ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

ADDIS ABABA INSITUTE OF TECHNOLOGY

DEPARTMENT OF CHEMICAL ENGINEERING

Optimum Production of Biogas from Bio-Municipal Solid Wastes Using Two Stages Anaerobic Digester

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By

Gizachew Assefa Kerga

JUNE 2011

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A thesis Submitted to the Research and Graduate School of Addis Ababa University, Addis Ababa Institute of Technology, Department of Chemical Engineering in partial fulfillment of the requirements for the attainment of the Degree of Masters of Science in Chemical Engineering under Environmental Engineering Stream.

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List of Abbreviations

AD	Anaerobic Digestion
BMSW	Biodegradable Municipal Solid Waste
COD	Chemical Oxygen Demand
C/N	Carbon to Nitrogen ratio
DCAD	Dry Continuous Anaerobic Digestion
DC	Developing Countries
HS	High Solids
ISWM	Integrated Solid Waste Management
LFG	Landfill Gases
LS	Low Solids
M	Mesophilic
MBT	Mechanical Biological Treatment
MC	Moisture Content
MS	Multi Stage
MSW	Municipal Solid Waste
MS-OFMSW	Manually sorted Organic Fraction of Municipal Solid waste
n.a.	No data available
OFMSW	Organic Fraction of Municipal Solid waste
OLR	Organic Loading Rate
OM	Organic Matter
RT	Retention Time
SRT	Solid Retention Time
SS	Single Stage
SS-OFMSW	Source-Sorted Organic Fraction of Municipal Solid Waste
STP	Standard Temperature Pressure
SWM	Solid Waste Management
T	Thermophilic
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
VFA	Volatile Fatty Acids
VS	Volatile Solid

Abstract

Various aspects of Anaerobic Digestion (AD) have been became a promising technology for the management of Municipal Solid Waste. Thus, the objective of this study was to optimize the production of biogas from these wastes by using two-stage anaerobic digester. To achieve this, batch anaerobic digestion experiments were conducted to determine the optimum methane produced from the digestion and co-digestion of Organic Fraction of Municipal Solid Waste (OFMSW) and Cow Manure (CM) in different mix operated under ambient condition. Moreover, this study presents several runs of experiment and analysis with the aim of substrate mix and the duration of overall stage of digestion process while optimizing the biogas generation with higher methane content. The physical and chemical characteristic of the sample done by analytical method, volume of biogas produced and methane composition of different mix of the feed stock were determined by water displacement and digital gas (SR2-BIO gas analyzer), respectively. The highest specific gas production observed was 353 L/Kg VS added for the digestions of 1:2 mix of OFMSW: CM. It was observed that the independent variable – mix ratio significantly affect (p-value $4.18*10^{-9}$) the dependent variable – amount of methane produced since the p-value is less than the chosen α-level usually 0.05. The calculated volume of methane in the biogas produced per Kg of volatile solid removed were 510L, 355L, 375L, 435L and 600L for OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM, respectively. In general From the digestion of 1:2 mix of OFMSW: CM: 0.47 $m^3/d/m^3$ biogas with 51.5% methane content was produced; 0.45 $m^3/d/m^3$, 0.35 $m^3/d/m^3$, 0.26 $m^3/d/m^3$, and 0.23 $m^3/d/m^3$, biogas with 31.4%, 42.9%, 20.3% and 33.7 % methane composition is produced from 1:1 mix of OFMSW: CM, CM alone, OFMSW alone and 2:1 1 mix of OFMSW: CM respectively. The mix ratio significantly affects the biogas production (p-value<<0.05).

1. INTRODUCTION

1.1 Background

Biogas is a clean, environmental friendly and renewable form of energy generated when micro-organisms degrade organic materials in an oxygen free environment. The formation of biogas can occur either in natural environment or controlled conditions in constructed biogas plants, so called anaerobic degradation/Digestion (AD). The potential feedstock for the production of biogas include; municipal solid waste, industrial organic waste, garden waste, agricultural waste (manure and crop residue), energy crops, cellulose rich biomass, algae and seaweed (water based), by-products of ethanol and bio diesel production (Lantz et.al, 2007; Demetriades, 2008; Börjesson and Mattiasson, 2007).

Conventionally, the digestion is accomplished by using thermally insulated, air tight tank with agitator unit and heating system. The gas collected in the digester tank above the slurry and can be piped for use or storage. At optimum temperature (35^0C) complete of animal or human faces takes around 20 days. Gas yield depend critically on the nature of the material feed to the digester and maintenance of nearly constant operating condition, which is difficult to control by common people. However, the new techniques remove the potential cause of variation from getting contact with methanation process which is very sensible to environmental change, and improves the stability and productivity of the plant. Among the technologies stage wise anaerobic digestion system which is employed for better stability of the process.

1.2 Problem Statement

Rapid population growth and urbanization have led to an enormous increase of solid waste generation per unit area. Particularly urban areas of low and middle – income-countries are confronted with great challenges concerning appropriate Solid Waste Management (SWM) in order to minimize the risk to human health and avoid environmental degradation (Lohri, 2009). For example, Addis Ababa, capital city of Ethiopia has solid waste generate of 0.4 kg/capital/day, the sources of waste generated is estimated to be 76% households, 18% institutions ,commercial, factories, hotels, and 6% is street sweeping. Of which the total waste generated in the city organic fraction estimated to be 60% (Tessema 2010, Tadesse 2004).

Coming together both the increasing trend in energy costs and troubles associated with incineration of municipal solid wastes (MSW), there have been propose many technologies that can partially solve the problems. Biological conversion of biomass to methane has received increasing attention in recent years.

A given amount of volatile solids of a particular organic waste can be converted to a maximum amount of biogas at a given temperature provided optimum conditions are expected. This conversion can be accounted by two factors i.e. biodegradability at a specified temperature and operating conditions that depend on kinetics, reactor configuration, the flow pattern within the digester, digestion stage as well as the presence of the inhibitory substances such as Volatile Fatty Acids (VFA) and ammonia concentration.

Creating favorable condition for the microorganisms responsible for the generation of biogas is a critical and important point to be taken seriously. Since digestion process is a complex and series of different reactions are performed, if these occurred in single reactor it would lead to inefficiency and poor performance of many biogas facilities can be attributed to failure to meet

this requirement. The living atmosphere of the microorganism can be disturbed due to different factors. Those problems like configuration of the reactor, type of input material to be biodegraded, temperature, PH, degree of mixing or agitation, etc. can greatly affect the process.

1.3 Objective of the Research

The overall objective of this thesis is to optimize biogas production from the bio-organic fraction of solid (OFMSW) wastes using two-stage anaerobic digestion process.

The specific objectives will be:

- Determination and characterization of selected bio-organic fraction of MSW for their nutrient content and establish optimum blend for feed
- Development of laboratory scale two stage biogas production unit;
- Determination of optimum hydrolysis time and pH for stage one unit and methanation time in stage two unit;
- Investigate the effect of feed composition, organic loading rate and biogas production rate and composition

Scope of the study

- The solid wastes are collected from some selected areas of Addis Ababa city.
- The digester was operated at room temperature in Environmental engineering laboratory, Chemical Engineering Department.
- The study is with selected substrates (cow dung and fruit &vegetable wastes)

2. LITERATURE REVIEW

2.1 Historical Background:

Historical evidence indicates that the AD process is one of the oldest technologies. Biogas was used for heating bath water in Assyria during the 10th century BC and in Persia during the 16th century. AD advanced with scientific research and, in the 17th century, Jan Baptista Van Helmont established that flammable gases evolved from decaying organic matter. Also, Count Alessandro Volta in 1776 showed that there was a relationship between the amount of decaying organic matter and the amount of flammable gas produced. In 1808, Sir Humphry Davy demonstrated the production of methane production by the anaerobic digestion of cattle manure (Lusk, 1997).

The industrialization of AD began in 1859 with the first digestion plant in Bombay, India. By 1895, AD had made inroads into England where biogas was recovered from a well-designed sewage treatment facility and fueled street lamps in Exeter. Further AD advances were due to the development of microbiology. Research led by *Buswell* and others (Lusk, 1997) in the 1930s identified anaerobic bacteria and the conditions that promote methane production.

Prior to 1920, most of the AD took place in anaerobic ponds. As the understanding of AD process control and its benefits improved, more sophisticated equipment and operational techniques emerged. The result was the use of closed tanks and heating and mixing equipment to optimize AD. The primary aim of waste stabilization in due course led to the basic municipal sludge digester. This design then spread throughout the world. However, methane production suffered a setback as low-cost coal and petroleum became abundant. AD systems made a comeback during World War II with fuel shortages hitting Europe but after the war AD was once again forgotten.

Another factor that led to declining interest in AD was increased interest in aerobic digestion systems. While the developed world shunned AD except as a wastewater sludge digestion technique, developing countries such as India and China embraced this technology. These countries saw gradual increase in small-scale AD systems used mostly for energy generation and sanitation purpose. In the developed countries, industrial expansion and urbanization coupled with low-cost electricity resulted in aerobic composting and landfilling to become the choice technologies for waste treatment, until recent times.

The energy crisis in 1973 and again in 1979 triggered renewed interest in development of simple AD systems for methane production as an energy source. India, China and Southeast Asia countries responded to the crisis with marked expansion of AD (Lusk, 1997).

Most of the AD systems were small digesters using combined human, animal and kitchen wastes. Many community digesters were installed to produce large volumes of biogas for village electrification. Also, Europe, North America and the Soviet Union became involved with research in AD for methane production from animal manure. The U.S. established renewable energy programs, emphasizing the AD of biomass for energy production (Lusk, 1997). The rush for deployment of AD systems to meet energy needs also led to many foreign-aid projects. Unfortunately, the knowledge on AD was still in a fledgling state and there were numerous failures. China, India and Thailand reported 50% failure rates. Failures of farm digesters in the U.S. approached 80%. Europe and Russia also experienced high farm digester failure rates (Lusk, 1997). Nevertheless, those designs that succeeded furthered the interest in research and development of AD. Apart from biogas production, AD found wider acceptance as an inexpensive technology for waste stabilization, nutrient recovery, reduction in biological oxygen demand (BOD), and sludge treatment. The dominant application of AD technology has been in farm-based facilities.

About six to eight million family-sized, low-technology digesters are used to provide biogas for cooking and lighting fuels with varying degrees of success. China and India have now adopted a trend towards larger, more sophisticated farm-based systems with better process control to generate electricity. With time, AD systems are becoming more complex and not limited to agriculture or animal waste treatment. The technology is now being applied for municipal waste treatment as well as industrial waste. Taiwan flares most biogas from waste treatment and has cut down river pollution, caused by direct discharge from the animal production industry, by simply using standard AD systems that serve 5,000 farms (Lusk, 1997).

2.2 Biogas composition

Biogas has approximately the following compositions.

Table 2.2. Approximate composition of Biogas

Methane	40-75%
Carbon dioxide	25-55%
Steam	0-10%
Nitrogen	0-5%
Oxygen	0-2%
Hydrogen	0-1%
Ammonia	0-1%
Hydrogen Sulphide	0-1%

In the above table, the values shown are reference values. The True values can deviate from the values shown in the table depending on the type of substrate used during digestion.

2.3 Biogas characteristics

Table 2.3.1 Physical and Chemical Characteristics

Density	1.2kg/m^3
Calorific value	4-7.5 kWh/m^3 (Depending on methane content)
Ignition temperature	700 ^{0}C
Ignition concentration gas content	6-12 %
Smell	Rotten eggs

Biogas potentials for different types of organic wastes

Most forms of organic matter containing proteins, fats, or carbohydrate cellulose (except mineral oils and lignin) can be microbiologically transformed into biogas (methane and CO_2). These three groups of organic material theoretically lead to different compositions of methane and carbon dioxide as shown in table 2.3.2

Table 2.3.2 Theoretical CH_4 and CO_2 composition in three main organic groups

Substrate	Liter gas per kg of dry substance	CH_4 %	CO_2 %
Protein	700	70	30
Fat	1200	87	33
carbohydrate	800	50	50

2.4 Advantages and Disadvantages of AD System

There are number of benefits resulting from the use of AD technology which are described in Table 2.4.

Table 2.4 Advantages of anaerobic digestion process

1.**Waste treatment benefits**	**Environmental benefits**
• Natural waste treatment process Requires less land than aerobic composting or landfilling • Reduces disposed waste volume and weight to be landfilled.	• Significantly reduces carbon dioxide and methane emissions • Eliminate odor Produces a sanitized compost and nutrient-rich liquid fertilizer • Maximizes recycling benefits potential to treat the OFMSW in countries considering banning landfilling of waste
Energy benefits	**Economic benefits**
• Net energy producing process • Generate high quality renewable fuel • Reduce CO2 emissions, by displacement of fossil fuels • Biogas proven in numerous end-use Applications	• More cost-effective than other treatment options from a life cycle perspective

Disadvantages of AD system

- Cost: this is a major barrier, as AD is (slightly) more expensive than composting in many cases.
- AD of MSW does not treat whole waste, only a fraction of it.
- Information on economic and practical issues is not widely disseminated
- Wastewater may need to be treated before disposal
- There are persistent materials handling problems

2.5 General Anaerobic Digestion (AD) process description

Generally the overall AD process of OFMSW can be divided into three stages: pretreatment, anaerobic digestion, and post-treatment.

2.5.1 Pretreatment of feed stock

The pre-treatment of feedstock consists in separating the recyclable or non-digestible wastes from the municipal solid wastes. Source separation has a significant effect upon the quality of the digestate. Mechanical pre-treatment leads to a lower quality digestate. The removal of all contaminants is not possible especially for the smaller fraction such as heavy metals. The resultant fraction is thus more contaminated. There are a variety of pretreatment processes that are chosen based on the characteristics of the incoming waste and the effects they have on digestion. Separation technologies for metals, glass and plastic are usually necessary. This section will focus on pretreatment processes unique to the AD process. The pretreatment of feedstock for AD involves:

- Providing a uniform small particle size feedstock for efficient digestion
- Removing the non-biodegradable materials
- Protecting the downstream plant (if it used series of treatment operations) from components that may cause physical damage
- Removing materials which may decrease the quality of the digestate

Most digestion systems require pre-treatment of waste to obtain homogeneous feedstock. The preprocessing involves separation of non-digestible materials and shredding. The waste received by AD digester is usually source separated or mechanically sorted. The separation ensures removal of undesirable or recyclable materials such as glass, metals, stones etc. In source separation, recyclables are removed from the organic wastes at the source. Mechanical separation can be employed if source separation is not available and the resultant fraction is then more contaminated leading to lower compost quality.

The waste is shredded before it is fed into the digester in order to enhance the digestion rate.

2.5.2 Process and mechanism of bio-methanation

In anaerobic digestion, different groups of micro-organisms work in sequence at four different stages. Figure 2.4.2 below illustrates the four main stages of anaerobic digestion of organic material. These are hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis (Jarvis, 2004).

2.5.2.1 **Stage 1-Hydrolysis**

The hydrolysis is considered to be the key step in the biodegradation of complex wastes (Mata-Alvarez, 2003). Fundamentally, this process involves the breaking down of complex organics into monomers or simple compounds by the action of hydrolytic microorganisms (Poland, 1992). This step involves the enzyme-mediated alteration of insoluble organic compounds with high molecular mass, i.e. proteins, fats, lipids, and carbohydrate etc, into soluble organic components such as amino acids, fatty acids, monosaccharide, and other simple organic compounds (Yadvika et al., 2004). The insoluble large molecules consist of many small molecules joined together by chemical bonds and thus need to be hydrolyzed before entering the bacterial cell. The hydrolysis step is carried out by several different anaerobic and facultative bacteria (Yadvika et al., 2004). Hydrolytic enzymes include *cellulase*, *cellobiase*, *xylanase* and *amylase* for degrading carbohydrates into sugars, protease for degrading protein into amino acids, and lipase for degrading lipid into glycerol and long-chain fatty acids (Parawira *et al.*, 2005). The microorganisms producing these enzymes can be obligate or facultative anaerobes. It is commonly found that hydrolysis is the rate-limiting step in degradation when the substrate is in the particulate form (Ghaly et al., 2000 and Borja et al., 2003).

2.5.2.2 **Stage 2-Acidogenesis**

In this stage, soluble compounds produced in the first stage are further degraded by a diversity of different facultative anaerobes through different fermentation processes by a second group of microorganisms called the fermentative acidogenic bacteria, e.g., *Clostridium spp* (Veeken et al., 2000; Pena-Varo, 2002). The fermentation results in the production of carbon dioxide, hydrogen gas, organic acids, alcohols, some organic-nitrogen compounds and some organic-sulphur compounds etc (Gerardi, 2003). The most important acid here is acetic acid as it is the principal organic acid used as a substrate material for the methane-forming organisms. The acidogenic microorganisms prefer a slightly acidic environment (pH 4.5-5.5) and are less sensitive to changes in incoming feed stream (Ostrem, 2004).

2.5.2.3 **Stage 3-Acetogenesis**

In this stage, the other intermediate products and acids than acetate that were formed in the fermentation are further converted to acetic acid as well as carbon-dioxide and hydrogen by different anaerobic oxidation reaction involving so called acetogenic bacteria (Jarvis, 2004).

In a stable anaerobic digestion, the acetate and H_2-producing bacteria called acetogenic bacteria transform the products of acidogenesis into acetate, hydrogen and carbon dioxide which are substrate for the methanogens. Approximately, 72 per cent of their fluent COD is converted to acetate. There may be formation of carbon dioxide or hydrogen along with the acetate depending on the oxidation state of the original organic matter (Veeken, et. al., 2000; Pen-Varo, 2002).

Acetogenic bacteria, also known as acid formers, convert the products of the first phase to simple organic acids, carbon dioxide and hydrogen. The principal acids produced are acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), and ethanol (C_2H_5OH). The products formed

during acetogenesis are due to a number of different microbes, e.g., syntrophobacter wolinii, a propionate decomposer and sytrophomonos wolfei, a butyrate decomposer. Other acid formers are clostridium spp., peptococcus anerobus, lactobacillus, and actinomyces (www.biogasworks.com- Microbes in AD).

2.5.2.4 **Stage 4-Methanogenesis**

Finally, the last group of microorganisms, the methanogenic, produces methane from acetic acid, hydrogen, and carbon dioxide as well as directly from other substrates of which formic acid methanol are the most important (Veeken et al., 2000). Methanogenic micro-organisms convert acetic acid, hydrogen and carbon dioxide to methane and carbon dioxide i.e. biogas. The remaining compounds like alcohols, organic-nitrogen compounds which methanogens cannot degrade will be accumulated in the digestate (Gerardi, 2003).

The methane is produced from a number of simple substances: acetic acid, methanol or carbon dioxide and hydrogen. Among these, acetic acid and the closely related acetate are the most important, since around 75% of the methane produced is derived from acetate (Evans, 2001).

Methane production is higher from reduction of carbon dioxide but limited hydrogen concentration in digesters results in that the acetate reaction is the primary producer of methane (Omstead, et. al, 1980). The methanogenic bacteria include *methanobacterium, methanobacillus, methanococcus* and *methanosarcina*. Methanogens can also be divided into two groups: acetate and H_2/CO_2 consumers. Methanosarcina spp. and methanothrix spp. (also, methanosaeta) are considered to be important in AD both as acetate and H_2/CO_2 consumers.

