Bio-methane Potential of Okra Calyx, Banana and Potato Peels through Anaerobic Digestion

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Session 2015-17

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Dedication

To,

My father who gave me the greatest gift " He trusted me"

My mother who was there to support when every else deserted me.

To my family who took my troubles as their own.

To my teachers who instilled the love of learning in me.

To my friends who are the most valuable asset I have. who supported me and helped me.

Acknowledgment

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AD	Anaerobic Digestion
BMP	Biochemical methane potential
BOD	Biological oxygen demand
COD	Chemical oxygen demand
CSTR	Constinous stirred tank reactor
FAO	Food and Agriculture Organization
FS	Fixed solids
FVW	Fruit and vegitable waste
GC	Gas Chromatograph
HRT	Hydraulic Retention Time
MW	Mega watts
TS	Total Solids
UNDP	United Nations Development Programm
VFA	Volatile Fatty Acids
VS	Volaitle Solids

Abstract

Fruit and vegetable wastes (FVW) are major components of domestic waste. Utilizing this significant amount of organic waste can yield sufficient energy for heating, cooking or electricity generation.. This study primarily focuses on evaluation of anaerobic digestion of different types of fruit and vegetable waste including potato peels, banana peels, okra calyx,. The substrates were tested at different feed to inoculum (F/I) ratios of 1.5, 2.5, 3.5, and 4. Therefore, bio-methane potential (BMP) assay was set up for the conversion of these waste materials into useful biogas. The effect of feed to inoculum (F/I) ratio was tested on different ranges of 1.5, 2.5 and 3.5 and 4.5 was observed at mesophilic conditions (35°C). The physical and chemical parameters including Elemental Analysis, COD values, pH, of all three Substrates and inoculum were examined. Gas samples were tested using a gas chromatograph for methane determination. Most suitable F/I ratios were found as 1.5 for banana and okra calyx, and 2.5 for Potato peels. Maximum methane yield were obtained in range of 60-65% these optimized F/I ratios are useful for design and operation of large scale anaerobic digesters. The study shows that there occurs a great opportunity to recover green energy from fruit and vegetable waste. This will also be beneficial in minimizing the methane emissions from organic waste which is dumped in landfills.

Keywords: Characterization, fruit peel, organic waste, anaerobic digestion, biogas, energy,

Contents

CHAPTER 1

Introduction

World energy use is growing with increasing urbanization and population and the need of modern world is to find methods for energy production while reducing the environmental impacts of the projects. Sustainable energy development and climate change are the issues to be dealt with proper planning and policy making. For mitigating environmental impacts of energy production the part of renewable energy technologies has to be increased in world energy mix. Like many developing countries Pakistan is also facing immense energy crises and environmental threats. One of the key sources of environmental pollution in Pakistan is landfills. Massive amount of methane leaks occur from landfills and waste dumps every years. There is a huge gap of around 5000-6000 MW between the demand and supply of electricity in Pakistan. [1]

Sustainable and ample energy supplies cause significant impact on socioeconomic scenario of developing countries. Despite increasing development, Pakistan is an energy deficient country with a significant electricity shortfall within of around 6500MW. Primary energy sources are mainly thermal (70-80%), hydropower (10-15%) and nuclear power (1-5%). The highest share in energy mix is of natural gas which is around 48% followed by oil which is around 32%. The largest consumer of energy is the Industrial sector and it is accounted around 35 percent of the total energy mix while transportation sector consumes around 32% and balance is consumed by the domestic sector. Other than conventional energy resources Pakistan has a renewable energy potential of around 2,900,000MW of solar, 346000 MW for wind 3000MW for biogas through different substrates, 2000 for small hydropower and 1000MW for waste-to-energy, but due to inadequate planning its share is still at very low in total present energy scenario and it is around 1%. [1] Overcoming energy scarcity is crucial for any nation's development but environmental protection stays a key factor. The greenhouse gas emissions still remain a massive threat for the environment and around one third of these emissions are generated from transportation sector.

Release of carbon monoxide and methane from waste in open pits can exaggerate the environmental conditions [2].

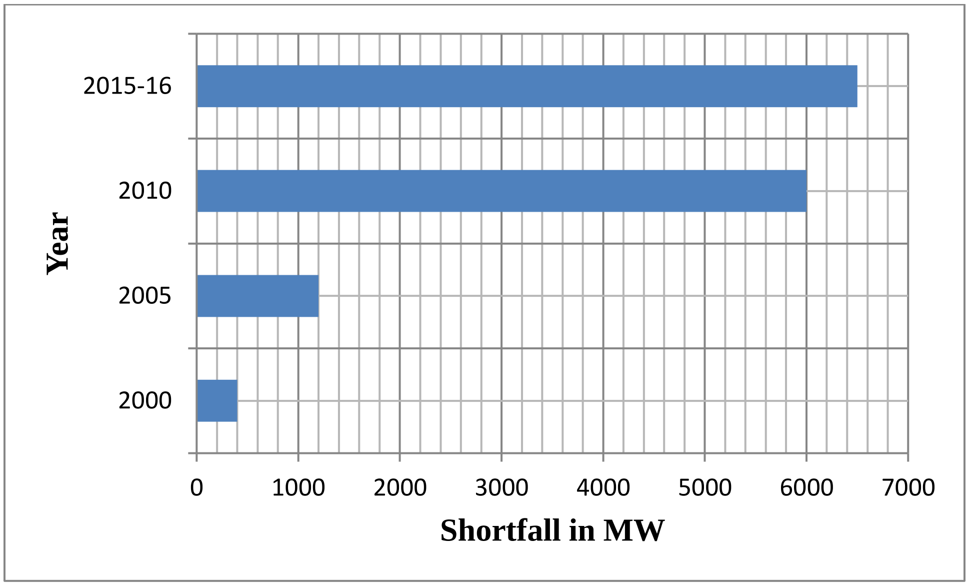


Figure 1: Electricity Shortfall in Pakistan

If exposed to oceans waste material can damage the ocean life.. The bio methane or bio gas does not contribute in increasing CO_2 content of atmosphere because it carries the same CO_2 which is absorbed from the atmosphere. Biofuels thus tends to reduce emissions to environment. Using organic wastes can contributes towards highest emissions savings while comparing with fossil fuels. By using biomethane there is an opportunity to reduce more than 85% of emissions from the automobile sector. [3] The greenhouse effect of methane is 25 times stronger than carbon dioxide; therefore the release of biogas into the atmosphere could reduce the emissions. Due to increased urbanization and inappropriate waste management techniques according to UNDP annual report 2015 Pakistan is third most vulnerable country to environmental impacts. With more development projects and coal based energy projects the environmental hazards are going to increase. Suitable and sufficient measures are required for overcoming emission problems in Pakistan.[4]

Anaerobic digestion is one of the microbial degradation techniques for organic waste. It is a renewable technology with ability of large COD and BOD reduction potential from municipal waste. Variety of microorganisms reacts with organic substrates under oxygen free conditions and produces up to 70 percent methane. This can be applicable in treating domestic food waste, agriculture waste and waste water. Being an agricultural based country availability of biomass waste is very extensive especially from agriculture and livestock sources, the amount of both agriculture and solid waste is around 300 thousand tons. After consuming the edible part of fruit the fruit and vegetables peels are separated and dumped into municipal landfills. This results in uncontrolled decomposition of organic material and produces methane in landfills. [5]

Although widely used anaerobic digestion requires experimental tests and tools to remove doubts that still hamper the full establishment of the anaerobic digestion. The optimization of the large scale plant is important to make it environmentally and economically most convenient and helpful process to treat organic solids. Among all available experimental methods, the bio-methane potential (BMP) tests are those that have been most successful, because of to their easy set up and conduction as well as the useful information obtainable from them. BMP assay allows the identification of the most appropriate feedstock waste, organic material, crops, pre-treatments, nutrient requirements etc. to achieve optimum biogas yield. [6] BMP testing is a small scale anaerobic digestion trial that supports Design of biogas digesters, Identification of potential feedstocks and co-digested blends, Testing of different pre-treatments, Identification of interfering substances and assessment of nutrient requirements [7].

Various factors effect performance of anaerobic digestion systems. Feed to inoculum ratio or f/I ratio is one of the most key parameters for stability of anaerobic digestion process. It is the ratio of mixing of bacterial source and organic substrate. The optimization of F/I is important because of the fact that increased amount of nutrients can cause bacteria to die. [8] This study focuses on optimization of feed to inoculum ratio for organic wastes including banana peels, potato peels, and okra calyx using BMP setup. The results can be further used to upscale the digestion system to pilot scale by using provided results

Research Objectives

- The overall objective of this research is to access most feasible Feed to inoculum ratios for Potato peels, okra calyx and Banana Peels to carry out anaerobic digestion.

- The objectives of this research include the physiochemical analysis of the substrate which will provide sufficient knowledge of elemental composition of substrates. The amount of micro and macro nutrients present in them
- The substrates assessment would be done using biochemical methane potential assay technique
- The gas produced will be characterized using gas characterization techniques such as gas chromatograph to determine the percentage of methane carried by the biogas
- . The results generated by the experimentation can further be used to optimize the existing plants for maximum biogas production. The study will also help up scaling the lab scale experiment to lab scale CSTR and then to pilot scale level. The substrate's performance will be assessed individually and also in comparison
- In a larger perspective the study focuses on utilization of waste to generate energy for domestic or industrial purpose which can be further upgraded and it serves as an alternative to natural gas running through the national gas grids.

CHAPTER 2

Literature Review

1. Substrate Selection and Availability

4.1. Potato peels

Potato is one of the largest producing food crops in the world with its wide application for consumption and starch production. According to FAO stats of 2017 around the globe potato production was around 3800 trillion tons. Pakistan produces large amount of potato during year 2016-2017. The cultivated area was 4 lac acres throughout Pakistan and nearly 3.3 million tons of potatoes are produced in Punjab only .The production of potato is raising through the country with increasing agriculture technology and hybrid seeds. So there is a huge potential of production of biogas from potato peelings as these are rich in starch and low in fibers so it gives greater digesting. Industries like Pepsi Co. Which produces large amount of potato products can get benefit from anaerobic digestion technology by producing biogas from their waste potato peelings to generate green energy. Waste sorting can be applied for gathering potato peels from local vegetable market or waste treatment plants. The chemical composition of potato peels is given in table below. The chemical composition may vary for different climate conditions throughout the globe. [8]

Moisture	84-85%
Protein	1-3%
Lipids	1-2%
Fiber	7-8%
Ash	8-10%
Carbohydrates	12-13%

Table 1: Chemical Compostion of Potato Peels

[9]

Figure 2: Physical Appearance of Potato Peels

4.2. Banana peels

Banana is one of the important fruit and is cultivated in more than 130 countries. In Pakistan the production of banana is around 1500000 tons per year by year 2016-2017. The banana peels is rich in fiber and carbohydrates and lower In protein content. Different studies have been carried out on biogas production form banana peels. *Nipon Pisutpaisal et al., 2014* has carried out a study for feasibility of banana peels for biogas production. In general the banana peels are discarded as waste and produce ammonia and hydrogen sulfide gas in landfills. According to American Chemical Society banana peels can be more beneficial for producing biogas from water treatment plants as it removes lead and copper from the waste water and river water very effectively. Utilizing banana peels for biogas production will not only produce energy but also removes the metal impurities from water in lower costs. Acording to studies Banana Peels contains about 6 to 12 % lignin and 6 to 10 % hemicellulose. These both materials make it difficult for organic material to degrade. [10]

Moisture	6-8%
Protein	1-1.5%
Lipids	1.7-1.8%
Fiber	30-32%
Ash	8.5-9%
Carbohydrates	58-60%

Table 2: Chemical Composition of Banana Peels

Figure 3: Banana peels physical appearance

4.3. Okra calyx

Okra also termed as ladyfinger is one of the widely produced seasonal vegetable in Pakistan. The stem part of okra is used for consumption while the leaves, flowers, and okra calyx is removed end up in waste bins. The literature shows suggest that major component of okra calyx are the carbs and protein. Okra calyx contains around 16-17% of fibers. According Pakistan agriculture Research Council to Nearly 200 thousand tons of okra is produced in Pakistan. Many Foods processing plants such as Fauji Fresh and Freez and Engro Foods process vegetables like okra calyx for cold storage and to use them in different products so the plants generate enough resource of okra calyx to consider it for biogas production. [11]

Moisture	10-11%
Protein	20-21%
Lipids	1.7-1.8%
Fiber	16-17%
Ash	6-7%
Carbohydrates	43-45%

Table 3: Chemical Composition of Okra Calyx

Figure 4: Okra Calyx physical Appearance

5. Anaerobic Digestion Process

Anaerobic digestion of organic matter is a complex process of reduction and number of biochemical reactions occurring under specific conditions. When provided necessary conditions, anaerobic bacteria convert large organic substances into simple, chemically stabilized compounds. The product produced as a result mainly consists of methane and carbon dioxide (Naik et al., 2010). The process involves hydrolyses and liquefaction of insoluble compounds while gasification of intermediate products into different gases. The anaerobic digestion technology refers to digestion of various feed stocks such as organic wastes, waste water, sewage waste, animal manure and organic parts of municipal waste. The schematic overview of anaerobic digestion process is given in Fig 1[12][13]

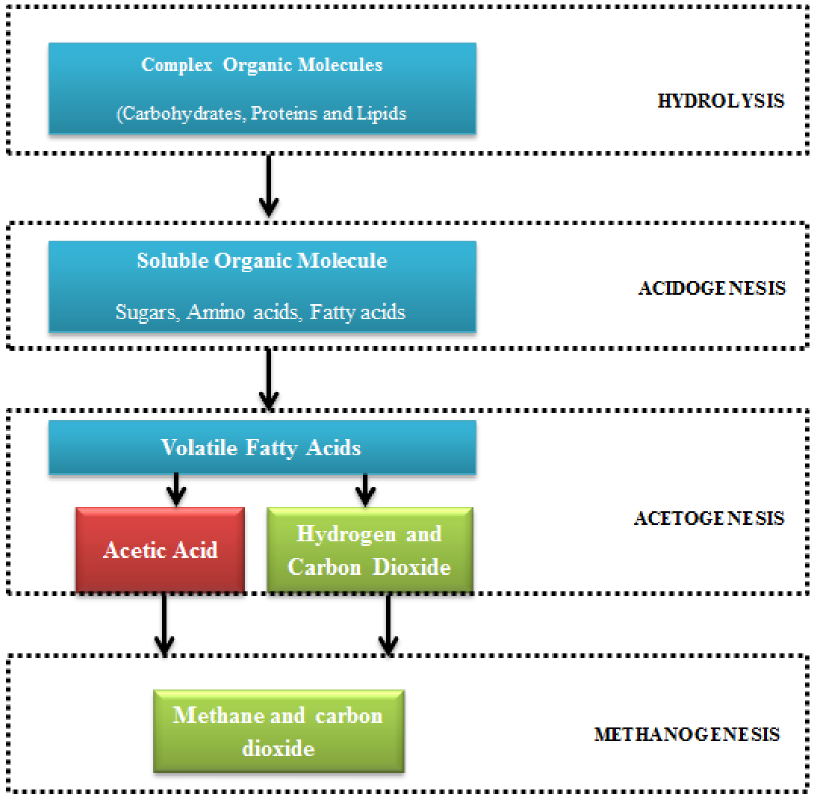


Figure 5: Anaerobic degradation process

a. Stages of Anaerobic Degradation

A specific characteristic of methane digestion is its phasing. Each of them accounts for degradation of a different type of compounds. Anaerobic transformation of organic wastes involves many different groups of bacteria, such as hydrolyzing, acidifying, acetogenic and methanogenic bacteria which in the final stage produce CO_2 and CH_4, that is, the main products of the process

i. Hydrolysis:

During hydrolysis process mostly insoluble organic compounds, such as carbohydrates, fats and proteins, are decomposed to soluble monomers and dimers such as mono-sugars, amino acids and fatty acids. In this stage of the methane digestion process hydrolyzing bacteria produces extra cellular enzymes such as amylases, proteases, lipases. The stage which limits the rate of reaction is during hydrolysis of hardly decomposable polymers, e.g. cellulose. Only 50% of organic compounds undergo biodegradation during solid wastes digestion and remaining portion remains in their initial state because of lack of appropriate enzymes which carry out their degradation. The process is manipulated by different process parameters such as pH, Particle size, adsorption and diffusion of different enzymes.

ii. Acidogenesis (Acidification phase)

During this stage, the acidifying bacteria convert water-soluble chemical substances, including hydrolysis products to short-chain organic acids such as formic acid, propionic acid, acetic acid, butyric acids, alcohols (methanol, ethanol), aldehydes, carbon dioxide and hydrogen. Amino acids and peptides produced from decomposition of proteins are used as a source of energy for anaerobic microorganisms. This process can be distributed further into two types: Hydrogenation and dehydrogenation. The products produced in acidogenesis which are usually carbon dioxide and hydrogen is effectively utilized by the methanogenic bacteria while other products remain unused. Among the products of this acidogenesis hydrogen sulfide and ammonia is also present which gives a intense and unpleasant smell to the phase. [14]

iii. Acetogenesis:

In this process, the acetate bacteria convert the acid phase products into acetates and hydrogen, pentanoic acid to propionic acid and then further convert this propionic acid to acetic acid which may be used by methanogenic bacteria. Acetegenesis is a phase which directly effects the biogas production in process and 65-70 % of the methane is produced in this process about 20 to 25 % is the aceteates and 10-12% of hydrogen is produced in this phase [15]

iv. Methanogenesis:

This phase is governed by methanogenic bacteria and methane is produced from the products such as acetic acids and hydrogen of previous phases. Methane in this phase of the process is produced from substrates which are the products of previous phases, that is, acetic acid, H_2, CO_2, formate and methanol, methylamine or dimethyl sulfide. The conversion of acetic acid is generally carried out by heterotrophic species of bacteria and nearly 30 % of methane generated comes from autotrophic species. Significant amount of H_2 is used up by acid forming bacteria which results low production of H_2 in acetogenesis phase and a gas rich in CO_2. [16][17]

6. Factors Effecting Anaerobic Digestion

Biological Processes are complex and sensitive to several factors. Slight variations in different parameters vigorously effect the organic degradation hence limiting the reactors performance. [18] These key parameters are listed as

- Carbon to Nitrogen Ratio
- pH
- Temperature
- Volatile fatty Acids
- Retention Time
- Feed to Inoculum Ratio

6.1.1. Carbon to Nitrogen Ratio

C/N ratio is one of the major threats to anaerobic digester performance. If the C/N ratio of feed and inoculum is not adjusted or optimized the increased nitrogen can form inhibitory ammonia within digester hence limiting the microbes performance. One of the techniques utilized for improving carbon to nitrogen ratio is Co-digestion of substrates. Studies revealed that animal manure contain high nitrogen values and can yield ammonia on digestion while the green and plant material are rich in carbon. So co digestion will balance the carbon to nitrogen hence increasing the reactor performance. [19]

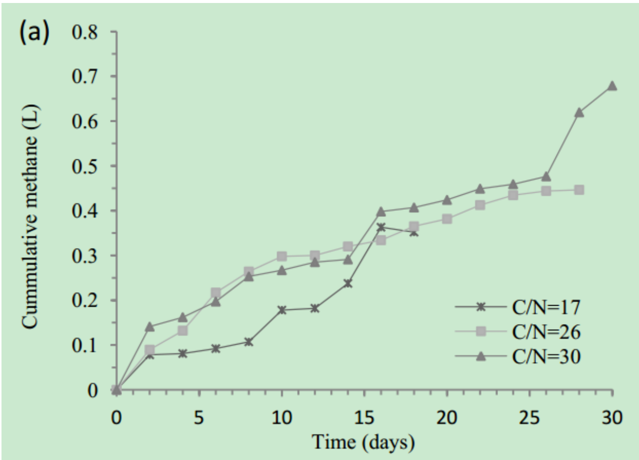


Figure 6: Effect of Carbon to Nitrogen ratios on biogas production form FVW

6.1.2. pH

Anaerobic Digestion Process is strictly sensitive to pH change. The organic acid produced during anaerobic digestion process tends to change pH of the reactor and expose bacteria to harsh acidic environment. These variations are generally caused by Volatile fatty acids, carbon dioxide or chemicals such as NaOH. Important stage in terms of pH is Acetogenesis where pH declines to 4-5 which causes death of methanogenic bacteria leading a digester failure. [20] (*Kun Wang* et al., 2014) studied effect of pH variation on food waste using different inoculum sources. The study proposed that the maximum performance of anaerobic bacteria was between pH ranges of 6.5 to 7. They observed that VFAs production was highest at pH of 6. Similarly study carried out by (*Lili Yang & Yue Huang in 2015)* stated that the pH adjusted digester produced more biogas then the unadjusted reactor. Leta Deressa et al., in 2015 studied the pH effect on biogas production from fruit and vegetable waste and suggested a pH range of 6.7 to 7.4 for maximum performance of digester [12]. A study carried out by (Simon Jayaraj et al., 2014) showd that biogas production from food waste was maximum at pH value of 7 among pH values of 5, 6 7, 8 and 9 . The COD, TS and VS removal efficiency was also maximum at pH range of 7 and lowest in 5. [21]

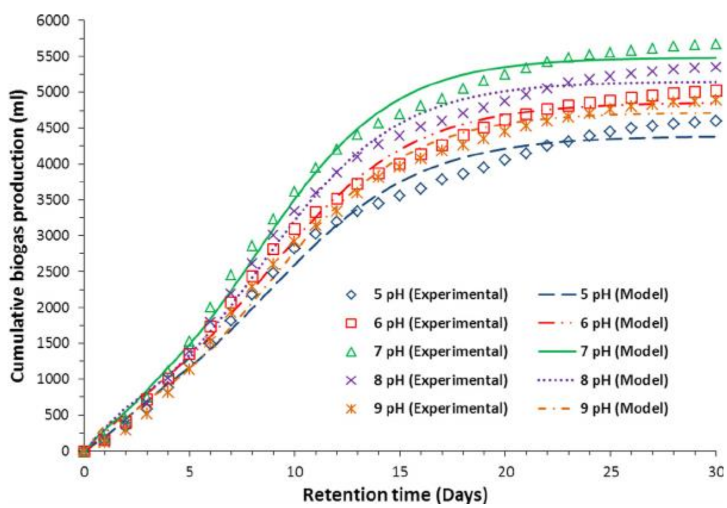


Figure 7:Effect of pH on Biogas Production

6.1.3. Temperature

One of the essential parameters to be considered for anaerobic digestion is temperature. Microbial Consortia requires optimum conditions of temperature for survival and maximum performance. There can be three types of conditions for bacteria to thrive. [22]

Psychrophilc Conditions	20-25°C
Mesophilic Conditions	30-40°C
Thermpphilic Bacteria	50-65°C

Anaerobic Digestion generally occurs at mesophilic and thermophilic conditions. Psychrophilic Conditions are under consideration for dry anaerobic digestion. Researchers have starting exploring and comparing the results of psychrophilic and mesophilic conditions. Stevens & Schule in 1979 reviewed the low temperature digestion and found methanogenic bacteria active at temperature as low as 4°C. The study averred that retention time of process increased while operating at lower temperatures. A Study carried out by Sutter &Wellinger in 1985 reported similar findings. Literature suggests that increasing the temperature drastically influences activity of methanogenic bacteria. [23]

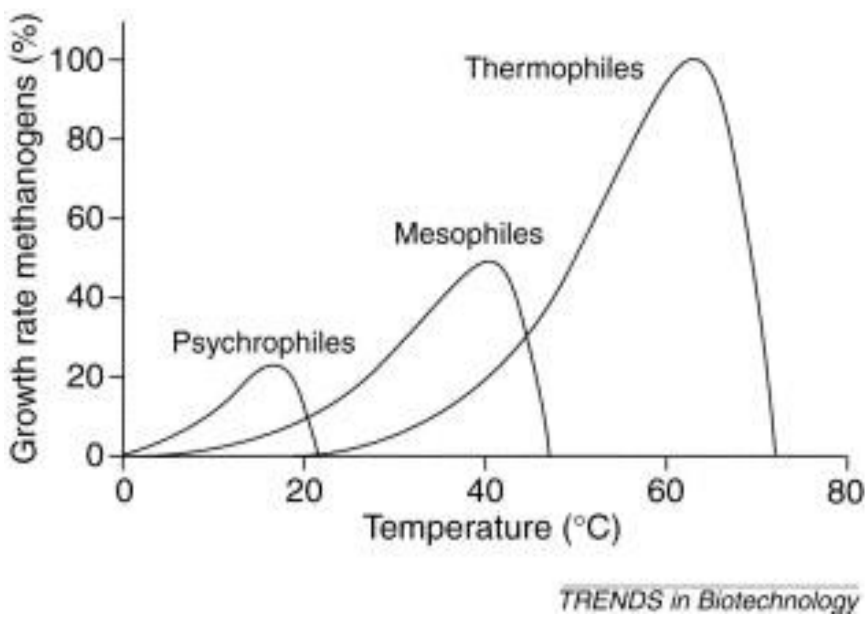


Figure 8: Effect of Temperature on Microbial Growth

Tropical regions provide mesophlic conditions for bacteria. As the picture suggest increasing temperature increases bacterial growth but to a certain level. Higher substrate destruction occurs at elevated temperature but gas producing bacteria die above a certain temperature level.

At temperatures above 65° C abatement of bacterial growth occurs. So provision of temperature control mechanism is much important in anaerobic digestion process for higher biogas and methane yields.[17][20]

6.1.4. Volatile Fatty Acids

Volatile fatty acids are the mid products in anaerobic digesters which are produced by both acidogenic and actogenic bacteria. Reactor's environment can get toxic for bacteria by acidification due to overloading. Parameter within reactor such as pH, temperature and VFAs are interlinked to each other and require a balance for better performance of digester. For example pH value decreases with increasing amount of VFA in reactor.[24] VFA is considered one the most important factor for accessing reactor performance. Studies suggested that accumulation of propionic acid at around 900mg/L caused a significant inhibition in reactor. Butyric and acetic acid accumulation upto 1800 and 2400mg/L did not caused significant process instability. [25]. The conversion of volatile fatty acids to acetic acids effect the quantity of methanogenic bacteria. A number of studies have been carried out to study effect of VFA on different reactors. Komemoto et al in 2009 studied the effect of temperature and VFA on performance of anaerobic reactor. [26]

6.1.5. Retention Time

Organic Molecules require a specific time for degradation which is often termed as retention time. There are two different types are discussed which are Hydraulic Retention Time and Solid Retention Time. The hydraulic retention time is defined by the mathematical equation as

$$\mathbf{HRT = V/Q}$$

where V is the reactor volume and Q is the flow rate of the influent. Solid Retention Time is the time required by bacteria to degrade solid fraction of organic waste over a certain time.

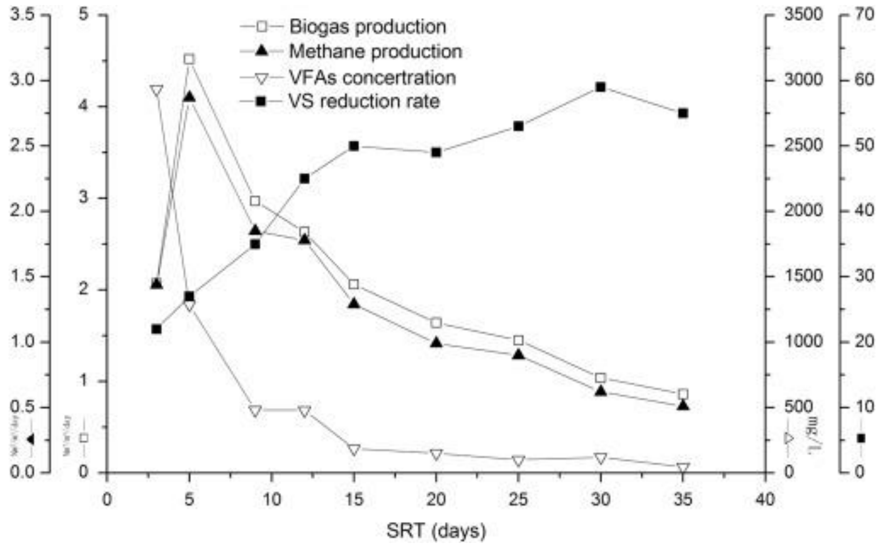


Figure 9: Effect of Retention Time on biogas production

6.1.6. Effect of feed to Inoculum ratio

Feed to inoculum ratio or f/I ratio is the ratio of mixing of bacterial source and organic substrate. It is one of the most key parameters for stability and enhancement of anaerobic digestion process. This can provide sufficient information for minimum requirement of inoculum to initial the biological reaction. The optimization of F/I is important because of the fact that increased amount of nutrients can cause bacteria to die. On the other hand reaction cannot start if sufficient amount of bacterial consortium is not present. Many researchers has carried out research for various substrates and inoculum types e.g Cunsheng Zhang et al ., 2013 worked in various feed to inoclumn ratios for green waste mixed with cow manure [27] . *Muhammad Rashed Al Mamun* worked on f/I ratios from 1 to 5 for cafeteria waste and found out that the highest production of biogas and methane content increased by decreasing the f/I ratio from 5 to 1.[28] *Xi-Yu Cheng et al ., 2014* worked in f/I ratio of 2 to 6 for anaerobic digestion of cotton stalks. A graphical trend observed by the study is shown below. [29]

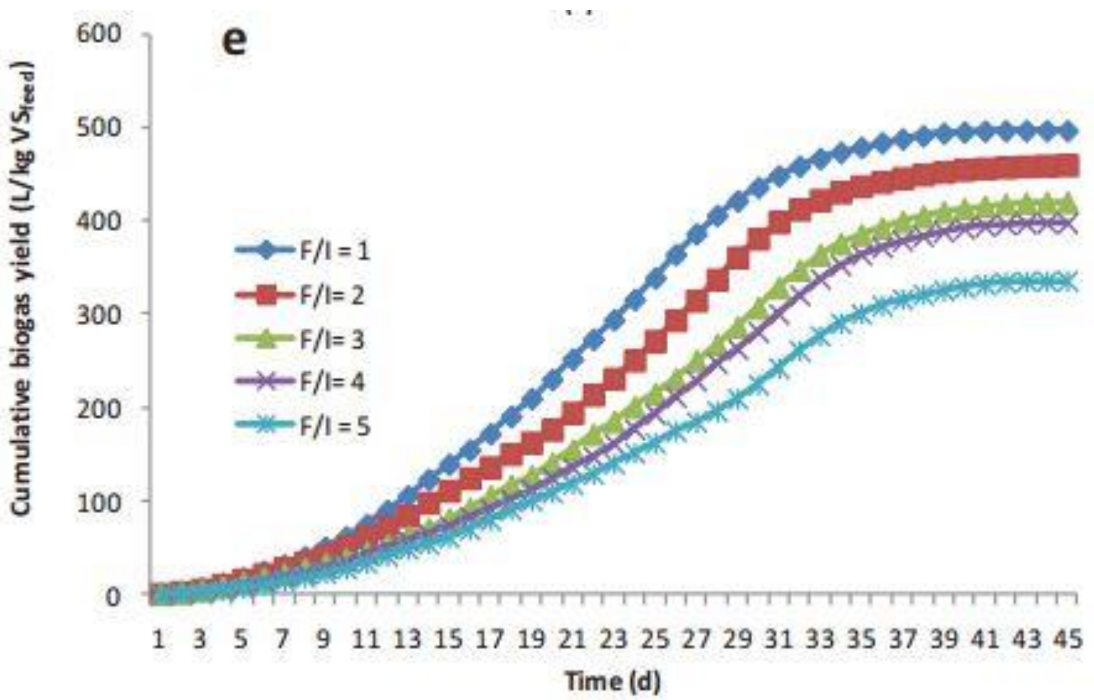


Figure 10:Effect of Feed to inoculum Ratio on biogas production from vegetable waste

