Evaluation of the Influence of Temperature and Co-digestion with Green Grocery Waste on Biogas Production from Cattle Manure.

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Inthenameof Allah Almighty,

the most Compassionate, the Merciful.

DEDICATION

I dedicated my this research work

to My Loving Parents.

Yasmin Saif

DECLARATION

The material contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Yasmin Saif

CERTIFICATE

This thesis by Miss. Yasmin Saif is accepted in its present form by the faculty of Biological Sciences, Department of Microbiology, Quaid-i-Azam University, Islamabad, satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

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CONTENTS

LIST OF ABRIVIATIONS

AMPTS	Automatic Methane Potential Test System
AD	Anaerobic digestion
CH_4	Methane
COD	Chemical oxygen demand
C/N	Carbon nitrogen ratio
HRT	Hydraulic retention time
H_2S	Hydrogen sulfide
NaOH	Sodium hydroxide
N ml	Normalized milli liter
NH_3	Ammonia
NL	Normalized liter
OLR	Organic loading rate
TS	Total solids
VFAs	Volatile fatty acids
VS	Volatile solids
WWTP	Waste water treatment plant

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Abstract:

In this study batch and continuous anaerobic digestion was carried out to evaluate the effect of co-digestion and temperature (controlled temperature and uncontrolled environmental temperature) on biogas production. Substrates used for co-digestion were cattle manure and green grocery wastes. In batch anaerobic digestion process anaerobic reactors containing different ratios of substrates were operated at controlled temperature (35°C) for almost 40 days to evaluate the full potential of substrates for methane production. Automatic Methane Potential Test System was used to record the methane yield. Results of batch anaerobic digestion process showed that co-digestion is more energy efficient process than single substrate digestion and methane production was enhanced to almost 10% by co-digestion of green grocery and cattle manure. To evaluate the effect of temperature on the continuous anaerobic digestion process,two independent 14 L anaerobic digesters with working volume of 10 L were selected. One digester kept at controlled temperature (37°C) and other placed at environmental temperature. For mono-digestion cattle manure was fed to both reactors at organic loading rate of 1 g VS/L.day and retention time was 20 days. It was observed that biogas yield was affected by temperature and its production was decreased with decrease in temperature (seasonal variation). As expected biogas yield in the reactor incubated at 37°C was higher than the reactor placed at environmental temperature. Duringwinter the methane production from cattle manure at uncontrolled temperature was almost 10 times lower than the biogas produced at 37°C. Then the substrate for both of the continuous reactors was replaced with mixture of cattle manure and green grocery waste for evaluation of the effect of co-digestion on biogas production. Since in Pakistan presently almost all anaerobic digesters use cattle manure solely as substrate and are operated at uncontrolled temperature. There was a significant increase in biogas yield by applying co-digestion. Biogas yield at controlled temperature with co-digestion (cattle manure and green grocery) was 20 times higher as compared to biogas yield in the reactor operated at environmental temperature and fed with cattle manure alone. This study shows that anaerobic digesters used in Pakistan, which are run at uncontrolled temperature and fed with cattle manure, can be significantly enhanced their production by applying co-digestion and controlled mesophilic temperature. The biogas can play a major role in the supply of energy source as alternate of natural gas in Pakistan, where natural gas has share of almost 50 % in the primary energy sources, which is expected to diminish in coming decades.

INTRODUCTION

Demand and price of the global energy of the world is continuously increasing, while the fossil fuel sources are depleting with the passage of time. This rapid consumption of fossil fuels will ultimately lead to eliminate natural energy sources in nearby future. So, alternate energy production sources are necessary to overcome the growing demands of energy and to reduce dependency on fossil fuels (Ermis etal., 2007). The fossil fuel reserves of the world are being depleted rapidly and it is expected that oil reserves will end in next 35 years, coal reserves in next 107 years and gas reservoirs will end in approximately next 37 years. This data shows that coal reserves are accessible up to 2112, and are the only type of fossil fuels lasting after 2042 (Shafiee et al., 2009). Use of fossil fuels is one of the causes of global warming, as burning of fossil fuels increase in amount of carbon dioxide in the environment and contributes to the accumulation of greenhouse gases (Saidu et al., 2013).

Methane produced by anaerobic digestion process reduces direct greenhouse gas emission by replacing the use of fossil fuels and also stops indirect greenhouse emissions by preventing emission of methane during waste storage or in landfills (Badshahet et al., 2012).Theaccumulation of larger quantity of organic wastes like animal manure, green grocery waste, food waste, industrial waste, increase in amount of organic waste and lacking its proper handling causes environmental pollution and different health related problems. So there is need for proper management of organic waste for the sake of environmental health.

Cattle manure is one of the major organic substancesand causes pollution due to its high content of nitrogen, phosphorus and organic matterand it is essentialto deal with it for its proper management (Rico et al., 2011). Anaerobic digestion is one of technologies for organic waste treatment like animal manure, food waste, municipal waste etc. Anaerobic digestion produces biogas and digestate as end products which can be usedfor energy and biofertilizer, respectively (Neves et al., 2008).

Pakistan has great potential to produce huge amount of biogas as it is one of the agricultural country and has giant amount of biomass resources in the form of cattle manure, green grocery waste, municipal waste, poultry litter,paper and pulp industry sludge. Which are worthy substrates used for biogas production. In Pakistan, natural gas has almost 50% of primary energy source and Pakistan has developed infrastructure for natural gas utilization. So biogas production and its utilization is one of the cost effective alternate energy sources. It should be noted that there is continuous increase in the share of natural gas in the primary energy source Pakistan has almost159 million cattle producing approximately 652 million kg of manure on daily basis from cattle and buffalo only; which can be used to generate 16.3 million m^3 biogas in one day and 21 million tons of bio fertilizer in one year. The digestate produced acts as a fertilizer for crops and plants (Amjidet al., 2011). So in this study cattle manure and green grocery wastes areused as substrate for biogas production.

Anaerobic digestion is a multistep process which involves breakdown of organic matter into biogas and digestate in the absence of oxygen. Biogas mainly consists of methane 60-75% and carbon dioxide25-40% (Zhong et al., 2012). Anaerobic digestion gives high energy recovery from organic wastes. The aim of anaerobic digestion is destruction and degradation of organic matter with the help ofa large number of bacteria and archeae (Kangle et al., 2012). Anaerobic digestion is possible with single type substrate as well as by more than one kindsof substrates. When more than one substrates are used in anaerobic digester, the process known as co-digestion. Co-digestion of different substrates may have greater biodegradability than that of single substrate digestion(Corral et al., 2008). Co-digestion is basically combined treatment of different kinds of wastes bearing complementary characteristics with the aim to enhance methane production, for the stability of the reactor or to overcome the seasonal shortage of a feedstock (Nges et al., 2012). In some cases co-digestion is more stable process.Co-digestion of different substrates produces a mixture with appropriate carbon and nitrogen ratio (C: N) as well as phosphorus ratio is also well-adjusted in case of co-digestion (Espositoet al., 2012).

A large amount of green grocery waste is produced every year in the world. These wastes comprise 8–18% of total solids (TS), bearing a total volatile solids (VS) concentration 86–92%. The organic portion contains about 75% of easily biodegradable material. Green grocery waste is one of thesuitable substrates for co-digestion. A major drawback of anaerobic digestion of green grocery waste alone is the quick acidification of these trashes. This acidification is caused due to carbon dioxide production and more volatile fatty acids production and ultimatedeclining in the pH of reactor (Bouallagui et al., 2004). Green grocery waste and animal manure both may have limitations to operate separatelyin continuous anaerobic digestersdue to process instability. To increase the methane yield and process stability, it is better to treat these two kinds of wastes together (Esposito et al., 2008).

Anaerobic digestion process is affected by a large number of parameters like concentration of ammonia,volatile fatty acids, pH value and temperature. Temperature is one of the most important parameter and one should give consideration to temperature whileoperating anaerobic digester. Temperature variation affect the methane content in total volume of biogas produced. Anaerobic digestion can be carried out at vast temperature range. On the basis of temperature, microbial consortia present in digestersresponsible for carrying out anaerobic digestion can be psychrophilic, mesophilic and thermophilic (Mashad et al., 2004).

Thermophilic anaerobic digestion process has several advantages i.e. high metabolic activity of microorganisms and high rate of degradation of organic matter. Thermophilic anaerobic digestion also results in high biogas production. However process instability is the main limitation of the thermophilic anaerobic digesters. It is observed that in thermophilic anaerobic digesters diversity of microorganisms involved in anaerobic digester is lower than in mesophilic digester, which make the thermophilic reactors sensitive to shocks. Because more number of microbial communities can flourish at mesophilic temperature and only thermophiles stay alive at high temperature conditions. Mostlybiogas reactors are operated at mesophilic temperature due to process stability and better results for methane production (Nielsen et al., 2004).

The efficiency of anaerobic digestion process is dependent on number of parameters. Temperature is one of the most important parameters that affect the anaerobic digestion process and hence anaerobic digesters are operated at constant and defined temperatures. Thermophilic temperature is better for microbial activity, but mesophilic temperature is best for process stability (Alvarez et al., 2006). Abrupt environmental variations, e.g.

intense increase or decrease in temperature could be a reason of severe imbalance of all parameters of progression of the process, and then the process has to be stable again which is further time consuming (Bouskova et al., 2005). Low temperature causes mostly lower chemical oxygen demand (COD) removal efficacies and accumulation of volatile fatty acids (VFAs) and ultimately results in lower production of biogas (Ahn et al., 2002).

Aims and Objectives:

The aim of the present research study is the evaluation of the effect of temperature and co-digestion of cattle manure with green grocery waste on biogas production.

The objectives of the present study are:

1) To study the effect of mono-digestion of cattle manure and co-digestion of cattle manure with green grocery wastes on biogas production in batch reactor at controlled temperature (35°C).
2) To evaluate the efficiency of anaerobic digesters when cattle manure fed with different organic loads under continuous mode at controlled temperature (37°C).
3) To evaluate the efficiency of anaerobic digestion of cattle manure with green grocery waste under continuous mode at controlled temperature (37°C).
4) To assess the efficiency of mono-digestion of cattle manure and co-digestion of cattle manure with vegetable and fruits waste in a continuous anaerobic reactor at variable organic loads operated at environmental (uncontrolled) temperature.

REVIEW OF LITERATURE

Energy requirements of the world are increasing with the increase in population and industries. The energy crisis are even worse in the developing countries as fossil fuels are depleting from the world and getting more expensive with time. It is estimated that fossil fuels will be eliminated from the world in near future. The studies show that gas and oil reserves will run out in coming decades. So there is need to fulfill energy requirements by using alternates that will accomplish energy demands of the world.

2.1. Biogas a Sustainable Energy Solution in Pakistan:

Pakistan has great potential to produce huge amount of biogas as it is one of the agricultural country and has giant amount of biomass resources in the form of cattle manure, green grocery waste, municipal waste, poultry litter and paper and pulp industry sludge. Which are worthy substrates used for biogas production.In Pakistan, natural gas has almost 50% of primary energy source and Pakistan has developed infrastructure for natural gas utilization. So biogas production and its utilization is one of the cost effective alternate energy sources. It should be noted that there is continuous increase in the share of natural gas in the primary energy source (Amjidet al.,2011).

Another reason to switch to utilization of biogas is that biogas is a clean and cheap form of energy. Use of some conventional energy sources like petroleum is not safe for the environment and it causes environmental and ecological problems. It causes air pollution, emission of greenhouse gases and toxic gas emissions etc. So biogas is one of the best substitutes for fossil fuels. Anaerobic digestion prevents direct greenhouse gas emission, as in this practice waste material is used as substrate and prevents the waste material from dumping and directly reduce greenhouse emission. Anaerobic digestion also reduces greenhouse emission indirectly as fossil fuels are replaced by biogas.

There are a number of approaches by which energy is harvested by different substrates. Energy efficiency of the substrate depends on the way, it is harvested. Cattle manure is one of the most common substrate for biogas producing digesters. It gives varying amount of energy based on the method to harvest energy from it.Cattle manure, when burnt directly as fuel source after drying, its efficiency of conversion to heat is not significant and is 8% only. Instead 25% energy is achieved from the same amount of cattle manure when used after its conversion from biogas to electricity. The most efficient energy utilization of cattle manure however, is as heat by ignition of biogas which will enhance its energy efficiency up to 55%.

2.2. Anaerobic Digestion:

Anaerobic digestion is break down of organic compounds under conditions where oxygen is absent. It is one of the technologies for the production of energy by using organic solid material (waste) as substrate (Burton et al., 2003).A large number of microorganisms are responsible for catalyzing this process (Kangle, 2012).Anaerobic digestion basically deals with degradation as well as stabilization of organic compounds with the help of microorganisms and ultimate result of this process is biogas and digestate (Kelleher et al., 2000).