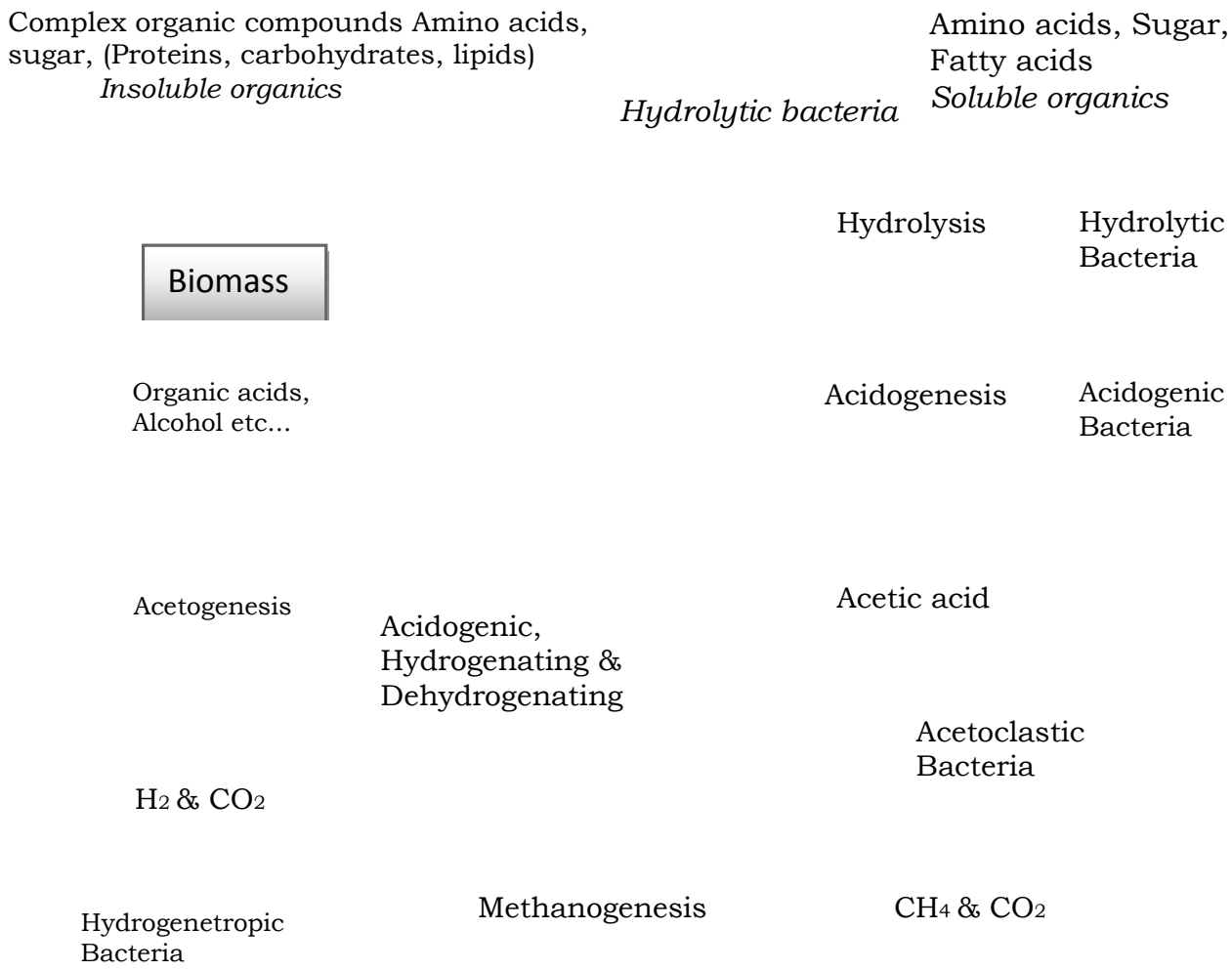


Figure 2.4.2 Anaerobic digestion process (Evans, 2001)

2.5.3 Post treatment of residual fraction from AD

After anaerobic digestion, the material usually requires refining before it can be used for fertilizer or soil amendment. If the feedstock is processed wet, the material may be spread directly onto farmland as slurry or it can be separated into a solid and liquid fraction. The solid fraction can be matured for about two to four weeks to provide dry and fully stabilized compost. Either the liquid fraction may be recycled for the dilution of fresh waste, applied directly to farmland as a liquid fertilizer, or sent to a wastewater treatment plant. If the

MSW is treated in a dry process, the digested material is usually dewatered and matured to compost.

Most of the liquor is recycled to moisten and inoculate the incoming raw MSW. The amount, quality and nature of digestate depend upon the quality of the feedstock to the anaerobic digestion process, the method of digestion, and the extent of the post-treatment refining process. As the digestate can be used as soil conditioner after post treatment, the energy consumption in fertilizer manufacturing could be reduced (Monnet, 2003). Application of digestate or liquor to farmland is dependent on digestate quality and local regulations.

The ability to utilize the residues of anaerobic digestion as soil amendments improves the economics and environmental benefits of the AD process. Use of this residue depends on its agronomic characteristics and pollution potential which can be accessed on the basis of the following physical, chemical and biological characteristics. The chemical characteristics of digestate are related to the presence of heavy metals and other inorganic contaminants, persistence organic contaminants and nutrients like Nitrogen, Phosphorus and Potash (NPK).

According to their origin, organic waste can contain hazardous matters, which can result in new routes of transmission of pathogens and diseases between animals, humans and environment. Thus quality control of this type of biomass is essential in relation to the biological treatment i.e. pathogens.
The presence of impurities in the digestate can cause a negative public perception of the AD technology, aesthetic damage to environment, increase the operational costs. The physical impurities that can be in the digestate are plastic and rubber, metal, glass and ceramics, sad and stones, cellulosic materials.

The contamination of the digestate inevitably depends upon the nature of the feedstock, the pretreatment applied and digestion itself. For the digestion of

MSW, source segregation is more efficient than mixed collection because the mechanical pre-treatments are not as effective in removing contaminants as is the elimination of potential contaminants at source.

2.6 Operational Parameters for Biogas Production

The rate at which the microorganisms grow is of paramount importance in the AD process. The complexity of anaerobic degradation reflects the biological part of an ecosystem that is also strongly influenced by chemical and physical parameters that are referred to as environmental factors (Björnsson, 2000).

The transformation of the organic matter to biogas is brought about by bacteria and these bacterial groups employ several kinds of enzymes to catalyze this reaction. All enzymes for normal activity require specific physio-chemical conditions (environmental conditions) under which the reaction rates are optimum.

The rate-limiting reaction in anaerobic digestion is usually the conversion of volatile acids to methane. Methane-forming bacteria obtain very little energy from the degradation of volatile acids. Most of the energy released from the volatile acids is transferred to the methane. Because of the low energy yield obtained from volatile acids by methane-forming bacteria, their growth rate is restricted, that is, the amount of substrate utilization per unit of organisms is high. Therefore, bacterial growth or sludge production is low and optimum operational conditions must be maintained for satisfactory rates of solids destruction and methane production. These factors are responsible for the rate-limiting reaction of the conversion of volatile acids to methane. However, if the substrates fed to the anaerobic digester were mostly slowly degrading particulate materials, then the rate-limiting reaction would be the hydrolysis of the particulate material.

The operating parameters of the digester must be controlled so as to enhance the microbial activity and thus increase the anaerobic degradation efficiency of the system. The production of biogas is factored by many operational parameters. Some parameters that affect the production of biogas include temperature, pH, pre-treatment, particle size, agitation, rate of organic load, retention time etc. Any rapid change in these parameters can adversely affect the production of biogas (Yadvika et al., 2004). Some of these parameters are discussed in the following section.

2.6.1 Temperature

Due to the strong dependence of temperature on digestion rate, temperature is the most critical parameter to maintain in a desired range. Methane production has been documented under a wide range of temperatures, but bacteria are most productive in either mesophilic conditions 25-40 ^{0}C, or in the thermophilic conditions, at 50-65 ^{0}C (Ahring, 1992 and Ostrem et al., 2004).
A thermophilic temperature reduces the required retention 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 than that of mesophilic bacteria (Kim & Speece, 2002b).

There are generally three temperature ranges under which the bacteria exhibit peak activity and they are:

- ✓ Psychrophilic (<15^{0}C)
- ✓ Mesophillic (15-45^{0}C)
- ✓ Thermophilic(45-65^{0}C)

2.6.1.1 **Psychrophilic**

Methanogenesis is possible under psychrophilic conditions (temperature below 15^{0}C), in order to achieve reasonable methane production, the temperature

should be above 15^{0}C, and this fact makes anaerobic treatment more attractive in tropical countries.

2.6.1.2 **Mesophillic**

The normal biogas plant operate within this operating range are the bacterial species that are involved (active) in this range are called mesophilic bacteria. The optimum temperature for mesophilic bacteria is 25-37 ^{0}C.The mesophilic bacteria which carry out the above reaction are generally hardy and withstand large temperature, pH and loading rate fluctuations when compared to thermophilic bacteria involved in biogas production.

2.6.1.3 **Thermophilic**

In biogas production there is also another group of bacteria which are active in this temperature. The optimum operating temperature for biogas production in thermophilic range is 55^{0}C. The rate of biogas production is higher compared to the other ranges (mesophilic and psychrophilic). The thermophiles are sensitive to temperature fluctuations and rapid fluctuations of order 2-3^0 can upset the biogas production.

The effect of thermophilic range is seen in the solubility of CO_2 and heavy metals. The solubility of CO_2 reduces with increased temperature and hence at lower temperatures greater amount of CO_2 dissolved in the liquid phase. Secondly for many heavy metals, solubility increases with temperature and hence at elevated temperatures their presence can be become toxic. Other disadvantage of thermophilic digestion is the biogas generated will have more H_2S content in it and consequently gives an offensive smell.

2.6.2 Hydraulic Retention time

The average time spent by the biomass inside a continuous biogas plant before it comes out from the digester is known as the hydraulic retention time, also abbreviated as HRT. The required retention time for completion of the AD

reactions varies with differing technologies, process temperature, and waste composition. The retention time for wastes treated in mesophilic digester range from 10 to 40 days. Lower retention times are required in digesters operated in the thermophilic range. A high solid reactor operating in the thermophilic range has a retention time is usually shorter (Demetrides, 2008).

The retention time is determined by the average time it takes for organic material to digest as measured by the COD and BOD of the exiting effluent. The longer a substrate is kept under proper reaction conditions, the more complete its degradation will be. The rate of the reaction, however, will decrease with increasing residence time, indicating that there is an optimal time that will achieve the benefits of digestion in a cost effective way. The appropriate time depends on the feedstock, environmental conditions and intended use of the digestate.

2.6.3 Type of substrates

The substrate determines the rate of the anaerobic degradation and must be taken into consideration in the process technology and process operation. If a substrate component of vital importance runs out, the microorganisms stop their metabolism. The wastes treated by AD may comprise a biodegradable organic fraction, a combustible and an inert fraction. The biodegradable organic fraction includes kitchen scraps, food residue, and grass and tree cuttings. The combustible fraction includes slowly degrading lignocellulosic organic matter containing coarser wood, paper, and cardboard. As these lignocellulosic organic materials do not readily degrade under anaerobic conditions, they are better suited for waste-to-energy plants. Finally, the inert fraction contains stones, glass, sand, metal, etc. This fraction ideally should be removed, recycled or used as landfill. The removal of inert fraction prior to digestion is important as otherwise it increases digester volume and wear of equipment. In waste streams high in sewage and manure, the microbes thrive and hydrolyze the substrate rapidly whereas for the more resistant waste

materials, such as wood, digestion is limited. Therefore, it is often necessary to feed possibly lacking substances (carbohydrates, fat, proteins, mineral substances, and trace elements) as well as the substrate.

Sugar, for example, hydrolyzes and acidifies within very short time. The degradation of cellulose proceeds considerably more slowly depending on the fraction in the form of lignin. At long residence times of 20 and more days, even medium - heavy and heavy degradable materials hydrolyze and are eventually metabolized to methane.

According to the composition of the substrates, intermediate products of the decomposition can also limit or inhibit the degradation. Thus, for example, the degradation of fats can give rise to fatty acids, which limit the further degradation. With the decomposition of proteins, methane fermentation can be restrained by the formation of ammonia and hydrogen sulfide.

The volatile solids (VS) in organic wastes are measured as total solids minus the ash content, as obtained by complete combustion of the feed wastes. The volatile solids comprise the biodegradable volatile solids (BVS) fraction and the refractory volatile solids (RVS). Kayhanian (1995) showed that knowledge of the BVS fraction of MSW helps in better estimation of the biodegradability of waste, of biogas generation, organic loading rate and C/N ratio. Lignin is a complex organic material that is not easily degraded by anaerobic bacteria and constitutes the refractory volatile solids (RVS) in organic MSW. Waste characterized by high VS and low non-biodegradable matter, or RVS, is best suited to AD treatment. The composition of wastes affects the yield and biogas quality as well as the compost quality.

Table 2.5.3 Maximum gas yield per kg VS for different organic wastes

Substrate	TS [%]	VS [%]	Retention time [days]	Biogas yield [m3/kg VS]	Source
Spent Fruits	25-45	90-95		0.4-0.7	Deublein
Vegetable waste	5-20	76-90	8-20	0.4	"
Market waste	8-20	75-90	30	0.4-0.6	"
Leftovers (canteen)	9-37	75-98		0.4-1.0	"
Bio waste	40-75	30-70	27	0.3-1.0	"
Overstored food	14-18	81-97	10-40	0.2-0.5	"
Fruit waste				0.2-0.7	Gunaseelan
Banana peels		86-94		0.2	"
Citrus waste		89-97		0.4-0.5	"
Vegetable waste				0.2-0.3	"
Mixed food waste (Korea)	26	90-95		0.3-0.5	Lee et al.
Bio waste	60-75	50-70		0.2-0.6	Eder & Schulz
Kitchen waste	9-37	50-70		0.2-0.5	"
Market waste	28-45	50-80		0.45	"
Liquid manure from cattle	6-11	68-85		0.35-0.55	Schiling & Tijmensen
Excreta from cattle (fresh)	25-30	80		0.6-0.8	Deublrin

2.6.4 pH:

The substrate's acidity is measured by pH, which is an important parameter affecting the growth of microbes during anaerobic digestion (Yadvika et al., 2004). Anaerobic bacteria, specially the methanogens, are sensitive to the acid concentration within the digester and their growth can be inhibited by acidic conditions. The acid concentration in aqueous systems is expressed by the pH

value, i.e. the concentration of hydrogen ions. At neutral conditions, water contains a concentration of 10^{-7} hydrogen ions and has a pH of 7. Acid solutions have a pH less than 7 while alkaline solutions are at a pH higher than 7. For optimal performance of the microbes, the pH within the digester should be kept in the range of 6.8 - 8.0. The pH value below or above this interval may restrain the process in the reactor since micro-organisms and their enzymes are sensitive to pH deviation (Yadvika et al., 2004).

During digestion, the two processes of acidification and methanogenesis require different pH levels for optimal process control. The retention time of digestate affects the pH value and in a batch reactor acetogenesis occurs at a rapid pace. Acetogenesis can lead to accumulation of large amounts of organic acids resulting in pH below 5. Excessive generation of acid can inhibit methanogens, due to their sensitivity to acid conditions. Reduction in pH can be controlled by the addition of lime or recycled filtrate obtained during residue treatment. In fact, the use of recycled filtrate can even eliminate the lime requirement. As digestion reaches the methanogenesis stage, the concentration of ammonia increases and the pH value can increase to above 8. Once methane production is stabilized, the pH level stays between 7.2 and 8.2.

2.6.5 Carbon: Nitrogen C/N ratio

The relationship between the amount of carbon and nitrogen present in organic materials is represented by the C/N ratio. Optimum C/N ratios in anaerobic digesters are between 20 – 30 in order to ensure sufficient nitrogen supply for cell production and the degradation of the carbon present in the wastes (Fricke et al., 2007). A high C/N ratio is an indication of rapid consumption of nitrogen by methanogens and results in lower gas production. On the other hand, a lower C/N ratio causes ammonia accumulation and pH values exceeding 8.5, which is toxic to methanogenic bacteria. Optimum C/N ratios of the digester materials can be achieved by mixing materials of high and low C/N ratios, such as organic solid waste mixed with sewage or animal manure.

For the optimal growth activity of bacteria, it is essential that many nutrients are available at the correct chemical form and concentration. Carbon in carbohydrate and nitrogen in protein, nitrates, etc are the main nutrients for anaerobic bacteria. While carbon supplies energy, nitrogen is needed for building up the cell structure. In order to provide an index for the presence of nitrogen in the correct concentrations, the concept of carbon to nitrogen ratio was extended to biogas substrates. Earlier this concept was greatly emphasized and C/N ratio of 15:1 to 25:1 was found to be optimum in the case of cattle dung. This was then considered universal for all substrates. However, with greater understanding in the field, it is clear that the ratio considered should be about the bio-degradability index. Bio-degradable carbon to available nitrogen ratio of 25:1 to 30:1 has been found to be ideal for biogas production. However, the various organic wastes used for biogas production differ unduly in their C/N ratio and hence an optimum mix of the input materials is necessary to get the optimum ratio.

Table 2.5.5 Typical C/N ratios of different materials

Raw materials	C/N ratio	Raw materials	C/N ratio
Animal waste		**Plant wastes**	
Duck dung	8	Vegetable wastes	11-19
Human excreta	8	Fruit wastes	20-50
Chicken dung	10	Maize straw	60
Goat dung	12	Rice straw	70
Pig dung	18	Wheat straw	90
Sheep dung	19	Saw dust	>200
Cow dung	24		
Water hyacinth	25		
Municipal solid waste	40		
Elephant dung	43		

Source: RISE-AT, 1998

2.6.6 Organic loading rate

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. This parameter has a significant influence on the process performance. It is expressed as the amount of organic matter (as COD or Volatile solids) per reactor volume. A higher OLR will demand more of the bacteria, which may cause the system to crash if it is not prepared. Under feeding the reactor could also lead to reduction in the digester performance due to insufficient nutrients for microbial growth. Organic loading rate (OLR) is a measure of the biological conversion capacity of the AD system. Feeding the system above its sustainable OLR, results in low biogas yield due to accumulation of inhibiting substances such as fatty acids in the digester slurry (Vandevivere, et. al, 1999). In such a case, the feeding rate to the system must be reduced. OLR is a particularly important control parameter in continuous systems.

2.6.7 Mixing

The close contact between micro-organisms and the substrate material is important for an efficient digestion process. This can be achieved in a number of ways. For example, daily feeding of the substrate instead of long interval provides the desired mixing effect. Installation of certain mixing devices such as propeller, scraper, or piston is also a mechanism for stirring (Yadvika et al., 2004).

The purpose of mixing inside the digester is to homogenize the material. Furthermore, mixing prevents scum formation and avoids temperature gradients within the digester. However excessive mixing can disrupt the

microbes so slow mixing is preferred. The kind of mixing equipment and amount of mixing varies with the type of reactor and the solids content in the digester.

2.7 Toxicity

In anaerobic degradation methanogens are most sensitive to the toxicity of NH, HS and VFA's. The process is pH dependent and ammonia is toxic at pH higher than 7, a free ammonia level of 150 mg/L can cause grows inhibition. HS and VFA's are toxic at PH bellow 7 (Björnsson, 2000).

Long-chain fatty acids are toxic to anaerobic cultures and give inhibitory effects on methane formation. Anaerobic bacteria also are sensitive to heavy metals but the concentration rate of those is normally low. Methanogenic bacteria are very sensitive to oxygen but facultative anaerobic bacteria present in the digester can consume any oxygen inside the digester.

2.8 Co- digestion

A combination of two or more substrates in anaerobic digestion is an 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.

The organic fraction of the MSW is mixed with animal manure and the two fractions are co-digested. This improves the carbon/nitrogen ratio and improves gas production. It also produces a sludge that is rich in nutrients and which is often usable as a fertilizer after composting or other treatment.

2.9 Seeding

To start up a new anaerobic process, it is critical to use an inoculum of micro organisms 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).

