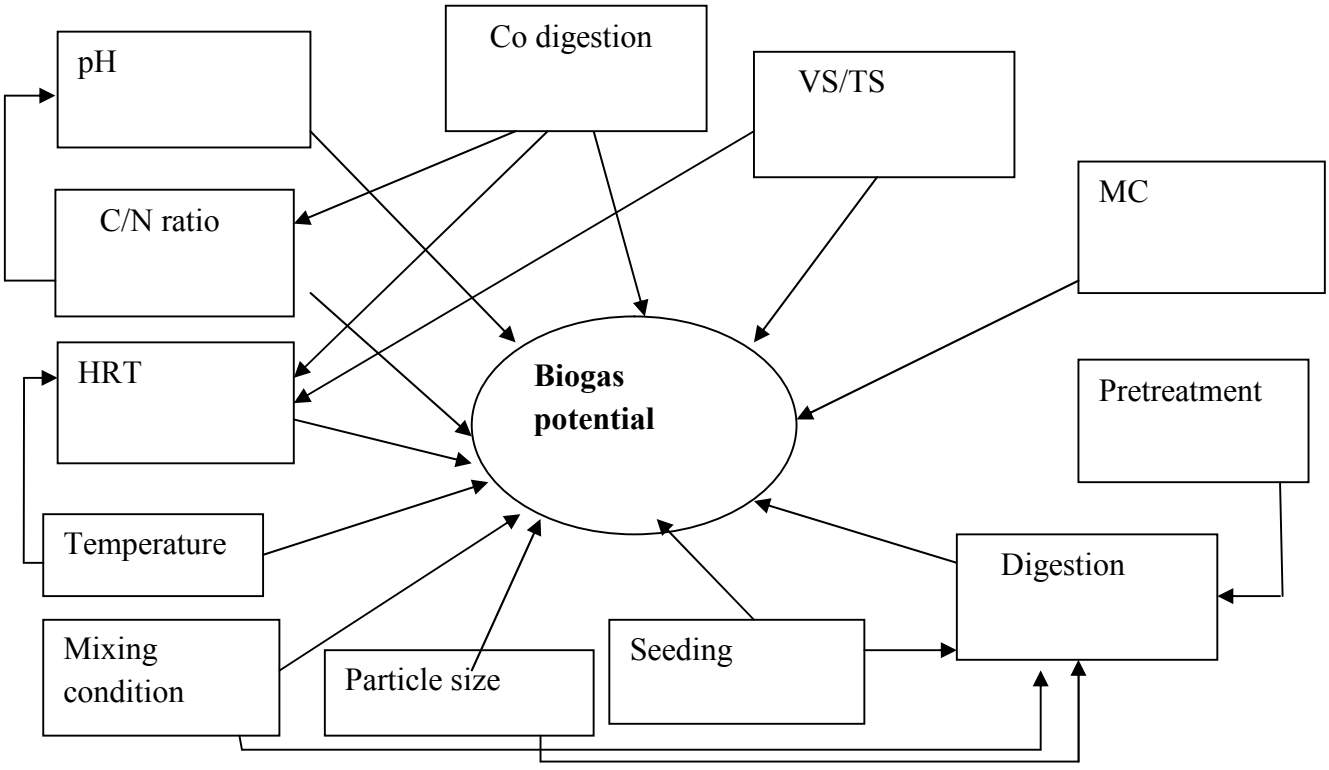
2.6 Co digestion

Co-digestion via the combination of two or more organic wastes is increasingly popular in AD technology (87). Particularly mixing organic substrates have resulted in the production a mixture with C/N ratio in the optimal range of 20 – 30. Co digestion of wastes can improve C/N ratio of substrate, pH adjustment, improve sludge quality,(5)improve digestibility, dilute inhibitory or toxic compounds, macro- and micronutrient equilibrium, biogas production, and process stability, improved buffer capacity of the process, providing economic and environmental benefits (86,87). The mentioned benefits have a positive effect on improved stability and performance of the process and leads to higher biogas yield and energy contribution.

Higher yield of biogas by co digestion is associated with the synergistic effect of the microorganisms. (5). The TS and VS reduction in AD is more pronounced on digestion of co substrates than mono substrate (88). The microbes involved in AD are sensitive to the inhibitors, which can reduce the growth rates and disable the microbial activities. At low pH, the main VFAs are acetate and butyrate that inhibit methanogenesis(76). Balanced C/N ratio is very crucial for successful operations of AD (5). To maintain the C/N ratio in optimum range apply co digestion of high carbon(low nitrogen) substrate with low carbon(high nitrogen) such as co

digestion of animal waste and human excreta with FW(40). A Korean study shows co-digestion of primary sludge and food waste leachate resulted in 40% higher methane production than mono-digestion of primary sludge(87). Similar study done in Iraqi reviled that digestion of co substrates of vegetable waste with butcher waste water gives 50% more biogas yield compared to sole substrates (89).Another study done in Thailand indicated 64.4%, 48.6 and 36% of methane was attained from 1:1, 1:0 and 0:1 mixing ratios of chicken manure to Napier grass (90).

2.7 Conceptual framework



3. OBJECTIVES

3.1 General objective

✓ To assess biogas production potentials from anaerobic co- digestion of food waste and human excreta

3.2 Specific objectives

❖ To determine physico- chemical parameters of food waste and human excreta

❖ To investigate biogas production potentials from co-digestion of food waste and human excreta

❖ To investigate methane production potentials from co-digestion of food waste and human excreta

❖ To evaluate selected pathogen removal efficiencies of anaerobic digestion process

4. MATERIALS AND METHODS

4.1 Study area and period

The study was conducted in Jimma University College of agriculture and veterinary campus medical entomology department in Jimma town, South west Ethiopia. Jimma is locally known as the town of Abba Juffar. It is situated at 353 km from Addis Ababa on the high way of Mettu – Gambella at an altitude of 1620m above sea level, the town is located at 7 ^{0}40'N latitude and 36 ^{0} 60'E longitude. The climate condition of the district is Woina Dega. The temperature of the town is high at March (30.4 ^{o} C) and low at January (8.5 ^{o} C) (31). The mean temperature ranges from 18 -26 ^{0}C(91). The study was conducted from May to July, 2018.

4.2 Study design and experimental setup

Lab based experimental study design was employed. One liter size plastic bottles were used as digester with a working volume of 3/4th of the digester. A 0.8 cm diameter hole was made at the center of the cover of each plastic bottle. Plastic tube with 12 cm length and 0.8 cm diameter inserted in to the hole and glued using super glue in order to ensure air tightness. The plastic tube served as gas conduit and transfer gas from digester to gas holder (1 L plastic bag). Each digester was painted black color galvanized paint for keeping the entrance of air and control algae growth inside the digester.

Biogas potential test was conducted in five duplicate mixtures of 100% FW, 75%FW + 25% HE, 50% FW + 50% HE, 25% FW + 75% HE and 100% HE. 20% TS was used to achieve the recommended moisture contents for AD processes(23,64). The digesters were charged once during the experiment duration for the retention time of 44 days with the mixtures indicated in table 1. The same amount of inoculum was inoculated in each digester. The digesters were operated at ambient (room) temperature with a retention time of 44 days. The experimental setup was at first set to monitor the biogas production potential of co digestion of human excreta and food waste and secondly to measure the volume of methane produced. The experimental set up is shown in figure 1.

Figure 1 Experimental setup

4.3 Sample collection and feedstock preparation

In this study human excreta (HE) and food waste (FW) was used as a substrate and approximately 819 g each of FW and HE was collected 9figure 2). FW was a blended mixture of food components like boiled rice, bread, kincha, miser, aterkik, enjera firfir, potato, meat and cabbage and tomato was used as a substrate. FW was collected from Jimma university student cafeteria and a one day age fresh deposited human excreta was collected from Jimma university student's hall of residence. After collection the substrates was brought to the laboratory and the investigation was carried out. Then undigested solid materials were separated from the sample and FW was grinded using pestle and mortar and it was homogenized.

The solid concentration taken in all the digester were 20% TS and for the entire experiments, 2167 ml of effluents was obtained from anaerobic digester of Jimma Degitu hotel and used as startup (10% of total volume). Weighting of the sample was done using a digital weight scale. Fresh wet food waste and human excreta presented in figure 2.

Figure 2 Fresh wet food waste and human excreta

Table 1Mixing ratio of substrates and main contents of digester

Digester	Main contents of the digester								
	Mix ratio FW%	HE%	TS of FW in (g)	TS of HE in (g)	% TS	% MC	Water (ml)	Inoculum(ml)	Total volume in (ml)
T1	100	0	162	0	46	54	343.8	216.7	722.5
T2	75	25	121.5	40.5	38	62	330.8	216.7	709.5
T3	50	50	81	81	37	63	329.2	216.7	707.9
T4	25	75	40.5	121.5	35	65	326	216.7	704.7
T5	0	100	0	162	26	74	311.4	216.7	690.1

FW- food waste, HE- human excreta, TS- total solid T1= 100% FW, T2=75%FW and 25% HE, T3 =50%FW and 50% HE, T4=25%FW and 75% HE and T5 100% HE.

