

**Utilization of food waste and pine needle for generation of
methane**

A Thesis

*Submitted in the partial fulfillment of the requirement of the award of
degree of*

MASTER OF TECHNOLOGY

IN

CIVIL ENGINEERING

With specialization in

ENVIRONMENTAL ENGINEERING

By

Amit Ratol (162755)

Under the supervision of

Dr. Ashish Kumar

(Associate Professor)

&

Dr. Sudhir Kumar

(Professor)



JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

WAKNAGHAT SOLAN – 173234

HIMACHAL PRADESH, INDIA

May-2018

CERTIFICATE

This is to certify that the work which is being presented in the report title "**Utilization of food waste and pine needle for generation of methane**" in partial fulfillment of the award of degree of Master of Technology in Environmental Engineering and submitted in Department of Civil Engineering, Jaypee university of Information technology, Wakhnaghat is an authentication record of work carried by **Amit Ratol** during a period from August 2017 to May 2018 under the supervision of **Dr. Ashish Kumar** Associate Professor, Department of Civil Engineering and **Dr. Sudhir Kumar** Professor, Department of Biotech & Bioinformatics, Jaypee University of Information Technology, Wakhnaghat, Solan.

The above statement is made correct to the best of my knowledge.

Date:

| | | | |
|-----------------------|---------------------|------------------|-------------------|
| Dr. Ashok Kumar Gupta | Dr. Ashish Kumar | Dr. Sudhir Kumar | External Examiner |
| Professor & H.O.D | Associate Professor | Professor | |
| Civil Engineering | Civil Engineering | BI & BT | |
| Department | Department | Department | |
| JUIT Wakhnaghat | JUIT Wakhnaghat | JUIT Wakhnaghat | |

DECLARATION

I hereby declare that the work reported in this project entitled”**Utilization of food waste and pine needle for generation of methane**” submitted at Department of Civil Engineering Jaypee University of Information Technology, Waknaghat is an authentic record of my work carried under the supervision **Dr. Ashish Kumar** AssociateProfessor, Department of Civil Engineering and **Dr. Sudhir Kumar** Professor, Department of Biotech & Bioinformatics. I have not submitted this work elsewhere for any other degree.

Amit Ratol

(162755)

Abstract

The main objective of this study is to examine the co-digestion of food waste, cow dung and pine needle. When we discuss about the main problems now days, we come across to the most crucial problem, which is depletion of non-conventional energy sources. Anaerobic digestion of food waste, cow dung and pine needle will not only solve environmental issues but also it emerged as one of the suitable way to generate energy. Besides, anaerobic digestion also helps in reduction in load on landfill sites and reducing risk of wild fires. The substrate are also studied for their physicochemical characteristics, such as pH, Total Solids (TS), Volatile Solid (VS), Total Organic Carbon (TOC), Total Kjeldahl Nitrogen (TKN), and C/N. Three-batch digester AD1, AD2 and AD3 of 45L are used, which consist of cow dung and pine needles. The retention time is 30 day in winter. Apart from that, this thesis includes another study, in which 1L digesters used in order to study difference in methane generation when digesters kept at ambient condition than digesters kept in incubator. Food waste and Pine Needles co-digested in this study. The retention time is 30 days. The initial and final values of change in characteristic of slurry mixture before and after the degradation process for various physiochemical parameter like pH, total solids, volatile solid, temperature, alkalinity, chemical oxygen demand were calculated. The result of this study shows that mixing of pine needle to food waste and cow dung increased the methane generation.

TABLE OF CONTENTS

| | |
|---|-------------|
| CERTIFICATE | I |
| DECLARATION..... | II |
| Abstract..... | III |
| LIST OF TABLES | VII |
| LIST OF FIGURES | VIII |
| Chapter 1..... | 1 |
| Introduction | 1 |
| 1.1 General | 1 |
| 1.2 Biogas..... | 2 |
| 1.2.1 Overview of Biogas components | 2 |
| 1.2.2 Substrate..... | 3 |
| 1.2.3 Merits of Biogas..... | 3 |
| 1.2.4 Demerits of Biogas | 4 |
| 1.3 Qualification of organic material as a substrate | 4 |
| 1.3.1 Pine needle as a substrate: | 4 |
| 1.3.2 Food waste as a substrate:..... | 5 |
| 1.3.3 Cow dung as a substrate: | 5 |
| 1.4 Anaerobic digestion | 6 |
| 1.4.1 Factors affecting the methane production..... | 8 |
| 1.5 Objectives;..... | 8 |
| Chapter2..... | 9 |
| Literature review..... | 9 |
| 2.1 General | 9 |
| 2.2 Literature on Biogas Production | 9 |
| 2.3 Summary | 15 |
| Chapter-3 | 16 |
| Experimental setup and methodology..... | 16 |
| 3.1 General | 16 |
| 3.2 Details of experimental setup..... | 16 |

| | | |
|------------------------------|--|-----------|
| 3.2.1 | Design 1 | 16 |
| 3.2.2 | Design 2 | 17 |
| 3.3 | Sample collection and material preparation | 18 |
| 3.3.1 | Inoculum source..... | 18 |
| 3.3.2 | Pine needle | 19 |
| 3.3.3 | Food waste | 19 |
| 3.3.4 | Cow dung..... | 20 |
| 3.4 | Characterization of substrate..... | 20 |
| 3.5 | Methodology | 21 |
| 3.5.1 | Experimental Procedure before and after for Design-1:..... | 21 |
| 3.5.2 | Experimental Procedure before and after for Design-2:..... | 22 |
| 3.6 | Standard testing methods | 24 |
| 3.6.1 | Temperature | 24 |
| 3.6.2 | pH..... | 24 |
| 3.6.3 | Determination of Total solid, volatile solid and Fixed solid | 24 |
| 3.6.4 | Determination of Total organic carbon (TOC)..... | 25 |
| 3.6.5 | Total Kjeldahl nitrogen determination..... | 26 |
| 3.6.6 | Determination of Chemical oxygen demand | 27 |
| 3.6.7 | Determination of Alkalinity..... | 28 |
| Chapter-4 | | 29 |
| Result and discussion | | 29 |
| 4.1 | General | 29 |
| 4.2 | Result of characterization of substrate | 29 |
| 4.3 | Comparison of biogas generation and different parameter used in winter | 30 |
| 4.3.1 | Effect of Variation in pH | 31 |
| 4.3.2 | Effect of COD on biogas production | 32 |
| 4.3.3 | Effect Alkalinity on biogas production..... | 32 |
| 4.3.4 | Effect of total solids and volatile solids..... | 33 |
| 4.3.5 | Effect of temperature | 34 |
| 4.3.6 | Biogas production in winter..... | 35 |
| 4.4 | Comparison of biogas generation during summer | 36 |
| 4.4.1 | Variation in pH with respect of time | 36 |

| | | |
|-------------------|---|-----------|
| 4.4.2 | Variation in Alkalinity | 37 |
| 4.4.3 | Biogas production in summer | 38 |
| Chapter-5 | | 39 |
| Conclusion | | 39 |
| 5.1 | General | 39 |
| 5.2 | Characteristics of substrate: | 39 |
| 5.3 | Biogas generation in winter | 39 |
| 5.4 | Conclusion of biogas generation in summer | 40 |
| References | | |

LIST OF TABLES

| | |
|--|----|
| Table 1.1 Composition of biogas | 2 |
| Table 4.1 Composition of biogas | 29 |
| Table 4.2 Parameter for digester before and after the digestion..... | 30 |

LIST OF FIGURES

| | |
|--|----|
| Figure 1.1 Stages of anaerobic digestion process | 7 |
| Figure 3.1 Anaerobic digestion design 1 | 17 |
| Figure 3.2 Anaerobic digester design 2 | 18 |
| Figure 3.3 Inoculum Source..... | 19 |
| Figure 3.4 Pine needles for biogas production..... | 19 |
| Figure 3.5 Digital Thermometer | 24 |
| Figure 3.6 pH meter | 24 |
| Figure 3.7 Device used in drying sample (a) muffle furnace (b) oven..... | 25 |
| Figure 4.1 Variation in pH in winter..... | 31 |
| Figure 4.2 Variation in COD | 32 |
| Figure 4.3 Variation in Alkalinity..... | 33 |
| Figure 4.4 Variation in total solids | 34 |
| Figure 4.5 Variation in volatile solids | 34 |
| Figure 4.6 variation in temperature in AD1..... | 35 |
| Figure 4.7 Variation in temperature in AD2..... | 35 |
| Figure 4.8 Variation in temperature in AD3..... | 35 |
| Figure 4.9 Overall biogas production (winter) | 36 |
| Figure 4.10 Variation in pH with time..... | 37 |
| Figure 4.11 variation alkalinity in all the digesters | 37 |
| Figure 4.12 Biogas generation in digester set 1 in summer..... | 38 |
| Figure 4.13 Biogas generation in digester set 2 in summer..... | 38 |
| Figure 4.14 Biogas generation in digester set 3 in summer..... | 38 |

Chapter 1

Introduction

1.1 General

World energy consumption increases day by day and it will be increased by a factor two or three in upcoming years. As indicated by the International Energy Agency petroleum products contributed 77.7% of the world's essential energy supply, while sustainable energy just contributes 22.3%. Therefore, there is a very much need for substitution of non-renewable sources of energy by a source that is never-ending, shoddy, and eco-friendly. Generation of biogas from waste like crop residues, food waste, animal waste, and human waste is very economical in a country like India. It is the best alternative to petroleum products, especially in a country like India where a large number of families depend on charcoal and fuel wood for their living, which leads to cutting down trees and promoting deforestation. By virtue of which they emit large amounts of harmful gases in the atmosphere like Carbon Dioxide and Methane. These gases aid in the depletion of the ozone layer by virtue of which the temperature of our environment increases day by day. The emission of carbon dioxide is 10.65 billion tons every year. This is the main reason for rising temperature and global warming. Mr. Piyush Goyal has given a detailed report on biogas plants and biogas generation in the New and Renewable Energy Ministry of India in the Lok Sabha. In 2011 to 2015, there was generation of 20,757 lakh cubic meters of biogas in India, which is 5% of the LPG used in India. Therefore, biogas is a very good alternative to non-renewable source energy.

The forest survey of India reported that 65.52% of the land in Himachal Pradesh is covered with forest. Lately, it is the by far best choice for an endless source of biomass accessible. Anaerobic digestion is the well-known process for the degradation of natural and inorganic matter.

1.2 Biogas

Biogas conceived by fermentation of organic matter by microorganisms in anaerobic digestion. In this process number gases produce, methane has the high composition followed by carbon dioxide and other gases.

Table1.1 Composition of biogas

| S.No. | Typical Biogas Composition | Concentration in terms of volume (%) |
|-------|--------------------------------------|--------------------------------------|
| 1. | Methane (CH ₄) | 55 to 60 % |
| 2. | Carbon dioxide (CO ₂) | 35 to 40 % |
| 3. | Water (H ₂ O) | 2 to 7 % |
| 4. | Hydrogen sulphide (H ₂ S) | 2% |
| 5. | Ammonia (NH ₃) | 0 to 0.05 % |
| 6. | Nitrogen (N) | 0 to 2 % |
| 7. | Oxygen (O ₂) | 0 to 2 % |
| 8. | Hydrogen (H) | 0 to 1 % |

1.2.1 Overview of Biogas components

The basic elements in biogas is given in above Table 1.1

1. Methane and carbon dioxide: The bigger portion of biogas occupied by methane and carbon dioxides and they depend upon various factors discussed below:

a. number of long chain hydrocarbon compounds.

b. The anaerobic digestion of different biomass that is rich in organic matter depends on the retention time.

c. Substrate in the digester is torn down homogeneously so the degradation of organic matter takes place at faster rate.

2. Nitrogen and oxygen: In biogas, the amount of nitrogen and oxygen is in the ratio of 4:1.

3. Carbon monoxide: The amount of carbon monoxide in biogas is 0.2% by volume.

4. Ammonia: The value of ammonia in biogas is very low about 1.5mg/m^3 .

5. Hydrogen sulphide: The concentration of hydrogen sulphide varies in according to process and waste type. The value of hydrogen sulphide is about 0.2% of biogas by volume without desulfurizing step. Because of harmful effects on digester parts it is kept lowest level.

6. Chlorine, Fluorine and Mercaptans: Their concentration in the composition of biogas is very low about 0.1mg/m^3 .

1.2.2 Substrate

Organic materials used as substrates in biogas generation are food waste, animal waste, crop waste, forest litter. Co-digestion of two different substrates enhances the methane generation, also help in balance the nutrient ration in the digester, thus the cumulative volume of biogas increased as compared to the biogas generation at individual level.