2.10 Compost

When the digestion is complete, the residue slurry, also known as digestate, is removed, the water content is filtered out and re-circulated to the digester, and the filter cake is cured aerobically, usually in compost piles, to form compost. The compost product is screened for any undesirable materials, (such as glass shards, plastic pieces etc) and sold as soil amendment. The quality of compost is dependent on the waste composition. Some countries have prescribed standards for compost quality. These standards are for compost treated by the aerobic process but may also be applied to AD compost product.

2.11 Types of anaerobic digestion systems

A wide variety of systems have been developed to anaerobically treat MSW. They can be split into different categories as following:

- Continuous versus batch process
- Mesophilic versus thermopilic digestion
- Single stage versus multi-stage digestion

Figure 2.10 Illustrate the classification of the anaerobic digestion system based on the operating criteria.

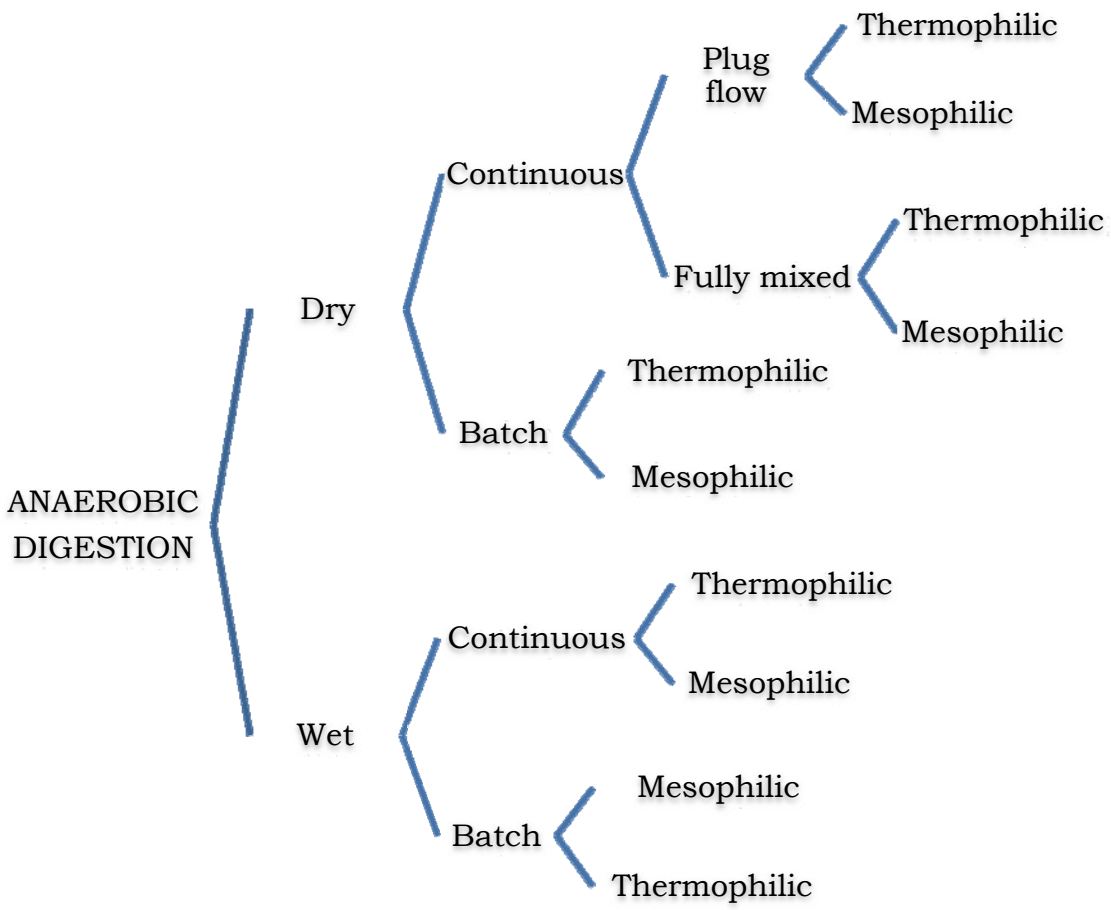


Figure 2.10 Classification of anaerobic digestion by operational criteria (Evans, 2001)

2.11.1 Continuous versus batch process

In a continuous process, the substrate is added to and removed from the digester continuously. Since fresh substrate is added continuously, all reactions involved in biogas generation will occur at a fairly constant rate. This results in a fairly constant biogas production rate. Usually, two digesters are used in the continuous process and the substrates are digested in two stages. The advantage of this process is that the digesters can be used as storage devices.

About 90% of the full scale plants, currently in use in Europe for the anaerobic digestion of sewage sludge and biowaste, rely on continuous one-stage systems. However, a considerable amount of literature has appeared concerning wastes treatment in two phases; first an acid forming phase followed by a methanogenic phase. A likely reason for this discrepancy is that two-and multistage systems afford more possibilities to the researcher to control and investigate the intermediate steps of the digestion process. Industrialists, on the other hand, prefer one-stage systems because of their simpler designs and lower investment costs.

In the batch process the substrate is fed into the digester and then the digester is sealed for the entire period without adding additional substrate until the decomposition process is near completion. Most of the digested substrate is then emptied and the digester is filled with new substrates, and then the digestion process starts again. In a batch process, the production of biogas is non-continuous. Gas production will peak at the middle of the process and will be low at the beginning and at the end of the process. Typically, in order to ensure a more steady supply of biogas, a number of batch digesters with substrates at different stages of anaerobic digestion are operated in parallel.

2.11.2 Mesophilic versus thermophilic digestion

The biodegradation of Hand Sorted Organic Fraction of MSW (HS-OFMSW) in a digesters at 35 ^{o}C resulted a maximum methane yield ranging from 0.39 to 0.43 m3kg-1 VS added without paper and wood and VS reduction ranged from 63 to 69 %. Furthermore, the methane yield of MS-OFMSW ranged from 0.11 to 0.16 m3/kg-1 VS added and VS reduction was found around 30 % due to its high ash value (Gunasselan, 1997). However, the quantity of biogas produced as a function of the quantity of introduced raw material will be variable according to several factors such as the quality of the organic matter and the environmental parameters.

In the thermophilic high solids anaerobic digestion, higher OLR and methane production rate can be achieved at reduced HRT. Gunaseelan (1997) studied that the methane yield was around 0.2 m^3kg-1 VS added. Digestion under thermophilic condition has many advantages such as higher metabolic rates and a high destruction of pathogens and weed seeds. On the other hand, thermophilic treatment has some drawbacks such as less stability compared to mesophilic conditions. Furthermore, the energy requirements of thermophilic systems are higher than those of mesophilic systems.

The effect of temperature is particularly important on the hydrolysis step. The hydrolysis rate of cellulose in thermophilic conditions is about 5 - 6 times higher than that observed in mesophilic conditions (Bouallagui et al., 2004).

The advantages and disadvantages of operating the anaerobic digestion process in mesophilic and thermophilic ranges are described in Table 2.6.2.

Table 2.10.2 Mesophilic and Thermophilic Anaerobic Digestion

Parameter	**Mesophilic**	**Thermophilic**
Temperature	25 - 40 ^{0} C	50 - 60 ^{0} C
Residence time	15 - 30 days	10 - 20 days
Total solids (wet) (dry)	 10 -15 % 20 - 40%	 10 – 15 % 20 – 40%
Advantages	More robust and tolerant process than Thermophilic	Higher gas production Faster throughput Process more sensitive to environmental variables
Disadvantages	Lower gas production rate, hence larger digestion tanks Separate sanitization stage	Needs effective control Separate sanitization stage

2.11.3 Single stage versus multi stage digestion

The advance of the High Solid (HS) technology resulted from research undertaken during the 1980's that established higher biogas yield in undiluted wastes. While most of the plants built until the 1980's relied on wet process, the new plants built during the last decade are evenly split between dry and wet systems. In dry systems, the fermenting mass in the digester has a solid content within a range of 20-40%. The equipment used in this system is robust and expensive than that of Low Solids (LS). Some of the examples of Single Stage High Solid (SSHS) systems are the Dry Anaerobic Composting (DRANCO), Kompogas, and Valorga processes. Due to the viscosity, plug-flow reactors are used. The advantages are that it is technically simple and no mechanical devices need to be installed inside the reactor. Because no mixing occurs within the digester, wastes must be mixed with digestate to provide adequate inoculation. With plug-flow digesters, no short-circuiting can happen as there are no moving parts. Feedstock is added at one end, thus pushing the digestate. The reactor is also smaller because no water is added.

Generally two reactors are used, the first for hydrolysis/liquefaction-acetogenesis and the second for methanogenesis. In the first reactor, the reaction is limited by the rate of hydrolysis of cellulose; the second by the rate of microbial growth. Two-reactor process allows a certain degree of control of the rate of hydrolysis and methanogenesis. For instance, microaerophilic conditions can be used to increase the rate of hydrolysis. The main advantage of the two-stage system is the greater biological stability it affords for very rapidly degradable wastes like fruits and vegetables (Verma, 2002).

Table 2.10.3 Advantages and disadvantages of single and two stages

Criteria	Digestion process		Significance
	Single stage	Two stage	
Flexibility	No	Yes	Optimization of each stage for improve yield and quality
Risk of beak down of methanogenesis	Yes	No	Uninterrupted biogas generation
Stability	No	Yes	Stable operation of the plant
Digester environment control	Difficult	Simple	Greater yield and quality
Digester volume	Higher	Lower	Cost efficient
Retention time	Longer >50	Shorter	Reduced cycle time
Foaming	Higher	Lower	Improvement in fermentation performance

2.12 Current Status of AD Treatment Processes

Anaerobic digestion is an alternative process to produce energy from solid organic waste. The technology is well proven and realized in many industrial plants all over the world. The anaerobic digestion is widely-used technology in Europe (Chavez-Vazquez & Bagley, 2002). In Europe, more than 36,000 anaerobic digesters are in operation, treating around 40-50% of the sludges generated (Mata-Alvarez et al., 2000). Many anaerobic treatment plants are in operation throughout the world. Some of them are operating in large scale (>2000 tones/yr) and some are in small scale (<2000 tones/yr) (RISE-AT, 1998). Table 6 shows the details of where the plants are situated and what

types of feedstock, pretreatment and post treatment are necessary for the respective AD plants.

Table 2.12 Methane yield from OFMSW (RISE-AT, 1998)

Substrate	**Operational parameters**	**Process**	**Yield (Nm3/day)**	**CH4 (%)**	**Degradation (% of VS)**	**References**
SS-OFMSW	5%TS,81-92%VS, 15 days HRT, 2.8 kg VS/m_3.d	T	275-410	62	81	Davidsson et al., 2007
OF-MSW	10% TS, 79% VS,	M	260 L CH4/kg VS	60	61	Nguyen et al. (2007)
MS-OFMSW + PF-OFMSW	20%TS, 62%VS, 13.5 days HRT, 9.2 kg VS/ m_3.d	T	230 m_3CH_4/ton VS	68.7	n.a.	Bolzonella et al.,2003
SS-OFMSW	30%TS, 20-55 days HRT	M	210-290	n.a.	n.a.	Fruteau de Laclos et al., 1997
Manually sorted -OFMSW	18%TS, 90%VS, 19 days HRT, 9.65 kg VS/ m_3.d	T	350	59	65	Gallert and Winter (1997)
MS-OFMSW	88%VS,	T	128-319	n.a.	36-50	Gunaseelan (1997)
MSW only	19 days SRT, 58%VS	M	380 m_3CH_4/ton VS	n.a.	50	Weiland (2000)

2.13 Two-Stage AD Systems

The rationale of two- and multi-stage systems is that the overall conversion process of OFMSW to biogas is mediated by a sequence of biochemical reactions which do not necessarily share the same optimal environmental conditions. Optimizing these reactions separately in different stages or reactors may lead to a larger overall reaction rate and biogas yield (Ghosh et al., 1999).

Typically, two stages are used were the first one harbors the liquefaction-acidification reactions, with a rate limited by the hydrolysis of cellulose, and the second one harbours the acetogenesis and methanogenesis, with a rate limited by the slow microbial growth rate (Liu and Ghosh, 1997; Palmowski and Müller, 1999). With these two steps occurring in distinct reactors, it becomes possible to increase the rate of methanogenesis by designing the second reactor with a biomass retention scheme or other means (Weiland, 1992; Kübler and Wild, 1992). In parallel, it is possible to increase the rate of hydrolysis in the first stage by using microaerophilic conditions (i.e., where a small amount of oxygen is supplied in an anaerobic zone) or other means (Capela et al., 1999; Wellinger et al., 1999).

The application of these principles has led to a great variety of two-stage designs. The increased technical complexity of two-stage relative to single-stage systems has not however always been translated in the expected higher rates and yields (Weiland, 1992). In fact, the main advantage of two-stage systems is not a putative higher reaction rate, but rather a greater biological reliability for wastes which cause unstable performance in one-stage systems. It should be noted however that, in the context of industrial applications, even for the challenging treatment of highly degradable bio-wastes, preference is given to technically-simpler one-stage plants. Biological reliability is then achieved by adequate buffering and mixing of incoming wastes, by precisely-controlled feeding rate and, if possible, by resorting to co-digestion with other types of

wastes (Weiland, 2000). Industrial applications have up to now displayed little acceptance for two-stage systems.

The low specific growth rates of some of the bacteria involved in anaerobic process is the main limitations. As a result, long retention times to avoid wash out of active biomass in conventional continuously stirred tank reactors. Biomass retention can be achieved by uncoupling the hydraulic and solid retention time thereby ensuring high solid content in the Methanogenic reactor. A possibility to achieve higher capacity and increased stability is to retain the slow growing microorganisms within the process. This can be accomplished by recirculation of sedimented microorganisms sludge, or by using process configuration that allows the formation of granulated sludge of high density, which is retained inside the reactor.

However, since most anaerobic digester problem is instability as a result of feed composition irregularity, thus the introduction of stages that buffer the problem and help to feed uniform and hydrolyzed materials to methanation unit is still attractive. Complication of the operation can be reduced by design the system derived by gravity or semi-continuous system in which the two stages may operated independently. Hence, in this work, different treatment to increase hydrolysis, rate of organic solid waste product mix, potential of their products is intended to be examined.

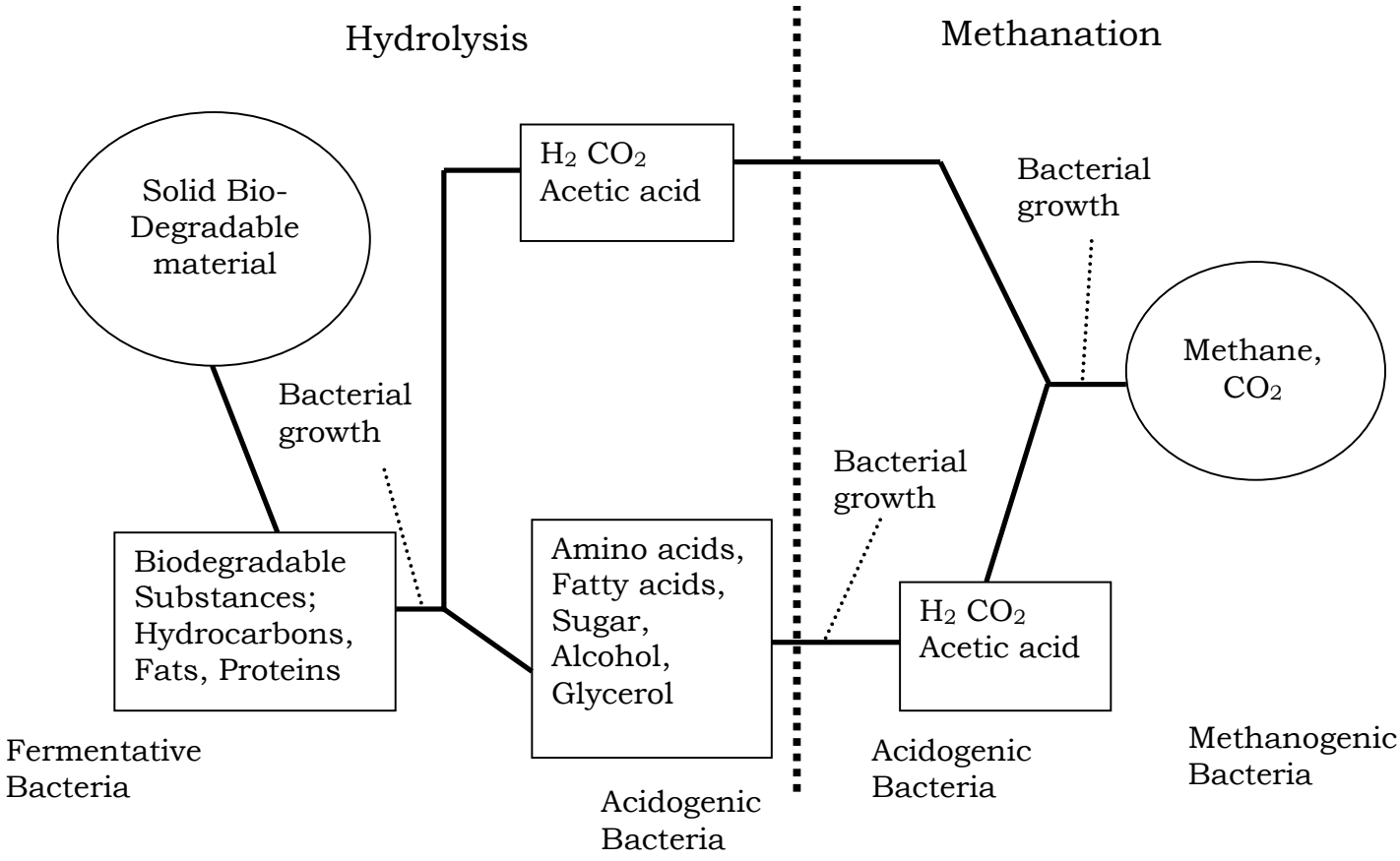


Figure 2.11 Two stage anaerobic digestion process

2.14 Performance Parameters

Gas and methane yields, rates, and reduction in organic matter:

The total gas and methane productions when related to organic matter are directly related to the extent and rate of conversion. Gas yields are related to organic matter added which is expressed as VS and this is also known as specific gas production. These data are typically reported as gas volume per weight of volatile solids. Gas yield is directly proportional to the process efficiency. However, it is also important to note that a low gas/methane yield does not necessarily indicate a deficient performance but it is simply due to a low biodegradability of the substrate used. The methane yield is preferred over the gas yield because pH changes in the reactor can cause change in release or uptake of carbon dioxide that are unrelated to degradation. The use of volatile solids permits the calculation of a material balance between the feed, effluent solids and gas.

Methane production rate is a measure of process kinetics and is determined as volume of methane per volume of reactor per day. This parameter is a product of loading rate (kg/m3/day) and methane yield (m3/kg VS added). Methane content of the gas is a good indicator of stability.

3. METHODOLOGY

The followings are the methodology used in the study to accomplish the objective of the study:

3.1 Materials

- Digester: 6 liters laboratory scale cylindrical anaerobic digester W8 issue 3 armfield model with temperature control and three 10 liters High-density polyethylene tanker.
- Containers for hydrolysis of OFMSW
- Substrates: The substrates used as feed stock materials for the generation of biogas in the laboratory were cow dung (CM) and organic fractions of municipal solid waste (OFMSW) which contains market and household wastes including vegetables, fruits and kitchen wastes. The cow dung used in the study was collected from private farm in Addis Ababa and fruits and vegetables wastes are collected from different areas in the city. ("Atikilt Tera", Dormitory canteen wastes, hotels, and households). Fruits and vegetables wastes were chopped in appropriate size to prepare for the experiments.
- Inoculum: The inoculums for mesophilic incubations were collected from the pilot scale two stage mesophilic anaerobic treatment plant treating distillery waste. This system exhibited ~40-60% VS removal, in which the gas composition, CH4, was detected as being 50- 60%.