2.3. Products of Anaerobic Digestion:

Biogas contains two major components methane and carbon dioxide (Sagagi et al., 2009). Methane in the biogas is the energy rich molecule that can be used for electricity production, heating, cooking purposes and transportation fuels. Biogas can be used as a substitute of natural gas. Biogas reduces diseases as its use is environment friendly. Digestate produced as a by-product of anaerobic digestion is used for agriculture purposes i.e. as a fertilizer. It improves the plants growth.

2.4. Feedstock Used for Anaerobic Digestion:

Feed composition is an important parameter to affect theperformance of anaerobic digester. Feed characteristics influence mainly biogas production potential and the extent of substratedegradability. The other factors affected by feed composition are kinetics of biogas forming process, content of methane in biogas and alkalinity of the digester as well. Some compounds like starch, cellulose, protein can be easily or moderately degradable, while substrates like lignocellulosic material and lignin are hard and resistant to degradation under anaerobic conditions. The level of degradability determines the rate of methane production (Badshah, 2012).

Theoretically any kind of organic material can be degraded by microbes used as substrate for production of biogas. Yield of biogas and rate of biogas production is dependent on different parameters of the process, as environmental and process parameters (Davidsson, 2007). Yield and rate of biogas produced during anaerobic digestion process is dependent on composition of feedstock. A large number of problems as instability of process and several inhibitors are related to anaerobic digestion process, which may be due to type of feedstock used (Chen et al., 2008). This process is also very sensitive to change in temperature and pH. Industrial organic wastes like paper and pulp sludges are considered good substrates for anaerobic digestion, as they comprise large amount of easily biodegradable substances.Basic main components in biomass used by microorganisms for biogas production are carbohydrates, proteins and fats. Biogas composition as well as amount of methane production depends on type of feedstock used as substrate and retention time. The content of fats, proteins and carbohydrates in substrate used to determine the theoretical biogas yield (Braun, 2007). Methane content of biogas produced by fats decomposition is more than the methane content produced by decomposition of carbohydrates and proteins (Biswas et al., 2006).

2.5. Co-digestion:

The term co-digestion is used for a process which deals with more than one type of substrates in anaerobic digestion. It gives better results for biogas production as it is suitably regulated for optimal C/N ratio, pH and dilution of toxicants in one of the substrates. Co-digestion also regulates content of nutrients present in the organic material which is degraded for biogas production (Alvarezet al., 2011).

C/N ratio is one of the most important parameters that influence the process of anaerobic digestion. A nutrient balance should exist in the reactor to maintain C/N ratio with in an appropriate range which in generally 20-30. Some studies showed that an optimum C/N ratio lies in the range of 15-20. If C/N ratio is not balanced (higher than required) it may result in lower methane production and process instability in case of continuous anaerobic digestion (zhanget.al., 2013). During the process of anaerobic digestion microorganisms generally consume more amount of carbon, 25-30 times faster than nitrogen. To fulfill the requirements of microorganisms, it is must to maintain 20-30:1 ratio of C and N. So that largest amount of readily degradable carbon is present in digester (Bardiyaet al., 1997).

When cattle manure and green grocery wastes are treated together in anaerobic digester, it gives better results for biogas production as well as increase the process stability as compared to mono-digestion. Co-digestion is one of the beneficial strategies to increase yield of biogas (Silvestre et al., 2013).

Sometimes trace elements (zinc, nickel, molybdenum, tungsten, iron and cobalt) are used as supplements for anaerobic digesters. These supplements are useful for microorganisms present in anaerobic digester systems. These supplements may be added to digesters in different combinations or single supplement may also be provided depending on the type of substrate used in digesters. These elements enhance the activity of enzymes required for methanogens, ultimately result in increased biogas production and process stability (Pobeheim et al., 2010).

Co-digestion carried out with cattle manure and food waste is one of the good alternate for improved biogas production. Total methane yield is increased by co-digesting cattle manure with food waste, in both batch experiment and semi continuous experiment. The mixture of the two substrate have good buffering capacity and maintain a balance between ammonia concentration and volatile fatty acids. So organic load of substrate may be increased because there will be no pH disturbance as well as a balanced C/N ratio exists in digester which is important for the process of anaerobic digestion to continue (Cunsheng et al., 2013).

Anaerobic co-digestion of food waste and cattle manure enhance methane yield because in this case of co-digestion microbial mass present in digester get more nutrients than a single substrate and helps to establish a positive synergism among microbes. As a result microbial mass and efficiency increases that increase the amount of biogas is produced (Mashadet al., 2010).

Xiao (2010) conducted study for co-digestion of milk with dairy manure. Batch reactors were run at controlled temperature (37°C). Each reactor contained different ratios of both substrates with varying content of milk. The results showed that co-digestion of dairy manure and milk is beneficial and the process is feasible if ratio of milk is up to 19% by wet weight. It gives significantly high amount of biogas production.

Alvarez (2008) studied the effect of co-digestion of manure, solid waste from slaughter house and vegetable and fruit waste. It was concluded that co-digestion is better for biogas production than digestion of pure substrates. As well as it showed that the co-digestion of two substrates gives significantly better results than the co-digestion of three substrates except the mixture of food and vegetable waste 50% and 50% by wet weight solid slaughter house.

Digestate:

Digestate is the remain after completion of anaerobic digestion process. It can be used as a fertilizer for growth of crops and plants. Digestate is beneficial source of mineral for growth of plants and crops.

2.6. Phases of anaerobic digestion:

Fermentation of methane during anaerobic digestion usually involves following phases i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis (Thauer et al., 2008).

The organic compounds (lipids, carbohydrates and proteins) are first broken down in to simpler components (oligomers, monomers) by bacteria present in digester, the process known as hydrolysis. Hydrolysis of polymeric compounds is carried out by extracellular enzymes. These simple organic substances are taken up by the microbes present in digester and the next process of forming volatile fatty acids takes place with in bacterial cells. Acetogenic bacteria perform an important role in converting volatile fatty acids in to acetate, hydrogen and carbon dioxide (Moritaet al., 2012).

2.6.1. Hydrolysis:

Hydrolysis is the first step for anaerobic digestion process. It involves break down of larger subunits known as polymers into simpler components and provides substrates that are easy to utilize for microorganisms to carry out further fermentative processes. Hydrolysis is an important step to start anaerobic digestion process because the substrates used mostly contain larger polymers which are not able to cross the microbial cell membranes (Madigan et al., 2008).

Hydrolytic enzymes (secreted by microorganisms) have key role to carry out hydrolysis. Hydrolysis involves liquefaction of complex organic material. Liquefaction is the conversion of insoluble material to soluble components with the help of hydrolytic enzymes secreted by microorganisms. End products of extracellular hydrolysis of protein liquefaction are peptides and amino acids and that of carbohydrates are saccharides in monomers or oligosaccharides. While the hydrolysis of lipids yields fatty acids and glycerol. Hydrolysis may be a rate limiting factor, if it is not producing enough monomers (Salminen et al., 2002). Therefore mostly the pretreatment is carried out for the enhancement of the biogas production yield and rate from recalcitrant biomass. The substrates that are in simple forms, do not involve this step.

2.6.2. Acidogenesis:

Next step of anaerobic digestion process is fermentation which is also known as acidogenesis. It involves the conversion of sugars present in organic matter into hydrogen, carbon dioxide, acetate, volatile fatty acids (VFAs) and long chain fatty acids. Volatile fatty acids include butyric acid, propionic acid, lactic acid, ketones, acetic acid and alcohols produced by different kinds of anaerobic microorganisms. Volatile fatty acids produced by acidogenesis are easily available substrates that methane forming bacteria can utilize (Angelidaki et al., 2007).

Hydrogen sulfide and ammonia are released as by-products during the fermentation of amino acids to volatile fatty acids and other products. These two by-products H_2S, NH_3) are toxic to microbes and have inhibitory effect on anaerobic digestion process (Salminen et al., 2002).

2.6.3. Acetogenesis:

In acetogenesis further conversion of some fermentation products takes place. Some volatile fatty acids, aromatic fatty acids and alcohols are converted to more simple organic acids, acetic acid, hydrogen and also carbon dioxide by microorganisms. Acetogenic bacteria present in this phase may be called as acid formers (Boe, 2006).

Major acids produced during acetogenesis involve acetic acid, propionic acid, butyric acid and ethanol. A large number of microorganisms including*syntrophobacterwolinii*, *peptococcusanerobus*, *lactobacillus*, and *actinomyces* carry out process of acetogenesis. Hydrogen forming acetogenic bacteria produce CO_2, acetate and hydrogen from short chain fatty acids (VFAs). Homo-acetogenic bacteria and alcohol forming bacteria produce acetate using CO_2 and H_2(Sterling et al., 2001).

2.6.4. Methanogenesis:

Methanogenesis is the last stage of anaerobic digestion and involves further biodegradation of end products of acetogenesis for methane production. A large number of methanogens are involved in this stage for methane production. Methanogens can utilize limited number of carbon sources. The substrates that can be utilized by methanogens are hydrogen, carbon dioxide, acetate, formate, methanol, methylamines, methyl sulphides, dimethyl sulphides etc. Methanogens belong toarcheae and include*Methanococcus*, *Methanosarcina*, *Methanobacillus,* *Methanobacterium*, *Methanobacteriumformicium,* and*Methanobrevibactorarboriphilus*.Methanogenesis can be of two types on the basis of substrate used in anaerobic digesters. Two groups of methanogens i.e. acetotrophic and hydrogenotrophic are involved in methanogenesis. acetotrophic methanogens utilizes acetate and hydrogenotrophic methanogens utilizes H_2/CO_2 to as a source of food to produce methane (Bitton, 2005).

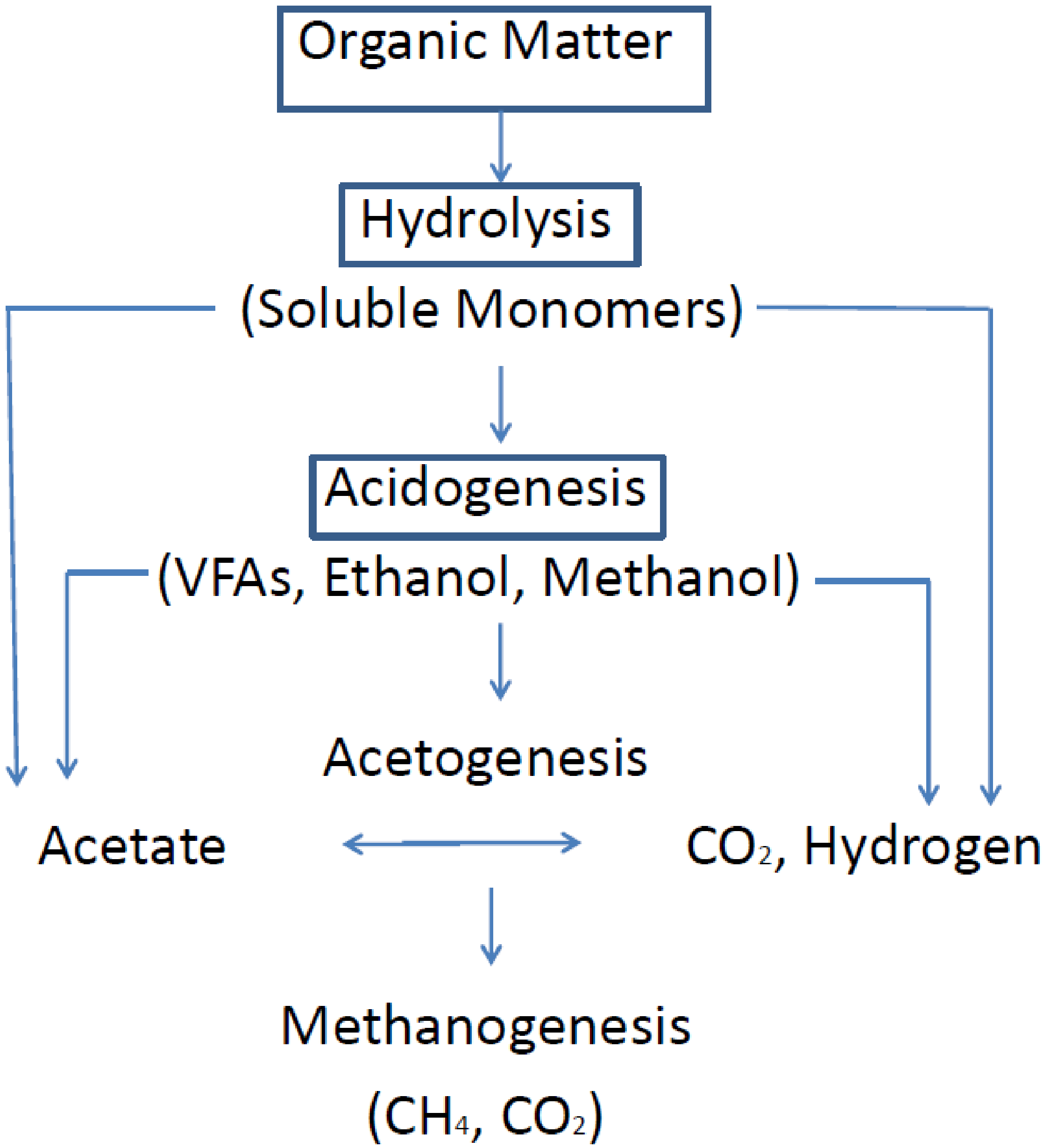

Fig.2.1. Anaerobic digestion of organic matter.