Biomass rich in carbohydrates, proteins, fats are the major components in the production of biogas. The selection of substrate depends upon the following factor:

1. No harmful pathogens are present in the substrate.
2. Biogas production depends upon the nutritional value of substrate.
3. Substrate is selected according to their availability in the area.

1.2.3 Merits of Biogas

1. It is renewable energy source so it is never ending source of energy.
2. It also reduce load on landfill sites.
3. It creates jobs for many peoples.
4. It is non-pollutant in nature and no harm to our environment.
5. It is economical in price. It can be used for electricity generation.

1.2.4 Demerits of Biogas

1. Lack of advancement in technology.
2. It is not suitable for densely populated area as of low generation rate of energy.

1.3 Qualification of organic material as a substrate

The main characteristics to be kept in mind for the selection of substrate are carbon and nitrogen. Carbon is the main source of energy while the nitrogen is responsible for growth microorganism. In order to determine the effectiveness of biogas plant we should calculate the value of C/N ratio. In relation with carbon and nitrogen present in organic matter, different substrate has different carbon availability. In household waste, the quantity of carbon is good and can easily be accessed. The ratio of nitrogen need for good growth of microorganisms is 20:30. The overdose of nitrogen i.e. ratio over 10:15 increase the ammonium stack and toxic the reaction. In case of deficiency of nitrogen i.e. above 30, then the retention time of substrate increased and degradation of substrate take longer period than usual. By performing experiment of TOC and TKN, we can calculate the value of C/N ratio of the material. In anaerobic digestion, the C/N ratio of substrate decreases because the carbon is converted into methane and carbon dioxide. For maintaining the balance in biogas process either carbon need to be added or nitrogen need to be removed. To solve this problem co-digestion of tow substrate is recommended.

1.3.1 Pine needle as a substrate:

Lignocelluloses most abundant source of biomass like Pine needle is a very good source of biomass. However, the degradation of pine needle in biogas plant is not done without any pre-treatment or co-digestion. Lignocelluloses have the most amount biomass accessible it has loosely 50% biomass present worldwide. Pine needles cannot be used as fodder for animal because of their lignin structure which very difficult to break that why it cannot be digested by animal, so it is used to be decomposed by burning without any recovery of energy from them. It is also responsible for wildfire in forest. The energy efficient degradation of pine needle in biogas production is very good alternative of nonrenewable source of energy, it also helps in protection over environment from pollution caused by burning of pine needle. In developing nation like India it very source of renewable energy, which is economical and eco-friendly, also invest in

environment protection. The degradation of pine needle alone is very difficult and it longer retention period. For solving this problem co-digestion with another substrate is done, which balances the process of digestion in biogas plant.

1.3.2 Food waste as a substrate:

Food waste covers very large part in our waste stream. In India very large portion of food wasted every day, which was then dumped into landfill site without any energy recovery and increases the load on landfill sites. The food waste mainly composed of uneaten food, food preparation leftover from houses, institution sources like college's canteen, restaurants, hotel and industrial sources like factory mess. As rapid increase in the cost associated with the energy supply and waste disposal and increases public concern with environmental degradation, conversion of food waste to energy is very good and economically in developing country like India. As the moisture content in food waste is very high it is very good substrate in anaerobic digestion. The C/N ration is also very good in food waste. That is very good plus point for biogas generation. The methane production depends upon the value of volatile solid in food waste. The food waste is mainly consisting of organic waste so it is easily degrade in anaerobic digester.

1.3.3 Cow dung as a substrate:

In a developing country like India where agriculture occupies a major and important role in the growth of economy, biogas generation from organic matter under anaerobic condition is a good source of energy. Cow dung is very good source renewable energy as oil prices increase rapidly in now a days. There is also a high risk with unsustainable wood fuel usage. The environment also get degraded by the pollution caused by cow dung as it is disposed openly like odor, air born ammonia, green house gases. These green house gases are responsible for the depletion of ozone layer. Cow dung is very rich in organic matter. C/N ratio is also very good in cow dung. The anaerobic digestion is best suited for cow dung disposal. In India where major portion of population depends upon agriculture and animal husbandry, cow dung is easily accessible. Therefore, cow dung is a very good substrate for biogas generation. Co-digestion of cow dung with other substrate elevate the methane generation in biogas plant.

1.4 Anaerobic digestion

Anaerobic digestion is one of the oldest processes used in generation of biogas generation. It can be used in decomposition of almost any kind of biodegradable organic waste such as plant, animal waste, food waste, waste paper, grass clipping, solids and bio solids.

Anaerobic digestion is collection of process in which organic matter of a substrate degraded by microorganism in the absence of oxygen. As the final product of anaerobic digestion, biogas generated. The composition of biogas mainly consists of methane, carbon dioxide, hydrogen sulphide, ammonia. The composition of biogas depends upon the substrate used and the functioning parameter of process used.

The key steps or processes in anaerobic digestion are:

1. Hydrolysis process
2. Acidogenesis process
3. Acetogenesis process
4. Methanogenesis process

1st Step- Hydrolysis: The hydrolysis is the first step anaerobic digestion process, in which large complex compounds like carbohydrates, lipids and proteins are break down into small compound like amino acids and fatty acids respectively. This is the slowest process in all the steps involve in anaerobic digestion. Cellulases, proteases and lipases exerted by facultative anaerobes are the enzymes in this process. Hydrolysis is slow process not because of less enzymes activity, it is due to the limited access to the surface of feedstock.

2nd Step- Acidogenesis: Acidogenesis is second step in the process, in the end product from stage one are further degraded by facultative anaerobes. The final products at the end of acidogenesis are: volatile fatty acids, such as Butyric acid, propionic acid, acetic acid as well as hydrogen and alcohols. This is the fastest process in the anaerobic digestion. In this process microbial growth rate is very high.

3rd Step- Acetogenesis: In this step the final product acidogenesis is further degraded into methanogenic substrate by the acetogens. It is acid forming stage. In this acids from the acidogenesis stage like propionic acid, ethanol, butyrate are degraded by acetogens for further use as substrate in methanogenesis process in final step of the anaerobic digestion.

4th Step- Methanogenesis: It is the final step in the process of anaerobic digestion in which the production of methane and carbon dioxide taken place by methane forming bacteria using the end product from acidogenic and acetogenic reaction.

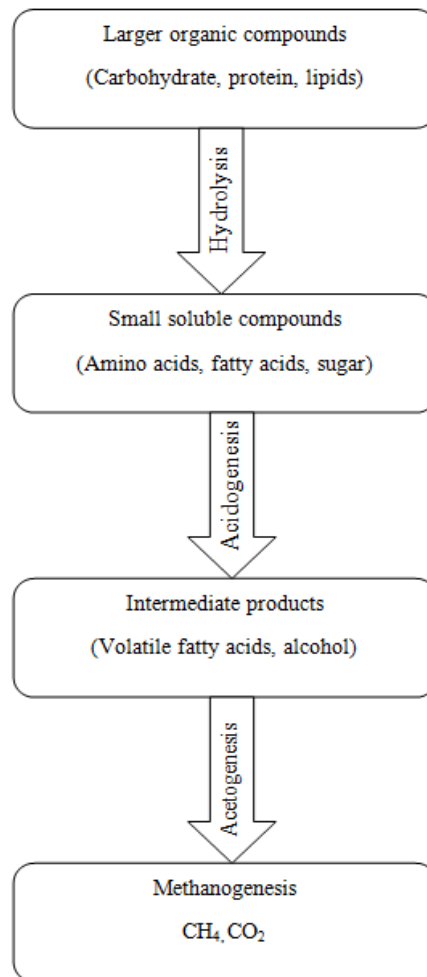


Figure 1.1 Stages of anaerobic digestion process

1.4.1 Factors affecting the methane production

Temperature: Temperature is a very important factor in biogas generation. The best temperature range for biogas process is 30-35°C, because responsible for methane generation are mesophilic which activate at temperature between 30-35°C. Biogas generation can occurs over a wide range of temperature. If the temperature goes below 32°C, more attention to alkalinity and volatile ratio acid is given.

pH and alkalinity: Anaerobic bacteria can be classified into two categories i.e. acidogens and methanogens. The growth of acidogens takes place in pH range is 5.5 to 6.5 and for methanogens, the pH ranges between 7.8 to 8.2. Therefore optimum pH range for biogas production is 6.8 to 7.4. Methanogens is very sensitive to pH factor as compared to acidogens. If pH increases, there is increase in ammonia toxicity. This instability in pH is due to the accumulation of ammonia and volatile fatty acids. The regular monitoring of pH value and taking control measure within the optimum growth of microorganisms reduce the risk of ammonia toxicity.

1.5 Objectives;

The main objective of this thesis are ;

1. Characterization of substrate i.e. food waste, pine needles and cow dung.
2. To find the optimum ratio of volatile solid of food waste and pine needle for maximum production of methane.
3. To find the optimum ratio of volatile solid of cow dung and pine needle for maximum production of methane.
4. To study the influence of temperature on biogas production.

Chapter2

Literature review

2.1 General

Biogas production is a quit hot research topic now a day's therefore there is very large amount of literature is available. The literature review is a collection of study done on the topic, including research articles published in national and international journals. This study is on;

1. Characterization of substrate i.e. food waste, pine needles and cow dung.
2. To find the best suited ratio of volatile solid of food waste and pine needle for maximum production of methane.
3. To find the best suited ratio of volatile solid of cow dung and pine needle for maximum production of methane.
4. To study the influence of temperature on biogas production.

2.2 Literature on Biogas Production

Ruihong Zhang et.al.,(2007) conduct a study for the characterization of food waste for the feedstock for the anaerobic digester. In this experiment, the variation various properties of food waste on daily and weekly evaluated. The anaerobic batch digester used at 50°C for digestion and biogas generation. Average of daily moisture content and ratio of volatile solid to total solid calculated over the weeklong sampling were 70% and 80%, and weekly average of moisture content and ratio of volatile solid to total solid were 74% & 87%. Food waste is a balanced in nutrient for anaerobic microorganisms as evaluate by the nutrient content analysis. Methane generation after 10th and 28th days of digestion is about 348 and 435mL/g VS. 81%, of volatile solid was consumed after digestion for 28 days. Therefore, food waste is excellent substrate for anaerobic digestion with high biodegradation.[1]

Guangqing Liu et.al.,(2009) study the aftermath of inoculums ratio on the production of biogas. In this study, biogas generation from food waste and green waste were determined by using anaerobic digester at mesophilic temperature range (35±2°C)

and for thermophilic temperature range ($50 \pm 2^\circ\text{C}$). The laboratory scale anaerobic batch digester used for this experiment. The substrate mixture made up of 50% food waste and 50% green waste, based on volatile solid in them. In thermophilic temperature the test were performed on four different mixture ratio of inoculum and food waste (F/I) i.e. 1:6, 3:1, 4:0 and 5:0 respectively and for mesophilic range the test is performed for only one ratio of food waste and inoculum i.e. 3:1. The retention time for anaerobic digestion is 25 days. This study shows that the food waste and inoculum ratio has significant affect on rate biogas generation. In thermophilic temperature range the generation of biogas in all the four ratio of F/I was 778, 742, 784 and 396 mL/g VS for food waste , 631, 529, 524 and 407 mL/g VS for green waste. Similarly biogas generation for mix of food waste and green waste was 716, 613, 671 and 555 mL/g VS. The biogas generation in mesophilic temperature was 430, 372 and 358 mL/g VS. The biogas production is lesser in mesophilic temperature digestion of food waste and green waste as compare to thermophilic temperature digestion. [2]

Hamed El-Mashad et.al., (2010) study to determine the biogas production of different mixture of dairy manure and food waste and also evaluate biogas production alone by food waste. Also determine the difference in production by co-digestion and alone by food waste and dairy manure. Batch digester used for anaerobic digestion. The organic loading rate of food waste and the mixture is 2, 3 and 5gVS/L. The retention time for digestion is 30 days. The average percentage of gas generated was 62% and 59% respectively. The food waste co-digested with dairy manure elevate the methane generation. The kinetic model developed also applied to determine the biogas generation for different mixture of food waste and dairy manure. The mixture with 60% of food waste and 40% of dairy manure gives the maximum biogas production. [3]