3.2 Measurements and Equipments

Analytical reagents were used for the analysis of OFMSW and Cow manure. The materials were analysed for TS, VS, COD, BOD, TKN and biogas production including its composition using equipment and apparatus. Some of them are described hereunder:

- Cylindrical glass made and High-density polyethylene tanker type anaerobic digester,
- Digital Gas Analyzer, SR2-BIO, Gas analyzer,

  Directly connect to the gas holder/ digester to measure the gases (CH_4 in Vol %, CO_2 in Vol %, O_2 Vol%, H_2S in PPM)
- Nanocolor 400D Photometer: multiple parameter (MACHERY-NAGEL)

  For Analyses of drinking, surface, ground, process and waste water using photometer.

  (In this experiment the apparatus used to determine the COD (mg/L) value of the feedstock both for hydrolysis and methanation stage)
- Oven: (Drying oven), NEMMERT

  In this experiment drying oven is used to determine the Total Solid (TS), and Moisture content of the substrate and also a stepping stone to measure Volatile matter/solid VS.
- Hann digester:

  In this thesis work it is used to digest the required samples when analyzing the COD, Total Nitrogen over different temperature range
- Furnace: H JURGENS & CO.BREMEN:

  Used for the determination of the Volatile solid of the sample which operate at 550 ^oC

- Hach, DATALOGGING colorimeter (DR/870):

  Used to measure the total nitrogen (TKN) in the sample
- Digital BOD incubator, Lovibond BOD system oxidirect.
- Microscope,
- Rubber hose
- Measuring cylinder and
- Weighing scale,

The above equipments are used throughout the experiment. The most important equipment and apparatus used in the analysis procedure are described in annex 3.

3.3 Methods

The study was conducted in Addis Ababa University, in the Environmental Engineering, Chemical Engineering Department laboratory. The methods used in this study are described hereunder:

3.3.1 Study variables

- ❖ Hydrolysis rate in neutral and different acidic level of solution (using of different acid concentrations with time variation).
- ❖ Optimal biogas production at optimum solid retention time
- ❖ Characterization of the waste before and after digestion : pH, TS, moisture content, VS, Total Nitrogen, COD, BOD

3.3.2 Study design

The experimental is designed for 5 level of mix (i.e. OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM) in having a hypothesis of the response variable, amount and quantity of biogas, varies with the variation of mix ratio of the feed stocks. A single but vigilant run was conducted because of lack of laboratory chemical reagents, apparatus and

equipments (like Nanocolor and SR2-BIO Gas analyzer) were borrowed for short time.

3.3.3 Sample analysis

Optimization design conditions employed in the study:

- *Hydrolysis/pretreatment stage*
  - Micro aerophilic (slightly aerobic) conditions in the digester to facilitate the rate of liquefaction.
  - Different acid concentration to pretreatment of the OFMSW with time variation, as BOD increase in the leachate due to solublization of the waste.
  - 5 mm diameter sieve will be used to collect the leachate.

- Operation of the Reactor
  - *Feeding unit*: The feeding of OFMSW was after size reduction of particles to less than 25mm and mixed with water to have a ratio of 1kg to 2.5-3 L, the reactor was kept for seven and half days for hydrolysis. (Paramsothy, et al., 2004).

- *Methanogenic stage*

A batch anaerobic reactor under mesophilic condition ambient temperature was used for a digestion period of 27 days was used. The biogas production process was separated into two phases, i.e., hydrolysis and acidification in one reactor and methanogenesis in the other to facilitate the optimal growth for non-methanogenic and methanogenic bacteria, respectively.

Some of the important sampling technique and analytical parameters are presented in table 3.5.1.

Table 3.5.1 Sampling analysis and analytical parameters

Stage	Parameter
Hydrolysis/Acidification	BOD, Solid retention time, Acid concentration
Methanogenesis	COD, BOD, TS, VS, TKN Gas volume, Gas composition, Retention time

- ✓ Volume of biogas production was determined by water displacement.
- ✓ Methane and carbon dioxide in biogas was measured by directly using digital gas analyzer (SR2-Bio gas analyzer).
- ✓ Total solid was estimated from weight loss up on evaporation at 105 0c for 24 hours (standard method procedure 2540 B) [78].
- ✓ Total volatile solid content was estimated from weight loss upon ignition at 550 0c for 2 hours (standard method procedure 2540 E) [78].
- ✓ pH using Jenway model 3510 digital pH meter.
- ✓ Total nitrogen (Hach method 10072 TNT per-sulfate digestion).
- ✓ Total Phosphorus (Hach method 8190 molybdovanadate with acid persulfate digestion).

3.4 Statistical analysis

Student's t-test was used to investigate the statistical significance of the results obtained. The t-test gives the probability that the difference between the means of different groups of data is caused by chance. A 95% confidence limit to calculate T is commonly used, meaning that, if the probability is less than 0.05, the difference is 'significant' and not caused by chance [Anderson.D.R., Sweeney.D.J., Williams.T.A., (2000)]. In the present study, for selected feedstock mixture, the T-statistic was used to compare the means of the results

of the parameters obtained from the five reactor configurations with a 95% confidence limit. After the determination of the amount of biogas in each level or treatment ANOVA and multiple comparisons were performed.

Table 3.1 sample Analysis parameters

Parameter	Explanation/ Relevance	Method & Instruments
Total Solids (TS)	Residue upon water evaporation after 48 hours drying at 105°C represents the total solids and presents a raw estimation of all the organic and inorganic matter content in the original sample.	Oven (Toschnival) Precision Scale (Satorius 2355)
Total Volatile Solids (VS)	The fraction of solid matter that can be oxidised and driven off as gas at 550°C for 2h ours (constant weight) is an approximation of the organic fraction of the dry matter determined at 105°C (TS). The residue is the inert (mineral) fraction, mainly due to inorganic matter.	Muffel-Furnace (Vecstar, LF 3) Precision Scale (Satorius 2355)
Total Chemical Oxygen Demand (CODtotal)	The oxygen equivalent of the organic matter that can be oxidised. COD total is a measure of all the organic matter in the sample.	Nanocolor
Total Nitrogen TKN (Ntot)	Essential nutrient to growth of organisms. Organic nitrogen and ammonia are together referred to as Kjeldahl nitrogen.	Semi-Micr o - Kjeldahl Method Hach DR/800
Total Phosphorus (Ptot)	Essential nutrient to growth of Organisms include orthophosphates and organically bound phosphates.	Acid Persulfate - Digestion Method Photospectrometer (Hach DR/201 0)

3.5 Experimental set up

Experiments were carried out in two 6 liters laboratory scale cylindrical anaerobic digester W8 issue 3 armfield model with temperature control and three 10 liters High-density polyethylene tanker tightly insulated to keep the temperature at ambient state (Fig. 3.3), to determine biogas production from different mixing factor of OFMSW and Cow manure.

Table 3.3 Laboratory setup feed preparation (parenthesis are ratio of feed preparation)

Laboratory setup feed preparation			
Reactors	**Cow Manure (CM) %**	**OFMSW %**	**Reactors volume, L**
R1	0(0)	100(1)	6
R2	33.3(1)	66.7(2)	6
R3	100(1)	0(0)	10
R4	50(1)	50(1)	10
R5	66.7(2)	33.3(1)	10

Figure 3.3 Experimental setup

The gas off-take from each reactor is taken to a volumetrically calibrated collector vessel operating by water displacement. A constant head, liquid seal device ensures that the gas pressure in the reactor is maintained at a constant value throughout the test run. The collected gas can be exhausted from the vessel and the volume re-filled with water during a run without breaking the liquid seal.

Gas sampling points are located at all strategic points around the reactors. Non-return valves and liquid seal syphon breaks are included in the process pipe-work to ensure each reactor operates at a constant volume without the entrance of air or the danger of accidental symphonic action. Gas compositions are measured directly from the reactors gas sampling hose using gas analyzer (SR2-Bio).

4. RESULTS AND DISCUSSION

This section describes the results obtained during the laboratory scale experiment of two stage anaerobic digestion of solid wastes. The characteristics of the wastes are presented. The experiments were conducted in two phases i.e. the hydrolysis of OFMSW by using different acid concentration for several days in one reactor and gas production and organic solid reduction from AD system using substrate mixing factor (OFMSW and CM) in another reactor to achieve the objectives of the thesis.

4.1 Results and Discussion on Performance of hydrolysis stage

The results of the experiment carried out for the hydrolysis of OFMSW using neutral and different acid concentrations are shown in Figure 4.1.

The increase in soluble substance at the beginning of hydrolysis process depends on the nature of the substrate. Fruit and vegetable wastes are easily biodegradable (Pavan et al, 2000) confirms this fact by adding that acidogenic conditions were quickly removed the required days during hydrolysis of fruit and vegetable wastes. It is very important to mention that the hydrolysis operation is crucial for early volume reduction and degradation.

From the BOD profile in figure 4.1., it is observed that the BOD in mg/L increment because of spontaneous acidification of the substrates was observed in the first two to four days after the start of the experiment, hydrolysis of complex organic structure by different acid concentrations were analysed. For the test, the following acid concentrations are used i.e. 0 % (pure water), 1%, 3%, and 5% acid was used to leach the collected solid waste. Initially the BOD value for raw waste was 13,200 mg/L. it is expected to increase the value because of hydrolysis and dissolving of complex structure which increases the biodegradation of the waste meanwhile it increases the BOD content of the leachate. The result shows that the 5% acid concentrations took the shortest

time i.e. one to two days to reach maximum level of biodegradable materials contained in the leachate that is ~80,000 mg/L. But for the other concentrations, when the retention time increases the degradation also increases but the time it took was relatively higher compared to the 5% acid concentration. For 0% acid concentration for complete degradation of OFMSW in microaerophilic conditions it takes five to six days and the BOD value became around 54,000 mg/L.

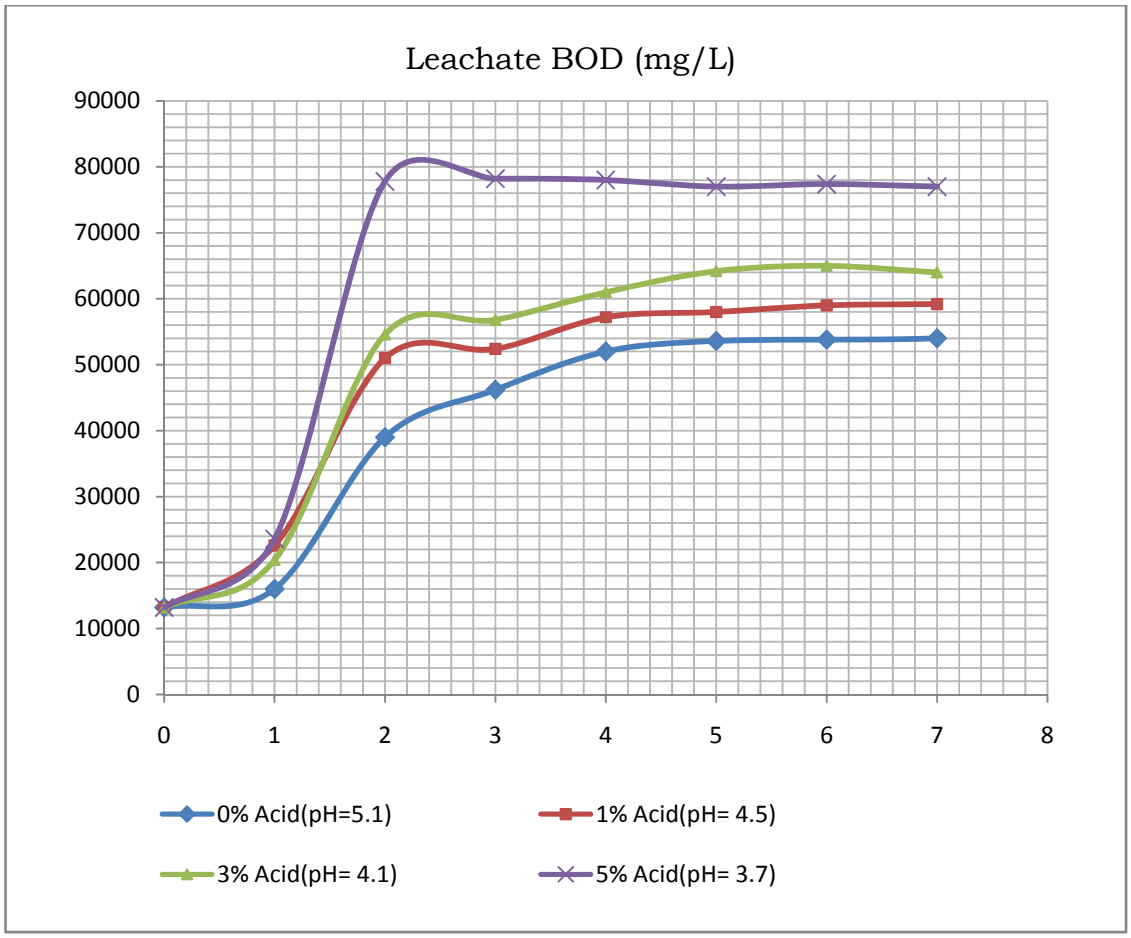


Figure 4.1 Leachate BOD (mg/L) increment per days

As shown in the analysis of variance (annex 2.2) the independent variable Acid concentration had not significantly affects (p-value 0.234) the dependent

variable-Leachate BOD. When the p-value of a coefficient is greater than the chosen α-level (usually 0.05), there is evidence of a no significant relationship between the predictor or factor level and the response variable when the retention time is increased, but as per the result it would significantly affect for the first hydrolysis periods.

4.2 Results and Discussion on Methanogenic stage

Chemical oxygen demand (COD), biological oxygen demand (BOD), volatile solid (VS), total solid (TS), total nitrogen (TN), pH, gas production rate and gas composition were determined at Environmental Engineering laboratory, Chemical Engineering Department; and IGNIS project biogas pilot plant.

4.2.1 Characteristics of the substrates used in the batch tests

Experiments were carried out in two 6 liters laboratory scale cylindrical anaerobic digester and three 10 liters High-density polyethylene tanker tightly insulated to keep the temperature at ambient, the substrate filled 90% of the container 10% left for gas collection, from 10% active inoculum(refer materials in methodology section) was used for all reactors for the total feedstock input.

The characteristics of OFMSW and CM as main substrate and co-substrate mixed in different mix ratio were determined and the results are shown in Table 4.2.1 below. When the wastes mixed and characterized; the mean value of the total solid were 4.37%(R1), 5.21%(R2), 6.92%(R3), 5.45%(R4), and 5.5%(R5), for OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM mix by volume respectively. The volatile solid for the mixes were 97.17%(R1), 96.76%(R2), 94.52%(R3), 96.77%(R4), and 95.62%(R5) for OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM mix by volume respectively.

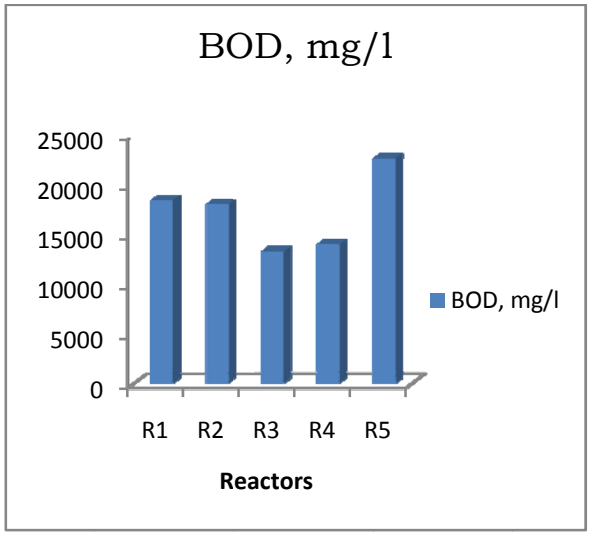
When considering the COD of the mix, OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM were 133,133.33 mg/L (R1),98,500 mg/L (R2), 79,933.33 mg/L (R3), 89,900.00 mg/L (R4) and 107,200.00 mg/L (R5) respectively.

Table 4.2.1 Characteristics of the substrates used in the batch tests

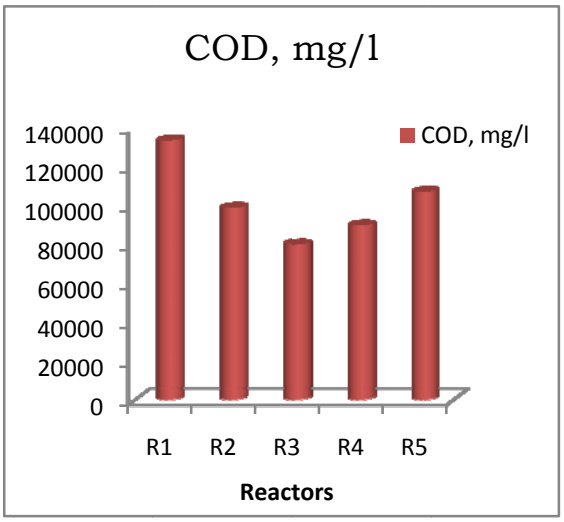
Parameter	**Batch Reactors**				
	R1	**R2**	**R3**	**R4**	**R5**
TS, %	4.37	5.21	6.92	5.45	5.50
VS/TS, %	97.17	96.76	94.52	96.77	95.62
BOD, mg/L	18,400	18,000	13,300	14,000	22,600
COD, mg /L	133,133.33	98,500.00	79,933.33	89,900.00	107,200.00
pH	6.8	6.7	6.2	6.5	6.4
TKN, mg/L	2475	2775	4650	2900	3975
C/N	53.79	35.49	17.18	31	26.96

When considering the TKN of the mix, OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM were 2475 mg/L (R1), 2775 mg/L (R2), 4650 mg/L (R3), 2900 mg/L (R4) and 3975 mg/L (R5), respectively.

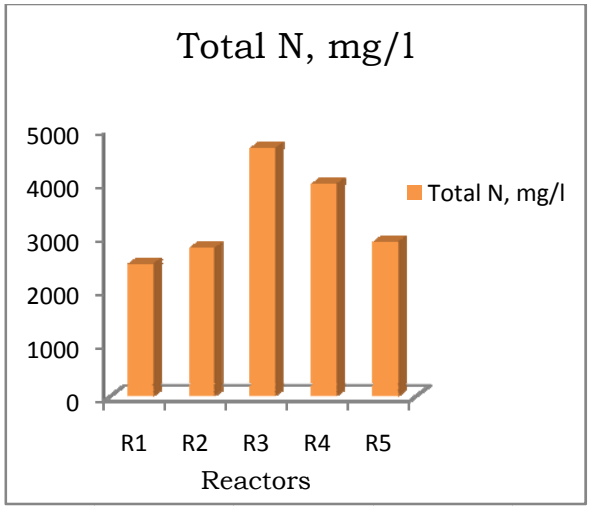
These two important parameters (COD, TKN) are important for the evaluation of biogas production and gas composition.



(A)



(B)



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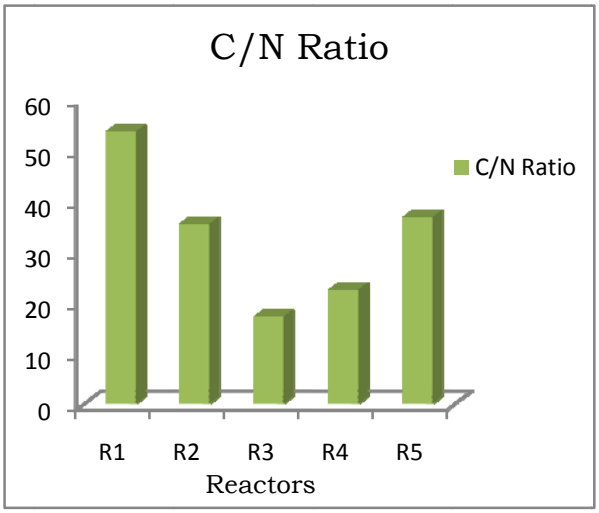

(D)

Figure 4.2 BOD input for the reactors (A), COD input for the reactors (B), Total Nitrogen (TKN) input for the reactors (C), C/N ratio of substrate input for the reactors (D).