2.7. Factors Affecting Anaerobic Digestion:

Anaerobic digestion is carried out by different groups of microbes which are sensitive to a number of factors which ultimately affect the amount of biogas production and time required for biogas production. As there may be less yield of methane, instability of reactor and some inhibitors also cause problem in process continuity and the process may undergo failure. Common toxicants that affect the biogas production include volatile fatty acids, ammonia, heavy metals, sulfide etc. Due to these problems this technique is not widely applied (Chen et al., 2010). So normally reactors are monitored regularly to avoid the failure of the process and may not be applicable for some substrates in their native forms and co-digestion could be solution to overcome some of the problems.

2.7.1. Volatile fatty acids:

Volatile fatty acids (VFAs) are acetic acid, butyric acid and propionic acid formed as a result of breakdown of organic compounds by microorganisms. These are essential mid-products for methane production process. The content of volatile fatty acids present in anaerobic digesters have great effect on fermentation process and also affect the process productivity. Among volatile fatty acids propionic acids have more adverse effects on microbial communities, mostly on methanogens present in anaerobic digester and as a result methane production is dropped (Wanget al., 2009).

In anaerobic digester all reactions (as hydrolysis, accidogenesis etc.) should occur in a balanced way, misbalance at any stage of anaerobic digestion may cause inhibition of the whole process. In the cases when organic loading rate is high, then the rate of two steps i.e. acidogenesis and acetogenesis is higher than the rate of methanogensis. Which results in accumulation of volatile fatty acids as their concentration increases with the increase in organic loading rate.

2.7.2. Ammonia:

Ammonia production takes place in digester by degrading action of microbes on nitrogen containing organic matter such as protein. Ammonia is one of the essentials for growth of microorganisms and biogas production increases with small increase of ammonia. But when its amount is present in excess then it causes of inhibition of anaerobic digestion process. This may be due to the reason that ammonia has direct inhibitory effect on those enzymes which are related to methane synthesis. More concentration of ammonia is also toxic for microbial cells when it enters in to the cell and become cause of proton imbalance and cause toxicity. High ammonia concentration inhibits the function of those microbial enzymes which are related to methane synthesis (Sung et al., 2003).

Ammonia inhibition is dependent on pH of the digester. Ammonia has inhibitory mechanism similar to that of volatile fatty acids, ultimately decreasing pH (Crine, 2006). Ammonia nitrogen has strong effects on biogas production and affects short chain fatty acids removal. A little increase of ammonia has positive effect on methane production but if amount of ammonia increases beyond a limit then it results in process inhibition and methane yield decreases. Ammonia when present in high concentration, it decreases yield of methane during anaerobic digestion up to 50% (Sterling et al., 2001).

Among the microorganisms present in anaerobic digester, methane producing bacteria are least tolerant to ammonia than other microbes present in digester. The growth of methanogens ceases due to presence of high ammonia concentration in digester (Kayhanian, 1994). Ammonia and ammonium ions are interconvertible, ammonium ions are beneficial for microorganisms present in anaerobic digester. As microorganisms use ammonium ions as source of nitrogen.

2.7.3. Effect of pH:

pH has great effect on anaerobic digestion. In methane producing bioreactors, it is necessary to maintain pH near neutral. Range of pH required by methanogens to perform their activities lies between (6.6- 7.6) (Sasaki, 2012). pH is one of the important factors, the pH of the digester affects the amount, rate and content of methane production during anaerobic digestion.

If pH is not maintained in proper range, it may lead to reactor failure. Sometime pH imbalance may be due to accumulation of ammonia in digesters. Because high ammonia concentration leads to process instability which results in accumulation of volatile fatty acids, which further causes decrease in pH and lowers methane yield by decreasing methanogenic activity of microorganisms. Some other elements like concentration of sulfate, sulfide, sodium, calcium, magnesium, potassium and aluminum may also have some inhibitory effects on anaerobic digestion practices (Chen et al., 2008). Some studies revealed that the amount of carbon dioxide present in the digester also affect pH value of anaerobic digester.

2.7.4. Effect of Temperature Variation:

Temperature is one of the most central parameters to affect the anaerobic digestion process. As temperature varies, the amount of biogas production also varies. Fermentation of methane takes place at different temperatures, mostly around 35°C and may also take place at high temperature conditions around 50°C and also at low temperature 4-20°C (Liu et al., 2008).

Suitable temperatures for anaerobic digestion process range from mesophilic to thermophilic temperature. Mesophilic temperature suitable for methane production range from (35–42 °C) while suitable thermophilic temperature range from (45–60 °C). To get better methane production, it is important to keep a constant temperature during the whole anaerobic digestion process, as temperature fluctuations affect the biogas production negatively. Most of the microorganisms prefer to work under mesophilic conditions and a large variety of microorganisms can be found at mesophilic temperature as compared to thermophilic temperature. So the digesters working at thermophilic temperatures have limited variety of microorganisms present there as compared to digesters working at mesophilic temperatures (Karakashev et al., 2005).

Thermophilic anaerobic practices are extra sensitive to the temperature variations and they need extended time for adaptation to a different temperature. Tolerance of mesophilic bacteria to the temperature variations is +/−3 °C. And this change will not significantly reduce biogas production. Thermophilic temperature is not preferred one for digestion because thermophilic process has many chances to be victim of ammonia inhibition and a greater risk of process imbalance. There is a direct relation between increase of temperature and ammonia toxicity, which is higher at higher temperature (Angelidaki et al., 2003).

It is noted that temperature variations have significant effect on pH value and ammonia concentration of the reactor. The reactors operated at higher temperature contain high concentration of free ammonia and also high values for pH as compared to the reactors which are operated at lower temperature (Mashadet al., 2004).

Wet fermenters are mostly operated at temperatures range from 38 to 42°C which is mesophilic temperature while a very short number of biogas plants are operated at thermophilic temperature between conditions 50 to 55°C (Angelidaki et al., 2003).

MATERIALS AND METHODS

3.1: Effect of Co-digestion on Anaerobic Digestion:

A number of strategies are used to increase biogas production during anaerobic digestion. Anaerobic digestion of cattle manure alone does not produce significant amount of biogas. So in this study, co-digestion of cattle manure with green grocery waste was applied to enhance biogas production.

3.1.1: Substrates:

The substrates used in this study were green grocery wastes (vegetable and fruit wastes) and fresh cattle manure i.e. (cow and buffalo manure mixed in equal proportion based on wet weight). In most of the farms in Pakistan, cow and buffalo manure are mostly present in mixed form not in separate form, so in this study mixture of cow and buffalo manure was used to produce biogas. Cattle manure was collected from local farms near Quaid-i-Azam University located in Islamabad Pakistan and was frozen prior to use. Food wastes were collected from Islamabad local markets and ground to smaller particles by using electric grinder and mixed completely to form homogeneous mixture and stored at freezing temperature. To assess the effect of co-digestion, batch reactors were used for incubation of substrates individually and in combination at different ratios under anaerobic conditions. The incubations were carried at controlled temperature (35 °C). Both substrates i.e. cattle manure and green grocery wastes were kept in freezer for almost 3 weeks before use.

Table: 3.1: Composition of green grocery waste:

Vegetable waste	Quantity	Fruit waste	Quantity
Ridge ground	0.25 kg	Mangoes	0.5 kg
Pumpkin	0.5 kg	Melon (with peels)	0.5kg
Brinjal	0.25 kg	Water melon	0.25kg
Carrot	0.25 kg	Banana peels	0.25kg
Spinach	0.5 kg		
Indian round ground	0.25kg		

3.1.2: Inoculum Development:

Inoculum was developed in lab under anaerobic conditions. Sludge used for inoculum development was collected from waste-water treatment plant (located at I-9 Islamabad, Pakistan). Sludge (mixed primary and secondary sludge) was collected from two different points of same waste water treatment plant. The moisture content of sludge from these two points varied from each other, one sample was in liquid form and other was almost in solid form. These two sludge samples were taken equal by wet weight (1 kg each) and total sludge was 2 kg. To homogenize it, the sludge was mixed with an overhead stirrer and added almost 3 liter tap water to sludge mixture for its dilution. After it the sludge was passed through steel sieve having pore size almost 3mm to remove larger particles.

Before incubation the sludge was placed in lab at room temperature for two days. Then 5 liter sludge sample was placed in two reactors (2.5 liters in each reactor), each reactor having size of 3 liters. Before incubation both reactors with sludge were flushed with nitrogen gas and closed with air tight stoppers to produce oxygen free conditions in the headspace of the reactors for proper inoculum development. Both reactors then were placed in water bath at temperature 35°C. The biogas produced in the reactor was passed through the tubing of almost half meter connected to the port in the rubber stopper to the inverted cylinder placed in a large beaker. Beaker and inverted cylinder were fitted with water and placed outside of the water bath. The biogas passing from reactor to the inverted cylinder causes water displacement in the cylinder. The amount of the water displaced in the cylinder corresponds to the amount of biogas produced during anaerobic digestion. However, the amount of biogas produced was not registered because it was out of the aim of study but this was done to keep the reactor in anaerobic conditions by avoiding the entry of air into the reactors. After accumulation of biogas in the cylinder, the biogas was removed by using a syringe attached to tubing reaching to the top of inverted cylinder. Sludge was incubated for almost 4 weeks and then reactors containing sludge were removed from incubator and placed at room temperature for 4 days, before use. Before using inoculum, its samples were taken for analysis of pH, total solids and volatile solids.

3.1.3: Total Solids and Volatile Solids:

Total solids and volatile solids of inoculum, green grocery and for cattle manure (cow and buffalo) were analyzed in triplicate using standard methods. For determination of total solids, standard method 2540 B (APHA, 2005) was used. Total volatile solids were analyzed using standard method 2540 G (APHA, 2005). In case of cattle manures i.e. buffalo and cow manure was individually analyzed for total solids and volatile solids. To find out total solids, china dishes were washed with tap water and dried at room temperature. Then these china dishes were placed in incinerator for 1 hour at temperature 550°C to remove organic matter if attached to china dishes otherwise this organic matter will end as organic matter of the sample. After incineration china dishes were allowed to dry at room temperature and weighed them separately. Then samples were added to each china dish and weighed. Amount of sample added to each china dish bears any value between 11 to 35 g. Then the china dishes with samples were placed in an oven at 105˚C to dry samples completely, for time duration of 20-24 hours. Then samples were cooled at room temperature and weighed to determine total solids in the samples. Readings were recorded and then the china dishes with dried samples were placed in incinerator for 2 hours at temperature 550°C. After 2 hours china dishes were removed from incinerator and allowed to cool at room temperature. When the samples were cooled, the china dishes with samples were weighed to determine volatile solids. The samples at this stage became ash, which was inorganic material while the organic material was volatilized. Formula to calculate values for volatile solids is as follows:

$$\text{Volatile solids (VS)} = \frac{\text{Weight (dried sample+cruicible)-weight of (burnt cuicible+ ash)}}{\text{Weight (dried sample+cruicible)-weight of burnt cruicible}} * 100$$

3.1.4: Setup for Batch Process Experiment:

The co-digestion was carried out in batch reactors using Automatic Methane Potential Test System (AMPTS) shown in figure 3.1. First of all the Automatic Methane Potential Test System was calibrated for gas measurement. For calibration, air was pumped through a syringe to each cell of the sensor chamber, until the cell lifts up by receiving full volume of gas. 10 readings were taken for each cell and average of 10 readings was used as the volume capacity of respective cell. Then 14 glass reactors having volume 1 liter were used for batch experiment. Cattle manure (buffalo and cow mixed in equal amount by weight) and food wastes in reactors were subjected to anaerobic digestion individually and in different ratios for co-digestion. The incubation of test and control reactors was carried out in duplicates. Two controls were run in parallel with test reactors as recommended by Angelidaki et.al. (2009). One control having inoculum to determine the methane production in the background and this amount of methane was subtracted from the test reactors to determine the net amount of methane from the test reactors. Other control run was used as reference having cellulose. The aim of incubation of the reference with inoculum is to confirm the activity of inoculum. In all reactors, inoculum to substrate ratio on the basis of volatile solids was 4:1. After adding substrates and inoculum in reactors, the reactors were flushed with nitrogen to remove air present in headspace of the reactor. And air tight stoppers were fixed on each reactor while flushing with nitrogen gas in order to avoid entry of air.