4.4 Experimental analysis

4.4.1 Measurement of Temperature and pH

Temperature and pH was measured in every other day until the end of the experiment using digital multi probe parameter (HACH, 4Q4od probe). Multi probe parameter was inserted in each digester to measure pH and temperature of slurry.

4.4.2 Measurement of Biogas and methane

The volume of biogas produced was measured by downward water displacement method using 5 L size plastic cylinder. During measurement gas collector plastic bag was connected with inverted measuring cylinder (5 L size) at the bottom. The measuring cylinder graduated and the divisions of the cylinder were used to measure the volume of gas in L and ml. The cylinder was completely filled with the water and kept upside down with a cap at its mouth. Then the plastic tube was fitted inside cylinder to keep its movement and gas collector bag was pressed and gas was moved into cylinder and water was displaced from the cylinder. The amount of water displaced is equally proportional to the volume of biogas produced (92).The yield of biogas was calculated based on the following formula (93):

$$Yield = \frac{\text{volume of gas collected (ml)}}{\text{Mass of input waste (g)}}$$

The volume of methane and carbon dioxide was determined through passing of biogas in 10% NaOH solution and the solution was used to absorb carbon dioxide in biogas and the remaining unabsorbed gas (methane) was passed and collected in gas receiving bag(100 ml plastic bag). Two plastic bags were used to measure the methane and carbon dioxide content in biogas. Sample gas was taken and injected into one of the two plastic bags using 50-60 ml syringe and the second bag remained empty. Then the sample containing bag was connected with the inlet of solution containing glass bottle and the empty bag was connected with the outlet of the bottle.

Once the two bags were connected with glass bottle , both bag valves was opened and sample gas containing bag was pressed and the gas was injected into the solution then, carbon dioxide was absorbed in the solution and the unabsorbed gas methane was passed in the solution and collected in empty bag. After completing this process the valves of the bottle and bags were closed, and then collected methane was measured by syringe. Finally the amount of methane collected in empty bag subtracted from the amount of sample gas taken gives the amount of carbon dioxide absorbed in the solution.

Figure 3 Biogas collector plastic bag

Sample gas containing plastic bag NaOH solution injecting of gas into solution

Figure 4 Methane gas measurement procedures

4.4.3 Moisture content

The percentage of moisture content of the sample was determined using the formula shown below after weighed 10 gram of sample(92) from each mixtures into a pre-weight dish and then, dried the sample in an oven at 105^{o}C about 24 hour(40).

$$\% MC = W - D \times 100/W$$

Where MC = Moisture content

W = initial weight of sample (g)

D = weight of sample after drying at 105^{o} C (g)

4.4.4 Total Solids

For the determination of TS, a clean evaporating dish (crucible) was used and firstly the dish was dried in an oven adjusted at 105^{o}C for one hour, then it was cooled in desiccators and weighted immediately before use. Then, 10 g of fresh collected samples of each ratio of food waste and human excreta was weighted using a weight scale, and it was placed in pre-dried and weighted evaporating dish. Then, the dish was putted inside oven which was maintained at 105 ^{o}C. Then the dish was allowed in the oven for 24 hours, and then taken out, cooled in desiccators and weighted(92,94) .

The percentage of the TS was determined by using the following formula:

$$\% TS = \frac{\text{MDS} \times 100}{\text{MFS}}$$

Where, %TS= percentage of total solids

MDS= mass of dry sample (final weight) in gram

MFS= mass of fresh sample in gram

4.4.5 Volatile solid

After TS was determined, the oven dried substrate was ignited at 550°C in furnace for two hours to determine the volatile and fixed solid content of the sample. The following formula was employed to calculate the percentage of volatile solids content of TS(94).

$$\% VS == \frac{\text{MDS} - \text{M(ash)} \times 100}{\text{MDS}}$$

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

The percentage of VS removal was calculated based on the following equation (94).

$$\%\, VSRemoval = \frac{\text{VSI} - \text{VSF} \times 100}{\text{VSI}}$$

Where, VSi = initial volatile solids (%) before AD

VSf =final volatile solids (%) after AD

4.4.6 Carbon content

The carbon content of the feed stock was measured by considering the content of volatile solids expressed in percentage using an empirical equation as reported by Menta T(2)

The total carbon content of the substrate was estimated as:

$$\%\, C = \frac{\%\text{VS}}{1.8}$$

4.4.7 Nitrogen determination

Kjeldahl UDK159 digestion, distillation and titration apparatus, code F30200150NemkeUS was used for analysis nitrogen in the sample. The analysis was done according to AACC method 46(2010) and EPA method 1687(2001) (95,96). The Kjeldahl procedures were followed in three major steps, these were: digestion, distillation and titration. For this purpose 10 ml of sample was measured using measuring cylinder and it was transferred into digestion flask and hydrolyzed by 15 ml 98% sulpheric acid solution and catalysts was added. The flask was putted on digestion chamber at 430 ^{O} C adjusted temperature for 60 minutes. And then the digested sample was neutralized by 50% sodium hydroxide solution to convert ammonium ion to ammonia gas and the ammonia gas was bubbled by steam and trapped by 4% boric acid solution and finally titration was made by 20% hydrochloric acid and nitrogen was determined. Detailed TKN analysis procedures were listed under annex 3.

Sample with H_2SO4 ⇨ Sample after digestion ⇨ Sample distillation and Titration

Figure 5 TKN analysis procedure

4.4.8 Microbial analysis

All microbiological analysis was done within 24h after sampling. In microbial analysis Fecal coliform and ova of S mansoni were investigated. Fecal coliform analysis was done based on standard method 9132 total coliform membrane filtration technique (97).For fecal coliform analysis 10 ml of sample were homogenized and serially diluted from 10-10 $^{-6}$ using standard laboratory practice. After dilution 100 ml diluted sample was filtered and membrane filter was inoculated equally in absorbent pad. Finally the Petri dish was incubated and enumerated after 24 hour incubation at 44.5 ^{o} C. Calculation was made based on the following formula(98).

$$\text{Coliform colonies/ml} = \frac{\text{colonies counted}}{\text{Volume of sample filtered} \times \text{dilution factor}}$$

Culture media preparation Sample dilution and filtration Incubation and colony enumeration

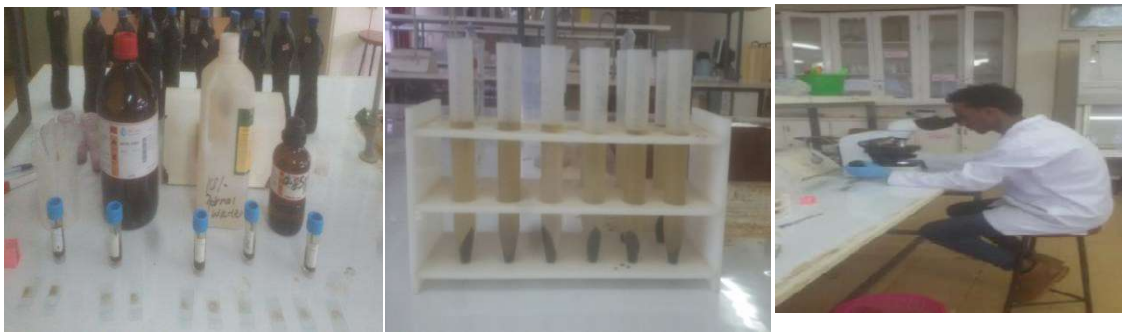
Figure 6 Fecal coliform analysis procedure

Saline wet mount and formol ether concentration techniques were employed for S mansoni detection. The investigation was carried out based on basic laboratory methods in medical parasitology (99). For saline wet mount method 1-2 drop of 0.9% saline was placed in the center of slide and 1 drop of sample was added and mixed it. The slide was covered by cover slip and putted on microscope, and then sample was examined on ×10 and ×40 object.

After wet mount analysis formol ether concentration technique was used. For this method 1 ml of sample was mixed with 8 ml of 10% formol water and added in centrifuge tube and mixed properly. The mixed sample was filtered and 7 ml of sample was taken and it was mixed with 3ml of ether. Then it centrifuged at 3000 rpm for 1 minute.

Finally the liquid suspension was discarded and the sediment was mixed and transferred into slide and after covered by cover slip examination was made in microscope. Detailed laboratory procedures are listed under annex 2.

Wet mount method of analysis is recommended if the number of parasite is high in the sample and to see the protozoan trophozoites because it usually destroyed by concentration method. While if the number of parasite is very low and the result of wet mount is negative concentration technique is recommended and the sample should be concentrated because parasites worm egg, larvae and protozoan cysts may be recovered by concentration method and it helps to detect schistosoma and Tania species(100,101).