2. Environmental Benefits of Utilizing Fruit and Vegetable Waste for Energy Generation

Environmental pollution is also a great issue and threat being faced by country. Increasing landfills and their emissions, untreated flue gas discharge from the power plants and other industrial chimneys have started to cause their impacts on climate in the form of acid rains and smog in major cities. [30] Recent studies suggest in Pakistan nearly 14500 MT of methane is emitted from landfills annually. This tremendous amount of energy goes unused while causing its drastic impacts on climate in form of greenhouse effect. This can be reduced around 88% by harnessing this methane and restricting its exposure to environment from landfills. Utilizing half these landfill materials in a controlled environment can contribute a significant amount of methane to produce around 85MW of electricity.[31] One of the major causes of severe environmental pollution in many countries is inadequately managed and dumped organic waste which often involves food waste, paper waste, and other from domestic sector and large amount of waste from industrial sector also. Anaerobic Digestion is one of the treatment processes used to degrade the organic waste by microbial activity. Replicating a natural process of microbial degradation in a controlled environment produces biogas which can be used for different purposes after further treatment. [32] [33][34]

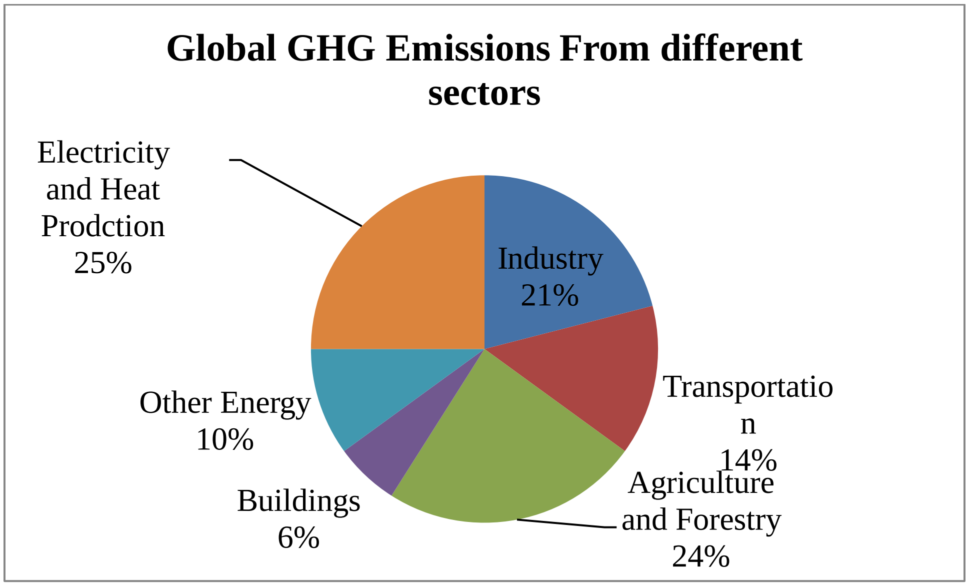
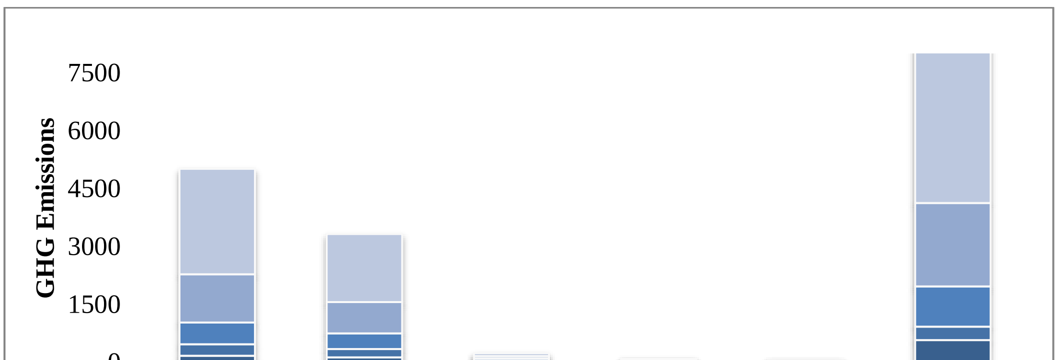


Figure 11:Global GHG Emissions by different sectors in around world 2015-2016



	Energy	Agriculture	Industry	Land use	Waste	Total GHG Emissions
2050	2730	1765	75	35	16	4621
2040	1250	812	61	20	13	2156
2030	560	408	52	15	11	1046
2020	295	210	30	13	9	347
2011	176	134	20	10	7	577

Figure 12: Projected GHG Emission in MT oil Equivalent for Pakistan

7. Classification of Anaerobic Digesters

Anaerobic digesters are the reactors used for carrying out anaerobic degradation of organic waste. These digesters are of various types and their selection depends on acute technical and economic analysis. A simplified and precise diagram of the classification of reactors is given in figure [35]

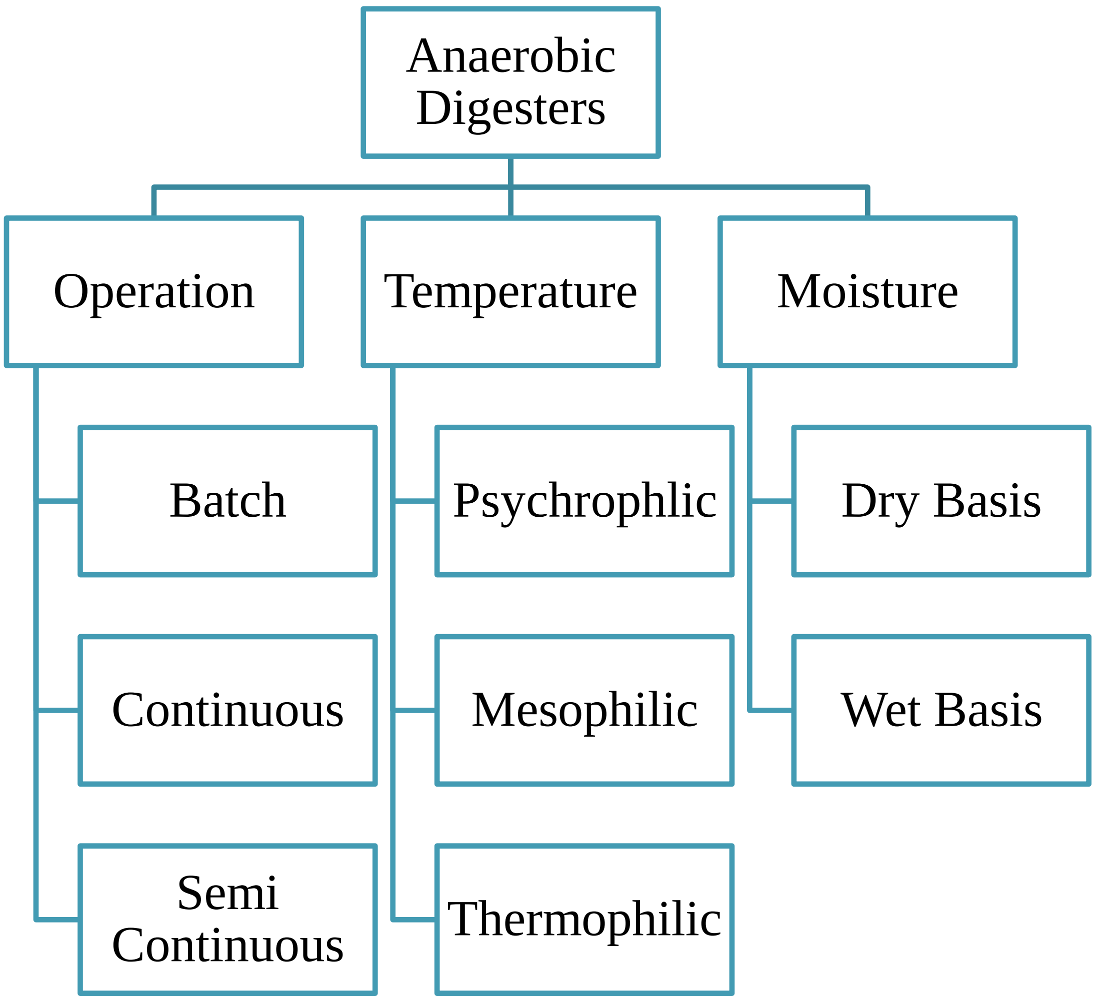


Figure 13: Classification of Anaerobic Digesters

The selection of reactor requires precise technical and economic analysis. Generally at smaller scale production which includes liquid phase requires batch and semi batch reactors. These are lower capital intensive than the continuous stirred tank reactors. When the process is scaled up, per unit production cost rise thus making it less viable for larger scale. These overhead costs includes material handling cost, labor cost safety measures costs and the costs of loss which occurs in the reactor emptying and refilling time.

Batch Digesters

Batch digesters are types of closed systems which is once inoculated and seeded gets not additional feed for reaction. The energy or material inputs strictly remain restricted and the waste products remain within the system instead of continuous removal. The stirring and agitation can be applied in the batch system depending on conditions to be provided. Nutrients and other materials like oxygen are declining and metabolic waste products are increasing. Nutrient broth culture tube is the most common and the simplest example of a batch reactor while the example of batch process includes microbial colonies growing on plates or slants. Table 4 represents the advantages and disadvantage of such digesters. [35], [36]

Criteria	Advantages	Disadvantages
Technical	○ Simple ○ Low-tech ○ Robust (no hindrance from bulky items)	○ Clogging ○ Need for bulking agent ○ Risk explosion during emptying of reactors ○
Biological	○ Reliable process due to niches and use of several reactors	○ Poor biogas yield due to channeling of percolate ○ Small OLR
Economical & Environmental	○ Cheap, applicable to developing countries ○ Small water consumption	○ Very large land acreage required (comparable to aerobic composting)

Table 4: Advantages and Disadvantages of batch digesters

8. BMP Assay

Bio methane potential assay is a lab scale test for assessment of methane and biogas content of organic wastes. Biochemical methane potential (BMP) was first used by Owen et al. in 1979 BMP test are carried out at initial stages of large scale reactor design for anaerobic digestion.

The results obtained from the assessment are used. Different design parameters can be tested at lab scale and the performance can be optimized efficiently. This test is much beneficial at initial design stage because of minimal cost and labor. During last decades numbers of publications had been done but still a standard procedure for performing this assay is missing. The changes have occurred in gas measurement and gas collection methods during last few years.[17] The effective BMP testing requires appropriate microbial community and optimum conditions for bacteria to grow. The test is generally performed in 50ml to 1L serum bottles. The organic waste is incubated in bottles and sealed appropriately. The pH is adjusted and the headspace of bottles is purged with any inert gas such as nitrogen. The bottles are then incubated at 35° C for mesophilic conditions and provides environment to bacteria to grow. One of the aspects of BMP test is availability of micro and macro nutrients to bacteria. There should be appropriate amount of nutrients available for BMP tests. Over feeding or underfeeding the digester with nutrients will tend bacteria to die hence limiting the performance of batch reactors. [37] *Kyoung et al*., 1995 used BMP assay for finding out the potential of methane and biogas from food and vegetable waste [38] *Chynoweth, D.P. et al*., in 1993 worked in biochemical methane potential of different biomass material including woody biomass, and municipal solid waste fractions. [39]. *Gunaseelan et al.,* in 2004 studied 54 different vegetables and fruit waste using BMP setup. Different parameter including biogas production and effect of temperature was studied and there occurred substantial difference in methane and biogas production and kinetics. [40] Owens, J. M. studied BMP of municipal solid waste and averted the methane conversion rates different substrates such as grass, paper waste. *Frantseska Maria et al*., in 2016 studied effect of substrate to inoculum ratio on biochemical methane potential of agro industrial wastes and studie different inoculum such as anaerobic sludge, landfill leachate and thickened anaerobic sludge was tested along with different feedstock such as fruit processing plant waste and cotton waste. [41] *Rodrigo A. et al*., in 2011 studied biochemical methane potential of an array of substrates and co digestion with dairy manure.

The results showed that methane content increased with co digestion instead of mono-digestion of dairy manure. 175 individual BMP assays and asserted that the substrates rich in lipids are degraded easily. [42] Yu Sheng et al., in 1994 studied the samples taken from California landfill plants using BMP assay. The test was carried out in 125ml serum bottles. Cellulosic and hemiscellulosic material were tested during the experimentation. [43] The graphical representation of BMP setup is given in figure below

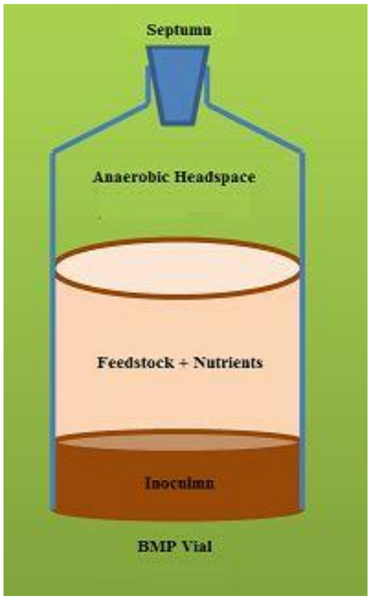


Figure 14: BMP Vial

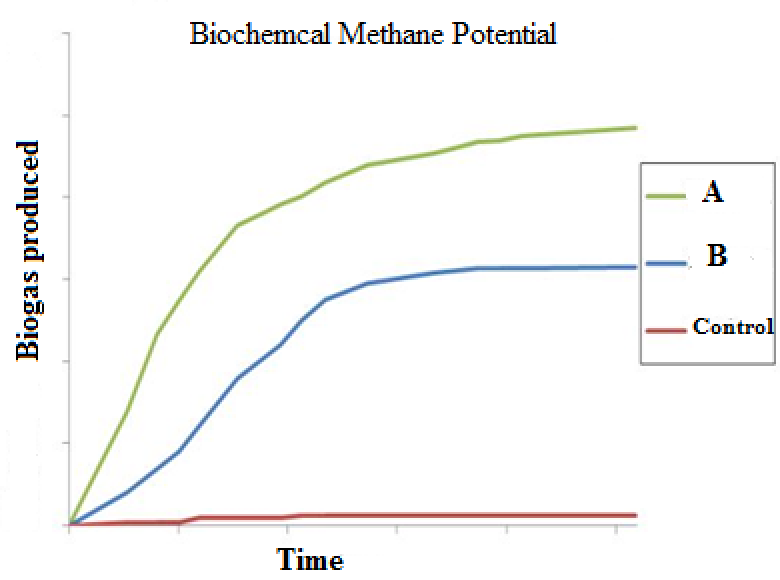


Figure 15 : Graphical representation of Biochemical Methane Potential

CHAPTER 3

MATERIAL AND METHODS

9. Experimental Scheme

The scheme of experimentation carried out in biofuel laboratory of United States Pakistan Center for advanced studies in Energy at NUST Islamabad below. Two trials of AD were carried out in a 280 ml working volume batch reactor bottles at 35°C ±2 °C. The experiment was carried out in steps as follows.

- Collection of Samples and storage.
- Collection of Inoculum and storage.
- Physio-chemical characterization and testing of the pre-treated samples;
- Experimental Setup and Temperature Control;
- Experimental Run at mesophilic conditions 35 °C.
- Measurement and analysis of the composition of the biogas produced for specific HRT.

a. Sampling of Substrates:

The substrate for the experiment involved, Banana Peels, Okra Calyx and Potato peels sample were collected from the kitchen of NUST hostels and stored in Plastic Storage Bottles

a. Around 2 Kg of waste was collected from kitchen and stored in Refrigerator at 4 °C
b. Banana peels were collected from the milk shake shops at Canteens of NUST Campus and stored in Plastic Storage Bottles in Refrigerator.
c. Potato Peels and Okra calyx were collected from NUST canteens and washed with distilled water and stored in plastic containers

Figure 16: Samples Collection and Sorting

b. Sampling of Inoculum:

The inoculum was collected from a manure digestion Plant in Fateh Jhang around 40 Km from Campus stored in two capped plastic bottles of 5 Ltrs Capacity. The bottles were properly Sealed and covered with wraps to prevent any leakage. The Conditions of the source provided by plant authorities are as follows.