The digestion was carried out in AMPTS, the schematic diagram of the AMPTS is shown in figure 3.1. All the reactors were incubated in water bath at temperature 35°C. A tube from each reactor was connected to the containers having 80-100 ml of NaOH (3M) solution. The alkaline solution (NaOH solution) was used for scrubbing CO_2 from biogas produced in the digester. The container of the scrubbing solution had two ports. One was inlet of biogas (methane and CO_2) generated in the reactor during anaerobic digestion and other was outlet of methane (as CO_2 is fixed by alkaline solution) passing to the respective cell in the sensor chamber. In the sensor chamber the volume of the methane is quantified and logged in a data acquisition system (through computer connected to sensor chamber). Reactors were manually shaken daily to mix the substrate and inoculum present in reactors. Setup of the reactors showing amount of substrate and inoculum used for co-digestion in each reactor is given in the table 3.1.

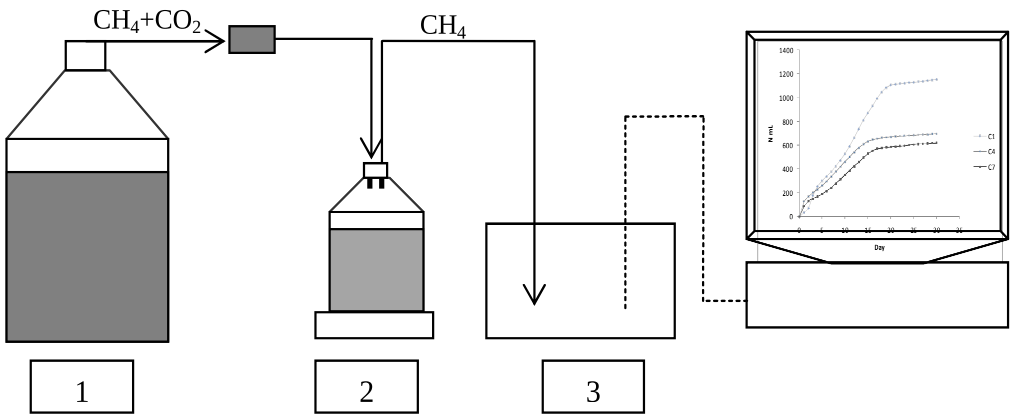

Figure 3.1: Schematic diagram of AMPTS. Biogas, mixture of methane and carbon dioxide, produced by batch anaerobic digestion process (1) passed through one-way valves and entered into respective scrubbing solution (NaOH solution) units where carbon-dioxide is fixed (2) and methane passes to the respective sensor chamber (3) where the volume is quantified and recorded by the software. Arrow shows the direction of gas flow.

3.2:Continuous Process of Anaerobic Digestion:

To evaluate the effect of temperature variation and co-digestion on anaerobic digestion, two independent anaerobic digesters were operated at same conditions but at different temperatures. The anaerobic digester is shown in figure 3.2.

Table 3.2: Setup for different concentrations of the substrates and inoculum used for co-digestion and mono-digestion under batch anaerobic digestion process

	Inoculum+ Cellulose+ Distill water	Inoculum+ Distill water	Manure+ Distill water	Vegetable + Manure+ Inoculum+ Distill water (25:75)	Manure+Inoculum+ Distill water	Vegetable + Manure+ Inoculum+ Distill water (50:50)	Vegetable + Inoculum+ Distill water	Manure + Inoculum
Inoculum (ml)	348	350	0	304	336	327	300	475.18
Cattle manure (g)	0	0	350	2.8	20	6	0	24.82
Water (ml)	150	150	500	150	150	150	150	0
Cellulose (g)	3.4	0	0	0	0	0	0	0
Vegetable waste (g)	0	0	0	43	0	17	53	0
VS of substrate (g)	3.4	0	56.91	2.615	3.25	1.9412	3.01	4.2
TS of substrate (g)	3.4	0	67.795	3.29	3.874	3.9099	3.3867	17.22

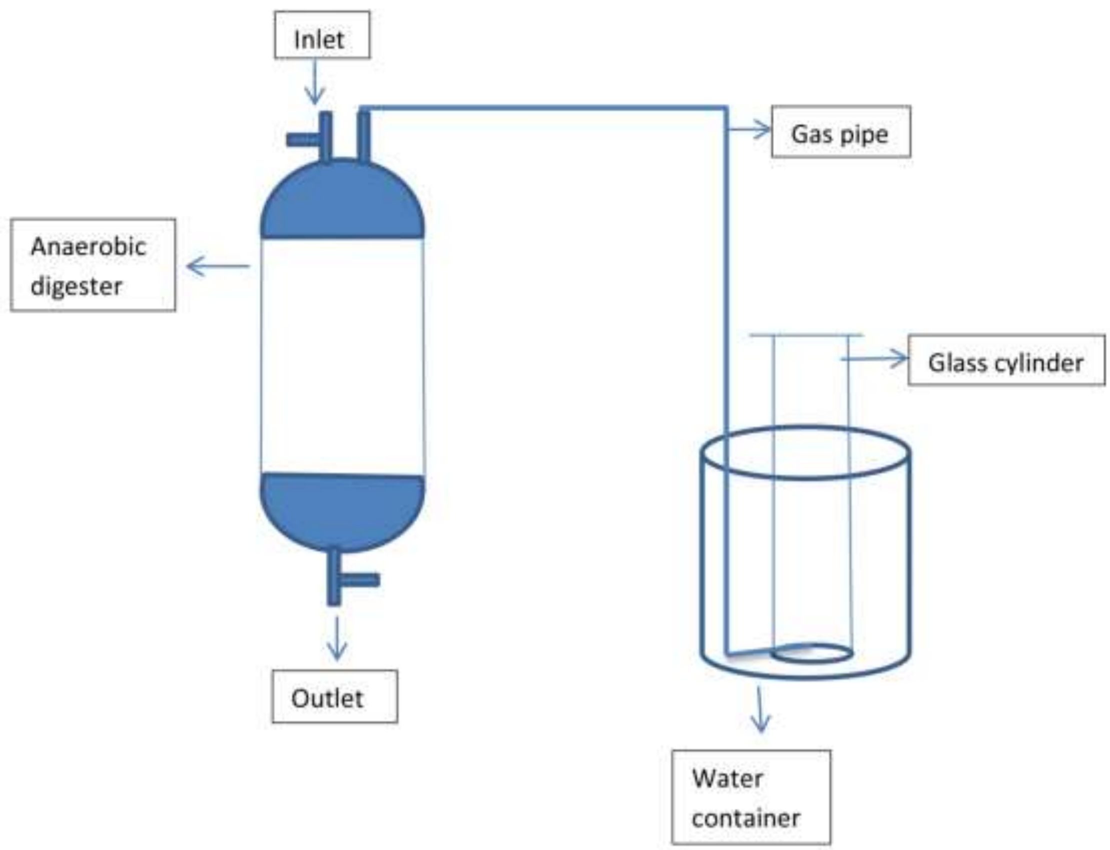

Figure 3.2. Setup of Continuous Anaerobic Digester

3.2.1: Sample Collection and Storage:

Substrates used for this study were cattle manure (cow and buffalo manure) and green grocery wastes. Cattle manure was collected from local farms in Islamabad (near Quaid-i-Azam University) Pakistan and frozen prior to use. Green grocery wastes were collected from Islamabad local markets, it was processed and stored at freezing temperature as given in section 3.1.1 (substrates).

3.2.2: Operational Conditions:

Hydraulic retention time provided to the reactor was 20 days. As this is normally time required for the decomposition of waste and this time is more than the doubling time of microorganisms. The effect of co-digestion was also studied in continuous reactors at controlled and uncontrolled temperature.

3.2.3: Inoculum:

Inoculum for continuous anaerobic digestion process was collected from a biogas plant. After collection inoculum was placed at room temperature for 2 days, before use. Total solids and volatile solids of inoculum were analyzed as mentioned in section 1.3 (total solids and volatile solids) before its use.

3.2.4: Reactor Setup:

Two stainless steel reactors of 14 liter with working volume of 10 liter were used for continuous anaerobic digestion of cattle manure in this study. Reactors were simple and with little monitoring. Both the reactors were of same design. Each digester (reactor) used had two outlets present at the top of reactor and one inlet present at the bottom of the reactor. One outlet of reactor used for gas collection and other to fed substrate. The inlet was used for collection of effluent from the reactor. Biogas produced during anaerobic digestion passed through a rubber pipe attached to the outlet for gas collection. Second end of the rubber pipe entered in to the cylinder. The cylinder was fixed in water filled container. Biogas produced by anaerobic digestion replaced water in the cylinder as mentioned in the section 1.2 (inoculum preparation). All the openings of reactors were made air tight to prevent gases exchange i.e. to prevent biogas leakage and oxygen to enter in the reactors.

During startup of reactors, 5 liters of manure in a composition of 20 g volatile solids per liter and 5 liter of inoculum was added to each of the reactors. To study the effect of controlled and uncontrolled temperature one reactor was incubated at environmental temperature where temperature fluctuates with day and night variation and the other reactor was incubated at controlled mesophilic temperature (37°C). Daily 1g VS/L.day of cattle manure was fed to both the reactors. The feed given corresponds to organic loading rate of 1 g of volatile solids per liter per day. Both the reactors, placed at controlled temperature and uncontrolled temperature were first fed with the cattle manure only. The reactors were considered quasi-steady-state, once the reactors were continuously fed at least one retention time (20 days) and difference in daily biogas production was less than 5%. After 3 retention times (60 days), when a steady state was achieved, a container having NaOH solution (3M) was attached to each reactor for calculation of methane yield, as NaOH solution has capacity to fix CO_2 as described in section 3.1.4. After reaching the quasi-steady-state of methane yield, the container having NaOH solution was removed and the feed was changed for both the reactors. The reactors were fed with the mixture of cattle manure and green grocery wastes to see the effect of co-digestion on biogas yield. Cattle manure and green grocery waste was given as feed in 1:1 ratio on volatile solids basis, to both reactors. For this composition of feed, both the reactors were fed for more than 20 days until the reactor reached quasi-steady-state.. When a quasi-steady-state reached, the container containing NaOH (3M) solution was attached to the reactor again to calculate methane yield. Calculations for hydraulic retention time and organic loading rate are given in the section 3.2.6.

3.2.5: Method for Biogas and Methane Measurement:

Gas production was registered each day by measuring water displacement in cylinder by the biogas passed from anaerobic digester. pH of the effluent and of feed was recorded on daily basis using wet tip meter, for both the reactors. Daily temperature changes for the reactor placed at uncontrolled temperature were also noted, as gas production changes with temperature change. Methane content in the biogas was determined once the process reached the quasi-steady-state condition as described in section 3.2.4.

3.2.6: To Calculate Flow Rate and Concentration:

Flow rate and organic loading rate were calculated as follow:

Flow rate (L/Day):

Flow rate= volume of reactor / HRT

Flow rate = 10 L /20 days

Flow rate = 0.5 L / day

Organic loading rate (OLR):

OLR = Concentration* (flow rate / volume of reactor)

Concentration = (1 g VS/ L. day) * (10 L)/ (0.5 L sample/day)

Conc. = 1 * 10 / 20 (g VS / L. day)

Conc. = 20 g VS / L sample

Formula to calculate total solids (TS):

TS = Weight of dried sample / weight of wet sample*100

Formula to calculate volatile solids (VS):

$$VS = \frac{\text{Weight (dried sample+cruicible)-weight of (burnt cuicible+ ash)}}{\text{Weight (dried sample+cruicible)-weight of burnt cruicible}} * 100$$

RESULTS

Batch Anaerobic Digestion Process:

Two types of substrates, cattle manure and green grocery wastes were used in the process of anaerobic digestion to evaluate the effect of co-digestion and temperature on biogas yield. The effect of co-digestion of cattle manure with green grocery wastes in different concentrations, on biogas yield was evaluated at mesophilic temperature (35°C) in batch assay. The setup for batch assay is shown in table 3.1. The incubation was carried out for about 100 days except for manure. Anaerobic digestion of manure (with use of inoculums) was carried out for 24 days almost. Methane production after 45 days of anaerobic digestion was constant in all the reactors, so the methane production shown in the results is only for 45 days of incubation. The batch anaerobic digestion was carried out in Automatic Methane Potential Test System.