Zehra Spaci., (2013) conduct a Study to determine biogas generation by pretreatment of agricultural residual. Barley, spring wheat, winter wheat and oat straw was used as substrate in biogas digester. To determine the changes in physiochemical structure of straw on biogas generation, pretreatment of two sample groups are done. The first sample was subjected to include milled straw and the second sample was subjected to comprise milled wet straw that was formulated by addition of deionized water. Then both the sample were placed in microwave at temperature of 200 or 300°C for 15min. 66 identical lab scale anaerobic batch reactor were used in this experiment. The

retention period for digestion is 60 days. The microwave pretreatment of straw did not help in increase the anaerobic digestion. Inverse relationship between thermal conversion yield and overall biogas generation was observed.[4]

DenBrown et.al., (2013) determine the most effective ratio of feedstock and effluent for food waste and yard waste mixing ratio for maximum biogas generation. In this study co-digestion of yard waste and food waste were done at feedstock and effluent for different ratio of 1, 2, and 3 respectively. The percentage of food waste on the bases of dry volatile solid is 0%, 10% and 20%. As a result, methane generation increased. The best gas generation achieved in mix with 10% and 20% percentage of food waste. Co-digestion of food waste and yard waste at specific ratio enhance the digester operating characteristics and boost the biogas production.[5]

Iqbal Syaichurrozi et.al., (2013) study biogas generation of vinasse with total solids percentage of $7.015 \pm 0.007\%$ was determined for broad range of ratio for chemical oxygen demand and total nitrogen. Substrate doped with urea to balance COD/N ratio of 400/7-700/7. Laboratory scale anaerobic batch digester used for study at room temperature for 60 days. The result concluded at controlled COD/N ratio of 400/7, 500/7, 600/7 and 700/7 production of biogas was 107.45, 123.87, 133.82, 139.17, 113.24 mL/g COD and COD removal was 31.274 ± 0.887 , 33.483 ± 0.266 , 36.573 ± 1.689 , 38.088 ± 0.872 , $32.714 \pm 0.881\%$. The substrate with COD/N ratio of 600/7 has maximum biogas production and COD removal. By kinetic model of biogas generation from COD/N ratio of 600/7 had kinetic constant of A, μ and λ of 132.580 mL/g COD, 15.200 mL/g COD.day and 0.213 days.[6]

Xiang Chen et.al., (2014) conduct a study on biogas generation by co-digestion of food waste and green waste. Six mixing ratio of substrate are adopted for biogas generation. As the percentage of food waste feedstock increases results in significant increase in methane yield, while shorter retention time achieved by increasing the green waste percentage in the feedstock. 40:60 ratios of food waste and green waste is preferred for best result in methane generation. After digestion period of 24.5 days, 90% of methane yield was collected, with cumulative methane yield of 272.1 mL/g VS. On the bases of preferred ratio, the effect of total solid content on co-digestion of food waste with green waste was evaluated over a total solid range of 5-25%. The result obtained by this study showed that methane generation from high solid anaerobic fermentation (15-20% TS)

were higher as compare to the methane generation from liquid anaerobic fermentation (5-10%TS), while methanogenesis was constrained by further increase in total solid content to 25%.[7]

Xumeng Ge et.al., (2014) conduct a study for biogas generation from tropical biomass waste by anaerobic digestion. In tropical region anaerobic digestion is a very useful technology for generation of biogas from abundant biomass in tropical region which can further used in production of heat, electricity. The determination of anaerobic digestion for tropical forestry wastes. This study, tropical biomass were evaluate for biogas generation by liquid anaerobic digestion or solid-state anaerobic digestion, depending upon the feedstock's characteristics. Like when alibizia leaves and chips were used as substrate, liquid anaerobic digestion had better methane production (161 and $113 \text{ Lkg}^{-1}\text{VS}$) than solid state anaerobic digestion (156.8 and $59.6 \text{ Lkg}^{-1}\text{VS}$), while solid state anaerobic digestion achieved 5 time higher value of methane production than liquid anaerobic digestion. The Co-digestion and mono digestion of taro skin, taro flesh, papaya and sweet potato achieve biogas production of 345 to 411 Lkg^{-1}VS , reveal the toughness of anaerobic digestion technology. [8]

Garcia. K et.al., (2014) conduct a experimental study on anaerobic digestion of mix of cattle manure and sewage sludge. The main purpose of this study was to compute the suitable composition and temperature for the anaerobic digestion of mix of cattle manure and sewage sludge for maximum production of biogas at mesophilic and thermophilic temperature. As the result of this study the anaerobic digestion of mix of cattle manure and sewage sludge is very good for greater biogas generation. The ratio for maximum methane production is 25% of cattle waste and 75% of raw sludge with value of 62% and 75.7% of COD and DOC removal and methane generation is $306 \text{ CH}_4/\text{g VS}$. [9]

A. Serna-Maza .et.al., (2015) Conduct a study to know the efficiency for removal of ammonia from fresh food waste. Batch anaerobic digester was used for digestion at 35, 55 and 70°C temperature. 0.125 and $0.250 \text{ L}_{\text{biogas}}\text{min}^{-1}\text{L}^{-1}_{\text{digestion}}$ biogas generated with pH control and without pH control of slurry in the digester. For effective ammonia removal was achieve at higher temperature and higher alkaline condition were required, and at 35°C with and without pH control, and at 55°C without controlling pH there was little or no removal of ammonia. The result of this study shows that ammonia removal

from fresh food waste was difficult as compare to ammonia removal from stored food waste for digestion.[10]

Dong Li et.al., (2015) conduct a study to evaluate the effect of feedstock ratio and organic loading rate in anaerobic digestion for rice straw and cow manure as substrate at mesophilic temperature range in a batch digestion of 2.5L capacity. The ratio volatile solids in rice straw and cow manure were 0:1, 1:2, 1:1, 2:1 and 1:0. The organic loading rate of feedstock in the digester was 3.0, 3.6, 4.2, 4.8, 6.0, 8.0 and 12.0kgVS/(m³d). 1:1 was the optimal volatile solid ratio. 383.5L/kgVS biogas produced at organic loading rate of 6kgVS/(m³d). There was accumulation of volatile fatty acids instead of ammonia when the organic loading rate was 12kgVS/(m³d).[11]

Muhammad Rizwan Haider et.al., (2015) study the number co-digestion ratio of food waste and rice husk to overcome the accumulation of volatile fatty acids in digestion of food waste alone. In this study four mixing ratios of food waste and rice husk with C/N ratio value of 20, 25, 30 was taken. Anaerobic batch digester was used in this study at mesophilic temperature. The feedstock with C/N ratio of 20 gives the highest yield of biogas of 584L/kg VS. The result elaborate that as the food waste proportion in the mix increases the biogas yield decreases. Further, fresh cow dung was used as inoculum to determine the best-suited ratio of feedstock and inoculum. In the second experiment, substrate with C/N ratio of 20 was subjected to anaerobic digestion for five different ratios of selected feedstock and inoculum of 0.25, 0.5, 1.0, 1.5 and 2.0 respectively. The maximum output of biogas generation of 557L/kg VS was given by the S/I ratio of 0.25. The accumulation of volatile fatty acid was caused by higher S/I ratio because to higher organic loading.[12]

Seung Gu Shin et.al., (2015) conduct an experimental study on biogas generation to characterize food waste-recycling waste water and also determine the seasonal variation of it. Samples of food waste-recycling waste was collected over the year. The FRW has high chemical oxygen demand (i.e. 148.7±30.5g/L), with carbohydrate (15.6%), protein (19.9%), lipid (41.6%), ethanol (14%) and volatile fatty acids (4.2%). The fermentation of organic matter including carbohydrate the food waste-recycling wastewater was partially (62%) soluble. In this, 50% of organic were converted into volatile fatty acids in beginning of the process. The biogas generated from digestion of food waste-recycling wastewater was 0.562 LCH₄/g VS_{feed}.[13]

Bodius Salam et.al.,(2015) conduct study on biogas production to evaluate the biogas generation by cow dung in mesophilic temperature using silica gel as catalyst. Two Anaerobic batch digester used for experiment one with catalyst and one without catalyst. The digesters are fabricated with glass conical flask of 1liter. 390gm of cow dung was mixed with 310gm of water to prepare slurry for digester. The biogas generated by digester without catalyst was 27.3 L/kg of cow dung and digester with catalyst was 30.5L/kg of cow dung. The retention period for experiment was 76 days at ambient temperature 27-31 °C.[14]

Li-Jie Wu et.al.,(2015) study the feasibility of co-digestion food waste and de-oiled grease trap waste to enhance the production of biogas. For this experiment lab-scale mesophilic digester, a temperature-phased anaerobic digester and a temperature phased anaerobic digester with recycling used. Co-digestion of food and de-oiled grease trap gives biogas production of 19% more in mesophilic digester and temperature phased anaerobic digester with recycling as camper to biogas production alone by food waste. The alkalinity of slurry removed in co-digestion higher as camper to alone in food waste. The mesophilic digester is preferred over temperature phased anaerobic digester and temperature phase anaerobic digester with recycling for degradation of lipids and long chain fatty acids.[15]

Chuanyang Liu .et.al.,(2015)study the co-digestion of food waste with sewage sludge at two different total solid concentration. The biogas production in low-solid group of total solid 4.8% increases as the percentage of food waste in feedstock increased from 0 to 100%, but in this there is no synergetic effect observe between the two substrate. Moreover, the addition of more food waste results in accumulation of volatile fatty acids by result of which biogas production decrease. While the mixing ratio with 50% or less food waste in high solid group with total 14% is preferred. It also helps in weaken the alkaline environment with pH 7.5-8.5 avoiding the excessive acidification but high ammonia concentration is a potential risk. However, in high solid group good synergetic effects was found between the two substrate because of addition of more food waste. Thus, 50% of food waste is the most preferable ratio for the best synergetic effect.[16]

Abhiash Kumar Tripathi (2015) study the production biogas from pine needle and also design cost efficient biogas plant for domestic use for generation of biogas from

pine needles. Cellulose present in pine needle is around 55% making very good source of biomass for energy generation. By the evaluation of result the biogas produce in winter season was 1.4L/Day to 1.9L/day and for summer season it is about 7.3L/day. The concentration of volatile solids also decreases in summer about 64% as compared to winter season.[17]

E.Fathi Aghdam et.al.,(2015)conduct a study on mesophilic anaerobic digestion of organic part of civil strong waste, bio waste, sewage sludge and co-digestion of bio waste and sewage sludge. Cumulative biogas generation from organic fraction of municipal solid waste, bio waste, sewage sludge and co-digestion of bio waste and sewage sludge was about 386 ± 54 , 385 ± 82 , 198 ± 14 and 318 ± 59 L CH₄/kg Vs. The organic loading rate for digester was 1 and 2 kg VS/ m³d. By co-digestion of sewage sludge and bio waste the average biogas production was increased by 61%. By result evaluation, the methane yield alone by bio waste is 12% more than methane production by mechanically treated organic fraction of municipal solid waste.[18]

Paul Thomas et.al.,(2017) this paper evaluate the scope of biomass in Indian economy. Being a agriculture country, India has a very good scope and potential resources from agromonic sector like crop residue, animal waste, food waste etc. At which rate the population rising in India the demand for energy is also growing at very fast rate, so to overcome this demand biogas production from waste is very good alternative. The faster growth of economy of India lead to urbanization which in turn the increases the generation of municipal solid waste. The municipal solid waste is a very rich source of biomass, so it can be used in many innovative ways for conversion to energy like biogas generation.[19]

2.3 Summary

After studying lots of research papers on biogas production from number different substrate. It is clear that very large amount of literature is available on biogas production from cow dung, food waste, agricultural residual, and also on co-digestion with different substrate. However, there is very study is done on co-digestion of cow dung with pine needles and pine needles. So the main motive of this research work was to gain more knowledge about biogas production and providing renewal source of energy.

Chapter-3

Experimental setup and methodology

3.1 General

In this chapter, we explained about the experimental design used, as well as the characterization of substrate used in AD process. The determination of physico-chemical characteristics of substrate i.e. food waste, pine needle and cow dung were done. Batch digesters used for performing biogas production. Use of two type of batch digesters, floating bucket and 1L glass bottle. Physico-chemical parameters of digester slurry initial and final were calculated. Daily reading on pH, temperature and gas collection noted. The detailed experimental design and methodology explained in this chapter.