4.2.2 Determination of digestate characteristics

The digestate characteristics after the experiment carried out with digestion and co-digestion of OFMSW and CM to determine maximum biogas production with good methane quality were indicated in Table 4.2.2.

The total solid after digestion of OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM were 2.18%(R1), 1.76%(R2), 2.72%(R3), 1.82%(R4) and 2.22%(R5) respectively. The mean value of volatile solid of the feed after digestion were 99.0%(R1), 98.49%(R2), 97.15%(R3), 98.48%(R4) and 97.47%(R5) for OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM.

Table 4.2.2 Feedstock characteristics after digestion

Parameter	**Reactors**				
	R1	**R2**	**R3**	**R4**	**R5**
TS, %	2.18	1.76	2.72	1.82	2.22
VS/TS, %	99.0	98.49	97.15	98.48	97.47
BOD, mg/L	1543.00	2175.00	2567.00	1945.00	2500.00
COD, mg /L	23,983.00	26,400.00	45,866.00	28,833.00	31,416.00
COD:BOD	15.54	12.14	17.87	14.82	12.57
pH	7.5	7.4	6.8	7.1	7.2
TKN, mg/L	1400	1333.33	1750	883.33	666.67

4.2.3 COD: BOD ratio

In conventional biological treatment systems the ratio increases with each stage of biological treatment with ratios higher than 10:1 expected in the final effluent. In this research work the ratio of COD: BOD shows 15.54, 12.14, 17.87, 14.82 and 12.57 for OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM. The ratio between COD and BOD increases the expected value i.e. 10: 1 across the methane reactor system which comparable with the literature (Fruteau et al., 1997, Nguyen et al. 2007).

4.2.4 Rate of Biogas production

The production of biogas was observed and volumes of the biogas collected were recorded during the experiment period 27 days, and the production of biogas was used mainly as an indication of optimum production and the development of the digestion process.

The daily biogas production rates from the digestions of OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM are shown in Fig. 4.2.4. The co-digestion of 1:1(R4) followed by 1:2(R5) mix OFMSW: CM produced biogas much faster and higher rate per reactor volume than the others three digestion tests in the first periods of the experiment, this maximum rate because since the feed OFMSW was already hydrolyzed in separate reactor for this case the readily digested small-molecular components such as alcohol quickly consume by methagonic bacteria, in the preceding four days the rate and the gas composition decreased due to monomers and small molecular compounds are depleted and larger once take a long time to degrade as hydrolysis (Daniqu, 2010).

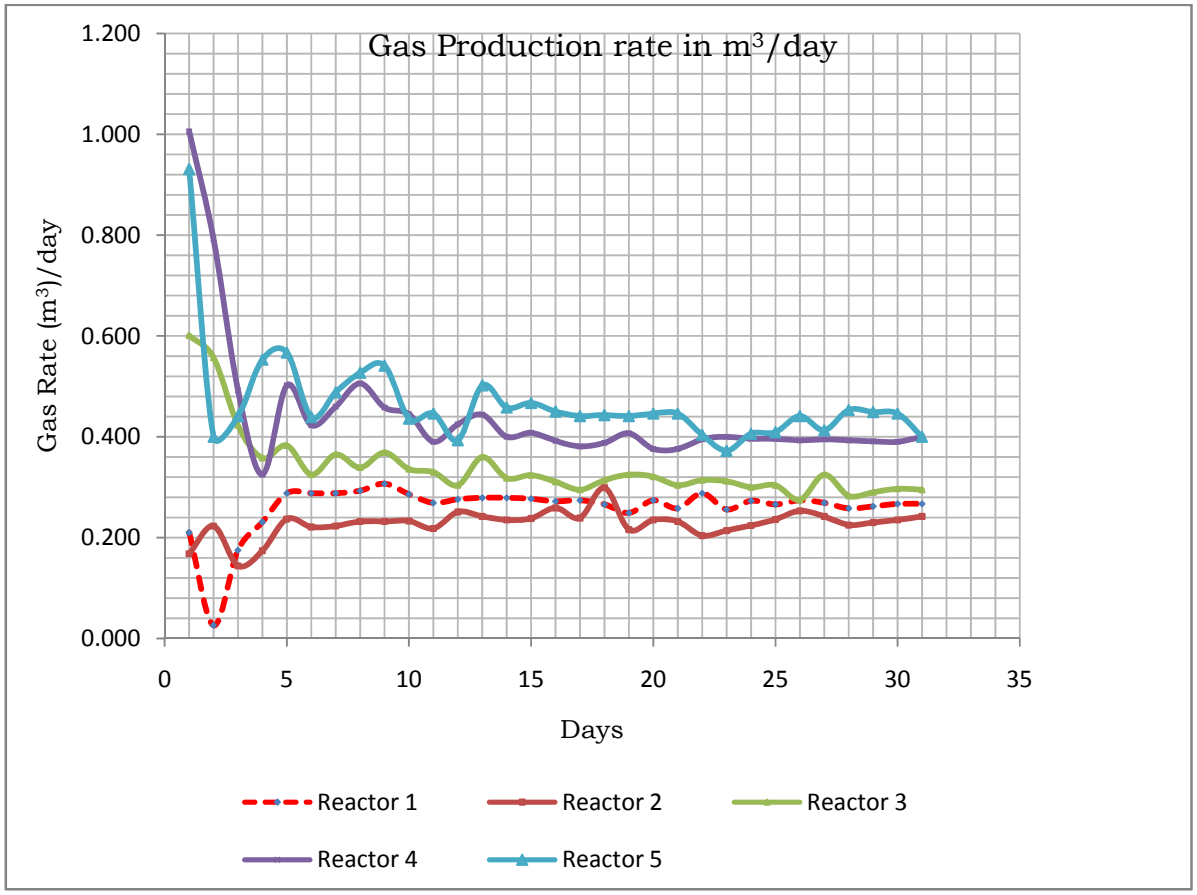

Figure 4.2.4 (A) Gas Production rate for different mix ratio (in m^3/day)

4.2.5 Identification of maximum mix ratio for highest biogas production

The mean cumulative biogas production for the digestion of OFMSW, CM and its co-digestion tests is shown in Fig. 4.2.4. In biogas production mixed digestion is better than digestion of substrates alone; it was observed in this experiment there is higher biogas production for R5 (1:2 mix of OFMSW: CM), R4 (1:1 mix of OFMSW: CM) and R3 (100% CM alone) i.e. 14.449, 13.745 and 10.557 m^3/Vol. of digester with comparable methane composition respectively. As shown in the analysis of variance (annex 2.1) the independent variable- mix ratio significantly affects (p-value $2.66*10^{-22}$) the dependent variable-amount of

biogas produced. When the p-value of a coefficient is less than the chosen α-level (usually 0.05), there is evidence of a significant relationship between the predictor or factor level and the response variable.

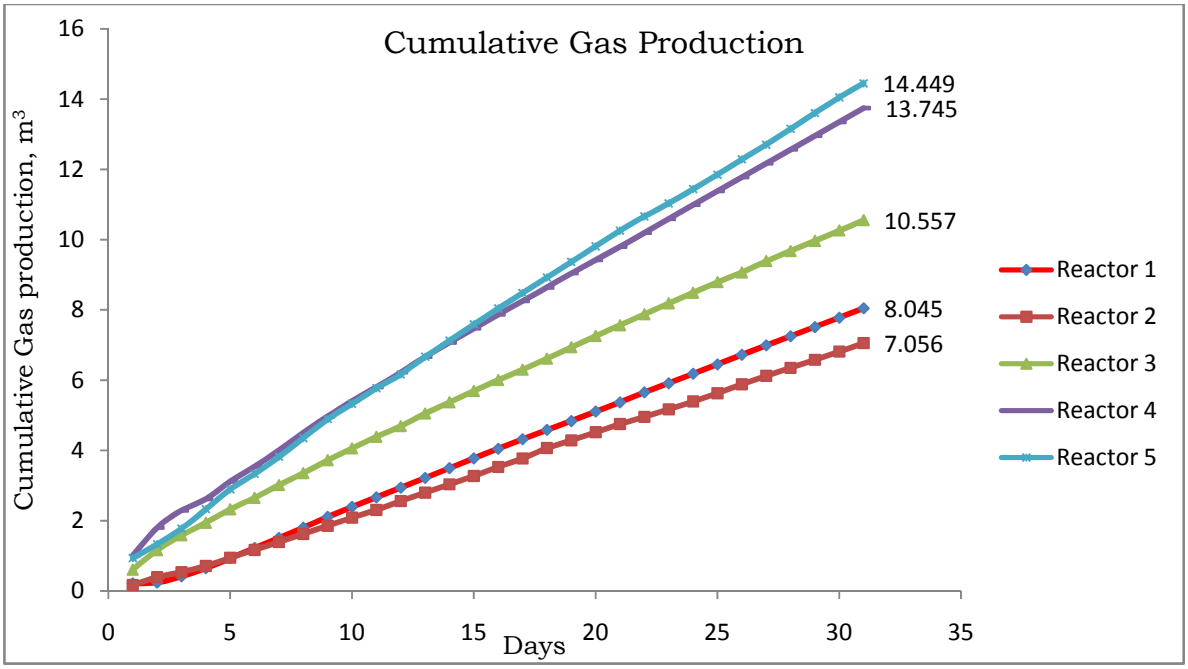

Figure 4.2.4 (B) Cumulative Gas Production for different mix ratio (in m^3)

Methane concentrations measured during the digestion period and mean values in different mix ratio are shown in table 4.2.5, A better methane composition with an average of 50% and 51.5 % was observed when digestion of CM alone and 1:2 mix of OFMSW: CM, respectively. When the OFMSW digested alone (R1) was used the percentage composition of methane decreases significantly by 46% followed by 18.44% and 26.21% for 2:1 mix of OFMSW: CM and 2:1 mix of OFMSW: CM, respectively. The reason behind for lower methane composition for the specified reactors mainly because of the deviations from the optimum range of C/N ratio i.e. 53.79 and 35.49 for the case of 100% OFMSW and 1:2 mix of OFMSW: CM. Since Optimum C/N ratios in anaerobic digesters are between 20 – 30 in order to ensure sufficient nitrogen supply for cell production and the degradation of the carbon present

in the wastes (Fricke et al., 2005). A high C/N ratio is an indication of rapid consumption of nitrogen by methanogens and results in lower gas production. On the other hand, a lower C/N ratio causes ammonia accumulation and pH values exceeding 8.5, which is toxic to methanogenic bacteria.

Table 4.2.5 Cumulative biogas production and composition

Feed stock	Biogas (m^3/Vol. of digester)	Methane (%)	CO2 (%)	Others (%)
R5	14.316	51.5	43.9	4.6
R3	10.557	50	44.4	5.6
R2	7.056	42	54.1	3.9
R4	13.745	38	55.4	6.7
R1	8.045	27.8	66.4	5.8

As shown in the analysis of variance (annex 2.2) the independent variable mix ratio significantly affects (p-value $6.97*10^{-5}$) the dependent variable-amount of methane produced. When the p-value of a coefficient is less than the chosen α-level (usually 0.05), there is evidence of a significant relationship between the predictor or factor level and the response variable.

4.2.6 Identification of maximum mix ratio for highest CH_4 production

Daily methane production rate, cumulative methane and methane content for the digestions OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM are shown in Fig. 4.2.5 (A) and (B). The digestion of 1:2 mix of OFMSW: CM had the maximum methane rate starting from the 13^{th} day and reached a maximum methane yield from the 13^{th} to 22^{th} days of digestion.

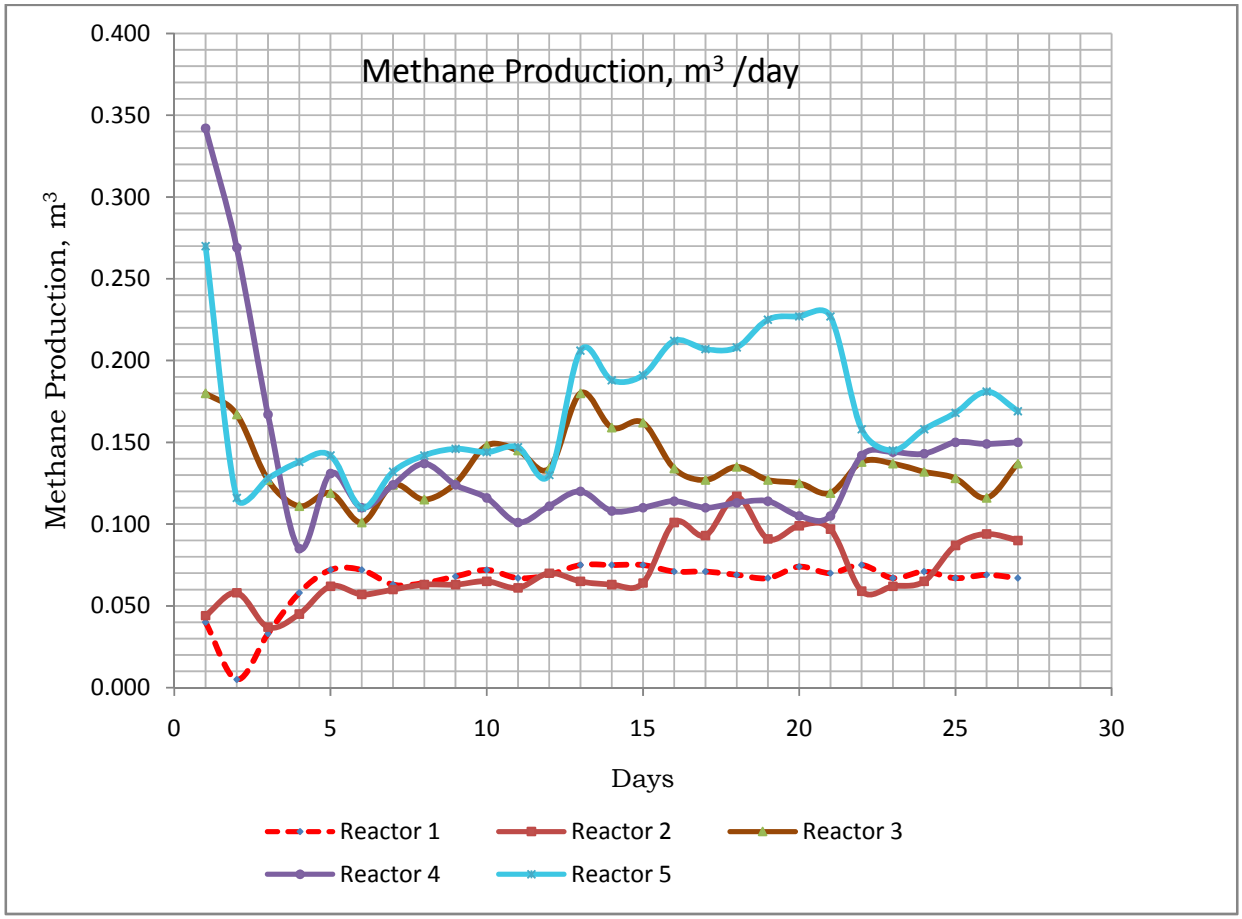

Figure 4.2.5 (A) Methane Production in m^3/day

The peak methane rate from the digestion of 1:1 mix of OFMSW: CM was 33.08 % higher than peak methane rate of 100% CM and 56.50% higher than peak methane rate for mix of 2:1 mix of OFMSW: CM. Not much methane was produced after 25 days for digestions of OFMSW (100%) and 2:1 mix of OFMSW: CM. The digestion of OFMSW (100%), 2:1 mix of OFMSW: CM did not produce much methane during the first 10 days then very little after 15 days instead of 5 days unlike the other mix (Fig. 4.2.5 (A) and (B)). The digestion of 1:2 mix of OFMSW: CM produced the highest cumulative methane (4.615 m^3), which was 20.8% more than CM alone and 20% more than 1:1 mix of OFMSW: CM (Fig 4.2.5 (C)).