Sample preparation Centrifugation Examination

Figure 7 Formal ether concentration technique procedures

4.5 Operational definition

FW –leftover foods generated in Jimma university student cafeteria including to enjera, cabbage, potato, tomato, onion, boiled rice, meat, bread, miser , aterkik and kincha.

Human excreta: one day age mixed human feces and urine obtained from Jimma university student's hall of residence

Total solids- the amount of solids remaining from 10 g of sample after heating for 24 hr at 105ºC.

Volatile solid –the amount of solid evaporated after igniting the dried sample at 550ºC for 2hr.

Moisture content -The amount of liquid evaporated after drying 10 g of sample at 105 ºC for 24 h

Substrate – Wet food waste and human excreta obtained from JU student cafeteria and resident hall before anaerobic digestion

Co substrate – the mixture of food waste and human excreta in 75:25, 50:50 and 25:75 ratios

Mono substrate- The mixture of food waste and human excreta in 100:0 and 0:100 ratios

Co-digestion- digestion of food waste and human excreta in 75:25, 50:50 and 25:75 ratios

Mono digestion - digestion of food waste and human excreta alone

Feedstock- the combined liquid (water and inoculum) with food waste and human excreta

Inoculum- an effluent obtained from active anaerobic digester of Jimma Degitu hotel which is used as a start up

Digester _ one liter size plastic bottle used as a fermenter

Glass bottle – a material which contains 10% NaOH (CO_2 absorbent solution)

Gas holder- one liter size plastic bag used for gas collection and measurement of biogas and methane generated.

Plastic tube – 12cm length and 0.8 cm diameter size tube, which is used to transfer gas from the digester to gas holder

Super glue- sticky material used to seal the opening of the digester to ensure air tightness.

Digestate- undigested solids and liquids remained in the digester after 44 days retention time anaerobic digestion.

4.6 Data Analysis

After conducting each of laboratory analysis and the result of MC, TS, VS, C/N ratio, pH, FC, and ova of S mansoni before and after anaerobic digestion, daily biogas and methane production were recorded. The data was entered and stored in excel spread sheet (Microsoft excel window 2016) and analyzed by (one way ANOVA) using SPSS version 21. Fisher least significance difference (LSD) was used to investigate statistical significance between treatments and Paired sample T- tests was used to investigate statistical significance within treatment. P value <0.05 was used as a significance difference. Data was presented in form of table, figure and graph.

4.7 Data quality management

To validate the data, the experiment was done in duplicate. All physicochemical and microbial analysis was determined based on scientific standard laboratory procedures. A microbial analysis was done with control group to avoid false positive results and to see the quality of reagents and efficiency of sterilization technique used. Sterilization of instruments was made based on standard procedure and PH meter was calibrated using 4, 7 and 10 buffer solution before the measurement was done.

4.8 Ethical consideration

Before conducting the study, ethical clearance from the institutional review board of Jimma University institute of health science and letter of cooperation was obtained from Jimma University.

4.9 Dissemination plan

A document of results will be submitted to Jimma University Postgraduate School. The result will be communicated with the stakeholders through presentations on meeting, workshops and scientific panels. Attempts will be made to publish the thesis in reputable scientific journals

5. RESULT

5.1 Physico chemical characterizations of food waste (FW) and human excreta (HE)

Table 2 summarized the TS, MC, VS, C/N ratio and pH before and after anaerobic digestion. As the table shown, there is a variation in composition between before and after digestion. The TS of the substrate varied from 26% to 46% before AD and 22% to 40% after AD and the VS percentage of the mixtures were found between 85% to 93.4% before AD and 75.2% to 85% after AD from 10 g sample of each treatment.

Highest VS reduction was observed in 50% FW + 50% HE mixing ratio, while the lowest VS reduction observed in 100% HE alone. C/N ratio of 75% FW + 25% HE**,** 50% FW + 50% HE and 25% FW + 75% HE were found in the optimum range (20-30). The highest initial pH values was recorded in 100% HE (7.5) and the lowest initial pH value recorded in 100% FW (6.6). TS and VS before anaerobic digestion were significantly higher than the values after digestion in all substrate mix ratios ($p<0.05$)**.** However the extent of decrement relatively appears more pronounced in co-substrates than mono substrates as indicated in the table below.

Table 2 Initial and final TS, VS, TS/VS, C/N ratio and pH of FW and HE.

Digester	TSi%	TSf%	MCi%	MCf%	VSi%	VSf%	%VSR	Ci%	Cf%	Ni%	Nf%	C/Ni%	C/Nf%	pHi	pHf
T1	46	40	54	60	93.4	85	9.9	51.9	47.2	1.5	0.9	34.6	52.4	6.6	5.49
T2	38	28	62	72	91	78.9	15.3	50.5	43.8	1.8	1.6	28	27.3	6.8	6.9
T3	37	25	63	75	90.2	75.2	20	50.1	41.7	2	1.8	25	23.1	6.94	7.2
T4	35	26	65	74	89.2	78	14.3	49.5	43.3	2.4	2.3	20.6	18.8	7.3	7.46
T5	26	22	74	78	85	79	7.6	47.2	43.8	3.5	3.2	13.4	12.5	7.5	7.7

C= carbon, TS= total solid, VS=volatile solid, N= nitrogen, MC= moisture content, C/N=carbon to nitrogen ratio, f= final, i=initial, T1=100%food waste, T2= 75% food waste + 25% human excreta, T3= 50% food waste + 50% human excreta, T4= 25% food waste + 75% human excreta and T5= 100% human excreta.

5.2 Daily and cumulative biogas production

The analysis result showed that there was a significance difference between biogas production and retention time ($p<0.05$). Daily biogas production depicted in figure 8 and the graph tells that all treatments were began fermentation and biogas production in day 2 and daily biogas production was low in the beginning and the end of the experiment period. As a result biogas production was low during the first 2 to 16 days and peak from18 to 29 days and eventually reached zero in day 40 (T1), 42 (T5) and others in day 44. However, the volume of gas varied between treatments. The experiment result confirmed that when retention time increased, the volume of biogas also increased. In the first 16 days of experiment only 19% of biogas was produced from the total gas production and in average 138 to 158 ml in T2, T3 and T4 and 98-114 ml in T1 and T5 biogas was produced in every other day.

Next 16 days to 30 days the volume of daily biogas production was reached to peak and 54% of biogas was produced, in this period about 580 to 603 ml from T2, T3 and T4 and 182 – 184 ml from T1 and T5 biogas produced in every other day. 30 days after 27% of biogas was produced to eventually reached 0 ml on day 40 (T1), day 42 (T5) and day 44 (T2, T3 and T4). Here, 182-259 ml (T2, T2 and T3) and 95 to 130 ml biogas produced in every other day in T1 and T5.

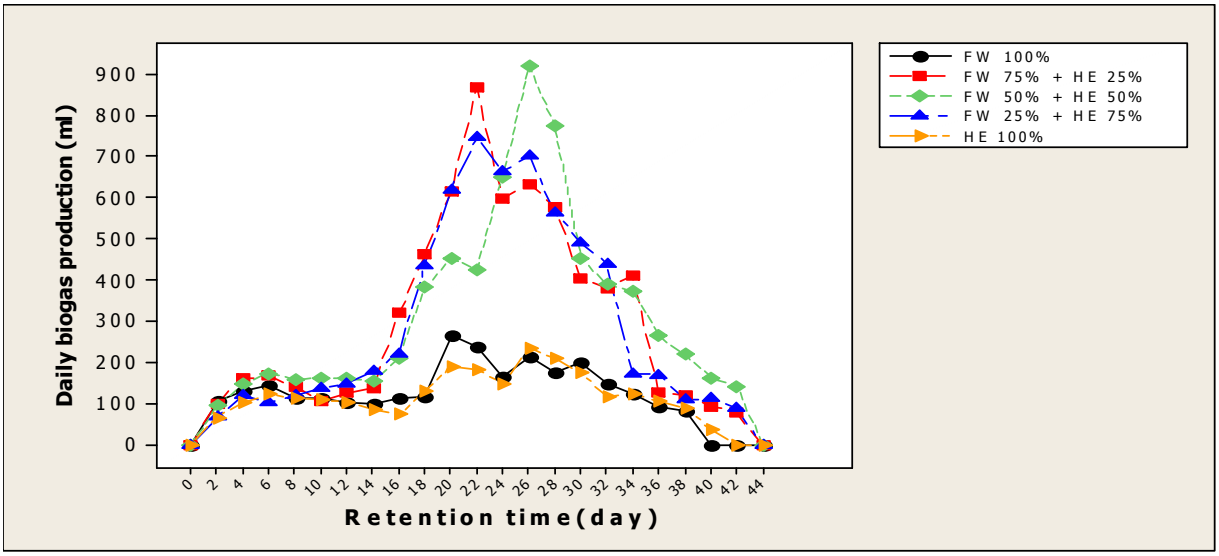


Figure 8 Daily biogas productions

The average mean values of biogas produced in T1, T2, T3, T4 and T5 presented in table 3.The average mean values of biogas produced in T1, T2, T3, T4 and T5 were 120, 288, 299.3, 279.4 and 110.4ml in every other day respectively in 44 day retention time.