- Plant Capacity: 10 Cubic ft
- BOD_5: 1.92mg/l
- COD: 7.3mg/l
- pH: 7.2
- Source Temperature: 37°C
- Waste Digested: Vegetable waste and Dairy Manure
- Plant Type and Agitation: Floating Head with Mechanical Motor
- Hydraulic Retention Time: 30 days

10. Physicochemical Characterization

The physiochemical characterization of substrates comprised the following parameters:

- Moisture content, Solids content, Total Solids (TS), Fixed Solids (FS) , Volatile Solids (VS)[44]
- Chemical oxygen demand (COD)
- Carbon, nitrogen, sulphur and oxygen content

10.1. Moisture Content

Samples were weighed on a weighing balance in a china dish. The weighed samples were kept in a drying oven at 105°C (±2°C) for 12 hours. The samples were taken out of drying oven and kept in a desiccator for cooling. Samples were weighed and change in mass was noted using following equation.

$$M_w = M_1 (M_2\text{-}M_c)/M_1 *1000$$

Where Mw is moisture content in grams

M_1 = mass of wet sample in grams

M_2= dry sample + mass of crucible in grams at 105°C (±2°C)

M_c= mass of china dish in grams**.**

Banana = 10.02(43.83-42.42)/10.02*100 = 85.92 ~ 86 %

Okra calyx = 10.02-(44.46-42.35)/10.02 *100 = 78.9 ~ 79%

Potato = 10.02-(35.91-33.40)/10.02 *100 = 74.96 ~ 75%

10.1. Solid Content

Solids Content was calculated by difference of weights after drying by eliminating moisture content from total mass of sample

Solid Content = Mass of wet Sample – Mass of moisture

Okra calyx = 44.46-42.35/10.02*100 = 21.01%

Banana = 43.83-42.42/10.02*100 = 14.07 %

Potato = 35.91-33.40/10.02*100 = 25.01 %

10.2. Fixed Solids, Volatile Solids , Total Solids

To determine the fixed solids previously dried and weighed samples were burnt in box furnace for 2 hours at 550 C. the fixed solids were calculated according to following equation .

$$M_F = M_2 - M_C / M_1 * 1000$$

Where MF is fixed solids content in grams

M_2= dry sample + mass of crucible in grams 550 °C (±5°C)

M_c= mass of china dish in grams**.**

M_1 = mass of wet sample in grams

Okra Calyx = 2.11-1.71/2.11 *100 = 19%

Potato = 2.51-2.21/2.51 *100 = 11.95 %

Banana = 1.40-1.26/1.40*100 = 10.1 %

The volatile solids were found by difference in mass of initial and final sample after burning and subtracting the amount of Fixed solids from initial weight

Volatile Solids = Mass of Initial Sample – Fixed Solids

Total Solids = Fixed Solids + Volatile Solids

Figure 17: Box Furnace

Figure 18: Sample Measurement on Wight Balance

Substrate	Wt of china dish (wd) In Grams	Wt of sample (ws) In Grams	Wt of sample + dish (w1) In Grams	Wt after drying at 105°C for 12 hours (w2) in Grams	Total Solids in grams (TS=w2-w1)	Wt of moisture in grams (w3=w1-w2)	Wt after drying at 550°C for 2 hours (w4)	Volatile Solids VS = W_2-w_4 In grams	Fixed Solids FS in Grams FS=TS-VS
Potato	33.40	10.02	43.42	35.91	2.51	7.51	35.61	0.30	2.21
Banana	42.42	10.02	52.44	43.83	1.40	8.61	43.69	0.14	1.26
Okra	42.10	10.00	52.10	50.5	1.6	8.38	42.24	0.25	1.35
Inoculum	42.66	10.02	52.68	44.16	1.50	8.51	44.01	1.35	0.15

Table 5: Total Solids, Volatile Solids and Fixed Solids of Substrates

Feedstock	Moisture%	TS%	VS% dry basis
Okra Calyx	84	16	15
Banana peals	86	14	10
Potato	75	25	12
Inoculum	85	15	10

Table 6: Volatile Solids Percentage

11. Chemical Oxygen Demand (COD)

One of the important physiochemical parameter is Chemical oxygen demand (COD) which is defined as "the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence". Standard APHA open reflux method was used for calculating COD. Open reflux method was used because it requires lesser homogenization and large particle size and COD values greater then 50mg of O_2 /ltr. The samples were converted into slurry using a lab scale blender and homogenized. Samples were diluted with 10 folds of water to lower the COD range. The final value was calculated using interpolation methods [45][46]

11.1. Reagents Preparation

12.26 g of potassium dichromate $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, was mixed in distilled water to produce a solution and diluted to 1 liter. For preparation of sulfuric acid reagent Ag_2SO_4 (reagent grade) crystals, were added to conc. H_2SO_4 at the rate of 5.5 g Ag_2SO_4 /kg H_2SO_4. And was stirred for 1 day . For preparing Ferrous Ammonium Sulphate Titrant (FAS) 98 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H2O$ was added in distilled water and diluted to 1 Liter. To check molarity of FAS titrant following equation was used

$$\text{Molarity of FAS} = \text{Vol. of } K_2Cr_2O_7 \text{ solution used / volume of FAS Used} \times 0.25$$

Figure 19: Prepared COD Reagents

Figure 20: Sulfuric Acid Reagent for COD

Figure 21: Open Reflux COD Digestion Apparatus

12. Elemental analysis using EDS and SEM

The elemental Analysis was done by a TESCAN VEGA3 SEM+EDS. The previously dried samples were further dried for 2 hours for complete moisture removal and to be used for EDS Analysis. The samples were gold coated for 5-10 minutes. Table 1 describes the obtained results for Detailed Elemental Analysis. The conditions provided for test are as follows [47]

Voltage Range = 10-15 KV

Working Distance= 15mm

Magnification = 184x

Scale = 200nm

	Banana		Potato Peals		Okra calyx	
Elements	**Weight %**	**Atomic%**	**Weight %**	**Atomic%**	**Weight %**	**Atomic%**
Carbon (C)	61.24	68.47	59.60	67.40	58.59	66.68
Nitrogen	0.52	0.24	3.62	6.79	9.94	9.70
Oxygen (O)	36.59	30.72	34.11	24.86	22.92	19.58
Potassium (K)	1.65	0.57	2.35	0.82	0.26	0.09
Maganesium (M)	--	--	--	--	0.13	0.07
Phosphorous (P)	--	--	0.24	0.10	--	--
Sulphur (S)	--	--	0.08	0.03	0.12	0.05
Chlorine (Cl)	--	--	--	--		

Table 7: Elemental Analysis of substrate using EDS

13. BMP Experimental Setup

The experiment was designed in glass bottles of 280ml volume with a working volume of 260 ml. feed and inoculum was added in measured quantities according to specific F/I ratio as given in table. The experiment was set for fixed HRT of 30 days and with variable Organic Loading Rates. Organic Loading Rate was determined by the equation

$$OLR = \text{Concentration of organic Matter added in } (kg.m^3) * \text{daily inflow of feed } (m^3.d^{-1}) / \text{Volume of Reactor in } m^3$$

The reactor bottles were used as batch reactors so the daily inflow of feed stays to zero. Bottles were purged with nitrogen for 2 minutes and sealed with septum and covered with steel caps and sealant. Temperature controlled convective incubator was designed to maintain 35°C ±2 by customizing a fish aquarium and insulating it with aluminum foil and styro-foam. K type Thermocouple was attached to the system and placed at equal distance from the temperature source which was bulb in this case. REX-C700 Temperature Controller with temperature range of 0-1300° C was used for temperature stabilization in system. [42]

F/I	Substrate (g VS)	Inoculum (g VS)	HRT (Days)
1.5	15	10	30
2.5	17.8	7.1	30
3.5	19.5	5.5	30
4	20	5	30
Control	25	0	30

Table 8: BMP Experimental Protocol

Figure 22: Prepared Batch Digester Bottles

13.1. pH Testing

pH was initially tested before purging the digesters with pH Strips. At start of setup Strips showed pH at neutral while deviation occurred after few days and pH deviation was controlled using appropriate neutralizing agent. pH was adjusted and neutralized as the experiment proceeded. The initial pH is important for setting up the batch reactors. Initial pH increase or decrease can create digester failure

Figure 23: pH testing Procedure

CHAPTER 4

Results and Discussions

14. Banana Peels

14.1. Volatile Solid Reduction

Volatile solids reduction is a performance indicator for anaerobic digestion of organic waste. The volatile solid reduction generally is associated with the active bacteria. The results for banana peels showed that maximum reduction occurred at ratio of 1.5 which relates the results of biogas production. The trend shows that increasing the feed decreases the volatile reduction in reactor. About 60 -65 % of reduction occurred in ratio of 1.5 and lowest occurred only 10 to 15 % in control reactor and a F/I Ratio of 4 at ratio 2.5 the percentage of reduction of VS is 55%. The major reduction phase occurred during first 15-25 days as the amount of biogas produced was maximum during this phase. The VS reduction is different in different ratios because of difference in amount of cellulosic and hemiscellulosic material.

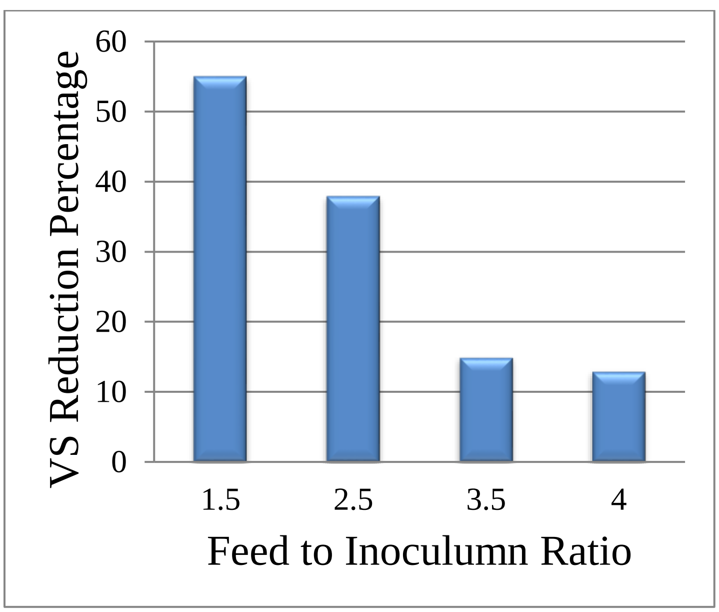


Table 9: Volatile Solids Reduction

14.2. Chemical Oxygen Demand Reduction

Chemical oxygen demand was calculated for the initial feeding and final effluent by APHA method. The COD results are generally more vulnerable to deviation because there are no specific methods for COD of solids substrate so COD was performed by APHA Method by applying multiple dilutions. The results showed value of COD maximum for the control reactor because control contains maximum amount of substrate. The COD reduction was maximum in F/I ratio of 1.5 because of greater activity of bacteria as it produced maximum amount of gas. The minimum COD reduction of around 1 to 5 percent occurred in controlled reactor because degradation of material didn't occur enough to reduce COD. The results are given in table

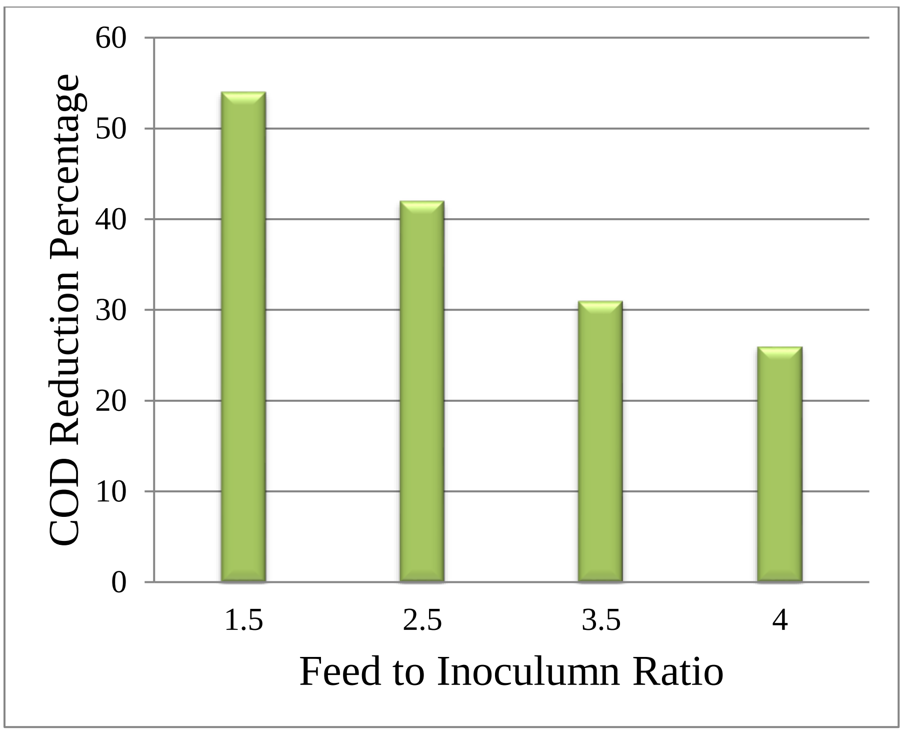


Figure 24: Chemical Oxygen Demand Reduction

14.3. Biogas Production

The biogas containing both methane and co2 and oxygen is shown in fig the maximum biogas produced is in ratio 1.5 and 2.5. The maximum amount of biogas nearly 80 ml produced on day 18^{th}. The biogas production exponentially rose from day 1 to day 19^{th} and then begins to stabilize and decrease from day 25^{th}. The control reactor showed activity in production of biogas because of naturally present methanegenic bacteria in feed. The biogas production is closely in relation with the pH. The decrease and increase in daily biogas production is because of pH Variations. Maximum amount of biogas in 2.5 is 60 ml. The biogas production in 3.5 and 4 showed a positive trend toward end of reaction which shows that increasing the retention time for these gas production values can increase overall biogas production. The cummulatve biogas production previosuly reported by *Nipon Pisutpaisal et al*., 2014 was 450 ml /g of VS added in reactor. [48]

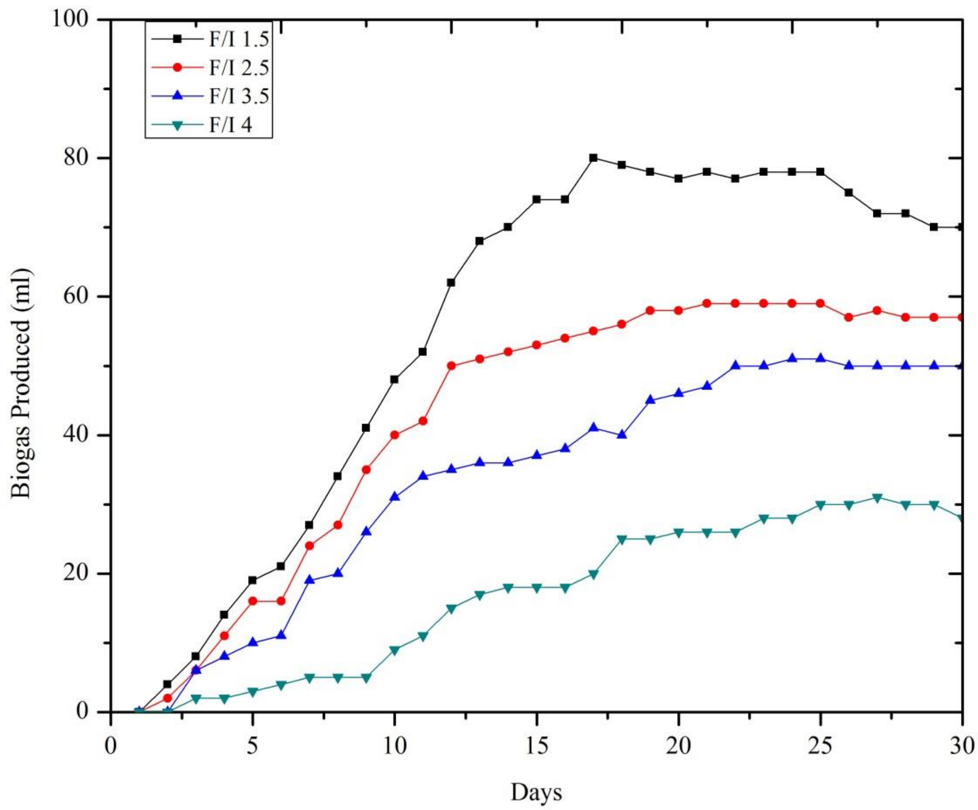


Figure 25: Biogas Production for Banana Peels

14.4. Methane Concentration

The result for banana peels shows that highest methane percentage of 62 percent occurred in ratio 1.5 the methane content started from 0.33 percent and rose to 62 percent. The highest methane content in ratio 1.5 occurred on day 21. The highest percentage in 2.5 occurred on day