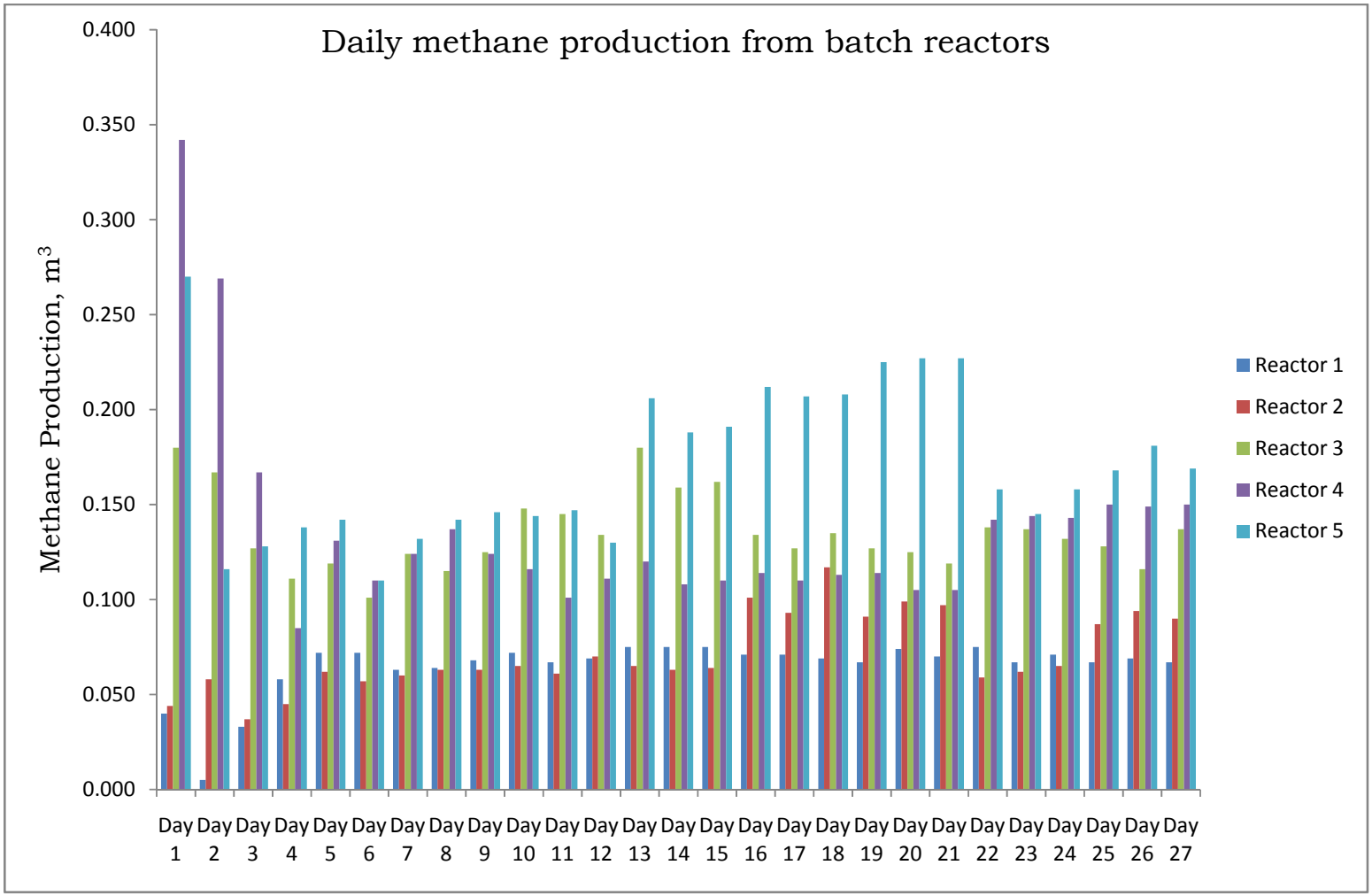

Fig.4.2.5 (B) Daily methane production from batch reactors m^3/day

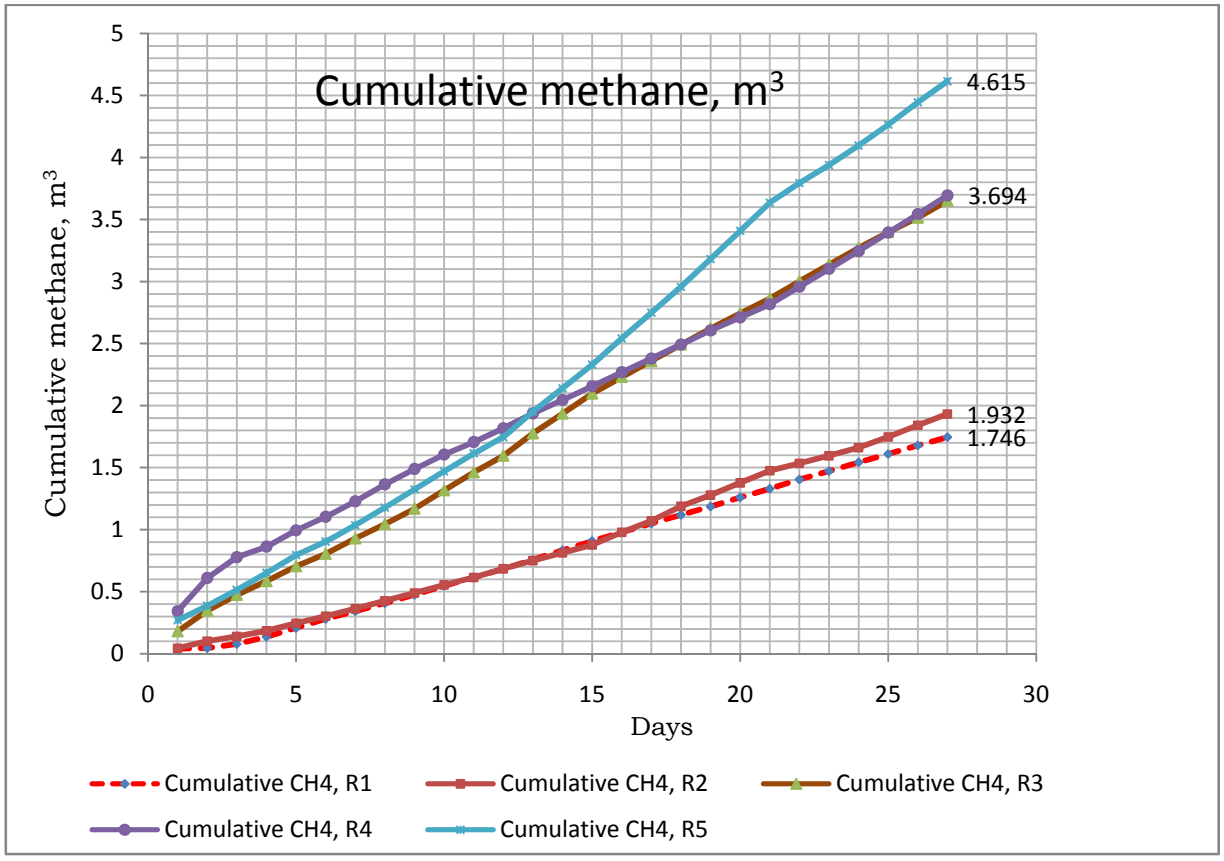

Figure 4.2.5(C) Cumulative methane Production for different mix ratio (in m^3)

4.2.7 COD, VS and TS Removal Efficiency

4.2.7.1 COD removal efficiency

Considerable removal efficiencies of COD were generally observed for OFMSW and cow manure mixture digestion with the average efficiency of 82%(R1), 73%(R2), 43%(R3), 68%(R4) and 71%(R5) for OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM, respectively. The COD removal efficiencies over the duration of the experiment were comparable to those reported in the literature (Callaghan et al., 1999) ranging from 60-75%. In general, the high removal efficiencies for COD are a good indication of the fact that the anaerobic digestion under proper operating conditions could

be used for the pre-treatment of wastes before the conventional waste treatment plant.

Table 4.2.5 (A) COD removal efficiency analysis for batch experiments

Reactors	COD Removal efficiency Analysis		
	Input COD(mg/L)	Output COD (mg/L)	Removal efficiency (%)
R1	133,133.00	23,983.00	82
R2	98,500.00	26,400.00	73
R3	79,933.00	45,866.00	43
R4	89,900.00	28,833.00	68
R5	107,200	31,416.00	71

4.2.7.2 **Volatile solid removal**

It was observed a significance variation in removal of volatile solid in the digestion substrate, (table 4.2.6 (B)). The mean removal efficiency of 66.01% is observed for digestion of 1:1 mix of OFMSW: CM, 65.66% for 2:1 mix of OFMSW: CM, 59.65% for CM alone, 58.84% for 1:2 mix of OFMSW: CM and 49.26 % for OFMSW alone. It is a very good indication of high uptake rate for the organic fraction of the total solids. The high VS removal efficiencies in R4 (1:1 mix of OFMSW: CM) digestion indicate the effectiveness of the anaerobic reactor in digesting under anaerobic digestion during proper operating conditions.

4.2.7.3 **Total Solid removal**

It was observed a significance variation in removal of Total solid in the digestion substrate, (table VGB). The mean removal efficiency of 67% is observed for digestion of 1:1 mix of OFMSW: CM, 66 % for 2:1 mix of OFMSW: CM, 61% for CM alone, 60% for 1:2 mix of OFMSW: CM and 50 % for OFMSW

alone. It is a very good indication of high uptake rate for the organic fraction of the total solids. The high TS removal efficiencies in R4 (1:1 mix of OFMSW: CM) digestion indicate the effectiveness of the anaerobic reactor in the breakdown and hydrolysis of the substrate during digesting under anaerobic digestion during proper operating conditions.

Table 4.2.6 (B) TS and VS removal efficiency analysis

Reactors	TS and VS Removal efficiency Analysis	
	Removal efficiency □ (%),TS	Removal efficiency □ (%),VS
R1	50	49.26
R2	66	65.66
R3	61	59.65
R4	67	66.01
R5	60	58.84

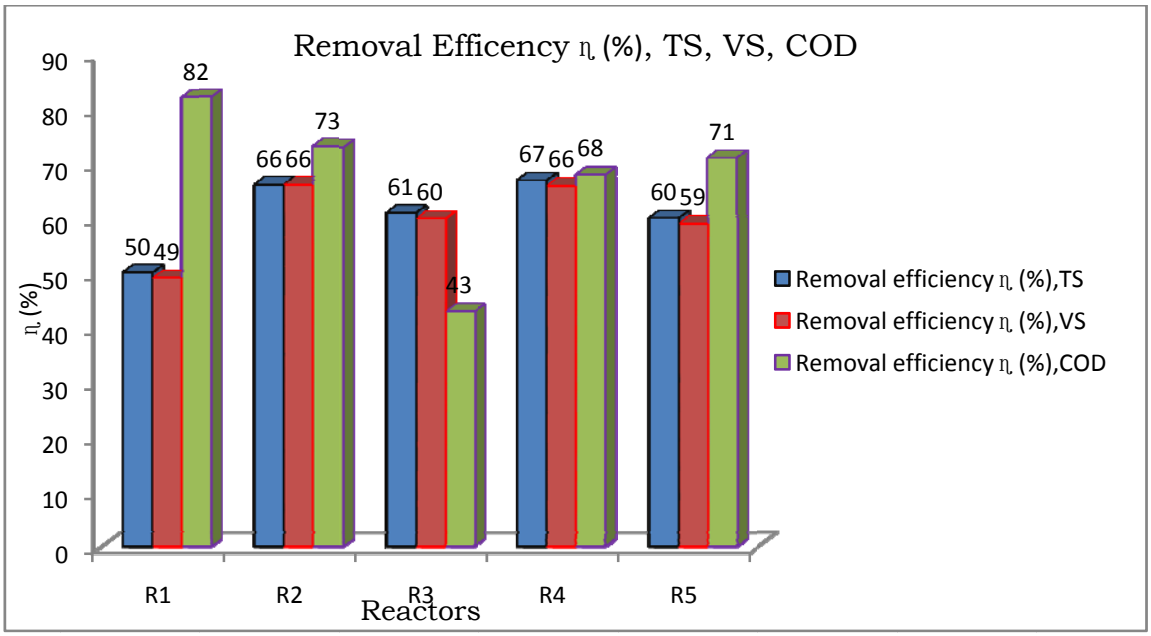

Figure 4.2.6 Removal Efficency (%), TS, VS, COD

4.2.8 Methane yield and VS removal

By comparing the methane yield based on the total VS_{added} in the reactors (Including inoculum), the digestions of 1:2 mix of OFMSW: CM (R5) had the highest average methane yields (0.353 m^3/Kg VS_{added}) (Table 4.2.7). With the optimum of the CM percentage mix, the methane yield based on total VS added increased except digestion of CM alone. For example, the methane yields from the digestion of 1:2 mix of OFMSW: CM (0.353 m^3/Kg VS_{added}) was 19%, higher than that of 1:1mix (0.286 m^3/Kg VS_{added}), 29% and higher than that 100% OFMSW and 34% higher than that of 2:1 mix of OFMSW: CM (0.233 m^3/Kg VS_{added}).

The result is comparable with the work of Callaghan et al., 1999, co digestion of cow manure with fruit and vegetable wastes at mesophilic condition (35 ^oC). They found an ultimate methane yield of 0.25-0.45 (L/g VS added) and 30-50 % of VS Removal Rate.

Table 4.2.7 Methane yields and VS removal rates for the batch tests

Feed composition	Average Methane Yield		VS removal rate
	m^3/Kg VSadded	m^3/Kg VS removed	%
100% OFMSW, R1	0.251(0.06)	0.509(0.12)	49.26
66.7% OFMSW, R2	0.233(0.06)	0.355(0.10)	65.66
0% OFMSW, R3	0.223(0.3)	0.374(0.05)	59.65
50% OFMSW, R4	0.286(0.11)	0.433(0.16)	66.01
33.3% OFMSW, R5	0.353(0.08)	0.600(0.14)	58.84

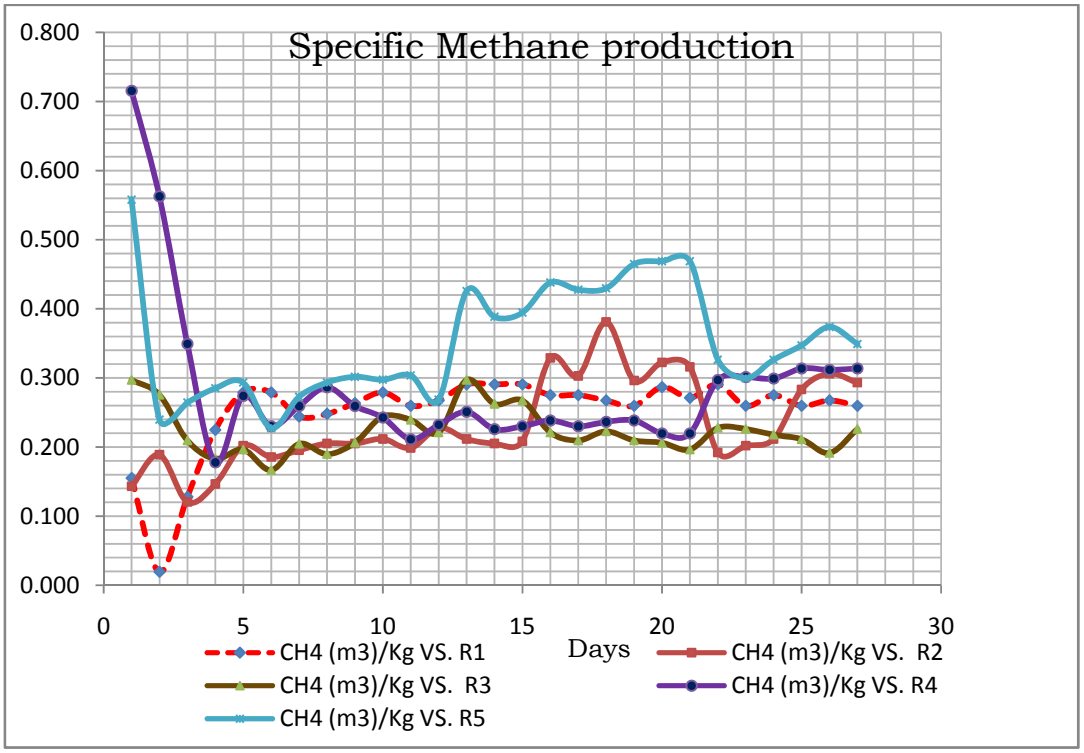

Figure 4.2.5(D) Specific Methane production for different mix ratio (in m^3)

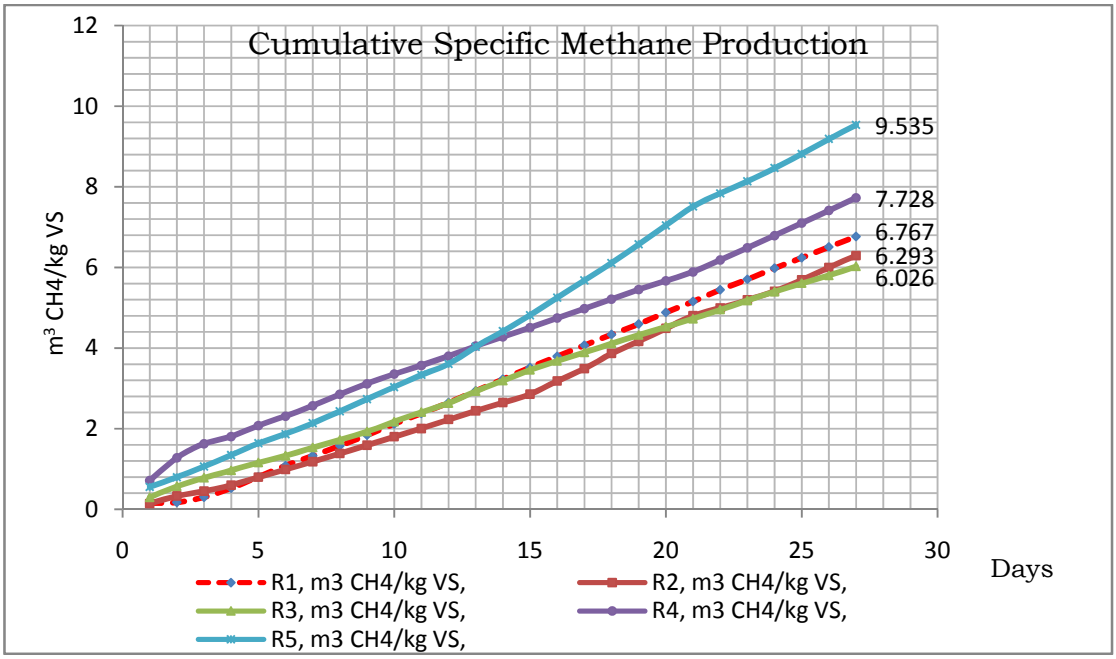

Figure 4.2.5(E) Cumulative Specific Methane Production for different mix ratio (in m^3)

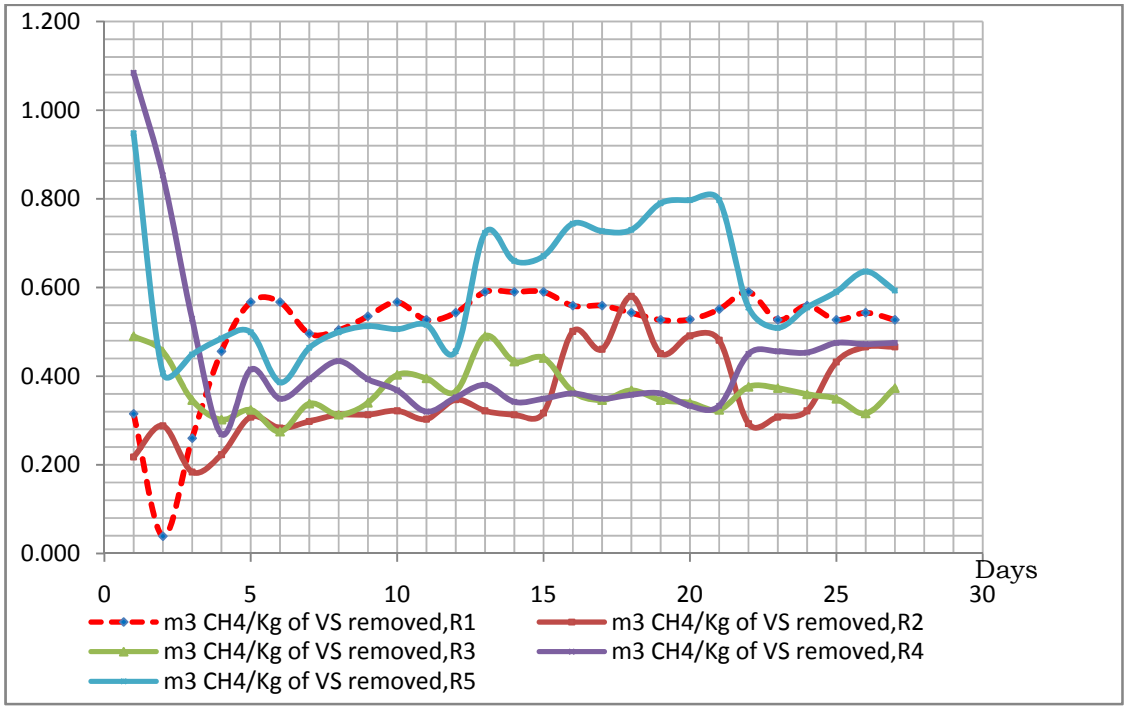

Figure 4.2.7 (A), methane production per VS removed

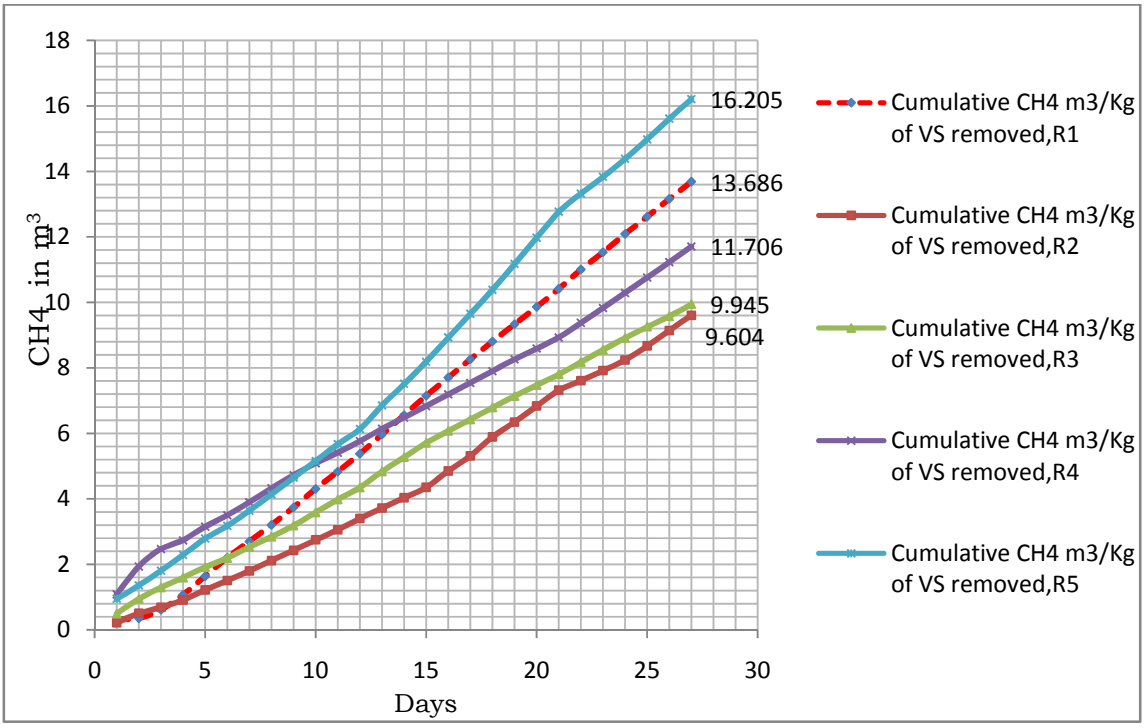

Figure 4.20.7 (B) cumulative methane produced per VS removed

4.2.9 Overall performance of the reactors

The total gas and methane production rate are correlated to the extent and rate of conversion of organic matter. Gas yields are related to organic matter added which is expressed as VS and this is also known as specific gas production. These data are typically reported as gas volume per weight of volatile solids. Gas yield is directly proportional to the process efficiency.