Table 3 Average biogas production in 44 days retention time

Treatments	Mean ± SD	SE	P -value
T1	120.2 ± 73	15	T2(0.003), T3(0.002) T4(0.005), T5(0.86)
T2	288.8 ± 240	50.2	T1(0.003), T3(0.85), T4(0.86), T5(0.002)
T3	299.3 ± 234	48.9	T1(0.002), T2(0.85), T4(0.72), T5(0.001)
T4	279.4 ± 241	50.3	T1(0.005), T2(0.86), T3(0.72), T5(0.003)
T5	110.4 ± 64	13.3	T1(0.86), T2(0.002), T4(0.001), T5(0.003)
Total	219.6 ± 205.6	19.1	0.000

The analysis result showed that there was a significance difference on biogas production between co digestion and mono digestion of FW and HE ($p<0.05$). This study revealed that co digestion of FW and HE affects the volume of biogas generated. The investigation observed that co digestion of FWwith HE provided more biogas yield compared to mono digestion of FW and HE as reported in figure 9. Accordingly two and half times greater biogas yield was obtained from co digestion of FW and HE compared to mono digestion of FW and HE. The highest and lowest biogas yield was attained in mix ratios of 50% FW + 50% HE (T3) and 100% HE (T5) respectively within 44 days retention time anaerobic digestion. The cumulative biogas produced in T1, T2, T3, T4 and T5 were 17 ml/g TS, 41 ml/g TS, 42.5 ml/g TS, 39.6 ml/g TS and 15.6 ml/g TS respectively.

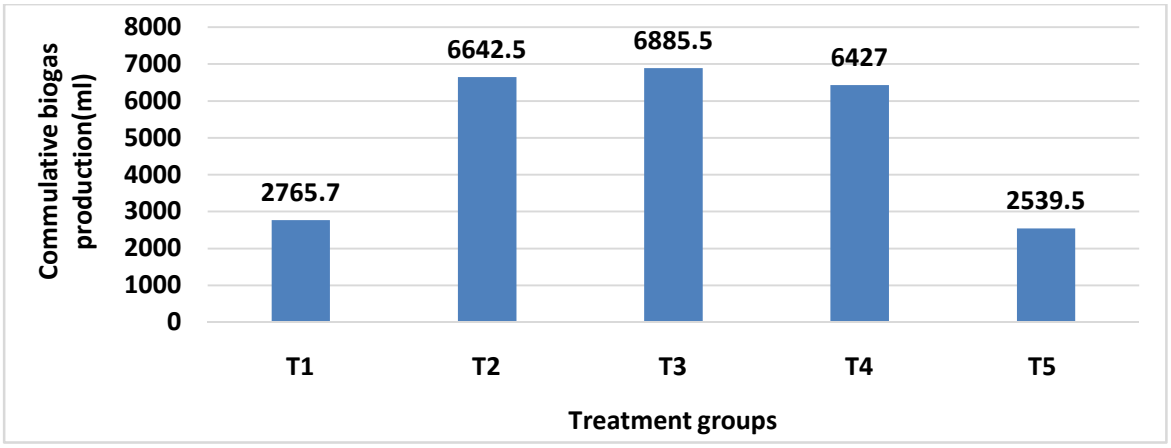


Figure 9 Cumulative biogas production VS treatment group

T1=100% FW, T2= 75% FW + 25% HE, T3= 50% FW + 50% HE, T-4= 25% FW + 75% HE and T5= 100% HE.

5.3 pH and retention time

pH and retention time relationship presented in figure 8. pH was significantly affected by retention time ($p < 0.05$). The ANOVA analysis result showed that there was a significant relationship between pH and retention time ($p < 0.05$). In all digesters pH was declined during the initial period of the experiment, where the pH value varies from 6.6-4.6, 6.8-5.3, 6.94-5.6, 7.3-6 and 7.5-6.88 in T1, T2, T3, T4 and T5.

The next figure depicted that the pH value during the first 16 days of operation was below 6 in T1, T2 and T3 and it started to increase from day 17 to 44. The lowest pH value of T1 and T2 was reported in day 16(4.6) and day 12(5.3) and finally reached to 5.49 and 6.9 at day 44. The lowest pH value of T3 (5.6) and T4 (6) was observed in day 8 and T5 in day 10 (6.88) and at the end of the experiment it was reached to 6.2, 7.46 and 7.7 respectively.

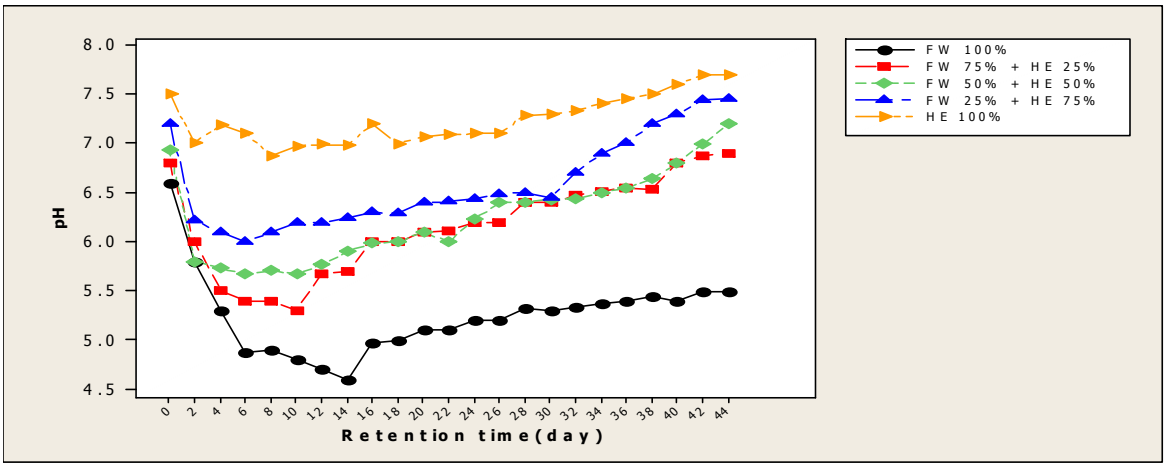


Figure 10 pH vs retention time

5.4 Biogas production and pH

The analysis of ANOVA indicated that there was a significant difference between biogas production and pH ($p = 0.000$) as presented in figure 11. In the present study the highest biogas production was recorded in the pH range between 6 and 7, compared to the pH value below 6 and above 7 (figure 9). The experiment result showed that in average 385.7, 442.7 and 362.8 ml of biogas was produced in T2, T3 and T4 at pH between 6 and7 compared to 362.3 ml and 436.1 ml in T2 and T3 at pH below 6 and 121 ml in T4 at pH greater than 7 respectively in every other day.

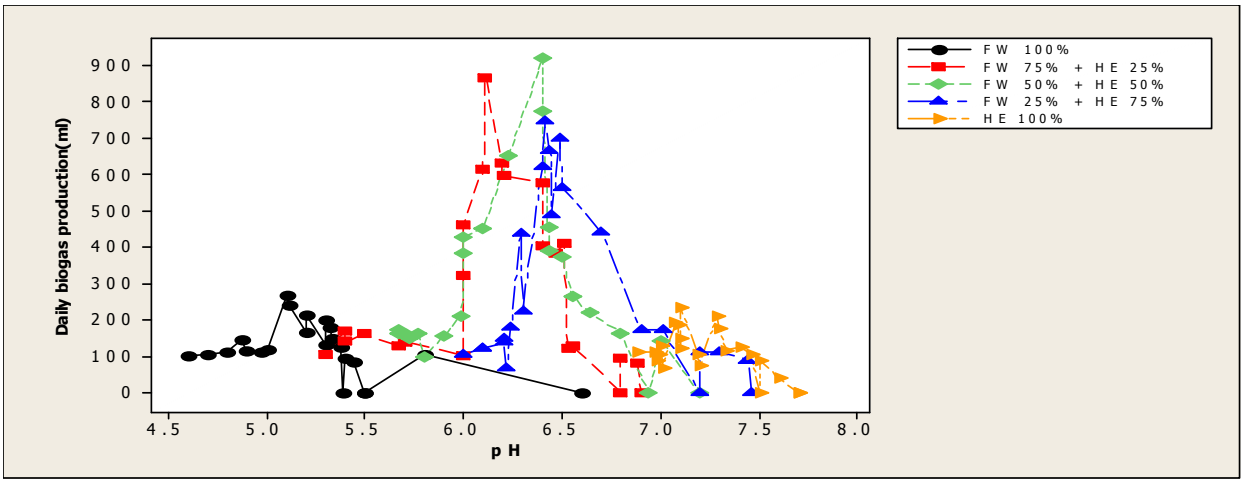


Figure 11 Biogas production vs pH

5.5 Cumulative biogas production and C/N ratios

The analysis result shown there was a significance difference between biogas production and C/N ratios of substrate (p=0.000). As figure 10 presented more biogas yield was recorded from optimum (20-30) C/N ratios of substrate compared to 13.4 and 34.6 C/N ratios. Most researchers recommended that an optimal C/N ratio between 20 and 30 is suitable for AD system (23). Similarly the present study confirmed that substrates with optimum C/N ratio produced more biogas yield than substrates without optimum C/N ratio. Highest biogas yield was obtained from 25 C/N ratio (50% FW + 50% HE), while the lowest biogas yield was obtained in 13.4 C/N ratio (100%HE) as depicted in figure 10.