3.2 Details of experimental setup

3.2.1 Design 1

Batch digester used for anaerobic digestion for lab scale study. The digester was self-fabricated from plastic bucket. The study be composed of three digesters named as AD1, AD2 and AD3 and each was made up of two plastic buckets, one act as the digester and the other as gas collection. The slurry for digester AD1 was composed of 100% of cow dung. The slurry for digester AD2 was composed of 100% of pine needle. Similarly, the slurry for digester AD3 composed of 50% cow dung and 50% pine needle. Then this slurry was tested for different physic-chemical parameter like pH, COD, Alkalinity. This experiment takes place at ambient temperature so temperature of slurry and outside temperature was also noted. The digester bucket capacity is 45L and the gas-collecting bucket is 20L. 40L is the working volume of the digester. The internal diameter of the digester bucket is 0.45m and 0.3m for the gas collector. The digester was fitted with galvanized iron fittings. The fitting consists of ½ inch nipple, ½ inch tank connection nipple, ½ valve and gas cork. The experimental time for study is 30 days in winter. The digester was placed inside the fluvial hydraulics laboratory at civil engineering department in Jaypee University of Information Technology, Waknaghat (H.P).

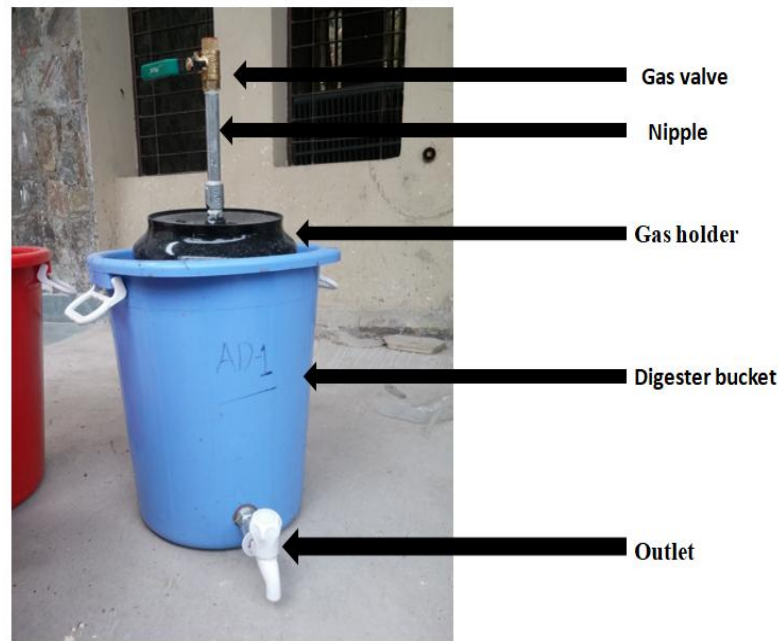


Figure 3.1 Anaerobic digestion design 1

3.2.2 Design 2

Batch digester used for anaerobic digestion for lab scale study. The digester was self fabricated from 1000ml brosil glass bottle. The study be composed of 18 digester, there in controlled temperature and the in ambient temperature. The digester named as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18. These 18 digester are further divided into three batch first, second and third. The first batch comprises of cow dung only. Second batch compose of co-digestion of food waste and cow dung. The third batch consists of cow dung and pine needle. The organic loading rate in the digester is calculated by the volatile solid content in substrate. The organic loading rate in first 4, 5, 6, 7, 8 and 9gmVS of cow dung. The organic loading rate in second batch of digester was 4, 5, 6, 7, 8 and 9gmVS of cow dung and food waste as a mix. The organic loading rate in third batch of digester was 4, 5, 6, 7, 8 and 9gmVS of cow dung and pine needle as a mix. The digester comprise of 100ml capacity. The bottle is fitted with rubber cork at the top and drip pipe. The gas collection is carried out by water raise method with 500ml measuring cylinder. Out of 1000ml, the working area for digester is 900ml. Three digester are placed inside the incubator at controlled temperature of 35°C, and the other three digester are placed inside the Fluvial Hydraulics Laboratory at the Civil

engineering department in Jaypee University of Information Technology, Wakhnaghat (H.P). The experiment time for study is 30 days in summer.



Figure 3.2 Anaerobic digester design 2

3.3 Sample collection and material preparation

The main purpose of this study is determining the biogas potential of co-digestion of two substrates to elevate the biogas production. The selection materials used as substrate in biogas production is mainly depend on the availability and are of no use. Pine needle was gathered from the forest fallen. The food waste is taken from the university mess anapurna. Cow dung is collected from the nearby village. The inoculum is prepared from the biogas plant installed in university.

3.3.1 Inoculum source

The inoculum was collected from the working biogas plant at Jaypee University of Information Technology. This biogas plant consists of two plastic tanks, digester and gas collector. First stir the digester tank for homogenous sample then collect the sample in closed container. Then perform test on it analyze the characteristics of inoculums. Addition of inoculum to the digester slurry was that inoculum has very high biodegradability. It also proved microorganism to start the degradation of substrate. It also helps in biogas production.



Figure 3.3 Inoculum Source

3.3.2 Pine needle

Pine needles co-digested with cow dung. Pine needles collected from surrounding of JUIT campus. Pine needles gathered from the forest and the impurities removed. Pine needles are evergreen trees. It is around 98-160ft tall. Pine needles bark generally red brown. Pine needles have many medicine value found in Himalayan range of Bhutan, Nepal, Kashmir, Sikkim, Tibet and other part of North India. Pine needles have very high lignin, cellulose and hemicelluloses i.e. it takes time to degrade. Pine needles subjected to physical pretreatment by chipping, grinding and milling to reduce cellulose crystallinity. Pine needles are sun dried and then converted into fine powders then sieve through 850 μ .



Figure 3.4 Pine needles for biogas production

3.3.3 Food waste

Food waste co-digested with cow dung. Cow dung was gathered from nearby village Wagnaghat. Food waste covers very large part in our waste stream. In India very large portion of food wasted every day, which was then dumped into landfill site without any

energy recovery and increases the load on landfill sites. The food waste mainly composed of uneaten food, food preparation leftover from houses, institution sources like college's canteen, restaurants, hotel and industrial sources like factory mess. As rapid increase in the cost associated with the energy supply and waste disposal and increases public concern with environmental degradation, conversion of food waste to energy is very good and economically in developing country like India. As the moisture content in food waste is very high it is very good substrate in anaerobic digestion. The C/N ration is also very good in food waste. That is very good plus point for biogas generation. The methane production depends upon the value of volatile solid in food waste. The food waste is mainly consisting of organic waste so it is easily degrade in anaerobic digester.

3.3.4 Cow dung

Cow dung collected from nearby village Waknaghat. It co-digested with pine needle with varying organic loading rate of volatile solid in biogas plant. Cow dung is a very good substrate for biogas plant. In a developing country like India where agriculture occupies a major and important role in the growth of economy, biogas generation from organic matter under anaerobic condition is a good source of energy. Cow dung is very good source renewable energy as oil prices increase rapidly in now a days. There is also a high risk with unsustainable wood fuel usage. The environment also get degraded by the pollution caused by cow dung as it is disposed openly like odor, air born ammonia, green house gases. These green house gases are responsible for the depletion of ozone layer. Cow dung is very rich in organic matter. C/N ratio is also very good in cow dung. The anaerobic digestion is best suited for cow dung disposal. In India where major portion of population depends upon agriculture and animal husbandry, cow dung is easily accessible. Therefore, cow dung is a very good substrate for biogas generation. Co-digestion of cow dung with other substrate elevate the methane generation in biogas plant.

3.4 Characterization of substrate

The determinations of physic-chemical characteristics of substrate are determined by standard experimental methods. pH, TS, VS,FS, are determine by APHA experimental

method. And other test like TOC are carried out by Walkley-black method, TKN determine by Micro Kjeldahl method and C/N calculated by dividing TOC and TKN.

3.5 Methodology

In this study there are two different design used for fermentation of substrate. Then the results were compiling to achieve the objective of study. The digesters consist of mixture of food waste, cow dung and pine needle.

3.5.1 Experimental Procedure before and after for Design-1:

In design-1, three anaerobic digesters used AD1 AD2 and AD3. In AD1, 4kg of cow dung mixed with 27lt of tap water and 1lt of inoculums from working biogas plant in JUIT campus. The water solid ratio in AD1 is 1:7 by weight. The chunk of cow dung is broken and mixed thoroughly to prepare slurry for digester in fermentation bucket. Gasholder bucket placed upside down on the fermentation bucket, and then the gas valve opened so the air in the fermentation tank escape out from fermentation bucket and the gasholder bucket sinks to the bottom of the fermentation bucket. The experimenting period is 30 days in winter at ambient temperature.

In AD2, there is 3kg of pine needle, 1kg of cow dung mixed with 27lt of tab water and 1lt of inoculums. The solid and water ration in AD2 is 1:7 by weight. The pine was grinded and sieves through 850 μ sieve. Then pine needle and cow dung mixed in water thoroughly to prepare slurry for digester in fermentation bucket. Gasholder bucket placed upside down on the fermentation bucket, and then the gas valve opened so the air in the fermentationbucket escaped out from the fermentation bucket and the gasholder bucket sinks to the bottom of the fermentation bucket. The experimenting period is 30 days in winter at ambient temperature.

In AD3, there is 1.5kg pine needle and 2.5kg of cow dung mixed with 27lt of tab water and 1lt of inoculums. The solid to water ratio in AD3 is 1:7 by weight. Pine needle and cow dung mixed thoroughly with water to prepare slurry for digester in fermentation bucket. Gasholder bucket placed upside down on the fermentation bucket, and then the gas valve on the top of gasholder bucket opened so the air in the fermentation bucket escaped out from the fermentation bucket and the gasholder bucket sinks to the bottom

of the fermentation bucket. The experimenting period is 30 days in winter at ambient temperature.

(a) Experimental observation before and after digestion in design-1

The experiment conducted before the digestion of slurry in the digester was evaluated in term of temperature, pH, total solid, volatile solid, fixed solid, alkalinity and COD. The generation biogas is noted on daily bases. The pH of slurry is checked after five day over the period of experiment using pH meter. The temperature of slurry and ambient temperature outside is also noted everyday with the help of digital thermometer.

(b) Biogas measurement

The evolution of biogas generation by anaerobic digestion is noted on daily bases. The measurement of biogas is calculated by the raise in height of the gas-holder by the gas generation this raise in height was then multiplied with $\pi/4d^2$ for the calculation of overall biogas generation. By biogass-5000 sensor, we noted down the percentage of methane and other gas generated by the anaerobic digestion.

3.5.2 Experimental Procedure before and after for Design-2:

In summer season, we uses 18 glass bottle of 1L capacity for anaerobic digestion for lab scale experiment at controlled temperature (35°C). The substrate used in this digester is cow dung, food waste and pine needles. There three batches of bottle comprises of 6 bottles in one batch with different mixture of substrate. The organic loading rate of First batch was 4, 5, 6, 7, 8 and 9gm VS. Cow dung was used as inoculum in the digester. In first batch digester are 1, 2, 3, 4, 5 and 6. The substrate used in first batch is a mixture of food waste and cow dung. The loading rate in digester 1 was 0.8gmVS of food waste and 3.2gmVS of cow dung. Similarly in digester 2 was 1gmVS of food waste and 4gmVS of cow dung. Similarly, in digester 3 were 1.2gmVS of food waste and 4.8gmVS of cow dung. Similarly, in digester 4 were 1.4gmVS of food waste and 5.6gmVS of cow dung. Similarly, in digester 5 were 1.6gmVS of food waste and 6.4gmVS of cow dung. Similarly, in digester 6 were 1.8gmVS of food waste and 7.2gmVS of cow dung. Then bottle are filled with tap water up to 800ml with headspace of 200ml for gas. The bottles are fitted rubber cork at the top. The rubber corks are further fitted with drip pipe for gas collection. The retention period of experiment was 30 days at 35°C.

The second batch of digester consists of 6 bottles. The substrate used in this digester was cow dung and pine needle. Cow dung was used as inoculum in digester. The organic loading rate of second batch was 4, 5, 6, 7, 8 and 9 gm VS respectively. The digester used in second batch was 7, 8, 9, 10, 11 and 12 respectively. The loading rate in digester 7 was 0.8 gm VS of pine needle and 3.2 gm VS of cow dung. Similarly loading rate in digester 8 was 1 gm VS of pine needles and 4 gm VS of cow dung. Similarly loading rate in digester 9 was 1.2 gm VS of pine needle and 4.8 gm VS of cow dung. Similarly, in digester 10 were 1.4 gm VS of pine needle and 5.6 gm VS of cow dung. Similarly, in digester 11 were 1.6 gm VS of pine needle and 6.4 gm VS of cow dung. Similarly, in digester 12 were 1.8 gm VS of pine needle and 7.2 gm VS of cow dung. The digester bottles are then filled with tap water up to 800 ml mark with headspace of 200 ml for gas. The bottles are fitted with rubber cork at the opening and further fitted with drip for gas collection. The retention period of experiment was 30 days at 35°C.