The overall performances of the reactors having different feedstock characteristic are presented in "Overall performance by total CH_4 production per kg of VS_{added}" (figure 4.2.8 (A)) and "Overall performance by total CH_4 production per kg of $VS_{Removed}$" (figure 4.2.8 (B)). From the two figures R5 (i.e. 1:2 mix of OFMSW: CM) had a cumulative of 9.535 CH_4 in m^3/Kg of VS added and 16.205 CH_4 in m^3/Kg of VS removed, indicates a good performance followed by R4 (1:1 mix of OFMSW: CM) had 7.728 CH_4 in m^3/Kg of VS added and 11.706 CH_4 in m^3/Kg of VS removed. For R1 (OFMSW alone), even if the methane production per volatile solid is the minimum (6.767 CH_4 in m^3/Kg of VS added) but it has a better performance on methane production per volatile solid removed (13.386 CH_4 in m^3/Kg of VS removed) when we comparing with R2 (2:1 mix of OFMSW), and R3 (CM alone).

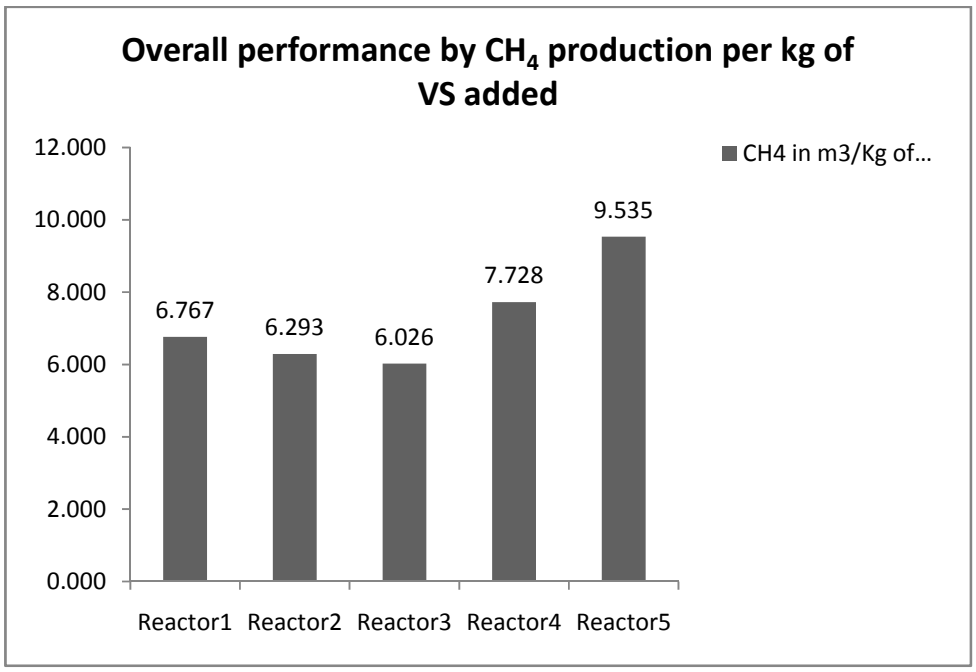

Figure 4.2.8 (A) Overall performance by total CH_4 production per kg of VS_{added},

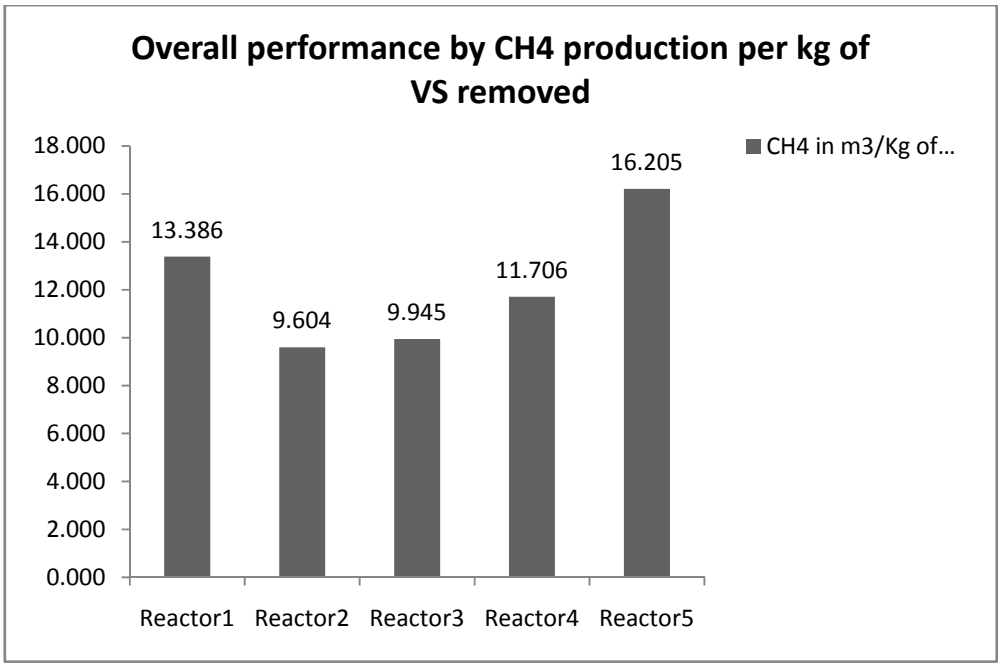

Figure 4.2.8 (B) Overall performance by total CH_4 production per kg of $VS_{Removed}$,

5. CONCLUSION AND RECOMMENDATIONS

For optimum production of biogas from Bio-municipal solid waste with optimum gas rate and methane composition a set of batch experiments were carried out. The conclusion and suggestion made from the study summarized hereunder:

5.1 Conclusion

- From the hydrolysis experiment it is shown that the creation of acidic environment favors the hydrolytic bacteria to enhance the biodegradability of the substrate so as minimize the hydrolysis time in biogas production process. But this is usually implemented to complex structured substrates like wood, garden wastes..., for this research the feedstock (OFMSW) easily biodegraded in a short period of time to the required level so that it is not economical to invest extra investment to create acid environment.

- Anaerobic digestion and co-digestion of Organic Fraction of Municipal Solid Wastes and Cow Manure were investigated using a two-stage Laboratory scale anaerobic digestion system. The biogas produced in the anaerobic co-digestion had a higher biogas rate and methane content than single substrate. The producing a biogas containing methane in the range of 27.8%-51.5% at a rate of approximately 0.251 -0.353 m3/kg VS for different mix ratio of OFMSW to CM. This result was satisfactory when we compare the previous research by (Gunaseelan. et al, 2004) and (Deublein. et al, 2008).

- An effective digestion with a production of biogas was quick in the first two days and this is might be the readily available free alcohols easily changed to biogas. The specific methane production rate of 0.251, 0.233, 0.223, 0.286 and 0.353 m^3CH4/kg VSadded were found for OFMSW

(100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM mix by volume respectively. Volatile solid reductions of 49.26 %, 65.66%, 59.65%, 66.01% and 58.84% were obtained for OFMSW (100%), 2:1 mix of OFMSW: CM, CM (100%), 1:1 and 1:2 mix of OFMSW: CM mix by volume, respectively.

- From the result obtained the quality and the quantity of biogas produced mostly depends on the optimum mix of substrate based on their C/N ratio value Therefore, co-digestion of OFMSW with other organic wastes, such as municipal sludge, animal manure may improve the biogas production. This is in line with the previous research (Bouallagui et al., 2009; Habiba et al., 2009; Nayono et al., 2009; Neves et al., 2009).

5.2 Recommendations for further study

The previous literature and the present study demonstrate that it is possible to use anaerobic digester to anaerobically treat OFMSW. It can offer the production of biogas as well as the potential economical value of residue byproducts. However, an attempt on the optimization of the process for this experiment was not completely achieved since the highest VS reduction obtained is only 58.81% and low methane content of 51.5% for the improved substrate mix (i.e. 1:2 mix of OFMSW: CM). Therefore, the new concept should be foreseen that may possibly improve the process for further study.

Before the system can be adopted on a large scale acceptance for the treatment of OFMSW in, it is crucial to carry out further investigation with multiple replicas and operate at pilot level operation. In this regard the following suggestions for further research work are made:

- From the result shows the biogas production and methane content from anaerobic digestion of OFMSW alone produce unsatisfactory so that different co-substrate should be searched and tested to increase the feasibility of the treatment; for instance anaerobic digestion of human waste, both on liquid and organic portion of solid waste.
- Source segregation of organic fraction of solid waste should be implemented in community for better performance of the AD system and also to have sustainable solid waste management.
- Pilot plant operation similar to the one undertaken in this research should be undertaken over a much longer period to establish steady state conditions that will enable correct assessments of long term treatment efficiencies, optimal loading rates, optimal hydraulic retention times, suitable dilution ratio, and gas production potentials among others.

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ANNEX

Annex 1: Laboratory procedures

1.1 COD

• Method

- ✓ Adaptation of the USEPA 410.4 approved method for the COD determination on surface waters and wastewater
- ✓ Oxidizable organic compounds reduce the dichromate ion (orange) to the chromic ion (green)
- ✓ The amount of remaining dichromate is determined

• Required reagent

- Reagent vial
- Deionized water

• Materials used

- C9800 Hanna reactor
- HI 740216 test tube cooling rack
- Nanocolor COD reagent

• Measurement procedure

- ➢ This method has its own calibrating vial so no need of a reagent blank correction like Hanna and Hach equipments
- ➢ A single calibration vial will be used more than once
- ➢ Choose a homogeneous sample
- ➢ Samples containing settle able solids need to be homogenized with a blender
- ➢ Preheat the Hanna reactor C9800 to 150 oc (32 0F)
- ➢ Remove the cap from reagent vials
- ➢ Add exactly 0.2 ml of sample to the vial (sample vial)
- ➢ Keep the vial at 45 degree angle while adding the water and the sample
- ➢ Replace the cap tightly and mix by inverting the vial a couple of times

- Insert the vial into the reactor and heat them for 2 hours at 150 0c
- At the end of digestion period switch of the reactor and wait for 20 minute to allow the vial to cool to about 120 0c
- Invert each vial several times while still warm, then place them in the test tube Rack
- Leave the vials in the tube rack to cool to room temperature
- Do not shake or invert any more otherwise the sample may become turbid
- Select the program number corresponding to oxygen demand, chemical HR(COD) on the secondary LCD by pressing program increase or decrease symbol
- Place the calibration vial into the holder
- Press zero and 'sip' will blink on the display
- Wait for a few seconds and the display will show '-0.0-' now the meter is ready for measurement
- Remove the calibration vial
- Place the sample vial into the holder and push it completely down
- Press M (measure) direct and measurement displays.
- Samples with higher chloride concentration should be diluted.

1.2 BOD

• Method

- Lovibond BOD system oxidirect
- preparing the sample
- estimate the measurement reagent and select the volume for the sample
- The sample volume is related to the expected BOD value. The oxidirect is designed to operate with the following ranges and samples volume allowing BOD measurement up to 0-4000 mg/L with out any dilution
- for 0-2000 mg/L expected value 56 ml sample volume with 3 drop ATH to avoid nitrification and 3-4 drop KOH

- for 0-4000 mg/l expected value 21.2 ml sample volume with 1 drop ATH to avoid nitrification and 3-4 drop KOH
- carry out the necessary pre treatment of the sample (setting pH value 6.5 to 7.5, filtering)
- the optimum pH value for BOD is between 6.5 and 7.5
- if higher or lower adjust by HCL or H_2SO_4 and NaOH
- mix well and advisable to settle or filter

• Required reagent
  - KOH to absorb CO_2
  - ATH to avoid nitrification

• Materials used
  - BOD bottle
  - BOD sensor
  - BOD bottle rack
  - Incubator
  - Measuring cylinder

• Measurement procedure
  - measure the sample precisely using appropriate over flow and if necessary add nitrification inhibitor (ATH)
  - insert magnetic stirring rod
  - place 3-4 drop of KOH solution into the seal gasket and insert gasket in the neck of the bottle
  - screw the BOD sensors to the sample bottle
  - place the bottle in the bottle rack
  - start the measurement
  - incubate the sample in accordance with the instructions BOD_5 for 5 days at 20 ^0C

1.3 Total Nitrogen

• Method

- Hach method 10072: TNT per-sulfate digestion
- Chromotropic acid method, a persulfate digestion converts all forms of nitrogen to nitrate. Then the reaction between nitrate and the reagents causes a yellow tint in the sample

• Required reagent

- Reagent vial-total nitrogen hydroxide reagent
- Total nitrogen per-sulfate powder pillows
- Total nitrogen reagent A powder pillows
- Total nitrogen reagent B powder pillows
- Deionized water

• Materials used

- Hann reactor
- Hach data logging colorimeter, spectrophotometer model DR / 870
- Measuring cylinder
- Pipette

• Measurement procedure

✓ Reagent blank correction

- This method needs a reagent blank correction
- A single vial may be used more than once
- The blank vial is stable up to one week (room temperature)
- For most accurate measurement, run a blank for each set of measurements and always use the same lot of reagents for blank and sample
- Choose a homogeneous sample
- Preheat the Hann reactor to 105 ^{o}C
- Prepare a sample to the required dilution factor
- Remove the cap from two digestion vials

- Add the content of one packet of total nitrogen per-sulfate reagent powder pillows
- Add exactly 0.5 ml of sample to one vial (sample vial)
- Add exactly 0.5 ml of deionized water to the other (blank vial)
- Replace the cap tightly and shake vigourously the vials for about 30 seconds until all the powder is completely dissolved
- Insert the vials into the reactor and heat them for 30 minute at 105 ^{0}C
- At the end of digestion period switch of the reactor and place the vial in test tube rack after digestion to cool at room temperature
- Select the program number corresponding to total nitrogen on the secondary LCD by pressing program increase or decrease symbol
- Remove the cap from the vials and add the content of one packet of total nitrogen reagent powder pillow to each vial. Replace the cap tightly and shake gently the vials for 15 seconds
- Wait for 3 minute without shaking the vials to allow the reaction to complete
- Remove the cap from the vials and add the content of one packet of total nitrogen
- Reagent B powder pillows to each vial. Replace the cap tightly and shake gently the vials for 15 seconds
- Wait for 2 minute without shaking to allow the reaction to complete
- Remove the cap from two other reagent vials
- Add exactly 2 ml of digested sample from the digested sample vial to one reagent vial (sample vial), and 2 ml of digested blank to other reagent vial (blank vial) while keeping the vial at 45 degree angle Replace the cap tightly and invert the vials 10 times
- Place the blank vial into the holder and push it completely down
- Press timer and the display show the countdown prior to the measurement, alternatively, wait for 5 minute and pres zero in both case 'sip' will blink on the display

- The display will show '-0.0-' now the meter is zeroed and ready for measurement Remove the blank vial
- Place the sample vial into the holder and push it completely down
- Press read direct and 'sip' will blink during measurement
- Instrument directly displays concentration in mg/l of total nitrogen on the liquid crystal display
    - To convert the reading to NH_3, multiply by 1.22
    - To convert the reading to NO_3, multiply by 4.43

- Interference
    - Bromide above 240 mg/L
    - Chloride above 3000 mg/L

TS and VS

• Method

- APHA Method, 2540G

• Apparatus used

- Evaporating dishes
- Desiccator, provided with a desiccant containing a color indicator of moisture
- Drying oven operating at 103-105 ^{0}C
- Analytical balance
- Graduated cylinder
- Beaker
- Muffle furnace for operating at 550 ^{0}C

• Measuring procedure

✓ Preparation of evaporating dish

- Ignite clean evaporating dish at 550 ^{0}C for 1h in a muffle furnace for VS
- Heat clean dish to 103 to 105 ^{0}C for 1h
- Store and cool dish in desiccator until needed

- Weigh immediately before use

✓ Sample analysis

- Pipet a measured volume of well mixed sample to a preweighed dish
- Evaporate to dryness on drying oven
- If necessary add successive sample portions to the same dish after evaporation
- Cool dish in desiccator to balance temperature, and weigh
- Ignite the residue produced by method 2540B in a muffle furnace
- Transfer to a desiccator for final cooling in a dry atmosphere

1.4 Moisture content, Total Solid (TS) and Volatile Solids (VS)

$$Moisture\ contect\ (MC)\ \% = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}}$$

$$Total\ Solid\ (TS)\ \% = \frac{\text{net mass of sample after drying}}{\text{net mass of sample before drying}}$$

$$Total\ solid\ \% = 100\% - Moisture\ contect\%$$

$$Volatile\ solid\ (VS)\ \% = \frac{\text{net mass of sample after ignition at 550 oc}}{\text{net mass of sample before ignition}}$$

Example Reactor 1 feed total solid content (%) can be calculated as:

Mass of crucible =22.865 gram

Mass of crucible +sample before drying = 40.102 gram

Mass of crucible +sample after drying = 23.619

Net mass before drying = Mass of crucible +sample before drying- Mass of crucible

= 40.102-22.865

=17.237 gram

Net mass after drying = Mass of crucible +sample after drying- Mass of crucible

= 23.619-22.865

=0.754 gram

$$Moisture\ contect\ (MC)\ \% = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}}$$

$$Moisture\ contect\ (MC)\% = \frac{17.237\ \text{gram} - 0.754\ \text{gram}}{17.237\ \text{gram}} * 100\% = 95.63\ \%$$

% TS = 100 – 95.63 = 4.37 % or

$$Total\ Solid\ (TS)\ \% = \frac{\text{net mass of sample after drying}}{\text{net mass of sample before drying}}$$

$$Total\ Solid\ (TS)\% = \frac{0.754\ \text{gram}}{17.237\ \text{gram}} * 100\ \%$$

$$=4.37\ \%$$

Weight of sample before 550 ^{o}C = 23.398 gram

Weight of sample after 550 ^{o}C = 21.657 gram

$$Volatile\ solid\ (VS)\ \% = \frac{\text{net mass of sample after ignition at 550 oc}}{\text{net mass of sample before ignition}}$$

$$Volatile\ solid\ (VS)\% = \frac{21.657\ gram}{23.398\ \text{gram}} * 100\%$$

$$= 92.55\ \%$$

Annex 2: Statistical analysis

Total gas production in m3/day with respect to mix ratio as co-substrate

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100% OFMSW, R1	27	6.991	0.258925926	0.002882456
66.7% OFMSW, R2	27	6.124	0.226814815	0.000875003
0% OFMSW, R3	27	9.392	0.347851852	0.005426208
50% OFMSW, R4	27	12.171	0.450777778	0.019275256
33.3% OFMSW, R5	27	12.701	0.470407407	0.010876866