27th. The trend shows that increasing the substrate to inoculum ratio increased the methane production time. For example as the result shows methane started to build up earlier in lower F/I Ratios while the methane concentration increased in ratio 3.5 from day 25. The ratio 4 and control showed the minimum amount of methane concentration because of drastic changes in pH and increased feed. Which generally limit the methanogenic bacteria activity. Control digester shows methane concentration because while feeding the digester there can be some microbial consortia present which digested the substrate and produced methane. Difference between gas production and methane concentration and pH can be due to certain leakges occurring while performing the test on gas chromatograph. Previous studies carried out by *Nipon Pisutpaisal et al., 2014* suggest that highest methane percentage was between range of 50-70% and study suggest that increasing the volatile solids increased gas production but to a certain limit of 10% VS further increase in VS solids decreased methane production. [48] Nirmala Bardiya et al., 1996 used banana peels for biomethane generation and achieved a methane concentration of 55-57% of methane using crushed and powdered banana peels. [49]

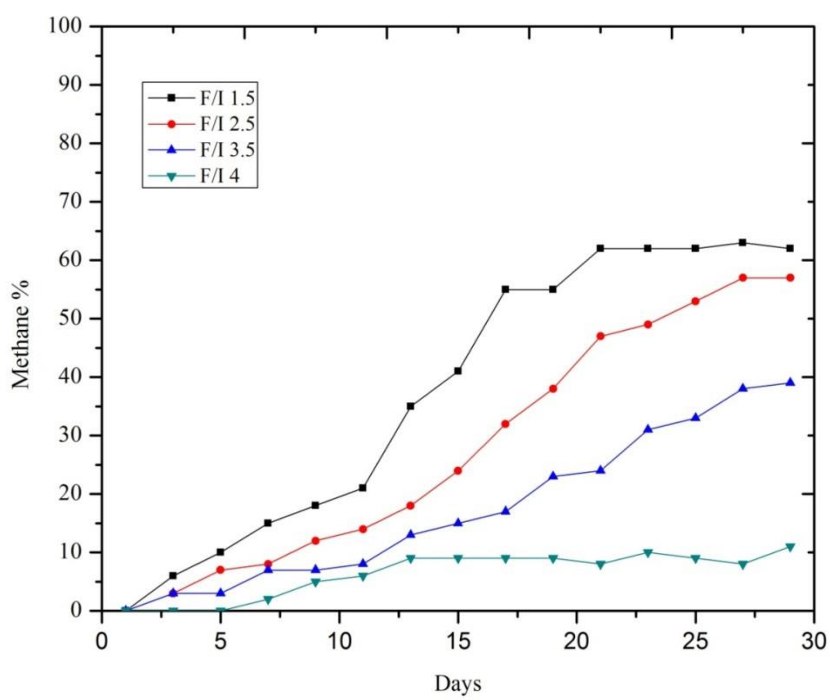


Figure 26: Banana Peels Methane Content

14.5. pH Variations

The results shows that average pH remained between minimum 6 to maximum 8.5. The pH values were more variable by increasing the substrate to inoculmn ratio for banana peels. The increased ratio accumulated more acids which drastically decrease the reactor performance as this can be seen that initially the pH increased to basic side which was adjusted with appropriate amount of acid solution. In Ratio 1.5 the biogas production decreased from day 16^{th} to 20^{th} because of pH drop which was adjusted by NaOH solution. For ratio 4 the pH varied between 6 to and minimum to 5.5 which decreased the gas production trends. Towards the end the pH was not adjusted and moved towards acidic nature which decreased the gas production also.

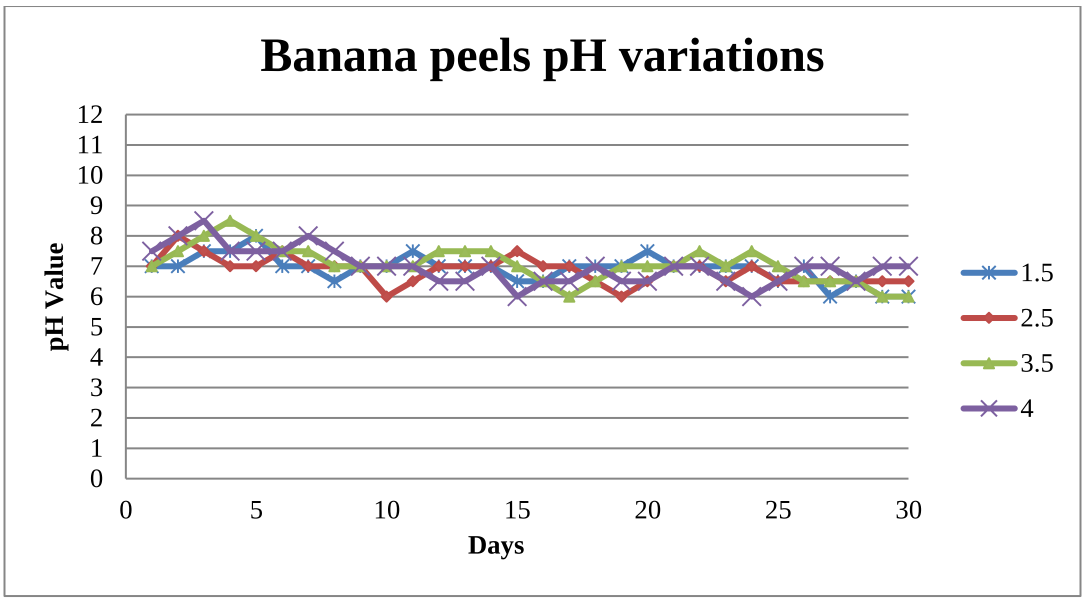


Figure 27: Banana Peels pH variations

15. Potato Peels

15.1. Volatile Solid

Figure 1 show that volatile solid reduction of potato peels was maximum in ratio 2.5 which is around 70 to 75 percent of VS reduction. The VS reduction for ratio 1.5 is 55 to 60 %. For ratios 3.5 and 4 the VS reduction is around 35-40% and 20 to 25 % respectively. The greater VS reduction in 2.5 is due to greater activity of microbes to reduce the substrate. This gives the fact that for maximum performance for potato peels for biogas production will be on 2.5. Increasing the substrate will increase total solids and volatile solids in the reactor which will require more time to degrade. In comparison with other substrates the above mentioned results show that maximum volatile solids reduction occurred in potato waste. The volatile solid reduction for ratio 2.5 can be greater because of slight difference in particle size. Smaller the particle size greater will be the reduction. Total VS reduction reported in previous studies is from 70-80 % which is near to this study. The study has reported greater protein content lower down the activity of microbe because protein gives rise to NH4-N which generally causes reactor failure. The reason behind the lower TS VS degradation by increasing F/I ratio is due to this increased protein content.

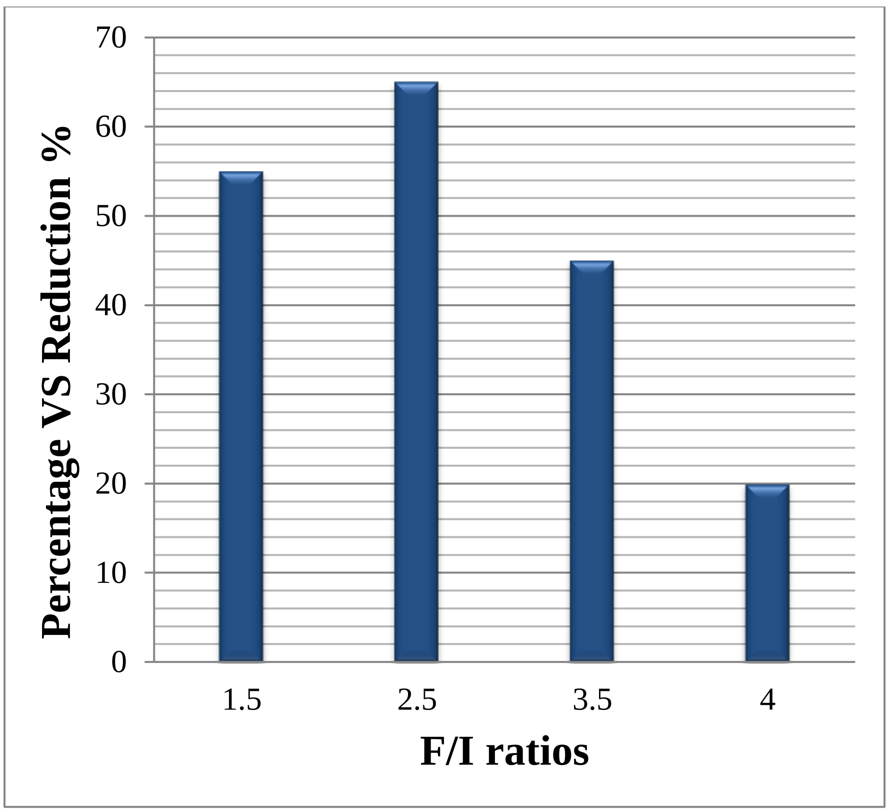


Figure 28: Volatile Solids Reduction for Potato Peels

15.2. COD Reduction

COD reduction is closely related to VS reduction of solids. This creates a direct relation between total solids and VS reduction. Greater the VS reduction greater will be the COD reduced. The results of potato peels in terms of COD reduction shows maximum COD reduction. Figure 2 shows that maximum COD is reduced in ratio 2.5 and minimum in 4. The reactor with ratio 4 was the most difficult one to measure COD because of greater amount of substrate. Control Reactor may have few methanegenic bacteria present which degraded the substrate. COD reduction is not only a measure for methane production but it is also helpful for measuring waterquality also.

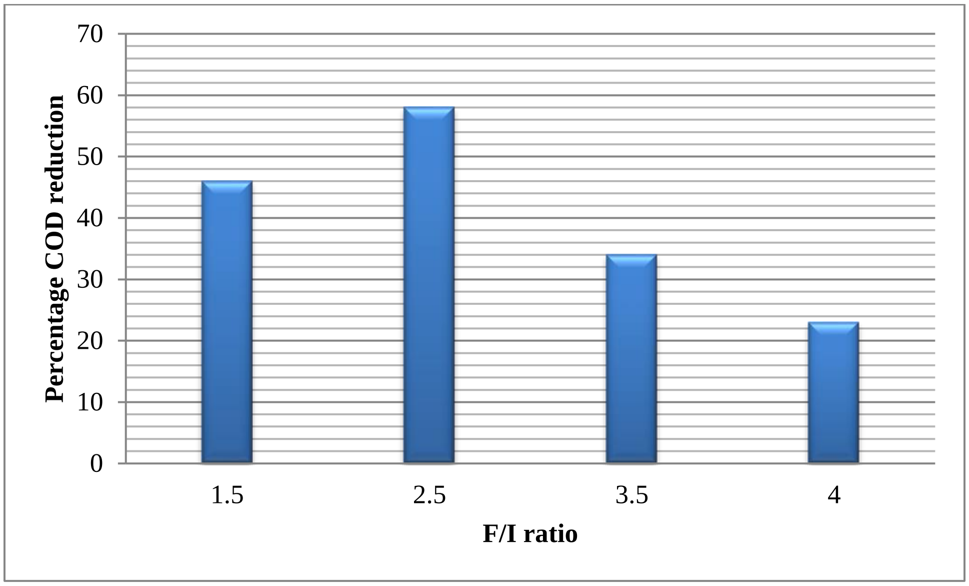


Figure 29: COD Reduction for Potato Peels

15.3. Biogas Production

The gas production for potato peels is greater than other substrates. Unlike other substrates the optimum ratio for biogas production for potato peels is 2.5. Although the values for ratios 1.5, 2.5, 3.5 are near to each other. The greater difference is between ratio 4. . The pH variations were increasing by increasing. The gas production was exponential from day 5 to day 15. This was different trend from other substrates where maximum biogas yields occurred after 20 days of incubation. Maximum biogas production for ratio 1.5 was 85 ml on day 20th. For ratio 2.5 maximum biogas produced was 97 ml on day 22nd and 23rd. For F/I ratio of 3.5 around 90 ml of biogas was produced from day 25 to 30. Cumulative biogas production for ratio 1.5, 2.5 and 3.5 and 4 are 2024 ml, 1829ml, 1380ml and 1450 ml while for control reactor cumulative biogas production was only 180ml. the results inferred that the biogas was decreased in ratio 4 reactors drastically after day 25th because of pH variations. The pH dropped drastically to acidic side the pH was adjusted using appropriate NaOH amount but still the effect of pH caused the digester failure. The biogas production previously reported by *Shaobo et al., 2014* is 239 L/kg VS fed which is greater than this study because of leakages occurring in the digesters. [8]

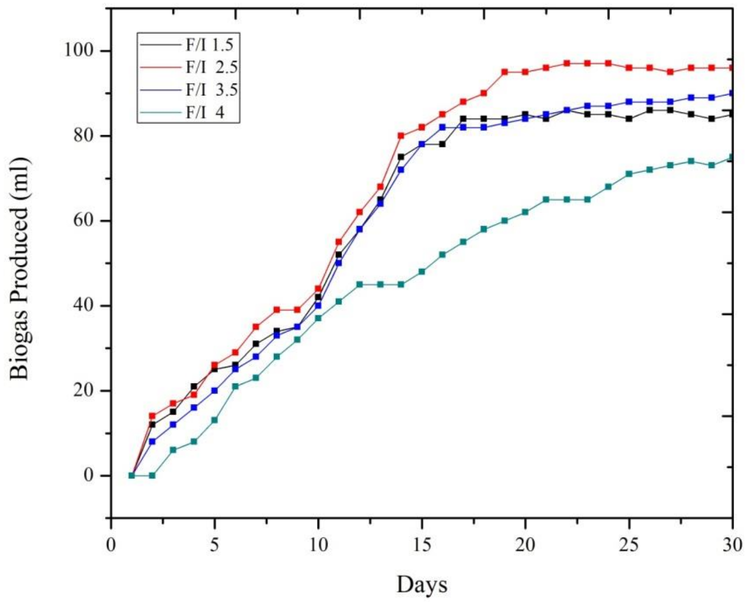


Figure 30: Cumulative Biogas production from potato peels

15.4. Methane Concentration

Figure shows the graph for methane percentage over time . The methane percentage trends show that maximum methane percentage was obtained in ratio 2.5 which is near to 70 percent of methane. For ratio 1.5 maximum amount of methane percentage was 62 and nearly 60 percent of methane was obtained from ratio 3.5. The trend shows that increasing the retention times increased the methane generated. The steps in the trends are variations due to pH which. pH causes direct effect on methane concentration. Maximum methane percentage for ratio was around 45 percent. This can be inferred from the result not even optimum ratio of 2.5 but other ratio like 1.5 3.5 can also contributes good amount of methane. Increasing the substrate further will yield more VFAs and pH variation hence producing less methane. *Parawira et al., 2004* worked on anaerobic digestion of potato peels and achieved a methane content upto 85 %. The reason behind this increased methane concentration is the study included greater amount of inoculum instead of substrates[50] . *Sahobo Lianget al.,* in 2015 showed that maximum methane percentage obtained for potato waste was 60-70 % and showed that pre fermented potato peels showd more methane percentage. [8]