The third batch of digester consists of 6 bottles. Cow dung is sole substrate used in digester. The organic loading rate for third batch of digester was 4, 5, 6, 7, 8 and 9 gm VS respectively. The digesters used in third batch of digester are 13, 14, 15, 16, 17 and 18. The loading rate in digester 13 was 4 gm VS of cow dung. Similarly, in digester 14 were 5 gm VS of cow dung. Similarly, in digester 15 were 6 gm VS of cow dung. Similarly, in digester 16 were 7 gm VS of cow dung. Similarly, in digester 17 were 8 gm VS of cow dung. Similarly, in digester 18 were 9 gm VS of cow dung. The digester bottles are then filled with tap water up to 800 ml mark with headspace of 200 ml for gas. The bottles are fitted with rubber cork at the opening and further fitted with drip for gas collection. The retention period of experiment was 30 days at 35°C.

(a) Experimental observation before and after digestion in design-1

The experiment conducted before the digestion of slurry in the digester was evaluated in term of temperature, pH, total solid, volatile solid, fixed solid, alkalinity and COD. The generation biogas is noted on daily bases. The pH of slurry is checked after five day over the period of experiment using pH meter.

(b) Biogas measurement

The biogas generation is measured with the help of water displacement method. In this one 1000ml capacity, measuring cylinder is filled with water; the it is placed upside down in a plastic tub half filled with water. Then drip pipe from digester insert in the cylinder. The gas from the digester replaces the water in the cylinder. The volume of gas in the cylinder is the volume of biogas produced. The percentages of gases present in biogas were measured with the help of biogas-5000 sensor.

3.6 Standard testing methods

3.6.1 Temperature

The measurement of temperature is done by digital thermometer.



Figure 3.5 Digital Thermometer

3.6.2 pH

pH measurement is noted with the help of digital pen type pH meter.



Figure 3.6 pH meter

3.6.3 Determination of Total solid, volatile solid and Fixed solid

1. Note the weight of empty crucible or dish.
2. Note down the weight of crucible with sample.

3. Place the crucible in oven at 103°C to 105°C for 24 hours for drying of sample.
4. Note down the weight of the crucible after drying in oven.
5. Place the crucible in a muffle furnace at 600°C for 15-20min.
6. Note down the weight of crucible after drying in muffle furnace.

$$\text{Total solid} = \frac{\text{Weight of dry sample} \times 100}{\text{weight of sample}}$$

$$\text{Volatile solid} = \frac{(\text{weight of oven dry sample} - \text{weight of sample after drying in muffle furnace}) \times 100}{\text{weight of oven dry sample}}$$



Figure 3.7 Device used in drying sample (a) muffle furnace (b) oven

3.6.4 Determination of Total organic carbon (TOC)

1. Take sample of 0.05gm of dry sample in 250ml Erlenmeyer flask.
2. Then add 10ml potassium dichromate solution of 1N concentration into the sample.
3. Then add 20ml of sulfuric acid into the sample, mix it properly for about 1min.
4. Then wait until the sample cools down.
5. Then dilute the sample with 200ml of deionized water.
6. Then add 10ml of phosphoric acid in the sample.
7. Then add 0.2g of sodium fluoride in the sample.
8. Then add 10 drop of diphenylamine indicator.

9. Then titrate sample with ferrous ammonium sulfate of 0.5N concentration until the color of sample change from green to turbid blue.

10. In the end prepare and titrate blank sample with same procedure.

$$\% \text{ TOC} = \frac{10(B-S) \times 0.003 \times 100}{B \times \text{weight of the sample}}$$

S is sample titration

B is blank titration

1ml of $\text{K}_2\text{Cr}_2\text{O}_7$ - 3mg or 0.003g organic carbon

Percentage of organic matter = $1.724 \times \text{TOC}$

3.6.5 Total Kjeldahl nitrogen determination

(a) Digestion

1. Take a sample of 0.5g in Kjeldahl Flask.
2. Then add 30ml of concentrated H_2SO_4 . Then mix the sample by shaking for 15min.
3. Add Hibbard's mixture of 10g.
4. Then add 1g of salicylic acid.
5. Then add 5g of sodium thiosulphate.
6. After that heat the sample at low temperature till there is no foaming. After that raise the heat until the sample in the flask change its color to grey or greenish yellow.
8. After that cool down the sample and add 100ml of water in it.
9. After mixing well transfer the sample into the 250ml volumetric flask.

(b) Distillation

1. Take 20ml of H_2SO_4 of 0.1N concentration in a conical flask.
2. Then add 2 drops of indicator prepared by the mixture of methyl red and bromo cresol green.

3. Then place the conical flask under the delivery tube of assembly of distillation.
4. After that take sample of 10ml of filtrate in the distillation assemblies flask.
5. Then add 10ml of NaOH solution of 45% in the flask by the funnel connected to the tube of distillation flask and distillation filter.
6. After the collection of 30ml of distillation in the conical flask after heating switch off the heater.
7. After that titrate the excess acid in the final sample by NaOH of 0.1N concentration until the colorless sample change its color to pink. Note down the reading of NaOH.

$$\text{Total Kjeldhal nitrogen percentage} = \frac{0.0014 \times \text{Titration Volume} \times \text{Total volume of liquid prepared}}{\text{ml of sample taken} \times \text{weight of sample}}$$

3.6.6 Determination of Chemical oxygen demand

1. Take two tubes for testing take sample of 2.5ml of sample in one tube and 2.5ml distilled water in other tube.
2. Then add 1.5ml of potassium dichromate in both tubes.
3. Then add 3.5ml of sulphuric acid reagent in both tubes very carefully.
4. Mix the solution very carefully.
5. Then close both the tube properly and place in the COD digester at 150°C for 2 hours.
6. Then after cooling titrate both the sample with freshly prepared ammonia sulphate until the color changes to reddish brown. Note down the reading of ammonia sulphate on the burette.

$$\text{COD (mg/Lt)} = \frac{(A-B) \times N \times 8 \times 1000}{\text{Volume of sample}}$$

A is the volume of ferrous ammonia sulphate use in titration of blank sample

B is the volume of ferrous ammonia sulphate use in titration of sample

N is the normality of ferrous ammonia sulphate

3.6.7 Determination of Alkalinity

1. Take a sample of 25ml in conical flask.
2. Then add 2 drops of phenolphthalein indicator, If the color of sample changes to pink by addition indicator then titrate it against the 0.02N of H₂SO₄.
3. If the color of sample does not change then add two drops of methylorange indicator. Then titrate it against 0.02N H₂SO₄.
4. Titrate the sample until the color changes from yellow to orange red, note down the reading of titration.

$$\text{Alkalinity} = \frac{\text{Normality of Sulfuric acid} \times 1000 \times 500}{\text{Volume of sample taken}}$$

Chapter-4

Result and discussion

4.1 General

The results from the study done are evaluated in this chapter. The evaluation of various physico-chemical characteristics of pine needle, cow dung and food waste. The calculation of generation of biogas in all the different ratio of substrate alone and in co-digestion.

4.2 Result of characterization of substrate

To calculate the biogas generation capability of the substrate food waste, cow dung and pine needle sample were experimented for characteristics like total solids, volatile solids, ratio of volatile solid and total solid, fixed solids, total organic carbon, organic matter, TKN and C/N ratio. The outcome from the results are evaluated and compared in the table 4.

Table 4.1 Composition of biogas

| Sr.NO | Parameters | Food waste | Pine needle | Cow dung |
|-------|--------------------------------|------------|-------------|----------|
| 1. | Total solids (%) | 28.276 | 82 | 18.3 |
| 2. | Volatile solids (%) | 13.254 | 70 | 14.2 |
| 3. | pH | 6.5 | 6.5 | 8.5 |
| 4. | Volatile solid/total solid (%) | 47.88 | 85.36 | 77.59 |
| 5. | Total organic carbon (%) | 45.7 | 49.06 | 18.7 |
| 6. | TKN (%) | 1.12 | 1.03 | 1.2 |
| 7. | C/N ratio | 40.8 | 48.02 | 15.74 |

The results are evaluated given in the above table. During the anaerobic process, the characteristics that needed to balance for better efficient working of biogas plant are carbon, nitrogen, total solids, volatile solids content and the substrate are torn down and mixed homogenously for production of good quality of methane. The co-digestion of two substrates also enhances the production of biogas as a whole.

Pine needles were characterized as lower in TKN percentage but rich in total solids, volatile solids, total organic carbon that is plus point in biogas production. While food waste and cow dung are lower in total solids, volatile solids, total organic carbon and C/N ratio. The total solids content depend upon the water added during dilution or preparing slurry for the digestion process. The part of water added through the process is measure accurately because it controls the digester composition as well as capacity of digester. The higher volatile solid content of pine needles when co-digested with cow dung has higher energy generation that is very appealing from economic perspective of biogas production.

For the effectiveness as a substrate C/N ration has very crucial role to play. This has been evaluated in the above section. C/N is an urgently important parameter for how good the biogas production can be. For best biogas production, the best-suited C/N ratio range is in-between 20 to 30. From the result given in the above table 4.1 C/N ratio of cow dung are lower 15.74 where as the C/N of food waste and pine needle is higher in contrast with cow dung i.e. 48.02 for pine needles and 40.8 for food waste. Therefore, due to lower C/N ratio preposition in substrate in either C or N will result in lowering the biogas production. For keeping the more biogas production, we must provide with either C or N to the digestion. This can achieve by adding substrate with suitable C/N ratio i.e. pine needles or food waste. For maintaining C/N, ratio co-digestion was done. Therefore, co-digestion of cow dung with pine needle and food waste is done in this study. By co-digestion, the production of biogas also improved. The percentage of organic matter is higher in pine needles. Organic matter also key point of co-digestion with pine needle higher the organic matter greater the degradation rate also increase the production of biogas.

4.3 Comparison of biogas generation and different parameter used in winter

The physic-chemical characteristic examined of substrate in digester at the beginning and at the end of the process are elaborated in the table 4.2

Table 4.2 Parameter for digester before and after the digestion

| Sr.NO | Parameter | Initial (AD1) | Final (AD1) | Initial (AD2) | Final (AD2) | Initial (AD3) | Final (AD3) |
|-------|------------------|------------------|----------------|------------------|----------------|------------------|----------------|
| 1. | Temperature (°C) | 15°C | 25°C | 15°C | 25°C | 15°C | 25°C |

| | | | | | | | |
|----|-----------------------|-------|-------|-------|------|-------|------|
| 2. | pH | 7.3 | 7.45 | 6.3 | 7.1 | 6.65 | 6.86 |
| 3. | Total solids (mg/L) | 15338 | 12568 | 13684 | 9846 | 12645 | 9856 |
| 4. | Volatile solids(mg/L) | 13245 | 9548 | 11265 | 7654 | 12568 | 9682 |
| 5. | VS/TS | 0.85 | 0.72 | 0.90 | 0.81 | 0.85 | 0.76 |
| 6. | Alkalinity (mg/L) | 1387 | 2727 | 1026 | 2754 | 1058 | 2056 |
| 7. | COD (mg/L) | 698 | 389 | 573 | 285 | 546 | 365 |

4.3.1 Effect of Variation in pH

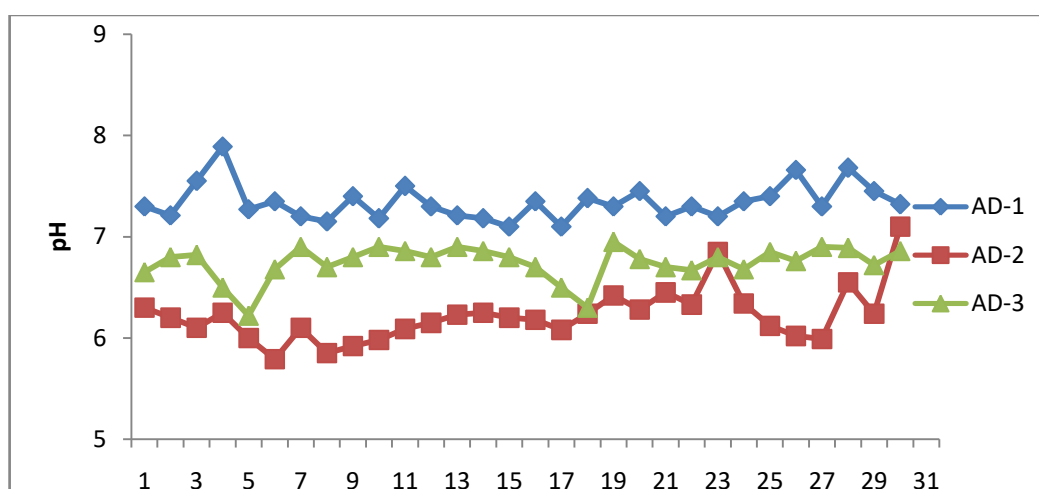


Figure 4.1 Variation in pH in winter

pH is an important factor that affect the biogas generation. So far the effective gas production depends upon the desire rang of pH for good growth of microbes for the degradation of substrate for methane generation. However, for maintaining pH in desire rang acid or base added in the slurry in the digesters per required. The desire range of pH for generation of biogas is in-between 6.5 to 8.5 respectively. The pH was observed in AD1 was 6.5 to 7.5, in AD the pH ranges around 6.2 to 7.5 and at last in AD2 the pH ranges in-between 5.5 to 7.4. In the starting pH of AD1 was around 6.1 and with the degradation of substrate the pH of the slurry decreases and the slurry become acidic to raise the pH adequate amount of CaCO_3 was added to slurry. Moreover, in AD2 the pH is around 8.1 as the degradation process goes on the value of pH goes down. And in AD3 the pH of slurry in the beginning was 6.65 it also goes down after some degradation of substrate and the slurry become acidic the adequate amount of CaCO_3 was added.