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.299327	4	0.324831804	41.28960045	2.66E-22	2.44135
Within Groups	1.022731	130	0.007867158			
Total	2.322058	134				

Methane production in m3/day with respect to mix ratio as co-substrate

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100% OFMSW, R1	27	1.932	0.071555556	0.000673487
66.7% OFMSW, R2	27	1.932	0.071555556	0.000396179
0% OFMSW, R3	27	3.652	0.135259259	0.000393892
50% OFMSW, R4	27	3.694	0.136814815	0.002824234
33.3% OFMSW, R5	27	4.615	0.170925926	0.001648994

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.209633	4	0.052408222	44.13854513	2.33E-23	2.44135
Within Groups	0.154356	130	0.001187357			

Specific Methane production m3/kg VS added with respect to mix ratio as co-substrate

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100% OFMSW, R1	27	6.768	0.251	0.003554538
66.7% OFMSW, R2	27	6.292	0.233	0.004190037
0% OFMSW, R3	27	6.026	0.223	0.001071157
50% OFMSW, R4	27	7.726	0.286	0.012354131
33.3% OFMSW, R5	27	9.536	0.353	0.007046541

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.299952	4	0.074988104	13.28803313	4.18E-09	2.44135
Within Groups	0.733627	130	0.005643281			
Total	1.033579	134				

Hydrolysis Performance Analysis by BOD increment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0% Acid Conc.	8	327800	40975	291639285.7
1% Acid Conc.	8	372600	46575	328485000
3% Acid Conc.	8	399200	49900	434480000
5% Acid Conc.	8	502200	62775	758062142.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.05E+09	3	683677916.7	1.508667907	0.233914	2.946685
Within Groups	1.27E+10	28	453166607.1			
Total	1.47E+10	31				

Methane composition with respect to mix ratio as co-substrate

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100% OFMSW, R1	9	2.22	0.246666667	0.000675
66.7% OFMSW, R2	9	2.81	0.312222222	0.003944444
0% OFMSW, R3	9	3.57	0.396666667	0.004525
50% OFMSW, R4	9	2.71	0.301111111	0.002136111
33.3% OFMSW, R5	9	3.33	0.37	0.0082

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.126276	4	0.031568889	8.102666477	6.97E-05	2.605975
Within Groups	0.155844	40	0.003896111			
Total	0.28212	44				

Specific Methane production m3/kg VS removed with respect to mix ratio as co-substrate

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100% OFMSW, R1	27	13.74	0.509	0.014626026
66.7% OFMSW, R2	27	9.581	0.355	0.009703746
0% OFMSW, R3	27	10.103	0.374	0.003011695
50% OFMSW, R4	27	11.704	0.433	0.028360028
33.3% OFMSW, R5	27	16.205	0.600	0.020326387

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.107112	4	0.276778085	18.20240638	6.79E-12	2.44135
Within Groups	1.976725	130	0.015205577			
Total	3.083837	134				

Annex 3: Experimental setups and Equipments

Direct measurements the composition of biogas using the apparatus SR2-BIO gas analyzer for different mix of substrate.

SR2-BIO, Gas analyzer,

- Directly connect to the gas holder/ digester to measure the gases (CH_4 in Vol %, CO_2 in Vol %, O_2 Vol%, H_2S in PPM)

Measurements of the COD value of the different mix of substrate using NANOCOLOR multiple parameters.

Photometer Nanocolor multiple parameter
MACHERY-NAGEL,

- In this experiment the apparatus used to determine the COD (mg/L) value of the feedstock both for hydrolysis and methanation stage

Oven: (Drying oven)
NEMMERT

- In this experiment drying oven is used to determine the Total Solid (TS), and Moisture content of the substrate and also a stepping stone to measure Volatile matter/solid VS.

Digital BOD incubator,

Lovibond BOD system oxidirect.

The method consists of filling with sample, to overflowing, an airtight bottle of the specified size and incubating it at the specified temperature for 5 day. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. Because the initial DO is determined shortly after the dilution is made, all oxygen uptake occurring after this measurement is included in the BOD measurement.

Hann digester:

- In this thesis work it is used to digest the required samples when analyzing the COD, Total Nitrogen over different temperature range.

pH meter:

The acid concentration in aqueous systems is expressed by the pH value, i.e. the concentration of hydrogen ions. At neutral conditions, water contains a concentration of 10^{-7} hydrogen ions and has a pH of 7. Acid solutions have a pH less than 7 while alkaline solutions are at a pH higher than 7

Furnace: H JURGENS & CO. BREMEN

- Used for the determination of the Volatile solid of the sample which operate at 550 ^{o}c

Measuring balance

- To weight the sample for analysis of TS, VS, and soon…

Hach, DATALOGGING colorimeter (DR/870)

- Used to measure the total nitrogen (TKN) in the sample.

Annex 4: Experimental Results

Total Gas rate (m3/day)					
Days	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5
Day 1	0.210	0.168	0.601	1.006	0.931
Day 2	0.026	0.223	0.557	0.792	0.400
Day 3	0.175	0.144	0.423	0.492	0.441
Day 4	0.231	0.174	0.358	0.325	0.553
Day 5	0.288	0.237	0.383	0.502	0.567
Day 6	0.288	0.221	0.325	0.423	0.439
Day 7	0.288	0.223	0.365	0.460	0.488
Day 8	0.293	0.232	0.339	0.506	0.527
Day 9	0.307	0.232	0.369	0.458	0.541
Day 10	0.286	0.233	0.336	0.445	0.436
Day 11	0.269	0.218	0.330	0.390	0.446
Day 12	0.276	0.251	0.304	0.425	0.393
Day 13	0.279	0.242	0.360	0.444	0.502
Day 14	0.279	0.235	0.318	0.400	0.458
Day 15	0.277	0.238	0.324	0.408	0.467
Day 16	0.272	0.259	0.311	0.392	0.450
Day 17	0.274	0.239	0.295	0.381	0.441
Day 18	0.266	0.299	0.314	0.388	0.443
Day 19	0.249	0.216	0.325	0.407	0.441
Day 20	0.274	0.235	0.321	0.376	0.446
Day 21	0.258	0.232	0.304	0.376	0.446
Day 22	0.288	0.204	0.314	0.395	0.404
Day 23	0.256	0.214	0.312	0.400	0.372
Day 24	0.273	0.224	0.300	0.396	0.406
Day 25	0.266	0.236	0.304	0.396	0.409
Day 26	0.274	0.253	0.275	0.393	0.441
Day 27	0.269	0.242	0.325	0.395	0.413

Cumulative Total gas production					
Days	**Reactor 1**	**Reactor 2**	**Reactor 3**	**Reactor 4**	**Reactor 5**
Day 1	0.21	0.168	0.601	1.006	0.931
Day 2	0.236	0.391	1.158	1.798	1.331
Day 3	0.411	0.535	1.581	2.290	1.772
Day 4	0.642	0.709	1.939	2.615	2.325
Day 5	0.930	0.946	2.322	3.117	2.892
Day 6	1.218	1.167	2.647	3.540	3.331
Day 7	1.506	1.390	3.012	4.000	3.819
Day 8	1.799	1.622	3.351	4.506	4.346
Day 9	2.106	1.854	3.720	4.964	4.887
Day 10	2.392	2.087	4.056	5.409	5.323
Day 11	2.661	2.305	4.386	5.799	5.769
Day 12	2.937	2.556	4.690	6.224	6.162
Day 13	3.216	2.798	5.050	6.668	6.664
Day 14	3.495	3.033	5.368	7.068	7.122
Day 15	3.772	3.271	5.692	7.476	7.589
Day 16	4.044	3.530	6.003	7.868	8.039
Day 17	4.318	3.769	6.298	8.249	8.480
Day 18	4.584	4.068	6.612	8.637	8.923
Day 19	4.833	4.284	6.937	9.044	9.364
Day 20	5.107	4.519	7.258	9.420	9.810
Day 21	5.365	4.751	7.562	9.796	10.256
Day 22	5.653	4.955	7.876	10.191	10.660
Day 23	5.909	5.169	8.188	10.591	11.032
Day 24	6.182	5.393	8.488	10.987	11.438
Day 25	6.448	5.629	8.792	11.383	11.847
Day 26	6.722	5.882	9.067	11.776	12.288
Day 27	6.991	6.124	9.392	12.171	12.701

Methane composition, CH4 Vol %					
Days	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5
3	0.190	0.260	0.300	0.340	0.290
6	0.250	0.260	0.310	0.260	0.250
9	0.220	0.270	0.340	0.270	0.270
12	0.250	0.280	0.440	0.260	0.330
15	0.270	0.270	0.500	0.270	0.410
18	0.260	0.390	0.430	0.290	0.470
21	0.270	0.420	0.390	0.280	0.510
24	0.260	0.290	0.440	0.360	0.390
27	0.250	0.370	0.420	0.380	0.410

Carbon dioxide composition, CO2 Vol %					
Days	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5
3 days	0.740	0.692	0.647	0.604	0.657
6 days	0.689	0.688	0.634	0.678	0.698
9 days	0.722	0.681	0.612	0.665	0.673
12 days	0.685	0.678	0.509	0.674	0.596
15 days	0.662	0.686	0.444	0.659	0.541
18 days	0.674	0.565	0.518	0.642	0.482
21 days	0.664	0.541	0.555	0.650	0.439
24 days	0.666	0.673	0.506	0.565	0.557
27 days	0.672	0.537	0.531	0.553	0.537

CH4 Production, m3/day					
Days	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5
Day 1	0.040	0.044	0.180	0.342	0.270
Day 2	0.005	0.058	0.167	0.269	0.116
Day 3	0.033	0.037	0.127	0.167	0.128
Day 4	0.058	0.045	0.111	0.085	0.138
Day 5	0.072	0.062	0.119	0.131	0.142
Day 6	0.072	0.057	0.101	0.110	0.110
Day 7	0.063	0.060	0.124	0.124	0.132
Day 8	0.064	0.063	0.115	0.137	0.142
Day 9	0.068	0.063	0.125	0.124	0.146
Day 10	0.072	0.065	0.148	0.116	0.144
Day 11	0.067	0.061	0.145	0.101	0.147
Day 12	0.069	0.070	0.134	0.111	0.130
Day 13	0.075	0.065	0.180	0.120	0.206
Day 14	0.075	0.063	0.159	0.108	0.188
Day 15	0.075	0.064	0.162	0.110	0.191
Day 16	0.071	0.101	0.134	0.114	0.212
Day 17	0.071	0.093	0.127	0.110	0.207
Day 18	0.069	0.117	0.135	0.113	0.208
Day 19	0.067	0.091	0.127	0.114	0.225
Day 20	0.074	0.099	0.125	0.105	0.227
Day 21	0.070	0.097	0.119	0.105	0.227
Day 22	0.075	0.059	0.138	0.142	0.158
Day 23	0.067	0.062	0.137	0.144	0.145
Day 24	0.071	0.065	0.132	0.143	0.158
Day 25	0.067	0.087	0.128	0.150	0.168
Day 26	0.069	0.094	0.116	0.149	0.181
Day 27	0.067	0.090	0.137	0.150	0.169

Cumulative CH4 Production, m3/day					
Days	**Reactor 1**	**Reactor 2**	**Reactor 3**	**Reactor 4**	**Reactor 5**
Day 1	0.040	0.044	0.180	0.342	0.270
Day 2	0.045	0.102	0.347	0.611	0.116
Day 3	0.078	0.139	0.474	0.778	0.128
Day 4	0.136	0.184	0.585	0.863	0.138
Day 5	0.208	0.246	0.704	0.994	0.142
Day 6	0.280	0.303	0.805	1.104	0.110
Day 7	0.343	0.363	0.929	1.228	0.132
Day 8	0.407	0.426	1.044	1.365	0.142
Day 9	0.475	0.489	1.169	1.489	0.146
Day 10	0.547	0.554	1.317	1.605	0.144
Day 11	0.614	0.615	1.462	1.706	0.147
Day 12	0.683	0.685	1.596	1.817	0.130
Day 13	0.758	0.750	1.776	1.937	0.206
Day 14	0.833	0.813	1.935	2.045	0.188
Day 15	0.908	0.877	2.097	2.155	0.191
Day 16	0.979	0.978	2.231	2.269	0.212
Day 17	1.050	1.071	2.358	2.379	0.207
Day 18	1.119	1.188	2.493	2.492	0.208
Day 19	1.186	1.279	2.620	2.606	0.225
Day 20	1.260	1.378	2.745	2.711	0.227
Day 21	1.330	1.475	2.864	2.816	0.227
Day 22	1.405	1.534	3.002	2.958	0.158
Day 23	1.472	1.596	3.139	3.102	0.145
Day 24	1.543	1.661	3.271	3.245	0.158
Day 25	1.671	1.748	3.399	3.395	0.168
Day 26	1.803	1.842	3.515	3.544	0.181
Day 27	1.932	1.932	3.652	3.694	0.169

CH4 production (m3) Per Kg of VS added					
Days	R1	R2	R3	R4	R5
Day1	0.155	0.143	0.297	0.715	0.558
Day2	0.019	0.189	0.276	0.563	0.24
Day3	0.128	0.121	0.210	0.349	0.264
Day4	0.225	0.147	0.183	0.178	0.285
Day5	0.279	0.202	0.196	0.274	0.293
Day6	0.279	0.186	0.167	0.230	0.227
Day7	0.244	0.195	0.205	0.259	0.273
Day8	0.248	0.205	0.190	0.287	0.293
Day9	0.264	0.205	0.206	0.259	0.302
Day10	0.279	0.212	0.244	0.243	0.298
Day11	0.260	0.199	0.239	0.211	0.304
Day12	0.267	0.228	0.221	0.232	0.269
Day13	0.291	0.212	0.297	0.251	0.426
Day14	0.291	0.205	0.262	0.226	0.388
Day15	0.291	0.208	0.267	0.230	0.395
Day16	0.275	0.329	0.221	0.238	0.438
Day17	0.275	0.303	0.210	0.230	0.428
Day18	0.267	0.381	0.223	0.236	0.43
Day19	0.260	0.296	0.210	0.238	0.465
Day20	0.287	0.322	0.206	0.220	0.469
Day21	0.271	0.316	0.196	0.220	0.469
Day22	0.291	0.192	0.228	0.297	0.326
Day23	0.260	0.202	0.226	0.301	0.3
Day24	0.275	0.212	0.218	0.299	0.326
Day25	0.260	0.283	0.211	0.314	0.347
Day26	0.267	0.306	0.191	0.312	0.374
Day27	0.260	0.293	0.226	0.314	0.349

Cumulative CH4 production (m3) Per Kg of VS added					
Days	**Reactor 1**	**Reactor 2**	**Reactor 3**	**Reactor 4**	**Reactor 5**
Day 1	0.155	0.143	0.297	0.715	0.558
Day 2	0.174	0.189	0.573	1.278	0.798
Day 3	0.302	0.121	0.783	1.627	1.062
Day 4	0.527	0.147	0.966	1.805	1.347
Day 5	0.806	0.202	1.162	2.079	1.640
Day 6	1.085	0.186	1.329	2.309	1.867
Day 7	1.329	0.195	1.534	2.568	2.140
Day 8	1.577	0.205	1.724	2.855	2.433
Day 9	1.841	0.205	1.930	3.114	2.735
Day 10	2.120	0.212	2.174	3.357	3.033
Day 11	2.380	0.199	2.413	3.568	3.337
Day 12	2.647	0.228	2.634	3.800	3.606
Day 13	2.938	0.212	2.931	4.051	4.032
Day 14	3.229	0.205	3.193	4.277	4.420
Day 15	3.520	0.208	3.460	4.507	4.815
Day 16	3.795	0.329	3.681	4.745	5.253
Day 17	4.070	0.303	3.891	4.975	5.681
Day 18	4.337	0.381	4.114	5.211	6.111
Day 19	4.597	0.296	4.324	5.449	6.576
Day 20	4.884	0.322	4.530	5.669	7.045
Day 21	5.155	0.316	4.726	5.889	7.514
Day 22	5.446	0.192	4.954	6.186	7.840
Day 23	5.706	0.202	5.180	6.487	8.140
Day 24	5.981	0.212	5.398	6.786	8.466
Day 25	6.241	0.283	5.609	7.100	8.813
Day 26	6.508	0.306	5.800	7.412	9.187
Day 27	6.768	0.293	6.026	7.726	9.536

CH4 production (m3) Per Kg of VS removed					
Days	R1	R2	R3	R4	R5
Day1	0.315	0.218	0.498	1.083	0.948
Day2	0.039	0.288	0.463	0.853	0.408
Day3	0.260	0.184	0.352	0.529	0.449
Day4	0.457	0.224	0.307	0.270	0.484
Day5	0.566	0.308	0.329	0.415	0.498
Day6	0.566	0.283	0.280	0.348	0.386
Day7	0.495	0.297	0.344	0.392	0.464
Day8	0.503	0.312	0.319	0.435	0.498
Day9	0.536	0.312	0.345	0.392	0.513
Day10	0.566	0.323	0.409	0.368	0.506
Day11	0.528	0.303	0.401	0.320	0.517
Day12	0.542	0.347	0.370	0.351	0.457
Day13	0.591	0.323	0.498	0.380	0.724
Day14	0.591	0.312	0.439	0.342	0.659
Day15	0.591	0.317	0.448	0.348	0.671
Day16	0.558	0.501	0.370	0.361	0.744
Day17	0.558	0.461	0.352	0.348	0.727
Day18	0.542	0.580	0.374	0.358	0.731
Day19	0.528	0.451	0.352	0.361	0.790
Day20	0.583	0.490	0.345	0.333	0.797
Day21	0.550	0.481	0.329	0.333	0.797
Day22	0.591	0.292	0.382	0.450	0.554
Day23	0.528	0.308	0.379	0.456	0.510
Day24	0.558	0.323	0.365	0.453	0.554
Day25	0.528	0.431	0.354	0.476	0.590
Day26	0.542	0.466	0.320	0.473	0.636
Day27	0.528	0.446	0.379	0.476	0.593

Cumulative CH4 production (m3) Per Kg of VS removed					
Days	**Reactor 1**	**Reactor 2**	**Reactor 3**	**Reactor 4**	**Reactor 5**
Day 1	0.315	0.218	0.498	1.083	0.948
Day 2	0.354	0.506	0.961	1.936	1.356
Day 3	0.614	0.690	1.313	2.465	1.805
Day 4	1.071	0.914	1.620	2.735	2.289
Day 5	1.637	1.222	1.949	3.150	2.787
Day 6	2.203	1.505	2.229	3.498	3.173
Day 7	2.698	1.802	2.573	3.890	3.637
Day 8	3.201	2.114	2.892	4.325	4.135
Day 9	3.737	2.426	3.237	4.717	4.648
Day 10	4.303	2.749	3.646	5.085	5.154
Day 11	4.831	3.052	4.047	5.405	5.671
Day 12	5.373	3.399	4.417	5.756	6.128
Day 13	5.964	3.722	4.915	6.136	6.852
Day 14	6.555	4.034	5.354	6.478	7.511
Day 15	7.146	4.351	5.802	6.826	8.182
Day 16	7.704	4.852	6.172	7.187	8.926
Day 17	8.262	5.313	6.524	7.535	9.653
Day 18	8.804	5.893	6.898	7.893	10.384
Day 19	9.332	6.344	7.250	8.254	11.174
Day 20	9.915	6.834	7.595	8.587	11.971
Day 21	10.465	7.315	7.924	8.920	12.768
Day 22	11.056	7.607	8.306	9.370	13.322
Day 23	11.584	7.915	8.685	9.826	13.832
Day 24	12.142	8.238	9.050	10.279	14.386
Day 25	12.670	8.669	9.404	10.755	14.976
Day 26	13.212	9.135	9.724	11.228	15.612
Day 27	13.740	9.581	10.103	11.704	16.205