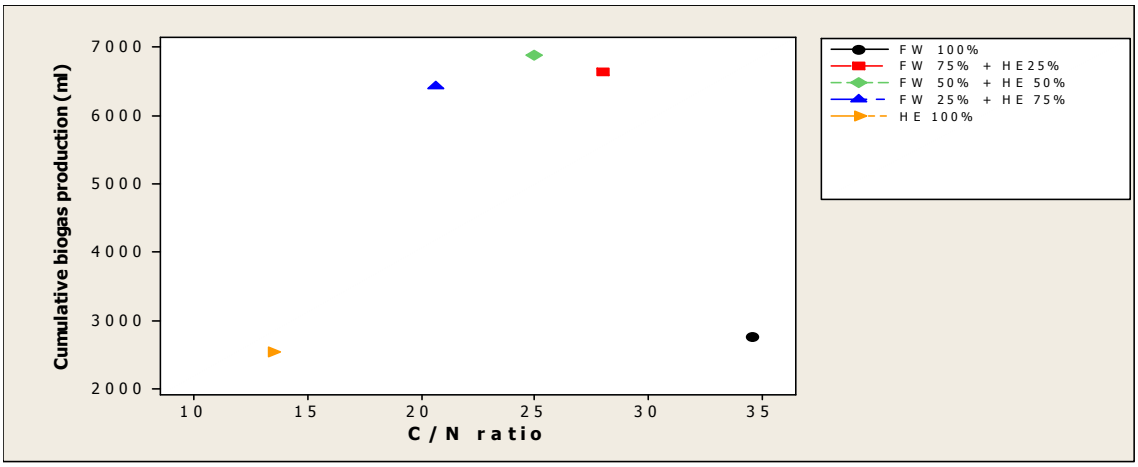


Figure 12 Cumulative biogas production vs C/N ratio

5.6 Daily and cumulative methane production

Figure 11 presented the daily methane percentages of five different mixtures of FW and HE. The percentage of methane after 44 days retention time indicated higher in co digestion of FW and

HE compared to mono digestion of FW and HE. The highest methane production was recorded in mixing ratio of 50% FW with 50% HE (72.6%), closely followed 75% FW+ 25% HE (71.4%), and 25% FW +75% HE (70.1%) compared to the lowest value 100% FW (59%) and HE (57.7%)alone.

The experiment data reported that during the initial time of the experiment (2-16 days) the percentage of methane in biogas was low. Since the main content of biogas was carbon dioxide and in average 46.5, 65,66, 66.4 and 50.6% of methane was produced in T1, T2, T3, T4 and T5 in every other day respectively. However, after 16 days 66.3, 74.5, 75.9, 72 and 60.8% of methane was produced in T1, T2, T3, T4 and T-5 in every other day respectively. The average methane percentage in all treatments from second day to 16 and 17-44 day were 59.8% and 70% respectively in every other day as reported in figure 11. In 44 days retention time 10.2 ml /g TS, 29.2 ml/g TS, 30.8 ml/g TS, 27.8 ml/g TS, 9 ml /g of TS methane were produced in T1, T2, T3, T4 and T5 respectively within 44 days retention time experiment.

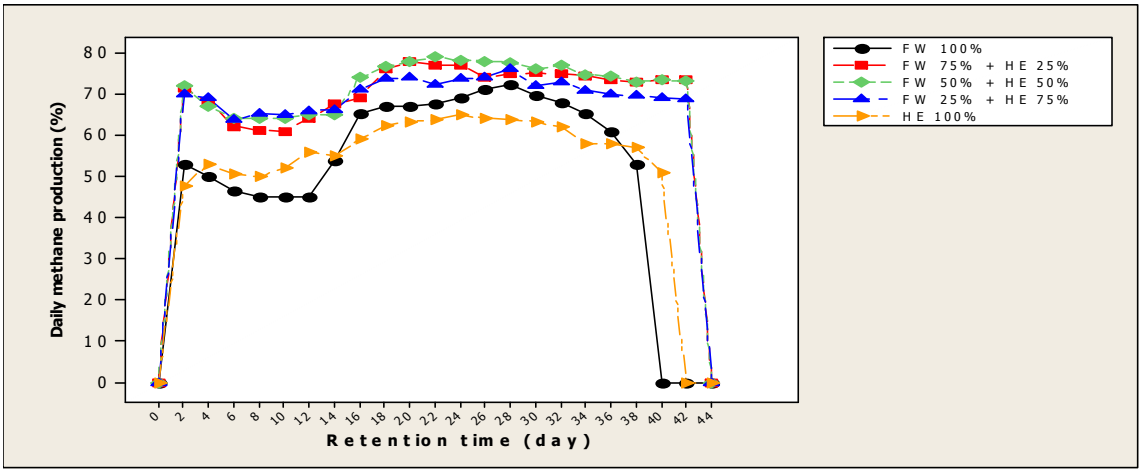


Figure 13 Daily methane percentage vs retention time

5.7 Methane production and pH

The ANOVA table showed that methane percentage was significantly affected by the pH of the slurry (p=0.04). Similar to biogas production the highest methane percentage was observed in the pH range between 6 and 7 compared to below 6 and above 7. The highest methane percentage was recorded at pH 6 (79% in T3), at pH 6.1(77.8% in T2), at pH 6.5(76.1% in T4) compared to the lowest methane percentage at pH 4.6 (45% in T1). In average 74.6 and 76% of

methane produced in T1 and T2 in the pH range between 6 and 7 compared to 66 and 65 % at pH below 6.

And in T4 70.7 % of methane generated in the pH range between 6 and 7 compared to 69% at pH greater than 7. This indicated that the pH range between 6 an7 was optimum for highest methane production than below 6 and above 7.

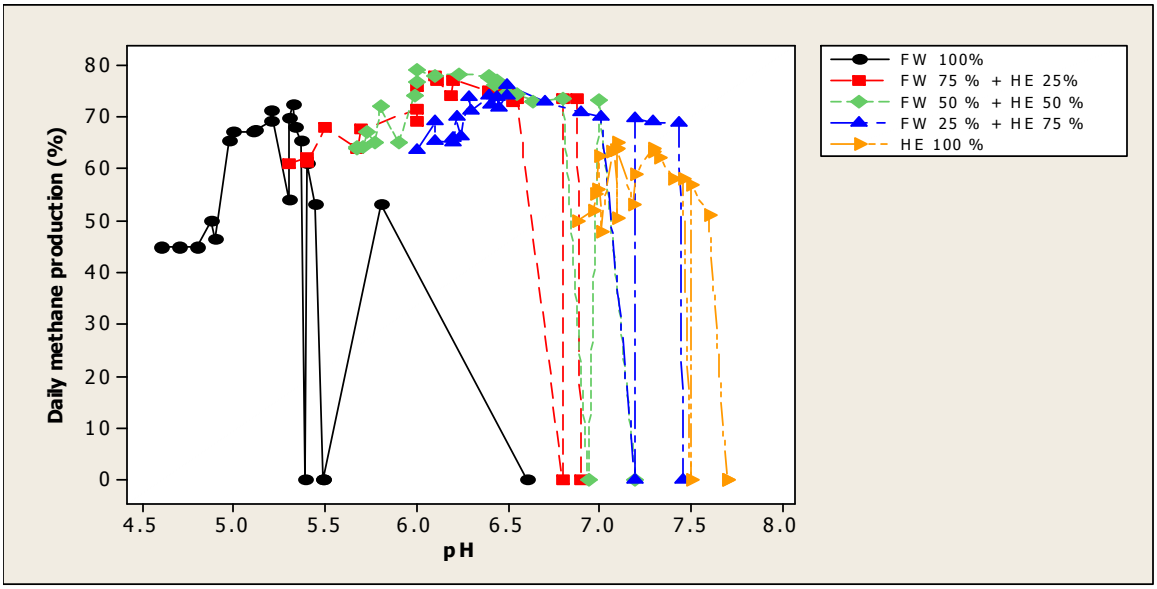


Figure 14 Methane production vs Ph

5.8 Operation condition

This study was operated at ambient temperature for 44 days retention time. Biogas generation at ambient temperature is ideal in terms of investment cost reduction. However, when biogas production is carried out in thermophilic temperature it needs additional energy source like electricity to adjust the required temperature. So biogas generation at ambient temperature promotes utilization of biogas technology in rural areas where, electricity is not available. This study confirmed that biogas generation is possible using ambient temperature without any additional energy input.