Cellulose was run as control in order to check the activity of inoculum. Fig. 4.1 shows the methane yield of cellulose in batch anaerobic digestion process. Methane production on the first day was 19.3 N ml/g of VS_{added} of the substrate. The production of methane reached maximum in first 10 days of anaerobic digestion process and the amount of methane production after 10 days was 325.6 N ml/g VS_{added}. Methane yield corresponds to 82% of the theoretical methane yield of cellulose. The results show that the inoculum was active and have enough number of microorganisms required to carry out the process of anaerobic digestion and for methane production. After first 10 days daily methane production was decreased. The maximum methane yield was obtained after 31 days, 342 N ml/g VS_{added}. Rate of methane production is shown in figure 4.2.

Methane yield from cattle manure (without use of inoculum) in mono-digestion under batch anaerobic digestion process is shown in Fig.4.3. Methane yield on the first day of anaerobic digestion process was 0.168 N ml/g of VS_{added}. However methane yield was quite lower throughout the study. Methane yield after first 10 days was 3.4 N ml/g VS_{added}. Figure 4.4 shows rate of methane production. After first 10 days the rate of methane production was slightly increased but the overall amount of methane produced was much less then green grocery waste and mixture of green grocery waste. The methane production reached to (39 N ml/g of VS_{added}) after 40- days and after 40 days of incubation methane production rate was decreased.

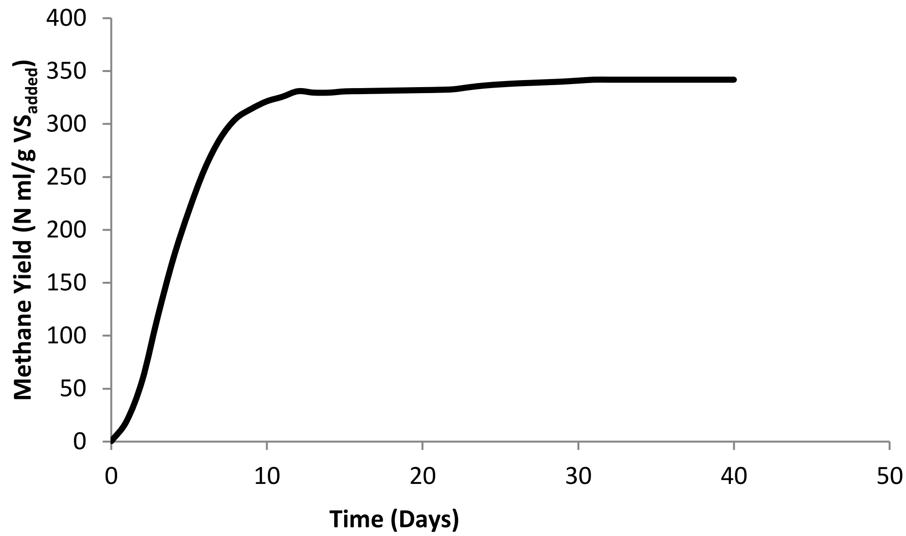

Fig. 4.1: Methane yield of cellulose in batch anaerobic digestion process**.**

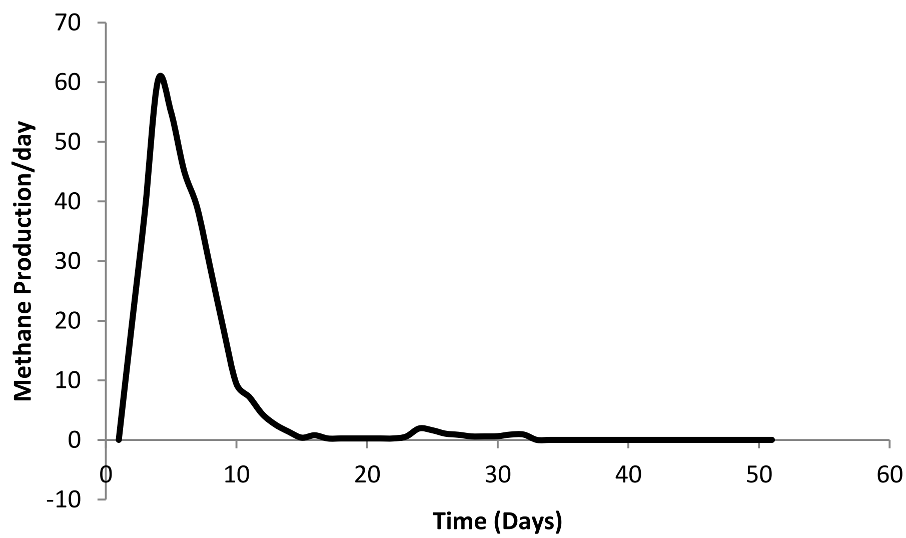

Fig. 4.2: Rate of methane production of cellulose in batch anaerobic digestion process**.**

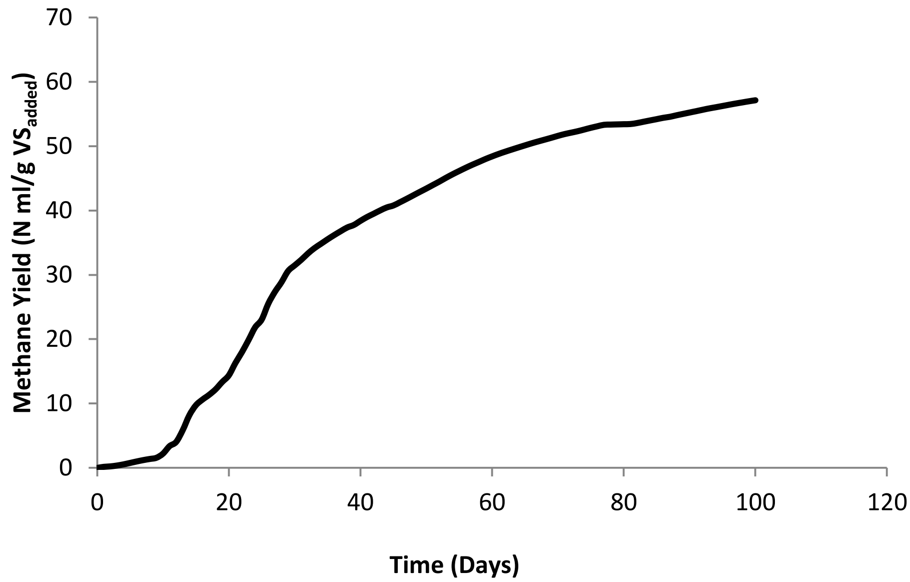

Fig. 4.3: Methane yield of manure (without use of inoculum) in batch anaerobic digestion.

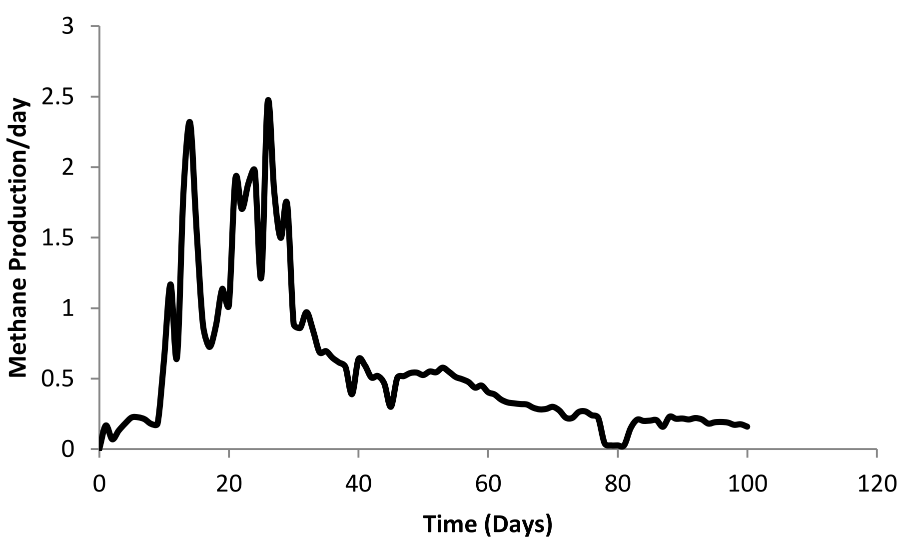

Fig. 4.4: Rate of methane production of manure (without use of inoculum) in batch anaerobic digestion process.

Methane yield from green grocery waste on the first day was 22.1 N ml/g of VS_{added} (Figure 4.5). Methane production was higher than the methane production by manure alone as well as from manure and inoculum. Its production was reached to maximum in first 10 days of incubation. Methane yield from the green grocery after first 10 days was 303 N ml/g of VS_{added}. After first 10 days methane production rate was decreased (Figure 4.6). Methane production was ceased after 32 days. Maximum methane production achieved after 32 days was 342 N ml/g VS_{added}.

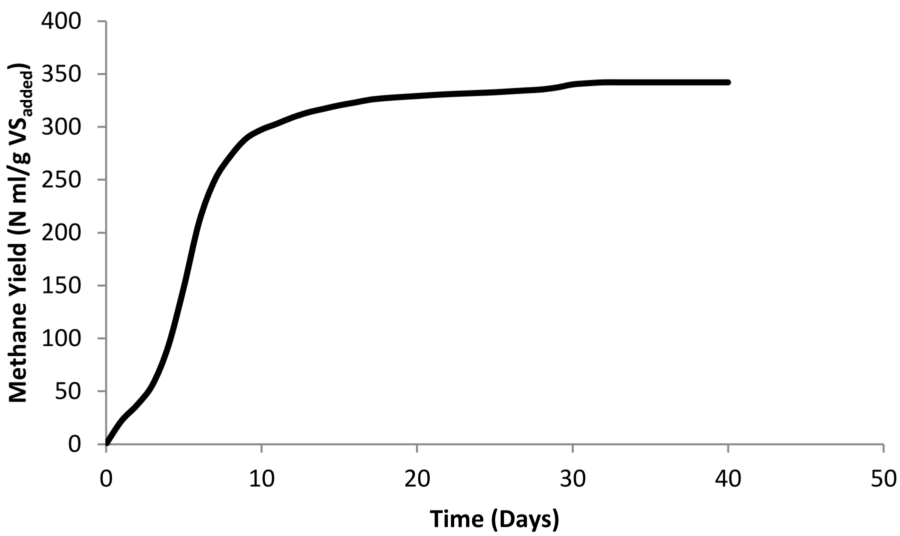

Fig. 4.5: Methane yield of green grocery waste in batch anaerobic digestion process.

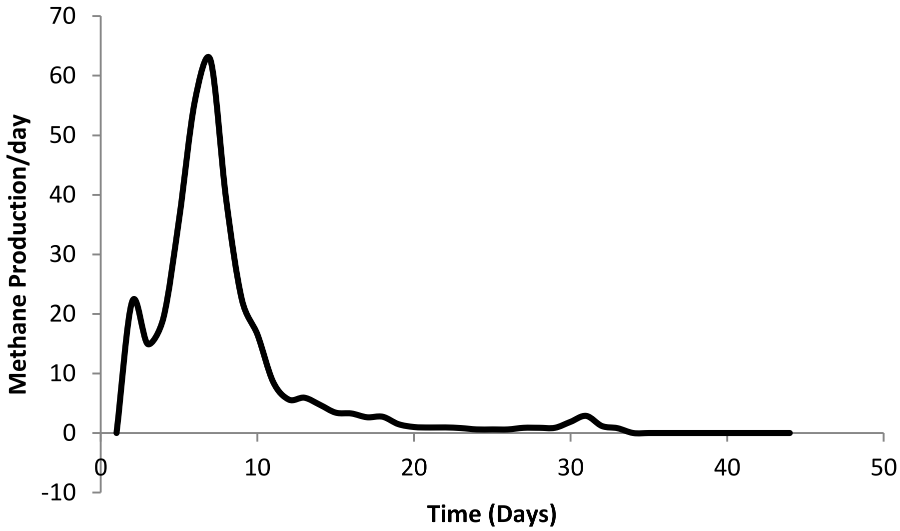

Fig. 4.6: Rate of methane production of green grocery wastes in batch anaerobic digestion process.

Figure 4.7 shows batch anaerobic digestion process of green grocery wastes and cattle manure mixed in ratio 75:25 respectively in terms of $VS_{added}$to evaluate the effect of co-digestion. Methane production was higher than methane production by cattle manure. On the first day of start of the study methane production was 17.3 N ml/g of VS_{added} and methane production rate was maximum in first 10 days. After 10 days methane yield was 259 N ml/g of VS_{added}. Onward from 10 days, the rate of methane production was decreased. The maximum methane yield was 297 N ml/g of VS_{added} obtained after 30 days of anaerobic digestion process. After 36 days methane production was ceased fig. 4.8.