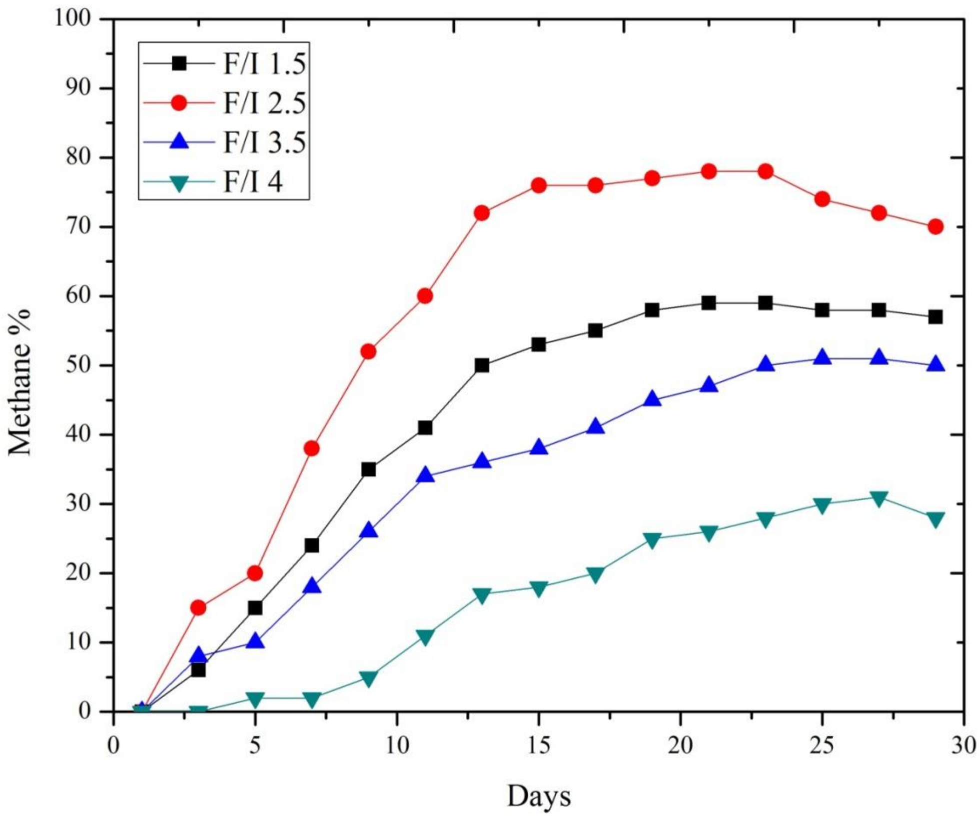


Figure 31: Methane Concentration of Potato Peels

15.5. pH Variations

pH variation and methane concentration are related to each other in a way that increasing or decreasing pH from range between 7 to 7.5 and below 6.5 causes intense conditions for bacteria. The accumulation of VFAs affects the pH value of digester. The result for pH shows that the pH value has been managed between maximum of 8 to minimum of 5.5. for ratio 2.5 which is the maximum yielding ratio in case of potato waste the pH effect occurred between day 7 and 10 . the pH dropped to acidic which was balanced with appropriate measure of adding NaOH solution. The pH dropped again between days 17 to 20. The smoother operation in terms of pH was in reactor with ratio 4. The first effect for ratio 4 occurred between day 22 and 25 where the pH dropped. Most varying pH values were of digester 3.5, where variation occurred day by day and it was adjusted with a difference of one day.

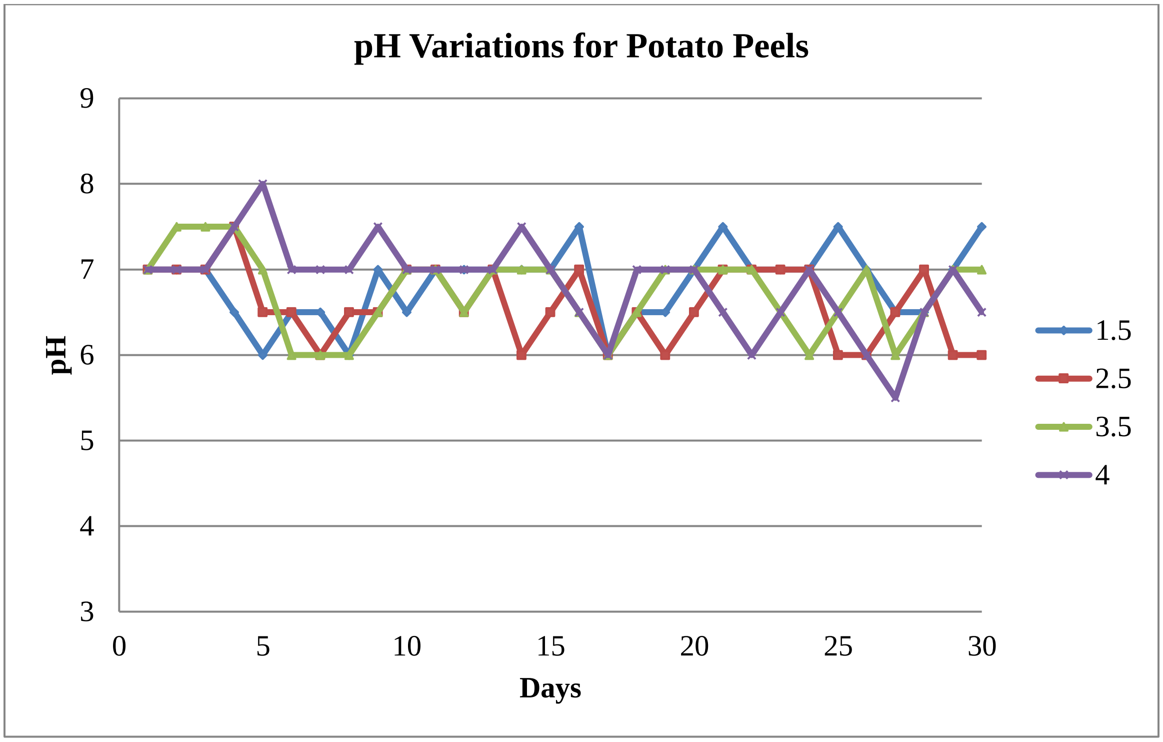


Figure 32: pH Variations in Potato Peels

16. Okra Calyx

16.1. Volatile Solid Reduction

Okra calyxes are rich in cellulosic and lignocellulose material which makes it difficult to degrade. The volatile solid reduction analysis showed that maximum reduction occurred in ratio of 1.5 where the reduction occurred around 60 to 65%. For ratio 2.5 the VS reduction is 40 to 45 percent. For ratio 3.5 and 4 the VS reduction is around 10-15 percent only. Because of greater cellulosic, hemi cellulosic and increasing carbon content with increasing substrate in digester the VS reduction is decreased. These okra calyxes contain polymers like pectin and covered with lignin. These polymeric materials are difficult to digest. Lignin is one of the strongest part of cell structure which has higher resistance of chemical and enzymatic degradation. To increase digestibility of okra calyx waste it must be reduced and pre-treated before anaerobic digestion.

The lower the F/I ratio for okra calyx greater is the VS reduction. Lower F/I ratio will have greater amount of inoculum and more VS will reduce.

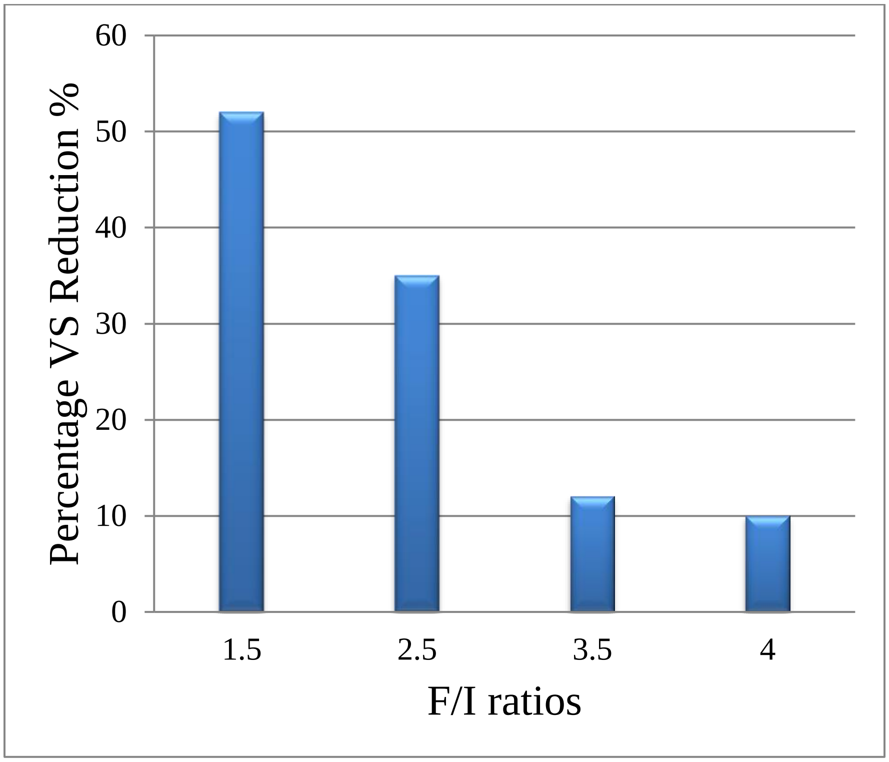


Figure 33: Volatile Solids Reduction for Okra Calyx

16.2. Chemical Oxygen Demand

Chemical oxygen demand for Okra pods was very difficult to perform as it contains large amount of COD values. Multiple times dilutions very applied for calculating COD by defined methods. About 3 to 4 experiments were performed to calculate COD values because the substrate COD range was greater than the defined methods. The results showed that the maximum COD reduction in1.5 ratio and minimum for control reactor and ratio 4. These results effect the overall performance and biogas production .Greater the COD reduction Greater will be the biogas production. COD reduction must not be confused by VS reduction as that is a different parameter.

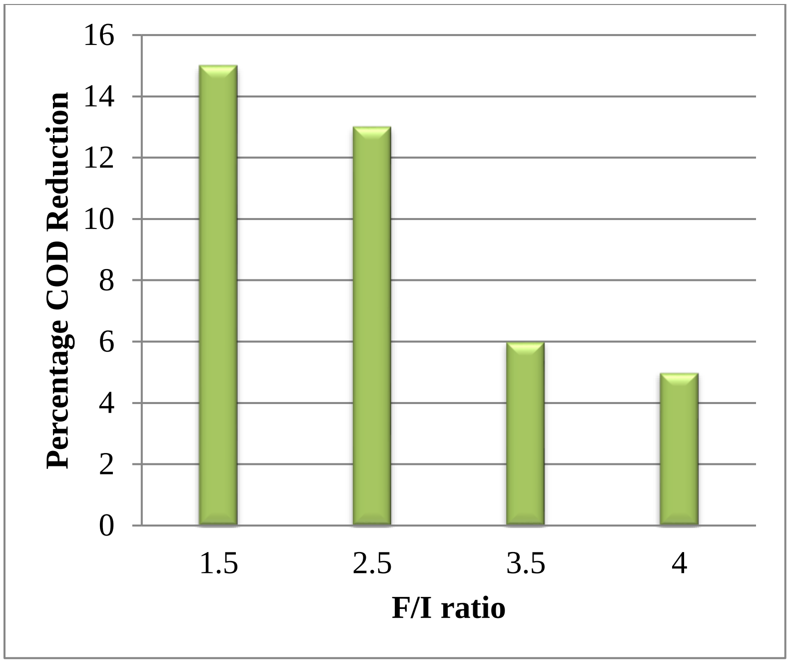


Figure 34: Okra Calyx COD Reduction

16.3. Biogas Production

The figure for biogas production showed that maximum biogas produced is in ratio 1.5 to 3.5. Further increasing the ratio is decreasing the overall biogas production. Because of over feeding of the digester the digester environment gets toxic with volatile fatty acids accumulation. The maximum biogas produced was between 15 to 25 days. For larger F/I Ratios the trend shows that amount of biogas produced is increasing because it requires greater time to digest and biogas production starts a bit late in these high F/I ratios. Another reason for lower biogas production in high F/I ratio is fluctuation in pH. Biogas is mixture of carbon dioxide and methane and other gases like oxygen so the graph shows the combined amount of gases produced during the experimentation period.

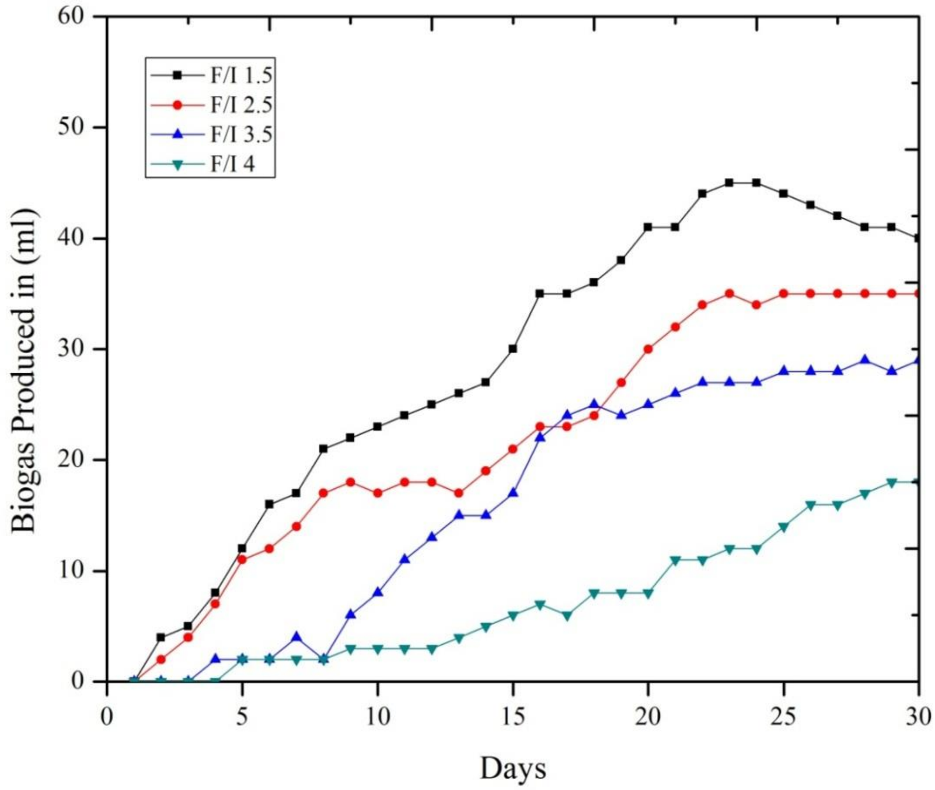


Figure 35: Okra Calyx Biogas Production

16.4. Okra Calyx Methane Concentration

It is definite from the results that methane concentration is greater in ratio 1.5 and 2.5 the lowest methane concentration is in digester of F/I ratio of 3.5 and 4. It is evident that increasing the organic feed in digester decreased the methanogenic activity. Overall methane concentration in okra calyx is lower than other substrates because of greater cellulosic and polymeric material in okra calyx. The polymeric fibers present in okra calyx makes it difficult for bacteria to digest the feed.