The analysis results reviled that no significance difference was observed between biogas production and daily ambient temperature variations ($p>0.05$).

As figure 13 shows the ambient temperature varied between 20.6 and 27.4 o C and the average mean temperature was 23.2 o C in 44 day retention time. The lowest and highest ambient temperature was recorded in day 16 and 34 respectively.

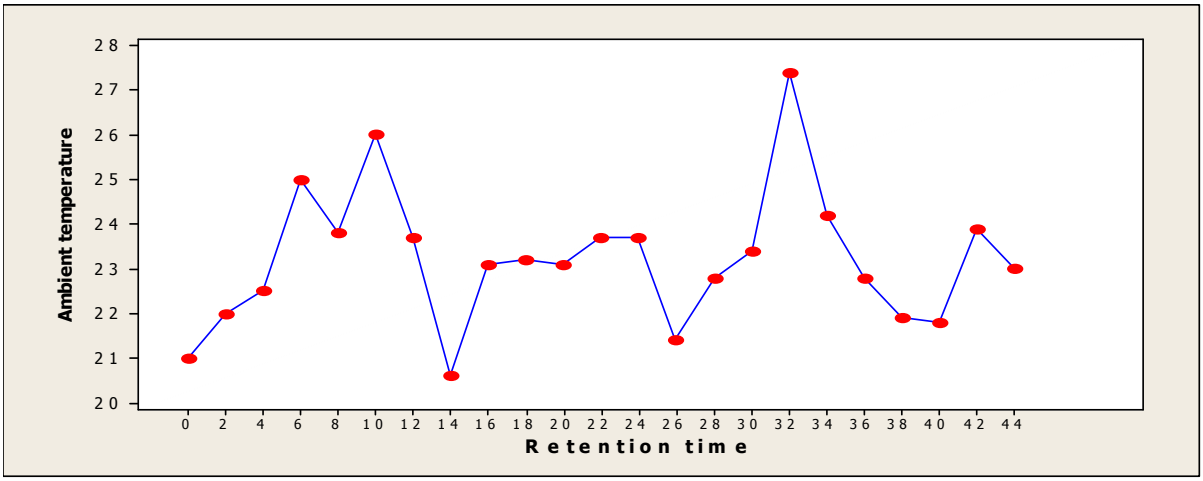


Figure 15 Ambient temperature vs retention time

There was a significant difference ($p<0.05$) between ambient and slurry temperature. The digester temperature varied between 21.4 and 31.7 **o** C and the average mean temperature was 27 **o** C in 44 day retention time. The lowest and highest digester temperature was recorded in day 1 and 34 respectively.

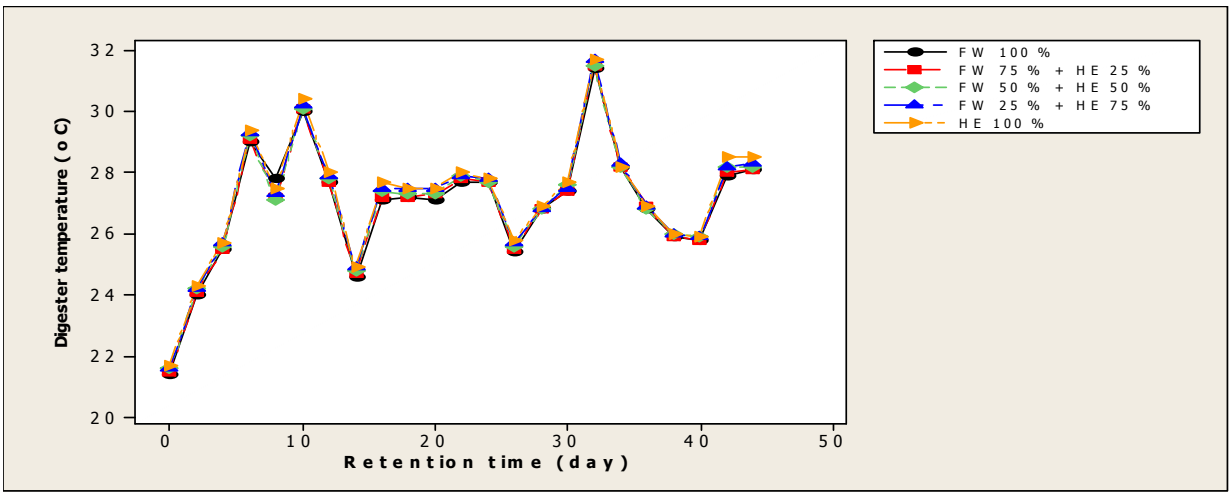


Figure 16 Digester temperature vs retention time

T1=100% FW, T2= 75% FW + 25% HE, T3= 50% FW + 50% HE, T-4= 25% FW + 75% HE and T5= 100% HE.

5.9 Microbial analysis before and after anaerobic digestion

Fecal coliform and ova of Smansoni before and after AD result were presented in table 5. As seen from the table all samples were positive for fecal coliform before and after AD. The ANOVA table shows there was a significant reduction of fecal coliform and ova of S. mansoni after 44 days' retention time AD ($p<0.05$). The experiment observed that 45.5% fecal coliform was reduced after 44 day retention time AD process. A mean value of 3± 2 of ova of S. mansoni was detected before AD per ml of sample, but no parasite has been seen in all treatments after 44-day retention time AD operated at ambient temperature. Ova of S. mansoni were significantly reduced after anaerobic digestion processes. However, it doesn't mean that all parasites can be removed by AD. Because this study was only evaluated the removal efficiency of ova of S. mansoni at room temperature anaerobic digestion. So, further study should be required to evaluate parasite cyst removal efficiency of AD operated at room temperature.

Table 4 Fecal coliform and ova of S mansoni before and after anaerobic digestion

Treatment	Fecal coliform (log CFU/ml)			Ova of S mansoni (No/ml)	
	Before AD	After AD	Reduction (%)	Before AD	After AD
T1	6.26	5	1.26(47.6%)	0	0
T2	6.51	5.3	1.21(47.6%)	2	0
T3	6.61	5.6	1.01(41.4%)	3	0
T4	6.72	5.78	0.94(46.1%)	5	0
T5	6.78	5.85	0.93(46.8%)	6	0
Mean ± SD	6.57± 0.22	5.5± 0.59	1.07± 0.4(45.9%)	3± 2	0
P value	0.000			0.000	

T1=100% FW, T2= 75% FW + 25% HE, T3= 50% FW + 50% HE, T-4= 25% FW + 75% HE and T5= 100% HE.

SD= standard deviation, CFU= colony forming unit, AD= anaerobic digestion

6. DISCUSION

The extent of TS, VS and MC reduction relatively more pronounced in digestion of co-substrate compared to mono substrates as indicated in Table 2. Highest TS and VS reduction was observed in T3 (50% FW + 50%HE), which was 6 % TS and 20% VS, while lowest TS and VS reduction was recorded in T5 (HE 100%), and it was 4% TS and 7.6% respectively. This is in agreement with the study done in Haramaya and who reported that highest TS and VS decrements was recorded in 1:1 mixture of banana fruit with poultry manure (6% TS and 30.8% VS) opposed to digestion of banana fruit alone (3.9% TS and 18% VS) respectively (2). Another research work reported in India also indicated that more VS reduction was observed in digestion of co substrates than mono substrates.

This might be due to proper C/N ratio found in co substrates compared to mono substrates. These possibly stable the pH of the slurry and it may create favorable condition for methanogenic bacteria. This can be used to speedup the digestion process and resulted for more TS and VS reduction in co digestions opposed to mono digestions. Or this could be due to the synergistic effects of microorganisms and richness of nutrients (89).

The TS and VS of 100% FW before AD was higher than other mixtures of FW and HE, but it produced lower bio gas yield than other treatment groups excluding toT5. This probably due to unfavorable condition of the substrate for methanogenic bacteria because of its lower pH values of the slurry compared to co digestion of FW and HE. FW has high content of organic matter, it may result for high acid accumulation due to hydrolysis of OM; this can causes for methanogens disability. Different studies confirmed that VFAs accumulation increased when the amount of FW increased in AD process (77). Biogas production was started in the second day of experiment period, which is similar with the study done in Sokoto, Nigeria (66). This probably resulted due to the presence of methanogenic microbes in the effluent (inoculum), because the inoculum obtained from anaerobic dig-ester of JimmaDegitu hotel was used as a startup.

The study done in Sokoto, Nigeria attributed that the inoculum could facilitate the fermentation process and helps to startbio gas production within a short period of time. Who deduced that biogas production rate was higher in substrates with inoculum than without inoculum.