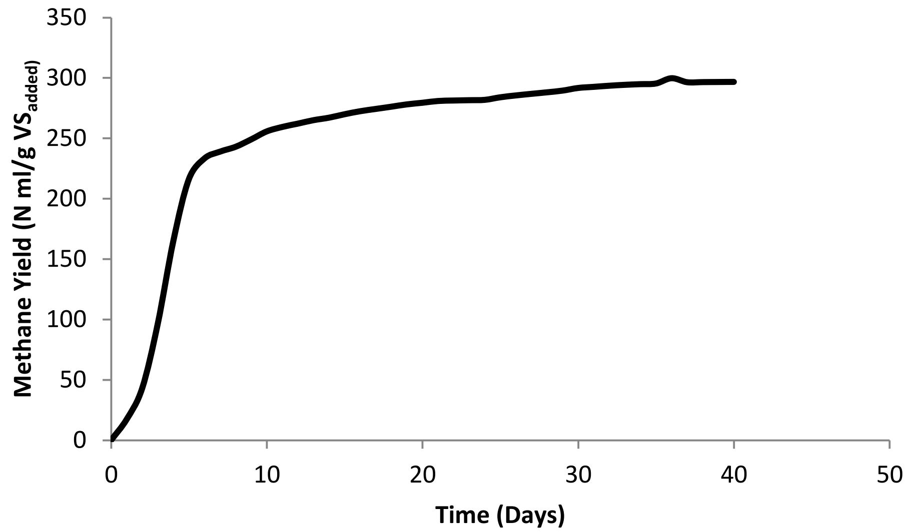

Fig. 4.7: Methane yield by co-digestion of cattle manure with green grocery wastes in ratio 25:75 respectively in batch anaerobic digestion process.

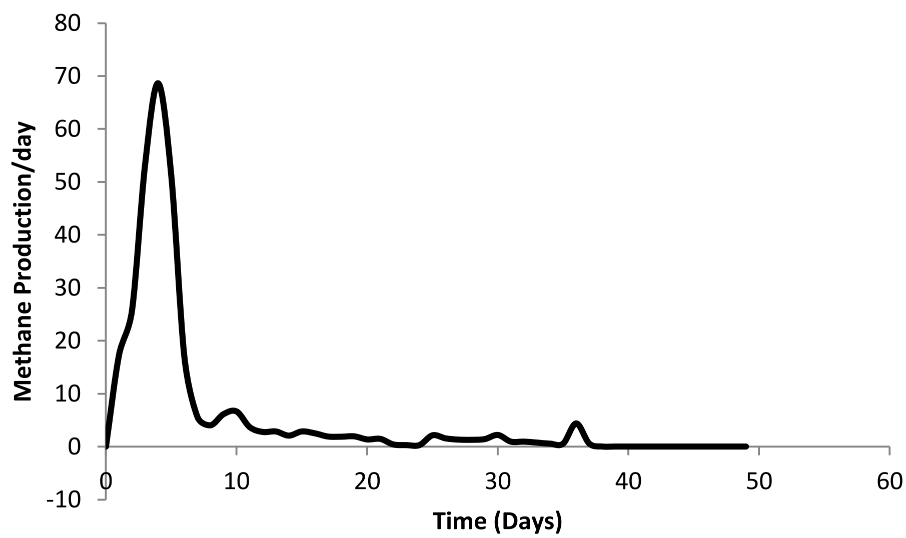

Fig. 4.8: Rate of methane production by co-digestion of cattle manure with green grocery wastes in ratio 25:75 respectively in batch anaerobic digestion process.

Fig. 4.9 shows methane yield from cattle manure and green grocery waste 50:50 under batch anaerobic digestion process. Methane yield on the first day was 31.5 N ml/g of VS_{added}. It shows that methane production in this combination is highest. Methane production reached to maximum in first 15 days of the start of the experimentand methane production after first 15 days reached to 355 N ml/g of VS_{added} (figure 4.9). Then methane production rate started to decrease and methane production after 30 days was 418 N ml/g of VS $_{added.}$ Methane production was ceased after 92 days and the maximum methane yield was 430 N ml/g of VS_{added}.

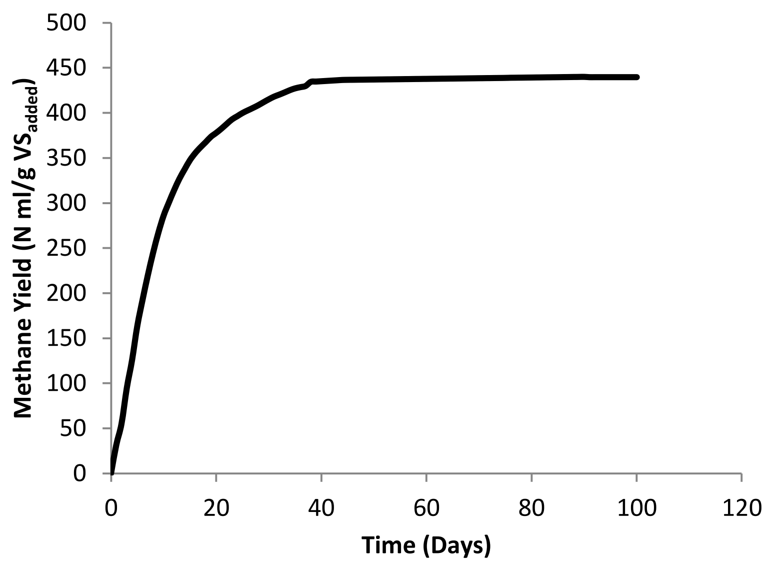

Fig.4.9: Methane yield by co-digestion of cattle manure with green grocery wastes in ratio of 50:50, under batch anaerobic digestion process.

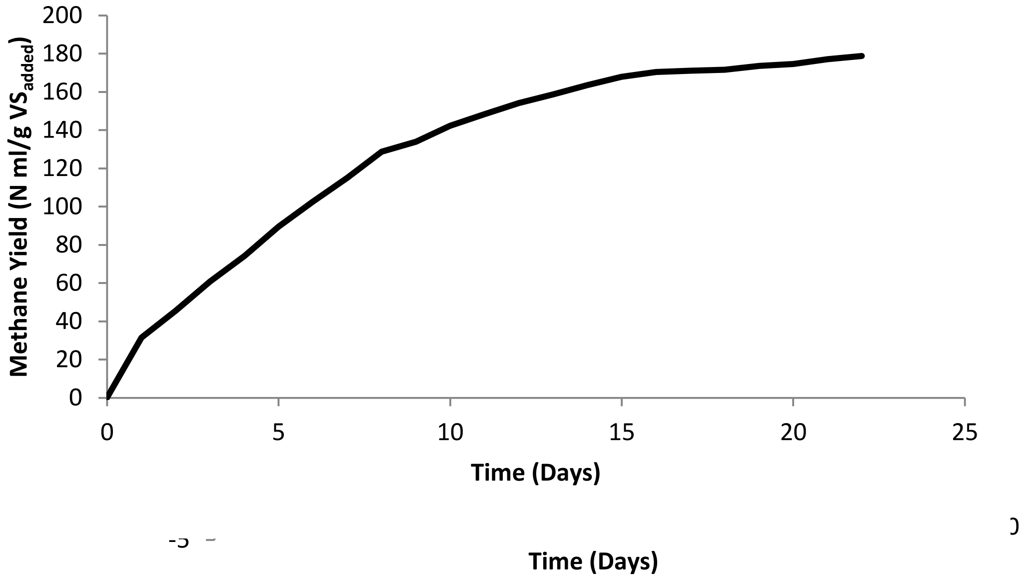

Fig.4.10: Methane production by cattle manure under batch anaerobic digestion process.

Methane production by cattle manure (with inoculum) was high than the methane production by cattle manure without use of inoculum. As fig. 4.10 shows that the methane production was lower than those of co-digestion combinations. On first day methane production was 31 N ml/g VS_{added}. And the amount increased with passage of time. The study for methane production by cattle manure was carried out for 23 days. Methane production was also lower than methane production by green grocery waste. Rate of methane production is shown in figure 4.11.

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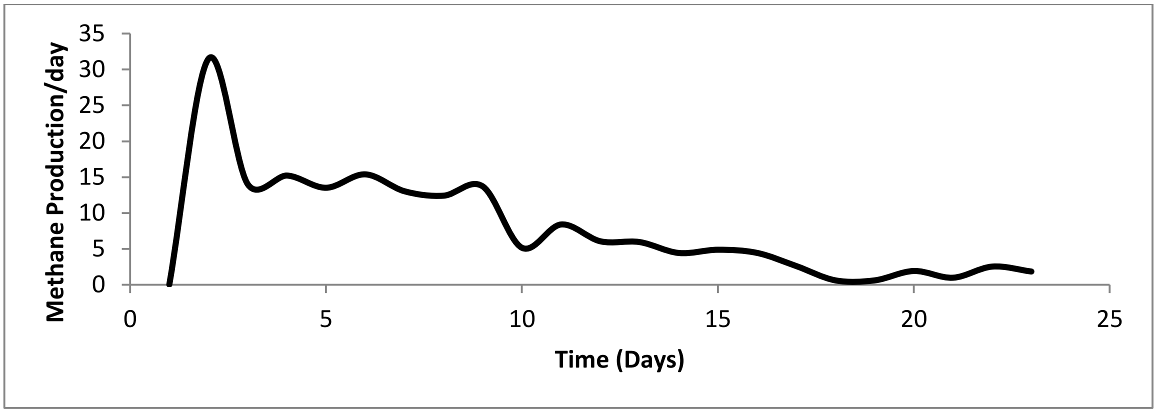

Fig.4.11: Rate of methane production by cattle manure under batch anaerobic digestion process.

Table: 4.1: Methane yield of different substrates during batch mode of anaerobic digestion process.

Days	Cellulose (N ml/g VS)	Manure (without inoculum) (N ml/g VS)	Green grocery waste and manure 75:25 (N ml/g VS)	Manure and green grocery waste 50:50 (N ml/g VS)	Vegetable (N ml/g VS)	Manure with inoculum (N ml/g VS)
day 1	19.3	0.168	17.3	31.5	22.1	31.5
day 5	218.1	0.777	216.3	163.7	148.2	89.6
day 10	321.3	2.2	255.8	285.9	297.5	148
day15	330.7	9.7	269.9	347.4	320.4	170
day 30	340.8	31.5	291.7	415	340	
day 100	341.7	57.2	298.9	899.7	342.2	

Continuous Anaerobic Digestion Process:

The effect of mono-digestion of cattle manure and co-digestion of cattle manure with green grocery waste was evaluated in continuous mode of anaerobic digestion. In continuous reactors the effect of temperature was assessed formono-digestion and co-digestion of cattle manure at controlled and uncontrolled temperatures. The operational conditions of both reactors were same, organic loading rate was 1 g VS/L.day for both of the continuous reactors, solid retention time was 20 days. Biogas yield during mono-digestion of cattle manure is shown in fig. 4.13. Biogas yield for both of the reactors was started from the first day of anaerobic digestion process. Biogas yield on the first day for mono-digestion with cattle manure was 1750 ml/day (1.57 N L/day), for the reactor placed at uncontrolled temperature. The production was increased with the passage of time. After 7 days of anaerobic digestion, maximum biogas yield was recorded to be 1850 ml/day (1.66 N L/day), when day temperature was around 35°C. With the decrease in temperature, the biogas production started to decrease. And after 30 days biogas production was around 250 ml/day (0.229 N L/day). After achieving steady state in 58 days, when consecutively 3 days biogas production was around 250 ml/day at temperature around 20°C, the amount of methane produced was measured by passing biogas through a container having NaOH (3M) solution. Methane yield was 150 ml/day (0.139 N L/day). It was 60% of the total volume of biogas i.e. methane content in the biogas was 60%. Fig. 4.14 shows the amount of methane in total volume of biogas.

Then reactor was changed from mono-digestion to co-digestion i.e. the reactor was fed with mixture of cattle manure and green grocery waste to study the effect of co-digestion. The composition of feed was 1:1 on volatile solids basis for green grocery wastes and cattle manure. Biogas yield gradually started to increase with the change of feed of the reactor and biogas yield was 300ml/day(0.274NL/day), and then increased gradually with time course. After 9 days of change of feed, biogas production reached to maximum, the amount of biogas was 500ml/day (0.474 NL/day). Fig. 4.13 shows clearly the increase in biogas yield due to co-digestion. Then again to calculate amount of methane in the total volume of biogas, the biogas produced in the reactor was passed through a container having NaOH (3M) and the amount of methane production during steady state was

300ml/day (0.274NL/day), which was almost 60% of the total volume of the biogas produced by the reactor. It was recorded that biogas production was increased 10 times by applying co-digestion for the reactor placed at uncontrolled temperature as compared to biogas production by mono-digestion at uncontrolled temperature. Fig. 4.7 shows the results of co-digestion on methane production.