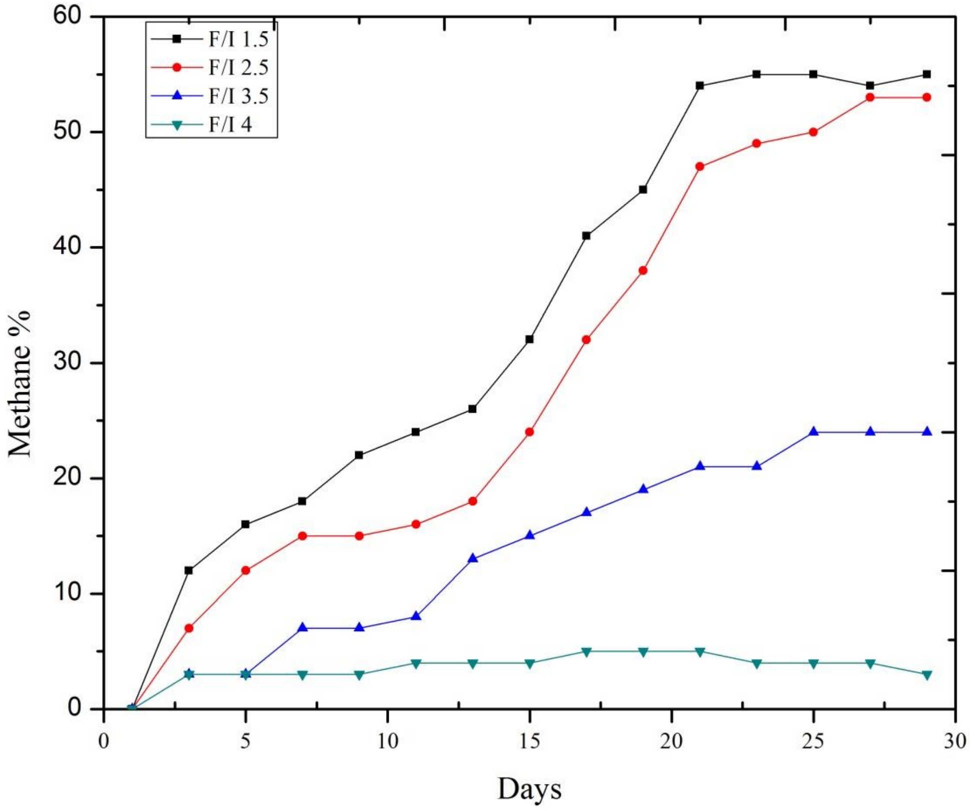


Figure 36: Okra Calyx Methane Concentartion

16.5. pH variations

pH is one of the most important driving factor for anaerobic digesters. The pH changes cause their effect directly on biogas production and methane concentration. As the results shows the average pH value throughout the experimentation remained between minimum five to maximum 8. The pH was adjusted regularly for both acidic and basic stage. As the result shows the change in pH effected the biogas production. First change occurred on day 5 for ratio 1.5 which decreased both methane concentration and biogas for the day. The pH was adjusted using dilute acetic acid solution. Similarly on day 7 for ratio 4 the pH rose to 8 which decreased the gas produced and upon adjustment of pH the next measurement was greater than the previous. As the reaction proceeded the pH tends to move towards acidity which was regularly adjusted using NaOH solution but upon completion of experimentation the value was not adjusted.

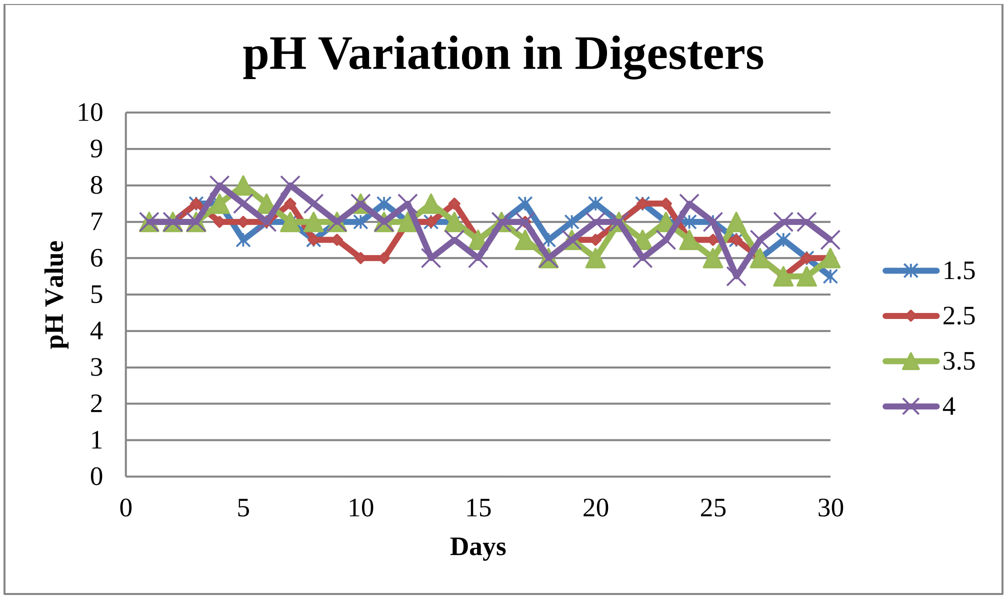


Figure 37: pH Variation for Okra Calyx

17. Cumulative performance comparison for ratio 1.5

The comparison of f/I ratio 1.5 is given below in figures 39 both in terms of biogas production and methane percentage with respect to days. The biogas production trend shows that the gas production is are exponential with days. The production has increased as the days increased until third week for both banana peels and potato peels. There occurs a greater difference between biogas production potential of okra waste at 1.5 f/I ratio while compared to other substrates. The gas production was exponential but the amount produced was much lower than that of potato peels and banana peels. If compared to the methane percentage the percentage methane was lower in banana peels than other substrate. This infers that initially the carbon dioxide was accumulated in banana peels digester at F/I ratio of 1.5.the maximum biogas produced for potato peels at ratio 1.5 was on day 21st which was nearly 82 ml of biogas. Similarly the highest production for banana peels occurred on day 17th and for okra on day 24th. The gas production in okra waste started at lower rate than other substrates. The graph shows that the biogas production reaches a stable value between 80 ml to 78 ml for 5 to 6 days because of decline of the microbial community or lowering of volatile solids.

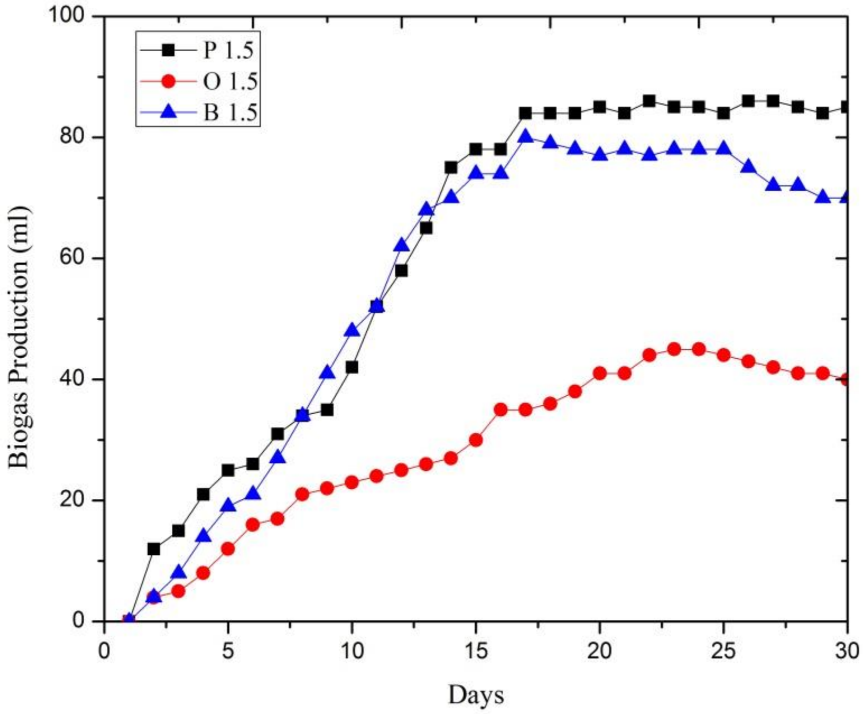


Figure 38: Cumulative performance of ratio 1.5 (Biogas Production)

Figure 40 shows the percentage of methane produced with increasing days in ratio 1.5. The graph shows that the methane content of potato peels and banana peels were near identical at day 18. The greater difference occurred between methane content of okra calyx. The performance of okra calyx was lower while compared with other substrates. The maximum methane percentage for potato peels occurred around. The methane content increased at a greater rate after day 15. For banana peels the methane content rise at a greater rate after day 10 and for potato peels the methane content rose exponentially. This is also shown in the figure below

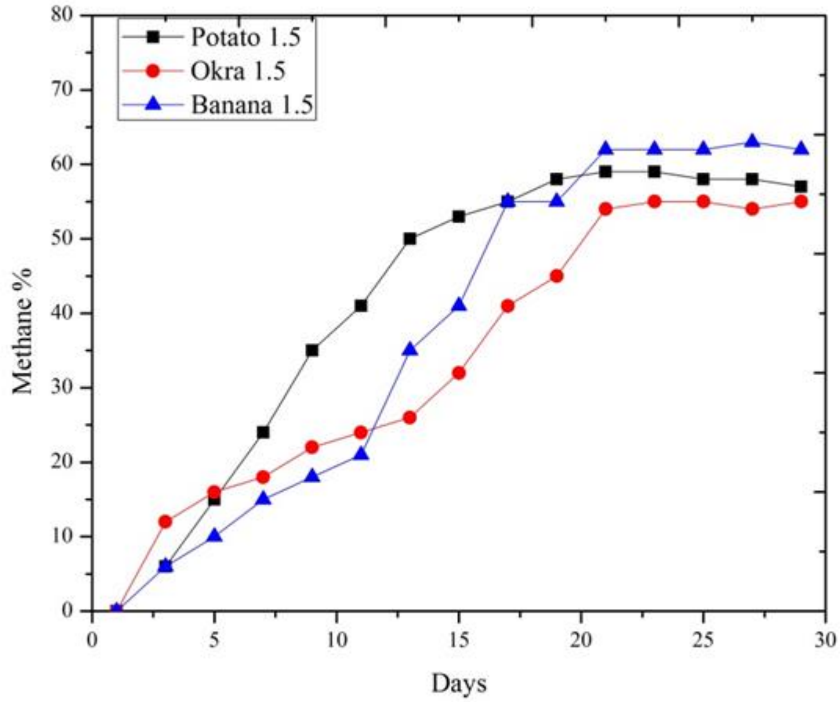


Figure 39: Cumulative performance of ratio 1.5 (Methane %)

18. Cumulative performance comparison for ratio 2.5

The ratio 2.5 shows a diverse trend for biogas production the figure shows that the leading producer remained the potato peels and it produced a maximum of 95 ml of biogas on day 24th. The banana peels were able to produce maximum of 60ml of biogas from day 20th to 25th while the okra calyx at f/I ratio2.5 was able to produce nearly 38ml of biogas.. The biogas production for potato peels kept on rising exponentially from day 3 to day 25th after which the production stabilized. The banana peels begin to produce biogas from day 2 to day 12 exponentially and then gradually begin to stabilize to around 60 ml. for the okra calyx the gas production was lower.

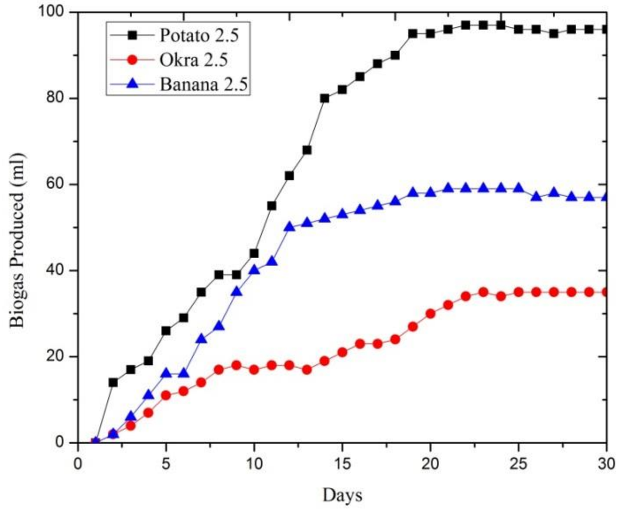


Figure 40: Cumulative performance comparison for ratio 2.5 (Biogas Production)

The methane production trend shows that maximum methane % occurred in potato peels while the methane % for okra calyx and banana peels had near values from day 15th to day 25th. The potato peels produced nearly 78 % of methane between day 15th and 21st while the methane % tend to rise for banana peels and okra calyx from day 25th. The exponential rise in methane % for potato peels suggests the performance of the digester is better among other substrates at this ratio. The performance of okra calyx and banana peels was lower than that of potato peels.

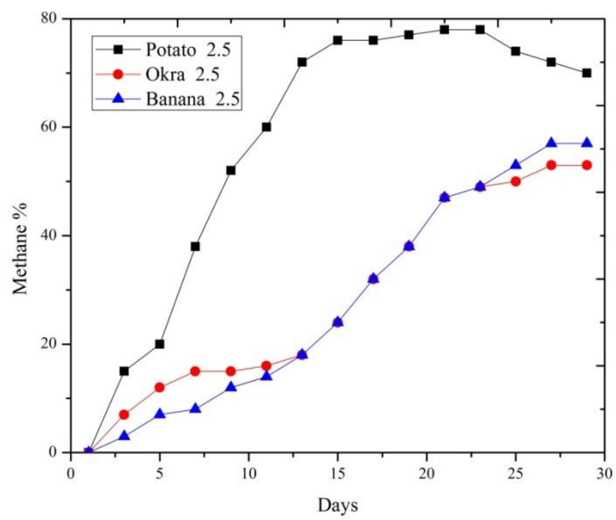


Figure 41: Cumulative performance comparison for ratio 2.5 (Methane %)

19. Cumulative performance comparison for ratio 3.5

The graph for F/I ratio shows a diverse trend between substrate. The maximum performance occurs in potato peels where the potential of biogas production reached to around 90 ml on day 25th. The individual comparison of ratios for potato peels i.e at 2.5 and 3.5 shows that the biogas production reached to its maximum earlier in ratio 2.5 while later in ratio 3.5. The biogas production for okra calyx was a nearly 25ml on day 28th which shows the process of degradation started later in okra calyx at ratio 3.5. Production for banana peels stood between maximum ranges of 40-45 ml between days 20 to 30th. The graph shows that the degradation started at a lower rate for ratio 3.5. This is because of the fact that greater F/I ratio would have greater amount of Total solids and requires greater inoculum.

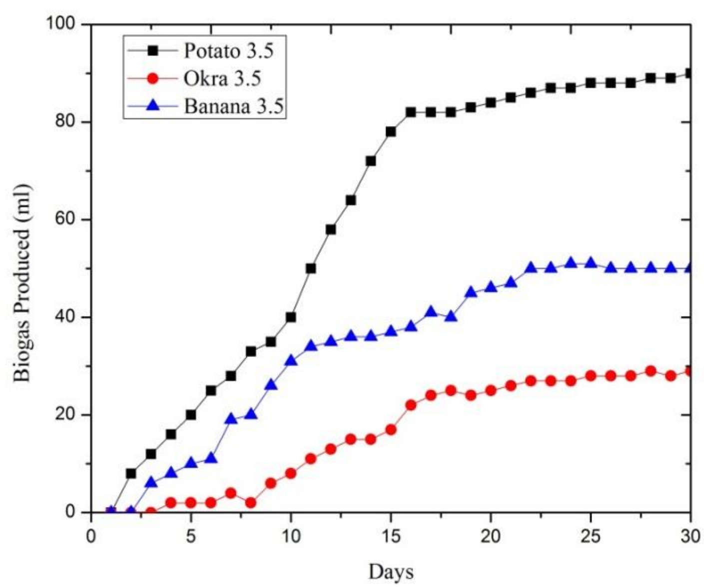


Figure 42: Cumulative performance comparison for ratio 3.5 (Biogas Production)

The methane % trend shows much relevancy to the biogas production trend in ratio 3.5. The maximum performance was of potato peels. The maximum methane percentage was between ranges of 50 to 55 % of produced biogas. The methane percentage in banana peels and okra calyx at this ratio was identical from day 1 to day 17th where the difference started to occur after day after day 17th. The methane percentage begins to rise to a maximum of 38 % in banana peels. The methane percentage begins to stabilize form day 24th in case of okra calyx. The methane percentage in banana peels was increasing at the end of the experiment upon pH adjustment. The figure shows the methane content of F/I ratio of 3.5.