By the examination of substrate co-digestion of two substrate cow dung and pine needle helps in balancing the pH of slurry. This contrast in pH is due to the alkalinity of the slurry. pH is main characteristics for the production as pH goes up or down it cause obstruction to methanogenesis bacteria in the production of biogas, The pH below 5 or above 8 cause hinder the methanogenesis process. The best-suited pH range for biogas production is 6.5 to 7.5.

4.3.2 Effect of COD on biogas production

COD is the characteristic by which we can evaluate the quantity of organic matter in the substrate and predict the biogas production. The substrate organic matter i.e. COD was change into biogas production by action of bacteria. The COD measure of degradation by bacteria known as COD removal. Higher the COD removal more will be the biogas production.

As given in the figure 4.2 initial value of COD was higher than the final value of the COD of the slurry as calculated. COD value of AD1 noted to be 698mg/L and after COD removal, the final value of COD was 389mg/L. The COD value of AD2 in the beginning was 573mg/L and after COD removal, the COD value was 285mg/L. The COD value of AD3 in the beginning was 546mg/L and after COD removal, the final value of COD was 365mg/L. the COD removal in AD2 is 33.56% higher as compare to digester AD1 and AD3 i.e. 27.37% and 19.86% respectively. Therefore, as the result the biogas generation AD2 is more in comparison to digester AD1 and AD2.

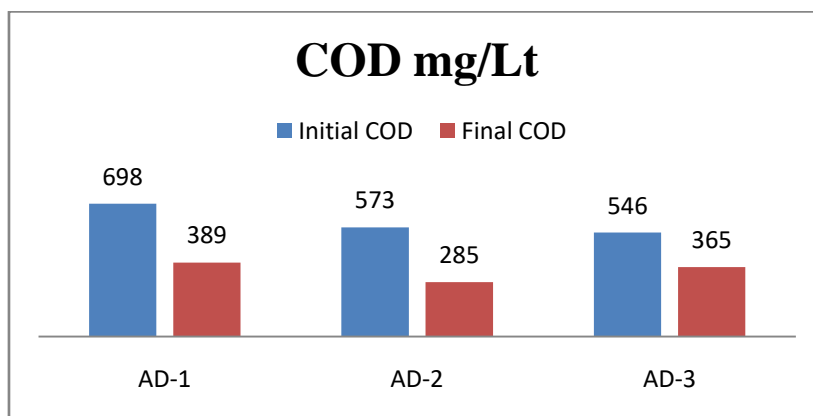


Figure 4.2 Variation in COD

4.3.3 Effect Alkalinity on biogas production

The alkalinity of the substrate is due to calcium, magnesium and ammonium bicarbonate present in them. The alkalinity in the slurry helps in maintaining the

buffering capacity of the digester. The alkalinity formed by torn down the protein of substrate. The alkalinity concentration depends upon the concentration of solid feed to a good extent. In biogas production, the range of alkalinity is around 2000mg/Lt to 5000mg/Lt for maintaining enough amount of burring capacity of the slurry. Alkalinity of the slurry increases as the digestion process goes on. The alkalinity of AD1 in the starting was 1456mg/Lt and after digestion, it was 2584mg/Lt. Similarly in AD2 the alkalinity of slurry was 1186mg/Lt and after digestion, it was 2865mg/Lt. Similarly, in AD3 the alkalinity of slurry was 1256mg/Lt and after digestion, it was 2363mg/Lt. So as the result shows the alkalinity of AD2 was better than AD1 and AD2.

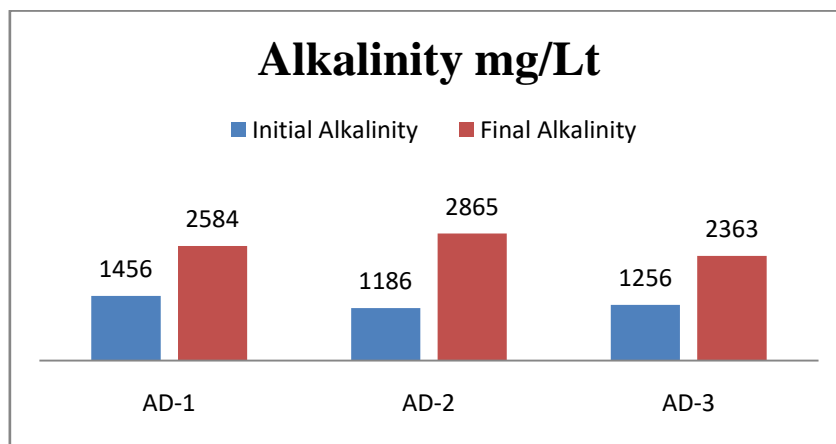


Figure 4.3 Variation in Alkalinity

4.3.4 Effect of total solids and volatile solids

Total solid represent the amount of inorganic and organic content in the feedstock. The total solid present in the slurry at the beginning of digestion was 14679, 13568 and 15694mg/Lt in digester AD1, AD2 and AD3 and at the end of digestion the concentration of total solid in the slurry was 11890, 10524 and 12963mg/Lt in digester AD1, AD2 and AD3. As the process of digestion, goes on the concentration of total solid in decreasing due to degradation of total solid by microorganism. Volatile solids are the solids that get evaporated when heated at 550°C. Volatile solids are the part of total solids that fed as a food for the microorganism. The volatile solid is the degrading portion of the total solids for production of biogas in anaerobic digester. Volatile solid has very crucial role in biogas production \. Out of all the substance in slurry volatile is the part that degraded by microorganism to produce biogas. Initial value of volatile solid was high but as the process goes on the degrading or digested by microorganism then the concentration of volatile solid decreases. The removal percentage of AD1, AD2

and AD3 was 27.66, 49.96 and 33.37 % . Therefore volatile solid removal percentage is higher in AD2. As the volatile solid removal goes high consequently the biogas production increases. Therefore, co-digestion helps in increasing the reduction rate of total solid and volatile solid.

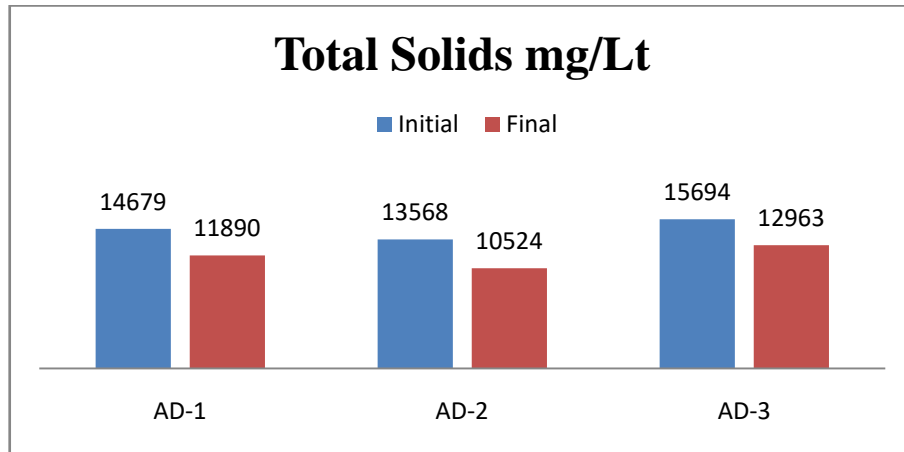


Figure 4.4 Variation in total solids

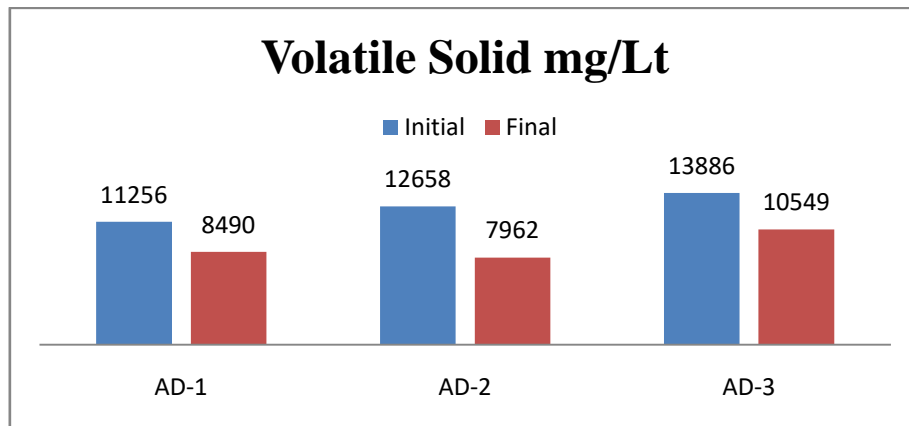


Figure 4.5 Variation in volatile solids

4.3.5 Effect of temperature

Temperature is very important parameter in biogas production process because all the microbial activity of bacteria depends upon temperature. The best result for biogas production is in mesophilic temperature range. As the temperature increases the biogas production also increases. Some of the processes in biogas production are very much sensitive to temperature so steady temperature needed like methanogenesis. The temperature of digester inside and outside varies from 16°C to 22°C inside and for out the temperature varies from 2°C to 19°C in winter. Variation in temperature in all the three digesters AD1, AD2 and AD3 given below.