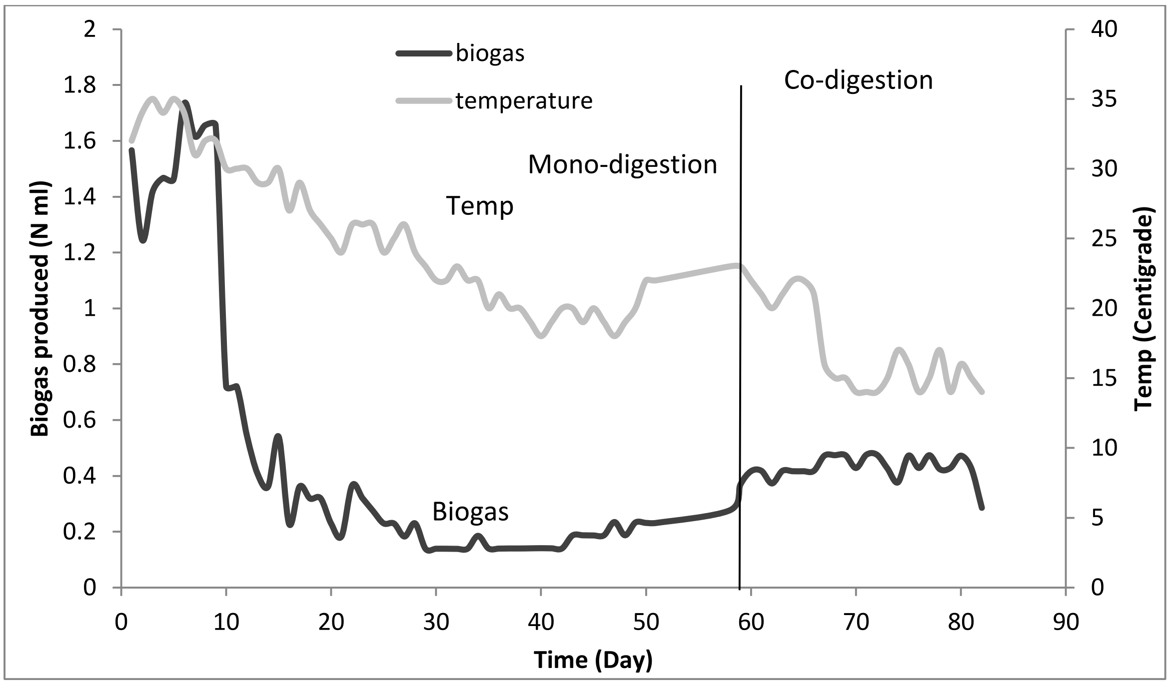

Fig. 4.12: Normalized biogas yield at uncontrolled temperature, by mono-digestion of cattle manure and co-digestion of cattle manure with green grocery waste under continuous anaerobic digestion process.

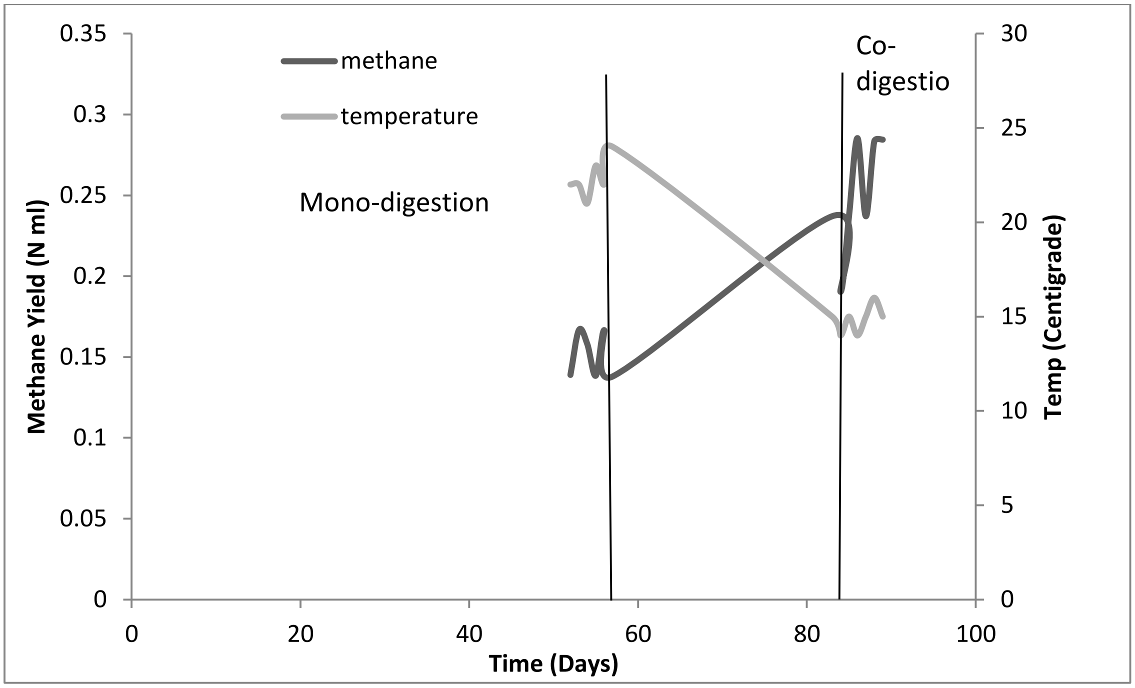

Fig. 4.13: Normalized methane yield at uncontrolled temperature, by mono-digestion of cattle manure and co-digestion of green grocery waste and cattle manure under continuous mode of anaerobic digestion process.

Reactor placed at controlled temperature (37°C), biogas production during mono-digestion of cattle manure on first day of anaerobic digestion was 2000ml/day (1.76 N L/day). Daily biogas production continuously increased and biogas production rate reached to maximum after 10 days and it was 3200 ml/day (2.818 N L/day) and onward biogas production was almost constant.Biogas production was 10 times more by the reactor placed at controlled temperature with mono-digestion than the reactor placed at uncontrolled temperature with mono-digestion. Then after 20 days when steady state was achieved, a reactor containing NaOH (3M) solution was attached to the reactor and daily methane production was measured. Average methane production rate of three days during steady state was 1850 ml/day (1.629 N L/day), which was 64% of the total gas volume produced by the reactor. Since biogas is composed of carbon dioxide and methane, so by passing the biogas through the alkaline solution the carbon dioxide is fixed and passed amount of gas which shows that the methane content is 64% . And methane content in total biogas volume was 4% high by the reactor placed at controlled temperature. When steady state was achieved, the feed of the reactor was changed and co-digestion of green grocery and cattle manure was carried out in the reactor. The cattle manure and green grocery was mixed in ratio of 1:1 on volatile solids basis. Biogas production started increasing gradually and became maximum after 13 days of feed change, and biogas production rate was its value was 5300 ml/day (4.667 N L/day). It shows that there is 10 times increase in amount of biogas produced than co-digestion at controlled temperature and 20 times increase in biogas production as compared to mono-digestion at uncontrolled temperature. And there is 10 times increase in biogas production by co-digestion at controlled temperature as compared to mono-digestion at controlled temperature.

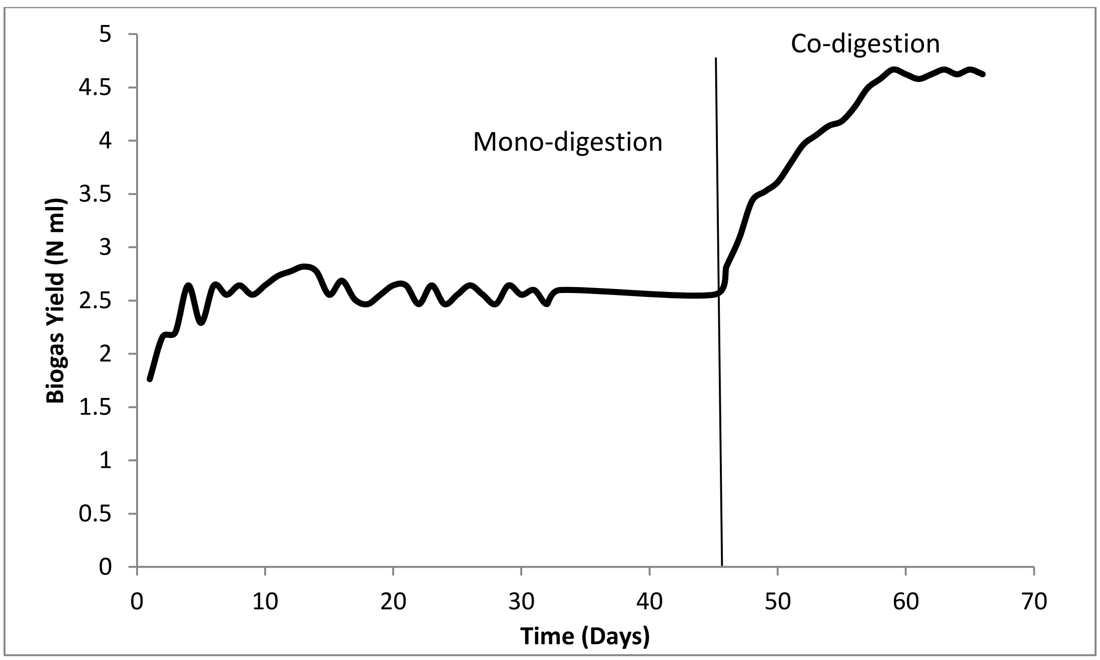

Fig. 4.14: Normalized biogas yield at controlled temperature (37°C), by mono-digestion of cattle manure and co-digestion of cattle manure with green grocery waste under continuous mode of anaerobic digestion process.

After achieving steady state, container having NaOH (3M) solution was attached to the reactor to calculate methane production. Amount of methane production was 3400 ml (2,994 N L/day), which corresponds methane content 64%. As shown in table 4.2 during mono-digestion and co-digestion the methane content in biogas produced in the reactor placed at controlled temperature was 4% high than the reactor placed at uncontrolled temperature. VS reduction was higher at controlled temperature than at uncontrolled temperature. VS reduction as uncontrolled temperature was 39.44% in mono-digestion and co-digestion. While VS reduction at controlled temperature was 46.44% for mono-digestion and co-digestion. VS reduction at controlled temperature was 7% higher as compared to the reactor placed at uncontrolled temperature.

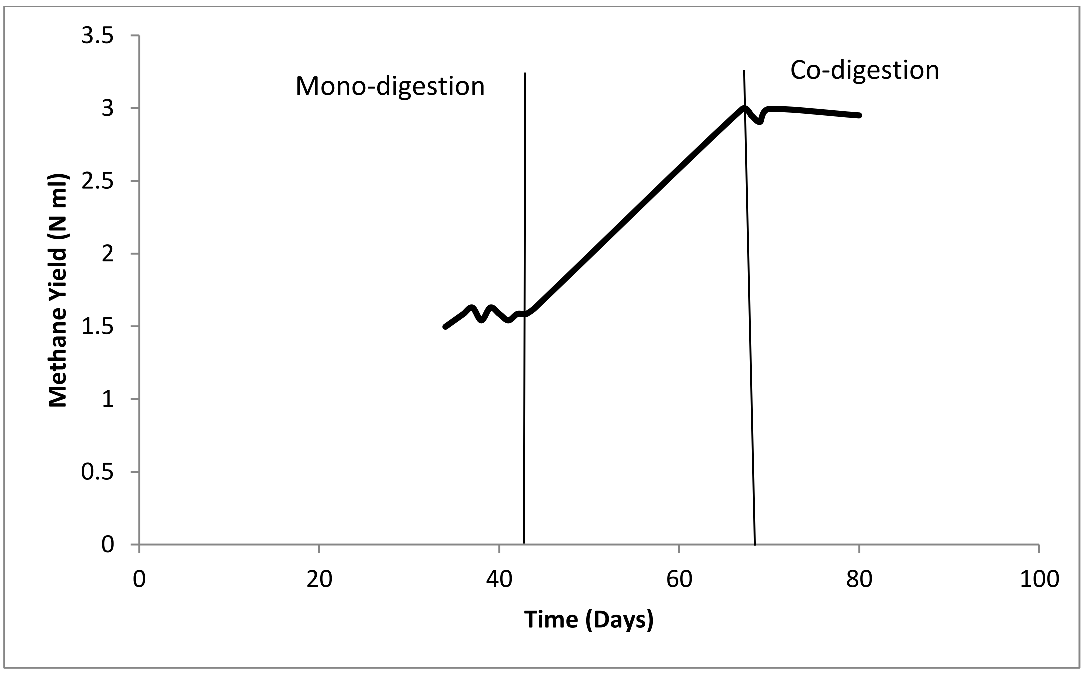

Fig. 4.15: Normalized methane yield at controlled temperature (37°C), by mono-digestion of cattle manure and co-digestion of cattle manure with green grocery waste under continuous mode of anaerobic digestion process.