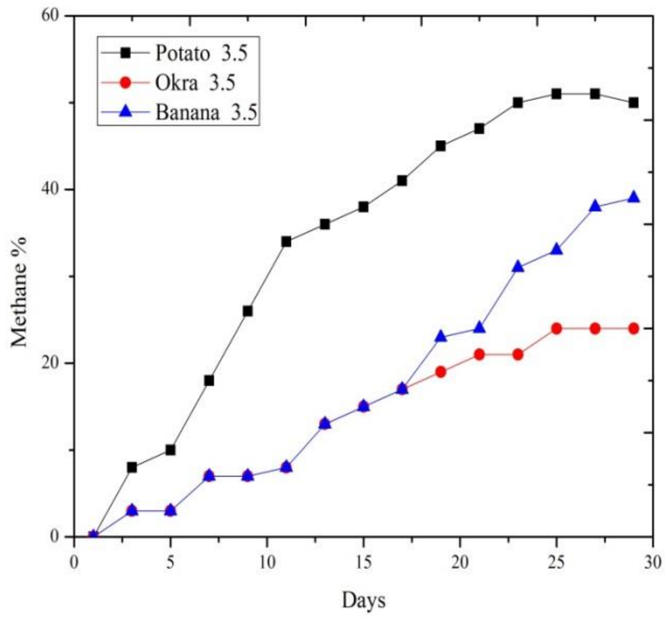


Figure 43: Cumulative performance comparison for ratio 3.5 (Methane %)

20. Cumulative performance comparison for ratio 4

It is now evident from the discussion of ratio 1.5 2.5 and 3.5 that potato peels were the maximum biogas yielding substrate. Similar trend occurred in F/I ratio of 4. The maximum biogas was produced in potato peels to a maximum range of 75 to 80 ml from day 24 to 30. The biogas production for potato peels showed similar values on day 11 to 13 and then 21 to 23. This could be because of pH variations which may have limit the reaction or because of leakages occurring Increasing the amount of substrate does cause its effect on all of the substrates. The potato peels outperformed other substrates on the basis of both biogas production and methane percentage The biogas production for okra calyx started from day 5 and kept on rising steadily to a maximum of 25-37 ml on day 30th. This avers that the degradation of okra calyx at a greater feed to inoculum ratio takes greater retention time. The biogas production started to rise for okra calyx towards the end of experimentation. The biogas production in banana peels was better than that of okra calyx but much lower than the potato peels. The plot for banana peels at ratio 4 shows do not shows a smooth exponential curve because of pH changes occurring. This can be inferred after comparison of biogas production and pH variations that where ever the production dropped or stabilized there was a change in pH. Upon adjustment the biogas production tends to rise. The production of biogas at a F/I ratio is shown in fig below

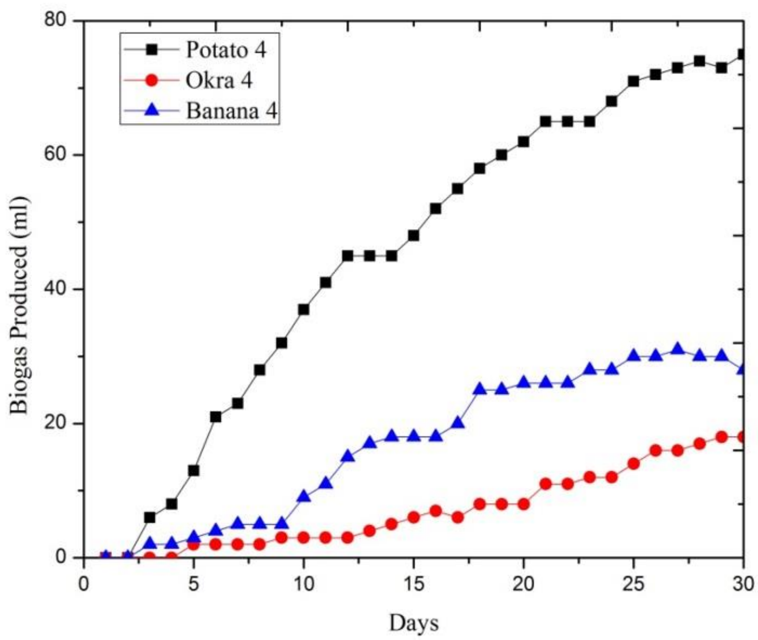


Figure 44: Cumulative performance comparison for ratio 4 (Biogas Production)

The maximum methane content for banana peels at ratio 4 was nearly 35 % for okra calyx. It stood at an extreme low of around 3 to 4 % and for banana calyx the value remained at maximum of 10 %. The F/I ratio 4 is the lowest yielding ratio for substrates in terms of both methane content and biogas produced. For okra calyx the values for ratio 4 were identical to control reactors. This could be because of digester failure due to excessive feeding.

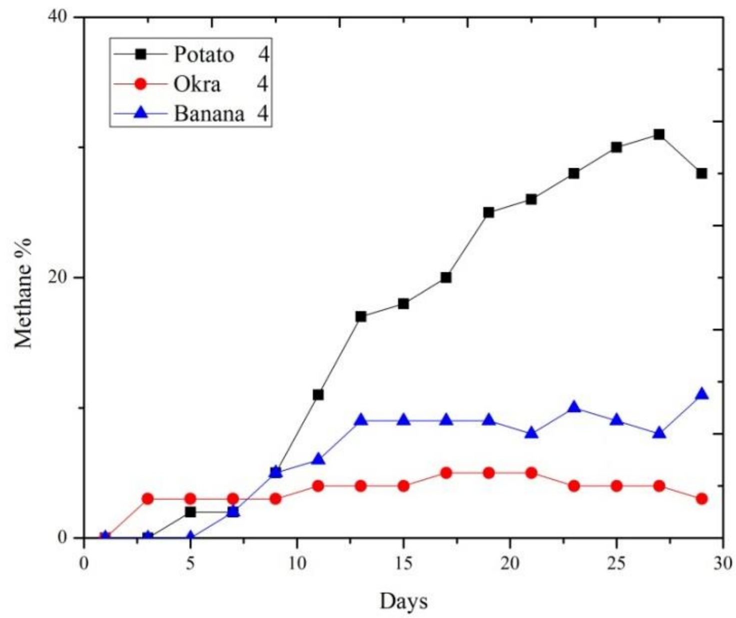


Figure 45: Cumulative performance comparison for ratio 4 (Methane %)

21. Overall BMP test performance

Figure 38 shows the overall comparison of biogas production from substrates. The overall results for biogas production shows that the maximum biogas was produced in potato of feed to inoculum ratio of 2.5 and for banana the maximum production was in ratio 1.5 and for okra the maximum yielding ratio is 1.5. Lowest biogas production was in okra calyx which is rich in cellulosic fibers. This can be asserted that as the starch increases The potato peels as mentioned in previous sections contains around 7- 8 percent of fiber while the fiber content in banana peels is nearly 30 % and okra calyx is 15-20 %.. As suggested Tsavkelova et al., in 2012 biogas production is hindered by presence of lignin and fibers.[51]. For lower lignin materials such as potato peels is more easily degraded and produces biogas in comparison with other substrates. Another aspect which drives the production of biogas is total solids. Greater the amount of total solids and volatile solids greater will be the biogas produced. For potato at ratio 2.5 there is sufficient amount of total solids present. While at ratios 1.5 for banana and okra calyx sufficient amount of total solids are present for bacteria to take action. Potato contains lower TS values than other substrates that's why a greater F/I ratio performed better for potato.

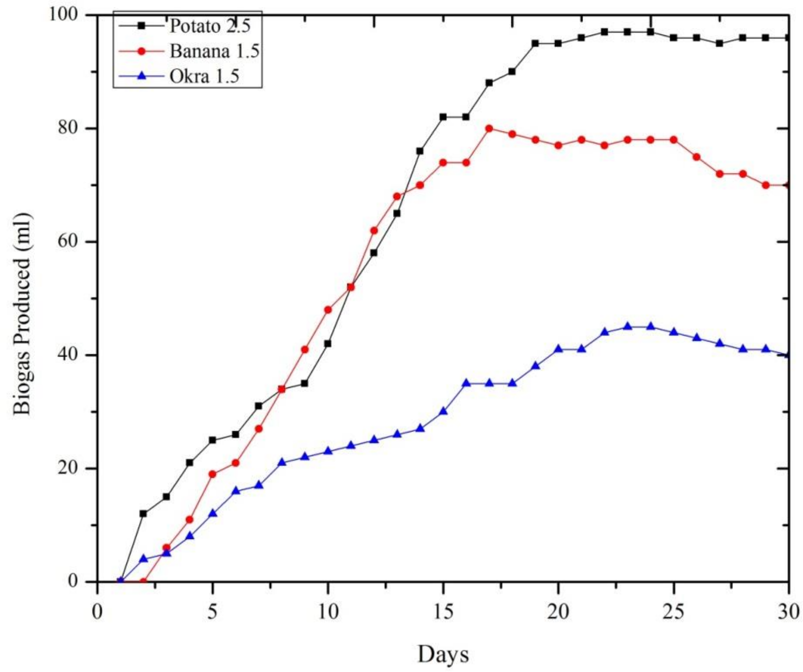


Figure 46: Performance comparison of high yielding ratios (Biogas Content)

Figure 39 shows the comparison between highest methane percentages obtained by substrates. The highest methane percentage was in potato peels of ratio 2.5 which was nearly 80 % and 60-65 in banana ratio 1.5 and nearly 55 percent in okra calyx. The most important driving force for methane in anaerobic digestion is protein content. The degradation of protein releases ammonia which limits the methane production. [52] Potato peels generally contains 1-2 percent of protein content similarly banana peels contains around 2-3 % of protein while the Okra calyx contains around 20-25 % of protein. This is also evident form the overall percentage of methane the maximum performing ratio for okra calyx yielded around 40-45 % of methane. The comparative results for overall performance measure are given in table 10. The VS reduction is a performance measure for BMP. The results suggest that the most VS reduction occurred in potato ratio 2.5 which is evident from the gas produced. In comparison to potato ratio VS reduced in okra calyx were around 60 percent. The gas produced from okra calyx were relative to that produced from banana peels but the difference occurred in methane content although 60% of the VS were reduced but the gas was not as rich in methane as the potato sample or banana peels samples primarily due to increased protein content .The overall pH of reactors remained between acceptable range for anaerobic digestion .

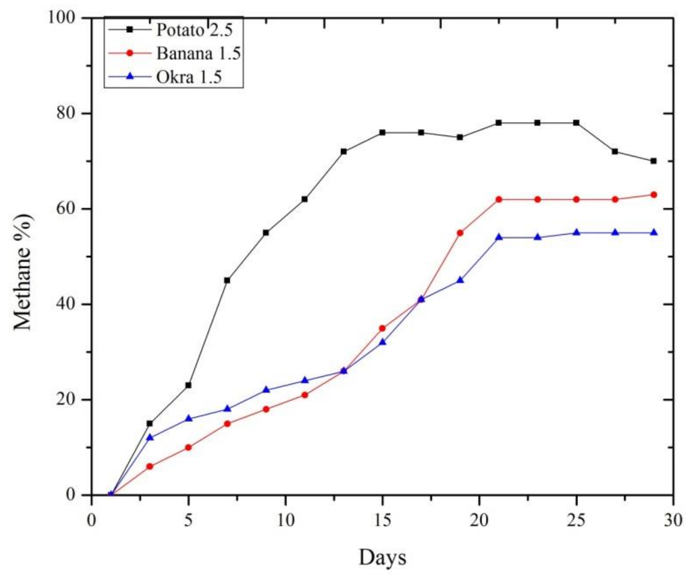


Figure 47: Performance comparison of high yielding ratios (Methane Content)

Subsrate	F/I ratio	VS reduction %	Initial pH	Final pH	Maximum biogas production ml	Maximum methane yield %
Banana	1.5	65	7	6	80	63
	2.5	38	7	6.5	59	57
	3.5	15	7.5	6	51	39
	4	12	7	7	30	11
Potato	1.5	60	7	7.5	97	78
	2.5	75	7	6	88	60
	3.5	40	7	7	89	51
	4	25	7	6.5	75	28
Okra	1.5	60	7	6.5	45	55
	2.5	40	7	5.5	34	53
	3.5	12	7	6	30	24
	4	10	7.5	6.5	18	5

Table 10: Cumulative experimental results of various ratios

22. Future Prospects of Current Research Work

- Biomass pretreatment and hydrolysis requires drastic improvement for economic production of biogas from complex organic matter such as lignocellulosic material. The hydrolysis process, where the complex insoluble organic materials are hydrolysed by extracellular enzymes, is a rate-limiting step for anaerobic digestion of high-solid organic solid wastes. Further studies are required to study specific pre-treatment methods for lignocellulosic material.
- Pre- treatment methods such as thermal pre-treatments are being studied further for their effect on various substrates.
- Trace elements plays vital role in anerobic digesters performance. The effect of different trace elemenets on methanogenic consortia is to be further studied and role of trace elements such as selenium and iron are under further consideration.
- The modeling and design of large scale anaerobic digestion plant requires initial inputs from the laboratory results. The current research can be further used to carry out pilot scale digester design and to simulate the digester using software like CHEMCAD and ASPEN Plus.
- Odors from anaerobic digester are one of the major issues with the farm scale digester. Post production treatment of gas has to be studied to treat the sour gas at the outlet or inside the digester so the user gets clean gas for domestic use.
- Improved reactor technology is important such as design for multistage anaerobic digester to separate phases of anaerobic digestion which will provide cover to methane producing methanogenic bacteria.
- Enhancement of biogas production from above mentioned substrates by Co-digestion with waste water sludge is further to be researched such as Okra calyx has not yet been studied at a larger scale.
- Biofilm reactors have high potential to be employed in biotechnology/bioconversion industry for viable economic reasons. The further research can be carried out along with banana peels for anaerobic digestion and water treatment at the same time.

Conclusion

The results for three substrates i.e potato peels, banana peels, and okra calyx are discussed in above section. The general trend in all graphs is that with increasing time the biogas production and methane concentration increased. The result showed that there is different amount of gas produced for different substrate at equal F/I ratio. For example gas produced by the okra calyx is different form the potato peels. The answer lies within the structure of material involved. The okra calyx is rich in cellulosic and semi cellulosic material the amount is greater than that of potato feels. So the VS reduction occurred in potato peels was greater than that of okra calyx. The greater the cellulosic material greater will be time required by the bacteria to degrade the material and produce biogas. Similar is the case with banana peels which contains fibers and lignin material which requires greater time of degradation. The results also suggest that for organic material which contains greater amount of Total solids requires lower F/I ratio so bacteria can perform actively. The results suggest that increasing the feed to inoculum ratio for banana peels and okra calyx makes it more vulnerable to pH variations. Increasing the substrate for these substrate accumulate more VFAs thus making it more vulnerable to digester failure. Another factor which causes a difference in gas formation and volatile solids reduction is the blending of substrates for experimental setup. There can be possibility that substrate is not uniformly crushed. The carbon to nitrogen ratio also required to be managed. Mixing of Feed with inoculum is also beneficial for managing the proper Carbon to nitrogen ratios. If the amount of carbon to nitrogen is disturbed the produced gas may decrease or production of ammonia can occur if nitrogen is increased. To eliminate the effect of cellulosic and lignin material the pretreatment can be applied. The organic can be further reduced to break lignin and cellulosic material to a lower value and smaller particle size which will enhance the biogas production and increase methanegenic activity. Greater volatile reduction in potato peels is an example of this phenomenon where the peels performed greater than other substrates because of lower ligocellulosic , fibrous and hemicellulosic material.

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