The experiments result tells that bio gas production was slightly low at the beginning and end the experiment period and it is in agreement with the study conducted in Nigeria. The study

attributed that biogas production rate is influenced by the distribution and growth of methanogens in the dig-ester and methanogens growth is similar with bacterial growth curve (59).

The initial biogas yield was low in each experimental group from second day until 16 day compared to the day after 17. In average 148 ml of biogas was produced in T2, T3, T4 and 106 ml in T1 and T5 in the first 16 days of experiment compared to 591.5 ml in T2, T3 and T4 and 183 ml in T1 and T5 from day 17 to 30 and 220.5 ml in T2, T3 and T4 and 112.5 ml in T1 and T5 from 31 to 44 days in every other day. This is strictly agreed with London, UK finding (102) and who reported 600-750 ml of biogas was produced in day two compared to 1700 to 2000 ml in day 7. Another study conducted in Japan indicated that averagely 500-1000 ml of biogas was produced starting from day three to 18 compared to 2000-4000 ml from day 21 – 48 per day. Furthermore, a research works in China (47) found that 70 ml/d of bio gas produced in the first seven days, but it increased to 230/d ml from seven to 20 days.

The growth of methanogenic bacteria lagging behind during initial phase of experiment period and unable to catabolize organic acid, due to this acid accumulation possibly increase and causes for pH decrement in the digester and it would be resulted for low biogas productions in the first 16 days of experiment. This is expected because in acidogenesis stage organic compounds broken down in to organic acid by acidogenic bacteria. In the early stage of fermentation carbonic acid production is higher than its consumption. Methanogenic bacteria cannot survive in extreme pH values (41,48).

A Norway study also clearly indicated that VFA accumulation was high in the beginning of fermentation and it resulted for low pH (81). The volume of biogas produced is directly proportional with digestion time (50).

However, 17 day after starting the experiment until 30 days biogas production was increased this could be due to increment of pH from acidic medium to neutral range. This is probably due to reduction of carbonic acid accumulation in the medium because of the enrichment of methanogens in the digester, it used carbonic acid as a substrate and convert it in to methane due to this the pH of the digester might be increased and it might be resulted for more biogas yield. The ability of acid metabolism became strong through time (47). The pH of the digester affects the growth of methanogens (40).

Then at the end of the experiment biogas production was reduced and eventually reached to zero at day 40, 42 and 44. This is might be due to depletion of nutrients and ammonia accumulation in the digester. If ammonia is reached to 2000 mg/l of the slurry, it is very toxic for microorganisms which are responsible for biogas production.

This study corroborates highest biogas yield was obtained from co digestion of FW and HE compared to mono digestion. Figure seven showed that co digestion of FW with HE resulted two and half times more biogas yield compared to the lowest value HE alone. This finding is supported by the study found in republic of Korea and who deduced that 1.8 to 2.9 fold times higher biogas was obtained in co digestion of sludge and food waste leachate than alone (88).

Similar study done in Vietnam indicated that five times more biogas yield recorded in 1:2 ratios of vegetable waste with cattle manure compared to vegetable waste alone. Another study done in Iraqi reviled that co digestion of vegetable waste with butcher waste water gives 50% more biogas yield compared to sole substrates (90). This could be due to proper nutrient balance (C/N ratio) and increased buffering capacity and reduced the effects of toxic compounds because of mixing of substrates (87,88). The variation of biogas yield between the present study and previous studies may be due to type and mixing ratios of substrates and operation temperature used.

In this experiment it was observed that the C/N ratios of co substrates of FWand HE found in the optimum range (20.6-28), while the C/N ratios of mono substrates were not found in the optimum range (34.6 FW and 13.4 HE) alone. More biogas yield was obtained from co digestion of FW and HE opposed to mono. Highest biogas yield was recorded in 25 C/N ratio followed by 28 and 20.6 than 34.6 and 13.4. This is in agreement with the study done in Abuja, Nigeria and the result showed that highest biogas yield was obtained in 24 C/N ratio (0.7 m^3biogas) compared to 10 (0.028 m^3) and 47 (0.2 m^3) C/N ratio and (9). Similar study conducted in Nigeria also found that 25 C/N ratio was described as optimum for biogas production (55). This increment of biogas production during co substrates of waste may resulted from improved C/N ratio (88). This probably leads to stable pH and it is hypothesized that the mixture was able to buffer itself prerequisite for proper biogas production.

If C/N ratio of the substrate is increase 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 and organic acid accumulation will increase. On the other hand, if the C/N ratio is

decrease, nitrogen will be liberated and accumulated in the form of ammonia, which is toxic to the bacteria and resulted for low biogas production (2,40).

The experiment denoted that 6.94 pH values substrates resulted more biogas yield compared to 6.6 and 7.5, which is similar with the study reported in India, and who indicated that highest biogas yield was obtained from pH seven (5675ml) than five (4594ml) and nine (4889ml) from anaerobic digestion of FW (51). In 44 day experiment period the highest and pick biogas was observed in the pH range between six and seven and 406 ml in T2, 432 ml in T3 and 349 ml in T4 biogas produced in every other day compared to pH below 6 (140 ml (T2), 158 ml (T3) and above seven about 121 ml of biogas was produced in T4. Optimum biogas production was achieved when the pH value in the digester between six and seven (59). This is possibly due to enrichment of acetogenic bacteria in the system and the ability to convert complex insoluble products in to methanogenic substrate may be increased in this range. Acetogenic bacteria preferred the pH range between 6.5 and 5.5 (40).

The percentage of methane attained from pure, HE and FW were 59.7 and 57.7 respectively, which is comparable with the study done in Sweden (64.1% FW and 60% HE) (64). It was observed that carbon dioxide production was very high during the initial stage of the experiment, while methane was reduced, but after two weeks later methane producing capacity gradually improved. In this study, the average methane gas produced in all treatments from second day to 16 and 17-44 day was 59.8% and 70% respectively in every other day. This is comparable with the study done in London, UK (102). Who reported 2.3% methane was obtained at day two compared to 55% at day 8.

Methane yields increased with an increasing retention time (102). Similar study done in Japan also deduced 3.5% of methane was recorded at day three compared to 59-61% from day 18-27. This might be due to the enrichment of methanogens in the digester, the rate of carbon to methane conversion became increased and the percentage of carbon dioxide decreased and the percentage of methane increased. The percentage of methane production is influenced by the pH of the growth medium (digester). If the pH is below 6.3 or above 8, the percentage of methane would be reduced, while the percentage of carbon dioxide or ammonia can be increased (1).

The highest cumulative methane percentage was obtained from T3 (72.5%), closely followed by T2 (71.3%) and T4 (70.1%) opposed to T1 (59.7%) and T5957.7%). This is similar with a study done in Thailand. They found 64.4%, 48.6 % and 36% of methane was obtained from 1:1, 1:0

and 0:1 mixing ratios of chicken manure to Napier grass (91). This might be resulted due to optimum C/N ratios of co substrates compared to mono substrates. The highest methane percentage was recorded in the pH range between six and seven than others.

This is probably due to enrichment of acetogenic bacteria in the digester which is used to broken down products do not directly converted to methane by methanogenic bacteria are converted to methanogenic substrate. So if the concentration of acetogenic bacteria increased in the medium the probability of methane production rate became increased.

The acetate serves as a substrate for methane – forming bacteria and acetogenic bacteria, which grows in a synergetic relationship with methane forming bacteria(7,10).

The microbial analysis result indicated that fecal coliform removal efficiency of AD at ambient temperature was 1.07± 0.4 log CFU/ml (45.9%), which is similar with a study done in SSA and who found that 0.7 log CFU/ml of FC reduced at 20-26 ^{o} C (37). Similar study done in Canada reported that 2.48-4.16log CFU/ml FC reduction was observed at 35 ^{o} C incubation temperature (71). Another study done in Mexico also showed 3 log MPN/g TS at 55 o C and 1 log MPN/g TS at 35 o C) fecal coliform reduced (72). The reduction variation between the present study and the previous study might be due to incubation temperature difference and accumulation of VFA and ammonia concentration in the digester. The present study was operated at lower temperature and lower reduction was observed compared to a study done in Mexico, Canada and New York.

It is obvious that more reduction is expected in higher temperature opposed to moderate (room temperature) temperature. In this study, ova of S. mansoniwas completely removed after 44- day retention time AD, which is strictly similar with the research work reported in Hannover Germany and the report showed that 100% ova of schistosoma reduction was observed within 7-22 retention time at 8-25 ^{o} C incubation temperature (74).

Volatile fatty acids, which are produced by acidogenic bacteria during AD , enhance the inactivation rates of pathogens and this effect is linked to the digester pH level(102). Organic acids acidify the cytoplasm of bacteria and, to overcome this, bacteria must use a specific mechanism known as H1-ATPase pump. This mechanism consumes energy. Moreover, organic acids act as protonphores and increase the inward leak of protons (H1) so that H1 efflux is not rapid enough to alkalize the cytoplasm. Another problem is the accumulation of the anionic form of the acid inside the cell. This accumulation becomes toxic to bacteria and leads to osmotic problems(103).