Table: 4.2: The biogas yield and VS reduction of cattle manure and green grocery waste during continuous anaerobic digestion process at different conditions.

Conditions	CH_4(%)	Biogas (L)	CH_4 (L)	VS Reduction (%age)	Biogas production rate (NL/vol of reactor)	Biogas yield(NL /VS added)	CH_4 production rate(NL/vol of reactor)	CH_4 yield(NL /VS added)
Mono-digestion (cattle manure alone) at controlled temperature	64	2.95	1.85 L	46.45	0.259	0.259	0.154	0.154
Co-digestion (cattle manure & green grocery) at controlled temperature	64	5.3	3.4	46.45	0.467	0.467	0.295	0.295
Mono-digestion (cattle manure alone) uncontrolled temperature	60	0.25	0.15	39.44	0.023	0.023	0.0138	0.014
Co-digestion (green grocery waste & manure) uncontrolled temperature	60	0.5	0.3	39.44	0.047	0.05	0.028	0.028

Table: 4.3: Minimum and maximum biogas yield under different conditions by cattle manure and green grocery waste in continuous mode of anaerobic digestion process.

	CH_4 %age	Niogas (L)	CH_4 (L)	VS reduction (%age)	Biogas production rate (NL/vol of reactor)	Biogas yield(NL/ VS added)	CH_4production rate(NL/vol of reactor)	CH_4yield(NL/VS added)
Co-digestion (cattle manure & green grocery) at controlled temperature	64	5.3	3.4	46.45	0.467	0.467	0.295	0.295
Mono-digestion (cattle manure alone) uncontrolled temperature	60	0.25	0.15	39.44	0.023	0.023	0.0138	0.0138

DISCUSSION

The Biomethane potential test shows that there was significant increase in biogas production during co-digestion of cattle manure with green grocery wastes compared to mono-digestion of cattle manure. The anaerobic digestion of single substrate is not much effective. Different ratios of green grocery wastes and cattle manure were subjected to anaerobic digestion in batch assay to study the effect of co-digestion at controlled temperature.

Methane production by cellulose was significant in the batch anaerobic digestion process. It shows that inoculum was active and it contained all the microorganisms in required number for methane production. Fig. 4.1 shows methane content of cellulose while rate of methane production is shown in fig. 4.2. Methane production was 82% of theoretical methane yield of cellulose which is good methane yield. The obtained methane yield shows that inoculum used in the batch assay had properly been developed and was suitable to be used for batch assay anaerobic digestion.

Methane production from cattle manure (without use of inoculum) in batch anaerobic digestion process was quite low in throughout the process showing that manure does not contain sufficient number of microorganisms for methane production and one must use inoculum to run anaerobic digestion process (Fig. 4.3).The methane yield of cattle manure is significantly low compared to green grocery and cattle manure when co-digested with each other as well as from that of manure containing inoculum. Methane yield from cattle manure by other researcher has been reported as by and has been lower than other substrates like (WWTP sludge, sludge from paper mill, used of anaerobic digestion for methane production was not significant. The reason may be that cattle manure does not contain easily biodegradable material. It is reported by Lehtomaki et al., (2007) that cattle manure has low amount of total solids and volatile solids, which results in low methane yield. It is also studied that methane production results were not significant by anaerobic digestion of cattle manure alone, because it has low C/N ratio. And low C/N ratio is the cause of increase in ammonia concentration and ultimately results in process inhibition (Alvarez, 2000). It is stated by Angeldaki et al., (2003), that cattle manure is not an attractive substrate to digest alone because it has high content of water over all. Instead manure is a good carrier substrate when degraded in combination with other substrates for co-digestion purposes. These results of AD of cattle manure alone show that even though methanogens are present in cattle manure but they are not in sufficient amount to carry out anaerobic microbial digestion process and so during start up, it is important to add inoculum in to the reactor. Instead the anaerobic digestion of cattle manure with use of inoculum resulted in higher methane production as compared to that of methane production by manure without use of anaerobic digestion. The results show that inoculum is necessary for methane production as fig 4.11 shows.

Methane yield from the green grocery waste was significantly high throughout the process (Fig. 4.5). The results show that green grocery waste is a good substrate as it is rich in easily biodegradable organic matter. As it is studied that green grocery waste is one of the favorable substrates for anaerobic digestion, as food wastes have all the nutrients in sufficient amount, which are required by microorganisms involved in anaerobic digestion (Zhang et al., 2006). Then daily methane production was started to decrease. This may be due to deficiency of nutrients and consumption of easily biodegradables. Ultimately methane yield was ceased. Amon et al., (2006) reported that green grocery waste is one of the suitable wastes to improve biogas yield.

Methane yield was significantly high in the start days of anaerobic digestion of green grocery waste and cattle manure mixture in ratio 75:25, respectively. Studies carried out by Nayono et al., (2010) showed that addition of food waste in the anaerobic digester enhances the methane production. Then biogas production rate was started to decrease. The co-digestion at commercial level is also desired because the co-digestion can overcome crisis due to the limited continuous supply of feedstock throughout the year (Nges et al., 2012). Fig. 4.7 shows methane yield from co-digestion of cattle manure with green grocery waste in ratio of 25:75 while fig. 4.8 shows rate of methane production.

Green grocery and cattle manure in ratio of 50:50was significantly high and results show that it is best combination for anaerobic digestion and for high methane yield. This may be due to better carbon nitrogen ratio and process stability. As it is reported by Mshad et al., (2004) that co-digestion of more than one substrates increase the process stability of anaerobic digestion process and maintain a balance between C/N ratio. Which exhibit more stable methane production. Callaghan et al., (2002) showed that green grocery waste and cattle manure are the best substrates for co-digestion (Fig. 4.9).

Continuous Anaerobic Digestion Process:

Continuous anaerobic digester fed with single substrate (cattle manure) placed at environmental temperature, when day temperature was around 35°C produced significant amount of biogas.

Biogas yield was increased with the passage of time and after 7 days of study its production reached to the maximum when the mid-day temperature was about35°C. It shows that anaerobic digestion is good at this temperature 35°C. When environmental temperature decreased, the biogas production rate also started to decrease with time. The temperature around 35°C is an optimum temperature for mesophilic biogas production. Same observations were reported by Angelidaki et al., (1994) in their study they have shown that biogas production has linear relation with increase in temperature but up to a certain limit. The rate of biogas production increases continuously, when the temperature of digester increases from 25-44˚C.

Different studies have shown that temperature has great effect on microbial activity and their metabolism. At high temperature microbial metabolism increases and the rate of substrate degradation also increases resulting in high biogas yield, while the decreasein temperature ultimately gives low biogas yield (Bouallagui et al., 2005). Mesophilic temperature around 35°C has advantage to maintain the process stability while low temperature is not suitable to obtain high biogas yield, as low temperature anaerobic digestion causes to fail the microbial activity and a large amount of suspended solids are buildup in the reactor. Data of this study showed that biogas production is affected by temperature change i.e. biogas yield by controlled temperature and uncontrolled temperature incubations vary and biogas yield by the anaerobic reactor incubated at uncontrolled temperature was not significant.

Including temperature, a balanced C/N ratio is also an important factor to affect biogas yield. Anaerobic digestion of cattle manure as a single substrate does not have a balanced

C/N ratio required for optimum biogas production. Optimum biogas yield by anaerobic digestion process can be achieved at optimum C/N ratio 25:1 (Gerardi, 2003). It shows that if balanced nutrients are present in anaerobic digester like more than one organic substrates are present in the same digester, it is beneficial for microorganisms present in anaerobic digesters. It is also possible that in case of very low C/N ratio, large amount of nitrogen prevails in the reactor, which results in formation of large amount of ammonia.

Methane yield of anaerobic digester placed at environmental temperature was determined only during steady state conditions, when biogas yield of continuously 3 days had difference less than 5%, the setup was changed to study the methane yield per day, at temperature was around 17°C. Volume of methane recorded was 60%of total biogas volume.

Then feed of the reactor changed, and cattle manure and green grocery waste was fed to the reactor to see effect of co-digestion. The composition of feed was 1:1 on volatile solids basis for green grocery waste and cattle manure. Biogas production gradually started to increase from the first day of feed change. It shows that instead of low temperature, co-digestion was beneficial to enhance biogas yield. Fig. 4.13 shows the effect of co-digestion and temperature variation on biogas yield at environmental temperature. It has been reported by Poschlet al., (2010) that co-digestion is more energy efficient and stable process for biogas production than the single waste digestion. Co-digestion gives high amount of biogas production due to process stability. The increase in biogas yield by co-digestion may be due to a balanced C/N ratio or due to optimumpH values. As pH increases by increase in ammonia concentration and inhibit methanogenic activity in case of cattle manure mono-digestion. As methanogens in comparison to other microorganisms present in anaerobic digesters consortia, are more sensitive to changes in pH. As pH changes, the most affected microorganisms are methanogens and their activity slows down (Salminen et al., 2002). There was no effect of mono-digestion and co-digestion on methane content in total volume of biogas was same for mono-digestion and co-digestion a well. The methane content was 60% in case of mono-digestion and co-digestion. Fig 4.14 shows the methane yield for mono-digestion and co-digestion at uncontrolled temperature.

For the reactor placed at controlled temperature, biogas yield was higher as compared to that of the reactor placed at uncontrolled temperature. This may be due to high mesophilic temperature of the digester as it is reported by Bolzonella et al., (2012) that at high temperature, volatile solids and COD removal is rapid resulting in high biogas yield as compared to lower temperature. After 20 days (one retention time) when steady state was achieved, a container having NaOH (3M) solution was attached to the reactor and daily methane production was measured. Methane production was which was 64% of the total gas volume produced by the reactor. It shows that the methane content of controlled temperature reactor was higher than the anaerobic reactor placed at uncontrolled temperature. It may be due to temperature difference. As Chae et al. (2008) showed in his study that volume of methane in total biogas production volume varied according to the temperature of digester. With the increase in temperature, methane content also increases, 3.79% more methane in biogas at controlled temperature anaerobic digestion. And methane content in total volume of biogas decreased with decrease in temperature. But this increase in methane content due to temperature difference is up to a small degree change. VS reduction at controlled temperature was higher because at controlled mesophilic temperature microbial metabolic activity is higher and more nutrients are utilized by microorganisms. While at low uncontrolled temperature microbial activity is lower, so low biodegradation of organic matter. Different studies show that at higher temperature biogas production is high due to high microbial activity as results show that VS reduction at uncontrolled temperature was 7% lower than VS reduction at controlled temperature.

Then the feed of the reactor placed at controlled temperature was changed and the reactor was fed with green grocery and cattle manure in ratio of 1:1 on volatile solids basis. Biogas production was measured on each day, it started to increase gradually and became maximum after 13 days of feed change. This may be due to nutrient balance and balanced C/N ratio in the digester. There may be high ammonia concentration in case of mono-digestion with cattle manure. It is reported that anaerobic digestion of cattle manure alone is cause of ammonia inhibition as high concentration of ammonia have direct inhibitory effect on methanogenic enzymes and cease methanogenic activity (Hansen et al., 1998).

Fig. 4.15 shows the difference in biogas yield in case mono-digestion and co-digestion. After achieving steady state of biogas production, container having NaOH (3M) solution was attached to the reactor to record the volume of methane in total biogas production.

CONCLUSIONS

This study carried out to evaluate the effect of temperature and co-digestion on biogas production shows that;

It can be concluded from the study that if the reactors used in Pakistan fed with cattle manure and placed at environmental temperature, are operated at controlled temperature and co-digestion with green grocery waste, biogas production can be enhanced by 20 times. While by applying co-digestion without control of temperature, biogas production is increased by 10 times as compared to mono-digestion. And biogas production at controlled temperature with mono-digestion also increases biogas by 10 times. It shows that microorganisms responsible for biogas production are sensitive to temperature changes and substrates used.Methane content of biogas also varies with temperature variation i.e. at mesophilic temperature around 37°C methane content was better than the reactor present at uncontrolled temperature. Continuous mode of anaerobic digestion gives high biogas production than batch anaerobic digestion process.

FUTURE PERSPECTIVES

The future perspectives of this research study are;

a) Co-digestion can be used with different substrates like cellulosic biomass, paper and pulp biomass and with other carbon rich material.
b) Effect of different organic loading rates with varying retention times on yield of biogas.
c) Types of changes can be studied which are occurring in methanogens as a result of changes in temperature and pH.
d) Pretreatment of manure to enhance biogas production.
e) Different kinds of microorganisms can be studied which are present in anaerobic digesters treated at different conditions.

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