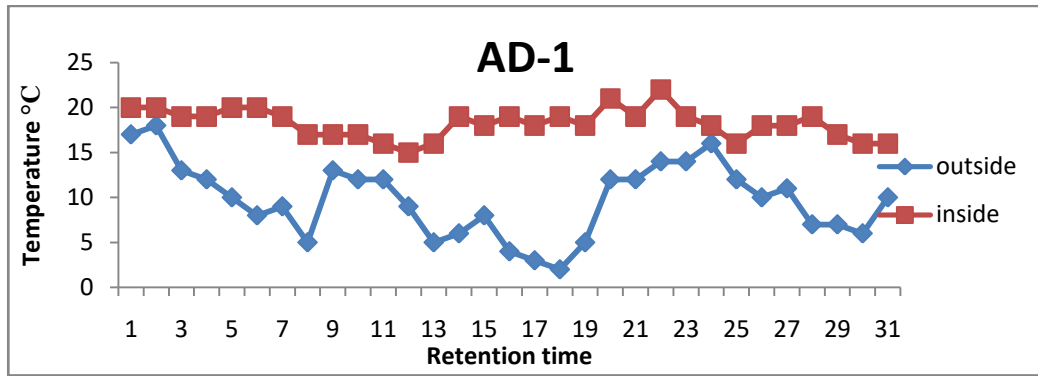


Figure 4.6 variation in temperature in AD1

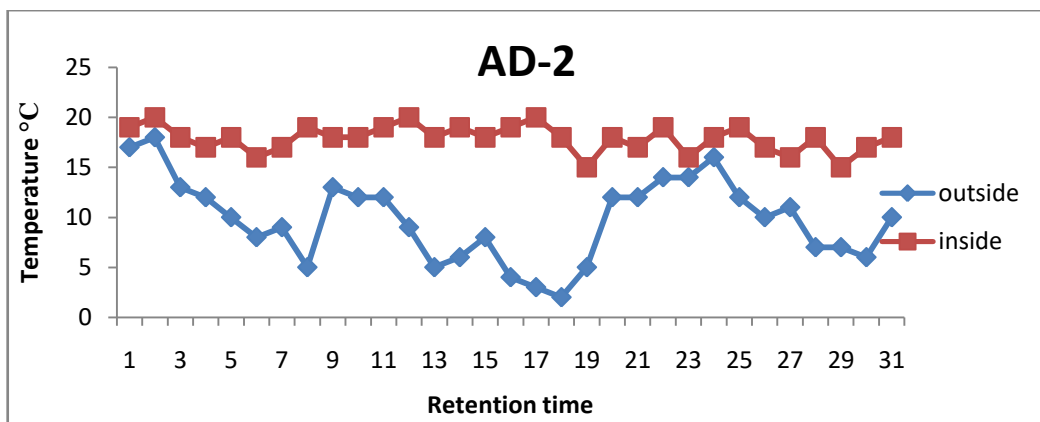


Figure 4.7 Variation in temperature in AD2

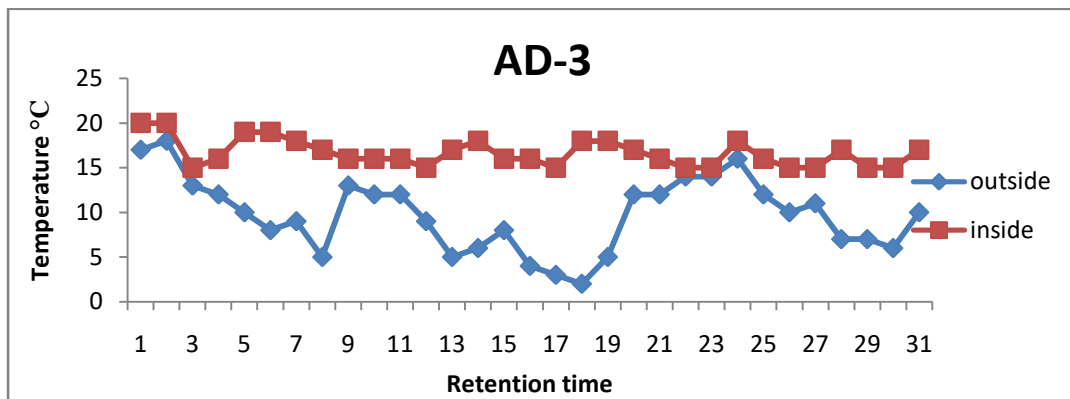


Figure 4.8 Variation in temperature in AD3

4.3.6 Biogas production in winter

The overall biogas generation in winter is shown in fig below. The gas collection checked on daily bases. The gas generation in digester, AD1 started on 8th day of digestion where in digester AD2 was on 6th day of digestion and in digester, AD3 was 12th day of digestion. The biogas generation in digester AD2 started earlier then other two digesters. Therefore, co-digestion also helps in fastening the digestion process and biogas generation. The result shows us the increment in biogas generation as the

digestion time increase. In the beginning of the digestion, there was no biogas generation in AD3 because of high pH. Then as the digestion goes on the pH lower in the digester and the biogas produce in AD3 as well. Where, as in digester AD2 the gas generation starts earlier due to balance pH. This also indicates overall biogas generation is also higher in AD1 because of co-digestion of pine needle and cow dung. Another reason for more gas generation in AD2 is embellish the C/N ratio and nutrient balance.

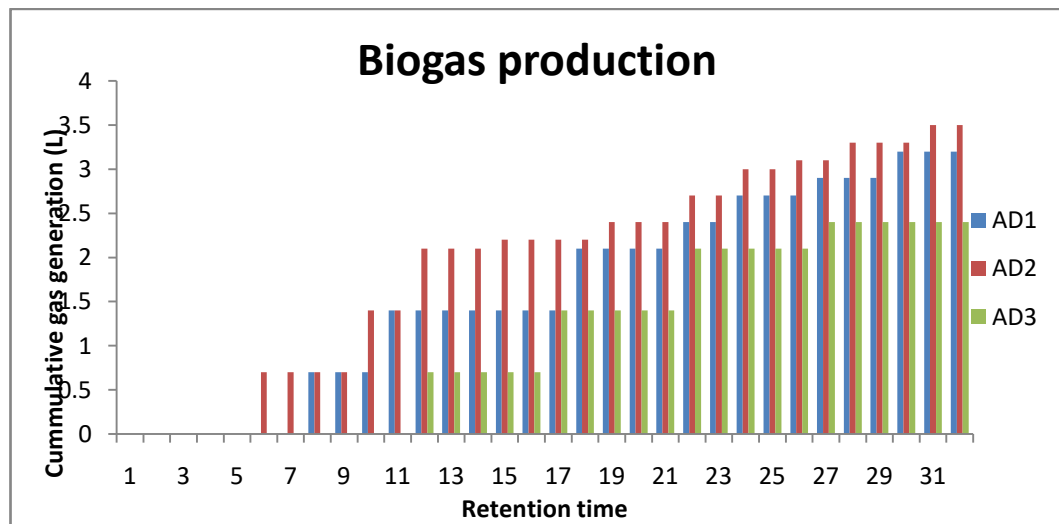


Figure 4.9 Overall biogas production (winter)

4.4 Comparison of biogas generation during summer

The physic-chemical characteristics examined of substrate in digester at the beginning and at the end of the process are elaborated below:

4.4.1 Variation in pH with respect of time

pH is an important factor that affect the biogas generation. So far the effective gas production depends upon the desire rang of pH for good growth of microbes for the degradation of substrate for methane generation. However, for maintaining pH in desire rang acid or base added in the slurry in the digesters per required. The desire range of pH for generation of biogas is in-between 6.5 to 8.5 respectively. The pH was observedat the beginning of the digestion is higher around 8.3 but as the digestion take place it value decrease and stable round 6.8 to 7.2.

By the examination of substrate co-digestion of two substrate cow dung and pine needle helps in balancing the pH of slurry. This contrast in pH is due to the alkalinity of the slurry. pH is main characteristics for the production as pH goes up or down it cause

obstruction to methanogenesis bacteria in the production of biogas, The pH below 5 or above 8 cause hinder the methanogenesis process. The best-suited pH range for biogas production is 6.5 to 7.5.

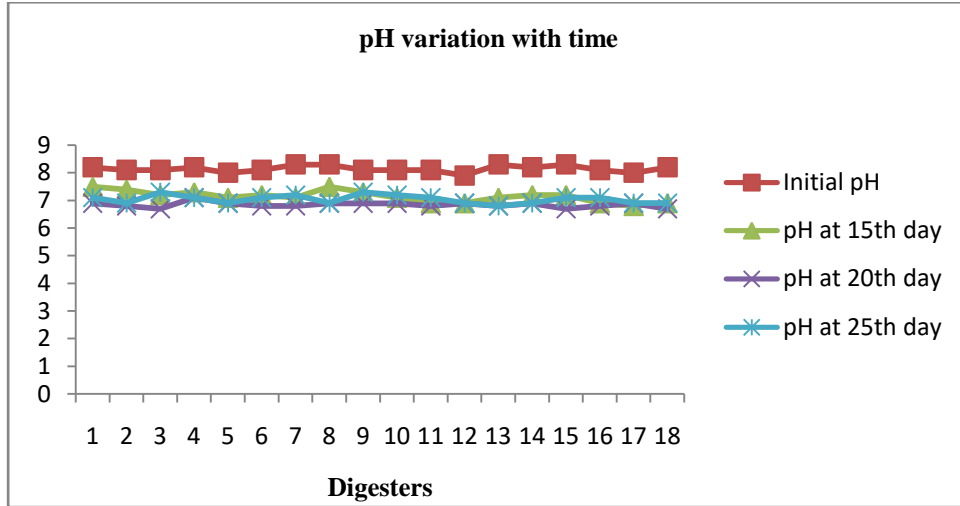


Figure 4.10 Variation in pH with time

4.4.2 Variation in Alkalinity

The alkalinity of the substrate is due to calcium, magnesium and ammonium bicarbonate present in them. The alkalinity in the slurry helps in maintaining the buffering capacity of the digester. The alkalinity formed by torn down the protein of substrate. The alkalinity concentration depends upon the concentration of solid feed to a good extent. In biogas production, the range of alkalinity is around 2000mg/Lt to 5000mg/Lt for maintaining enough amount of buffering capacity of the slurry. Alkalinity of the slurry increases as the digestion process goes on. The variation in alkalinity of all the digesters at beginning and the end of the digestion was given below.

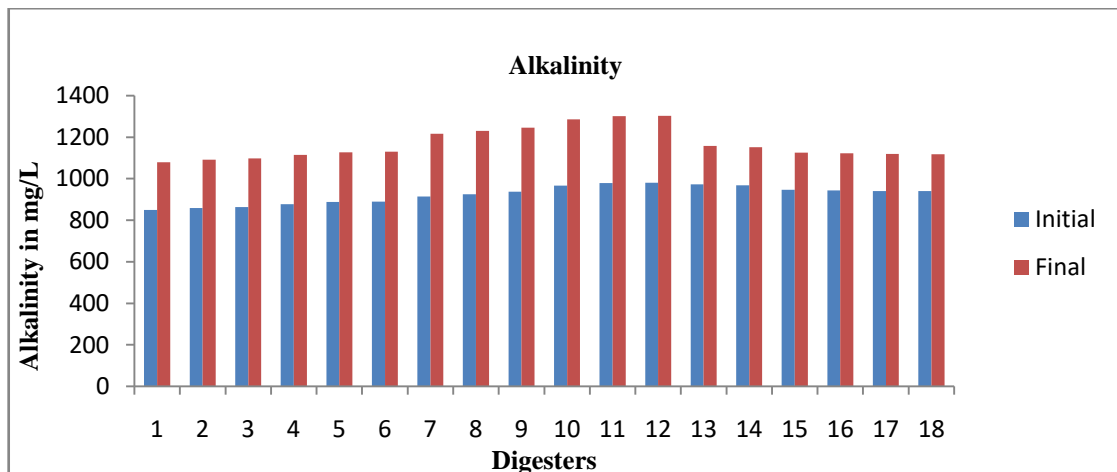


Figure 4.11 variation alkalinity in all the digesters

4.4.3 Biogas production in summer

The biogas collection first collected at 15th day digestion. Then biogas collection is collected after every 5 days. There are three set of different combination of substrate