Increased concentration of ammonia increases the inactivation of pathogenic organisms. It destroying the membrane potential, as well as denaturing bacterial membranes and cell proteins. When ammonia gas enters the cell it may cause damage by rapid alkalinization of the cytoplasm. Consequently, to maintain optimum internal pH, the cell takes up protons from the outside but sacrifices potassium ions (K+) instead and the loss of this essential substance eventually leads to death of the bacterial cell(104).

Limitation of the study

The major limitation of this study was lack of enough literatures regarding on co digestion of food waste and human excreta. It was difficult to get local research and well documented references. In this study, time constraint was one of the major challenges, due to this the fertilization potentials of digestate was not evaluated. The study also did not evaluate the biogas production potentials of co digestion of food waste and human excreta in psychrophilic and thermophilic temperature. Additionally the effect of retention time on pathogen removal efficiency was not studied.

7. CONCLUSION AND RECOMMENDATION

7.1 Conclusion

The objective of this study was to evaluate the potential of biogas production from co digestion of food waste and human excreta and the last has given us a clear direction on how to tackle problems related to thus wastes through bioconversion of wastes to energy like biogas. The C/N ratios of co substrates were found in the optimum range (20-30) compared to mono substrates. Biogas production from T1 (100%FW), T2 (75%FW + 25%HE), T3 (50%FW + 50%HE), T4 (25%FW + 75%HE) and T5 (100%HE) was recorded.

The experiment results denoted that co digestion of FW and HE is quite rich in producing biogas and methane opposed with mono digestion. Highest biogas yield and methane percentage was attained in T3, closely followed by T2 and T4 and the proportion of biogas yield increased two and half times as compared to T1 and T5. This indicated that mixing of FW and HE is able to improve biogas and methane production potentials. 45.5 % of FC was removed after 44 day retention time operated at ambient temperature.

7.2 Recommendations

To tackle food waste and human excreta related problems the country should look for sustainable waste management options and convert waste to valuable product like biogas. So, the government should encourage and implement anaerobic co digestion technologies at household and institution level.

In order to have more biogas production anaerobic co digestion of 50% FW + 50%HE mixture should be applied. Biogas production potentials of anaerobic co digestion of FW and HE at psychrophilic and thermophilic temperature should be investigated.

The effect of retention time on pathogen removal efficiency not studied, so further detailed investigation should be carried out. The fertilization potential of digestate was not evaluated, so further study should be conducted on it. Therefore, for developing countries like Ethiopia to tackle poor and unsustainable waste management practice and to find out alternative sustainable energy source the anaerobic co digestion process could provide the much awaited solution if given coveted attention.

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Annex I: Fecal coliform isolation and enumeration procedures

Chemicals and materials UsedCompany nameCountry origin

M- Lauryl sulfate broth	Sigma Aldrich	India
Vacuum pump	VWR international GmbH	Germany
0.45 µm size filter paper	-	-
Alcohol (97%)	Fine chem general trading	-
Autoclave	Dixons surgical instrument	-
Safety cabinet		
Filtration apparatus		
Funnel		
Incubator		

M- Lauryl sulfate broth compositions gram/liter

Peptic digest of animal tissue ----------40

Lactose----------30

Yeast extract----------6

Sodium lauryl sulfate----------1

Phenol red----------0.2

1. Media preparation: For fecal coliform isolation and enumeration M- lauryl sulfate broth was used. The media was prepared according to the instruction given on the package. Based on that 76.2 g powder was dissolved in 100 ml sterilized water and sterilized by autoclave at 121 ^{o}C for 15 minute.

2. All required equipments was sterilized

3. Dilution water was autoclaved and sterilized at 121 o C for 15 minute

4. Lift the lid of Petri dish was lifted and 0.2 ml of M- lauryl sulfate broth poured on the absorbent pad equally

5. Set up the membrane filtration apparatus

6. Sterile forceps was used to put a membrane filter in assembly.

7. Diluted sample inverted for 30 second (25 times) and sample mixed well.

8. Sample added on the funnel using a pipit and vacuum pump was applied until the funnel is empty.

9. Funnel was removed from the filter assembly and rinsed in boiled water two or more times

10. 0.45 µm diameter membrane filter lifted using sterile forceps

11. Membrane filter putted on 1.8 thickness absorbent pad and the lid putted on the Petri dish and incubated at 44.5 ^{o} C for 24hours.

12. Bacteria colonies counted and calculated based on the following formula

Annex II: S mansoni investigation procedures

Chemicals and materials UsedCompanynameCountry origin

Formalin	Follium pharmaceutical	Ethiopia
Ether	LOBA Chemie	India
Normal saline (0.9%)	-	-
Slide	UNI- Sci	-
Slide cover	-	
Microscope	OLYMPUS	China
Centrifuge tube	FUSA SA	China
Gauze	-	-

Saline wet mount investigation procedure used:

1. Labeling or coding of any necessary information was made using a pencil or pen or marker at the right or left hand sides of the slide

2. 1-2 drop of 0.9% saline was placed in the center of slide

3. Small amount of sample was added in slide and mixed with saline

4. After mixing mount was covered with cover slip and holed the cover at an angle, touched the edge of the drop, and lower gentled on to the slide. This will reduce the chance of adding air bubbles in the mount.

Examination procedure

5. Slide with the mounts was putted on the microscope stage and focused on the mount with the ×10 and × 40 power objective

6. Light was regulated in the microscope field with the sub stage diaphragm.

7. Sample was examined the entire cover slip area with the × 10 objective , × 40 object

8. When organisms or suspicious material are seen, switch the high-dry objective, and increase the light by opening the sub stage diaphragm to observe the detailed morphology.

Formol-ether concentration technique parasite investigation procedures performed

1. 1ml of sample with 8 ml 10% formol water was added in a centrifuge tube and stirred using applicator stick until a slightly cloud suspension made.
2. A gauze covered funnel was placed on the top of the centrifuge tube
3. Then sample suspension passed through filter into centrifuge tube until 7ml mark is reached
4. Filter and lumpy residue was removed and discarded
5. 3ml of ether was added and mixed for one minute
6. Centrifuge tube was centrifuged at 3000 rpm for one minute.
7. After centrifuge of sample four layers were formed a small amount of sediment in the bottom, formalin on the top of sediment, then fecal debris on top of formalin, ether at the top.
8. Then the supernatant was poured away by quickly inverting the tube
9. The tube was replaced in its rack and allowed the fluid on the slides of the tube to drain down to the sediment. Mixed well and transferred a drop to a slides for examination under cover slip
10. × 10 and ×40 objectives was used to examine the whole area under cover slip(99,100).

Annex- III Nitrogen determination procedures

Materials and chemicals usedcompany namecountry origin

Potassium sulphate	UNCHEM	India
Copper sulphateThechparmachem	India	
Sodium hydroxide	UNCHEM	-
Hydrochloric acid	BDH proLABO	-
Sulfuric acid	BDH proLABO	-
Methyl red	LOBA CHEM	India

Bromocresol green BDH -

1. Sample preparation and digestion

1.1 10ml of sample was measured using measuring cylinder and added into 500 ml digestion flask

1.2 Sample hydrolyzed by 15 ml of 98% sulfuric acid (H_2SO_4) solution

1.3 0.2 gram of copper (II) sulphate ($CUSO_4.5H_2O$) and 0.7 gram of potassium Sulphate (K_2SO_4) was added in digestion flask to speed up the digestion process

1.4 The sample was mixed thoroughly and the digestion flask was putted in digestion chamber and it is heated up for 40 minutes at 420 ^{o}C.

1.5 After digestion the digestion unit was switched off and cooled for 20 minute

And finally N from the sample converted to ammonium ion in the form of sulphate.

2. Distillation (transformation of ammonium ion to ammonia gas)

2.1 The Kjeldahl flask containing the digested sample was placed into the Kjeldahl distillation apparatus and 30 ml of distilled water was added

2.2 The sample was neutralized by addition 50 ml of 50% sodium hydroxide solution to convert ammonium ion to ammonia gas.

2.3 The formed ammonia was then distilled off by steam distillation and retained in the form of ammonium in a sample beaker containing 4% dissolved 400 g of boric acid in distilled water containing 70 ml 0.1 % alcohol solution of methyl red and 100 ml 0.1 % alcoholic solution bromocresol green diluted to 10 ml of distilled water.

When ammonia reacts with boric acid the solution turned to red violet color to green and simultaneously boric acid converted to borate ion and ammonium ion

3. **Then titration was made by** 20% of sulpheric acid standard solution by diluting 430.1 ml 38% HCl to 10 L with distilled water until the solution has a slight violet color and finally the volume of titrant used equivalent to the amount of nitrogen available in the sample and the result was displayed on chamber digital screen.