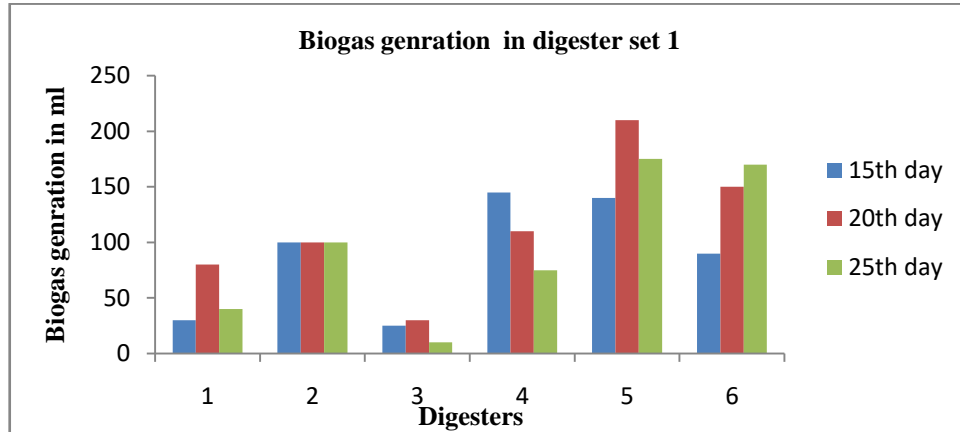


Figure 4.12 Biogas generation in digester set 1 in summer

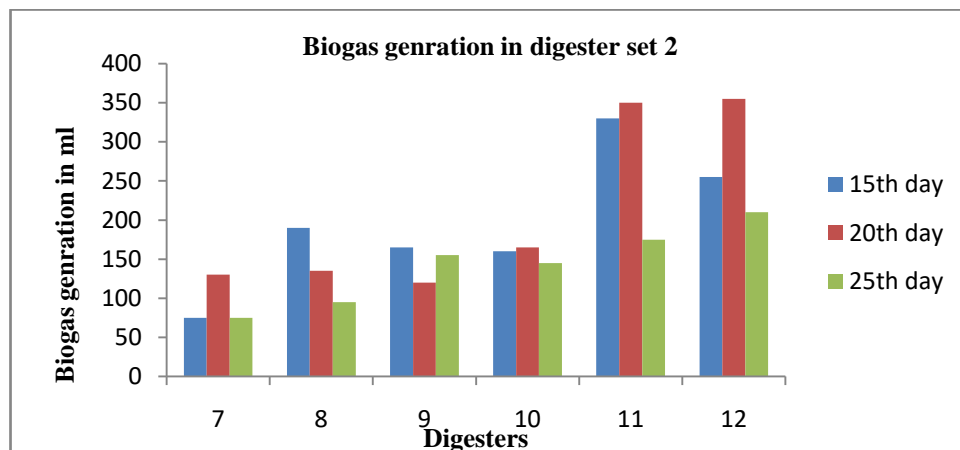


Figure 4.13 Biogas generation in digester set 2 in summer

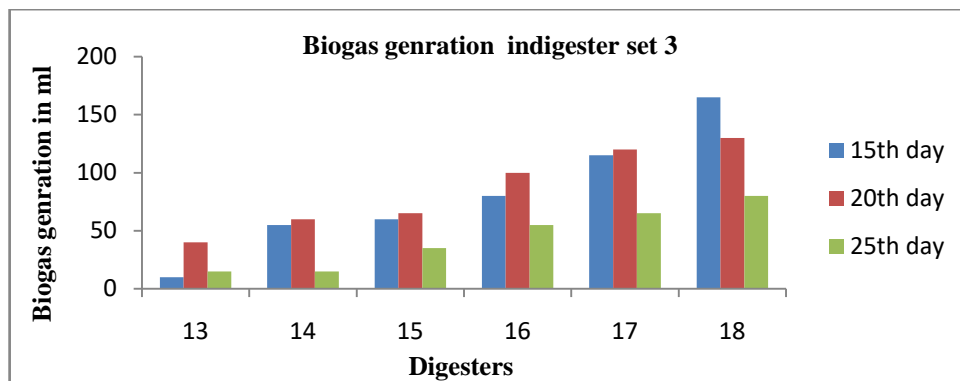


Figure 4.14 Biogas generation in digester set 3 in summer

Chapter-5

Conclusion

5.1 General

By evaluating results from the experimental study, it inferred that anaerobic digestion of pine needle, cow dung and food leftover is a very good source of renewable energy. Besides, it also helps in protecting environment from harmful gases generated during digestion of these biomasses in open. Co-digestion of two substrates also enhances the biogas generation.

The substrate used in this study was collected from JUIT campus and forest beside the campus. A significant amount of food is daily disposed at dumping sites without any utilization. Furthermore, by the decomposition of this waste in open produce harmful gases leading to accumulation of contaminants in the environment. On the other hand, pine needles have no use, moreover Pine Needles are also responsible for forest fire which leads to rise in pollution and responsible for demolition of wildlife. Therefore, this study gives us alternative use of pine needles and food waste to convert them into useful energy source as biogas generation. Furthermore, this study also helps in determining the optimal ratio substrate in anaerobic digestion process.

5.2 Characteristics of substrate:

- Food waste has lower TS, VS, total, TOC, and C/N ratio percentage as compare to pine needles. Therefore, by co-digestion of food waste with pine needles helps in balance the nutrient value in the substrate.
- Pine needles have lower percentage of TKN than that of FS.
- The substrate with higher volatile solid content has higher biogas generation.
- C/N is very important parameter in production of biogas generation. Co-digestion helps in balancing C/ N ratio in the digester.

5.3 Biogas generation in winter

- The substrate having pH less than 5 and greater than 8 are responsible for restraining the methanogenesis bacteria which is accountable for biogas generation.

- The value of total solids was higher in the beginning but as the time passes, TS concentration reduced gradually.
- The reduction in volatile solids was 27.66%, 46.96% and 33.37% in AD1, AD2 and AD3, respectively.
- The removal of COD by digestion is 27.37%, 33.56 and 19.86 in digester AD1, AD2 and AD3, respectively.
- The alkalinity of AD1, AD2 and AD3 in the beginning of the digestion was 1456, 1186 and 1256 mg/l and at the end of digestion was 2584, 2865 and 2363mg/l, respectively. Therefore alkalinity of the slurry in the digester increased as the digestion goes on.
- This experimental study took place in open at ambient temperature range. The temperature inside the digester was in range of 14°C to 22°C and outside the temperature in range of 2°C to 24°C. Because of degradation process, the temperature inside the digester was higher as compare to outside temperature.
- The overall biogas generation in the end of the process was 3.2liter in digester AD1, 3.5liter in digester AD2 and 2.4liter in final digester AD3. Therefore as the result shows the biogas generation is higher in AD2 due co-digestion of two different substrates.

5.4 Conclusion of biogas generation in summer

- From the results, it is observed that as OLR increased biogas yield increased however, after 8gmVS/l a drop is spotted.
- Food waste has yielded to more biogas than that of Pine Needles due to its lignocellulosic nature.
- Co-digestion of Food Waste with Pine Needles is further a research gap.

References

- [1] Ruihang Zhang, Hamed M. El-Mashad, Karl Hartman, Fengyu Wang, Guangqing Liu, Chris Choate, Paul Gamble,(2006) Characterization of food waste as feedstock for anaerobic digestion, *Bioresource technology*,.
- [2] Guangqing Liu, Ruihang Zhang, Hamed M.El mashad, Renjie Dang, (2009) Effect of feed to inoculum ratios on biogas yield of food waste and green waste, *Bioresource Technology*
- [3] hamed m.el mashad, Ruihang Zang, (2010) Biogas production from co-digestion of dairy manure and food waste, *Bioresource technology*,
- [4] Zehra Spaci, (2013) The effect of microwave pre-treatment on biogas production from agricultural straws, *Bioresource technology*,
- [5] Dan Brown Yebo Li, (2015) Solid-state anaerobic co-digestion of yard waste and food waste for biogas production, *Bioresource technology*,
- [6] Iqbal Syaichurrozi, Budiyano, Siswo Sumardiono, (2013) Predicting kinetic model of biogas production and biodegradability organic material: biogas production from vinasse at variation of COD/N ratio, *Bioresource technology*,
- [7] Yiang Chen, Wei Yan, Kuichuan Sheng, Mehri Sanati,(2014) Comparison of high-solids to liquid anaerobic co-digestion of food waste and green waste, *Bioresource technology*,
- [8] Xumeng Ge, Tracie Matsumoto, Lisa Kieth, Yebo Li,(2014) Biogas energy production from tropical biomass waste by anaerobic digestion, *Bioresource technology*,
- [9] Garcia K, Perez M, (2014) Anaerobic co-digestion of raw sludge and cattle manure influence of temperature condition and substrate composition, *Bioresource technology*,
- [10] A. Serna-Maza, S. Heaven, C.J. Banks, (2015) Biogas stripping of ammonia from fresh digestion from a food waste digestion, *Bioresource technology*,

- [11] Dang Li, Shengehu Liu, Li MI, Zhidang Li, Yuexiang Yuan, Zhiying Yan, Xiaofeng liu, (2015) Effect of feedstock ratio and organic loading rate on the anaerobic mesophilic co-digestion of rice straw and cow manure, *Bioresource technology*,
- [12] haider Muhammod Rizwan, Zeshan Riffat Naseem malik, Chettyappan Visvanathan, (2015) Effect of mixing ratio of food waste and rice husk, co-digestion and substrate to inoculum ratio on biogas production, *Bioresource technology*,
- [13] Seung G Shin, Gyuseang han, Joonyeab Lee, Kyungjin Cho, Eun-Jeong Jean, Changsoo Lee, Seokhwan Hwang, (2015) Characterization of food waste recycling wastewater as a biogas feedstock, *Bioresource technology*,
- [14] bodius Salam, Sumana Biswa, Md Sanaul Rabbi, (2015) Biogas from mesophilic anaerobic digestion of cow dung using silica gel as catalyst, *Elsevier*,
- [15] li-Jie Wu, Takuro kobayashi, Hidentoshi Kuramochi, Yu-You Li, Kai-Clin Xu, (2015) Improved biogas production from food waste by co-digestion with de-oiled grease trap water, *Bioresource technology*,
- [16] Chuangan liu, Yuyao Zhang, Can Liu, (2016) Improve biogas production from low organic content sludge through high-solids anaerobic co-digestion with food waste, *Bioresource technology*,
- [17] Abilash Kumar Tripathi, Mamta Kumari, Ashish Kumar, Sudhir Kumar, (2015) Generation of biogas using pine needle as substrate in domestic biogas plant, *International journal of renewable energy research*,
- [18] E. Fathi Aghdam, V Kinven, J.Rintala, (2015) Mesophilic anaerobic co-digestion of municipal solid waste and sewage sludge, *Bioresource technology*,
- [19] Poul Thomas, Nirmala Soren, Nelson, Pyanadathu Rumjit, Jake George James, M.P. (2017) Saravanakumar, Biomass resource and potential of anaerobic digestion in Indian Scenaria, *Renewable and sustainable energy reviews*,
- [20] Sihuang Xie, Faisel.I. Hai, xinmin Zhan, Wenshan Guo, Hao H Ngo, William E Price, Long D.Nghiem, (2017) Anaerobic co-digestion: A critical review of mathematical modeling for performance optimization, *Bioresource technology*,

- [21] Jun Zhou, Jun Yang, Qin Yu, Xiaoyu Yang, Xinxin Xie, Lijuan Zhang, Ping Wei, Hanghua Jia, (2017) Different organic loading rates on the biogas production during the anaerobic digestion of rice straw, *Bioresource technology*,
- [22] J.B Holm Nielsen, T.Al SEadi, P.Oleskowioz-Popiel, (2009) The future of anaerobic digestion and biogas utilization, *Bioresource technology*,
- [23] Yi Zheng, Jia Zhao, Fwqing Xu, Yedo Li, (2014) Pretreatment of lignocellulosic biomass for enhanced biogas production, *Progress in energy and combustion science*,
- [24] Peyman Salehian, keikhasro Karimi, (2013) Improvement of biogas production from pine wood by alkaline pretreatment, *Fuel Elsevier*,
- [25] Johan Lindmark, Eva Tharin, Rebei Bel Fdhile, Erik Dahlquist, (2014) Effect of mixing on the result of anaerobic digestion, *Renewable and Sustainable Energy Reviews*,
- [26] Hamed M. El MASHad, Wilka K.P van Loan, Grietje Zeema, (2002) A model of solar energy utilization in the anaerobic digestion of cattle manure, *Biosystem Engineering*,
- [27] T.M Alkhamis, R. El-Khanzali, M.M. Kabla, M.A Alhusein, (2000) Heating of a biogas reactor using a solar energy system with temperature control unit, *Elsevier*,
- [28] J.A Aluaroz, L. Otero, JM. Lema, (2009) A methodology for optimizing feed composition for anaerobic co-digestion of agro industrial wastes, *Bioresource technology*,
- [29] Rodrigo. A. Labotut, Lorgus, Angenment, Narman, R Scott, (2015) Biochemical methane potential and biodegradability of complex organic substrates, *Bioresource technology*,
- [30] H.I Owomath, O.C Izinyen, (2015) Optimal combination of food waste and maize husk for enhancement of biogas production. *Environmental Technology and innovation*,
- [31] G. Zhang, Y. Li, Y.J. Dai, R.Z Wang, (2016) Design and analysis of a biogas production system utilizing residual energy for a hybrid CSP and biogas power plant, *Applied thermal engineering*,

[32] Rangaraj Ganesh, Michel Torrijos, Philippe Sousbie, Jea Philippe Steyer, Aurelien Lugardon, Jean Philippe Delgenes, (2013) Anaerobic co-digestion of solid waste effect of increasing organic loading rate and characterization of the solubiliaed organic matter, *Bioresource technology*,

[33] Maryam M. Kabir, Karthik Rajendran, Mohammad J. Teherzadeh, Ilana Sarvari Horvath, (2014) Experimental and economical calculation of bioconversion of forest residues to biogas using organaosolve pretreatment, *Bioresource technology*.