Effect of Organic Loading Rate on the Performance of Acidogenic Reactor and Methanogenic Reactor during Two Stage Anaerobic Codigestion of Cattle Manure with Green Grocery Waste.

QUAID-I-AZAM UNIVERSITY

ISLAMABAD

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بسم الله الرحمن الرحيم

"IN THE NAME OF ALLAH THE MOST BENEFICENT, THE MOST MERCIFUL"

Read! And the Lord is Most Honorable and Most Benevolent, Who taught (to write) by pen, He taught man that which he knew not.

(Surah Al-Alaq 30: 3-5)
Al-Quran

DEDICATION

I dedicate this humble effort to my beloved parents

for their love, support and prayers.

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Muhammad Adil Nawaz Khan

DECLARATION

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

Muhammad Adil Nawaz Khan

Certificate

This thesis submitted by ***Muhammad Adil Nawaz Khan*** is accepted in its present form by the Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan; as satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

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LIST OF ABBREVIATIONS

%	Percentage
mg	milligram
VFAs	Volatile fatty acids
C/N ratio	Carbon to Nitrogen ratio
TS	Total Solids
VS	Volatile Solids
OLR	Organic Loading rate
AD	Anaerobic digestion
Aco-D	Anaerobic Co-Digestion
HRT	Hydraulic retention time
GHG	Greenhouse gas
pH	Power of Hydrogen ions
°C	degree centigrade
g	gram
kg	kilogram
ml	milliliter
L	liter
N L	Normalized liter

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ABSTRACT

Energy is a very essential part of our daily life but due to increase in human population the demand in energy is increasing. The fossil fuel is the primary energy source but the problem with fossil fuel is their rising demand with energy issues and GHG gas emissions. To overcome these problems biogas production through anaerobic digestion is the best alternative option. In two stage anaerobic digestion process at high organic loading rates the methanogenic reactor become unstable due to high VFAs accumulation while the acidogenic reactor still can form more VFAs. This can be overcome through constant VFAs loading rate in methanogenic reactor. The Aim of the current research was to study the effect of organic loading rate on the performance of acidogenic reactor and methanogenic reactor during two stage anaerobic co-digestion of cattle manure with green grocery waste. The experiment was performed in two stage reactors having working volume of 5 liters each at controlled temperature of 37 °C with retention time 10 days. Each OLR was run for more than two retention times and study the effect of OLR on the biogas production. The biogas yield at 5.5, 6, 6.5 and 7 OLR g VS $L^{-1}day^{-1}$ was 0.242, 0.292, 0.210 and 0.168 NL/g VS. While the maximum VFAs production at 7 OLR that was 13.3 g/L. After this the methanogenic reactor was fed at different VFAs loading rates in suspended growth reactor with biogas yield 0.07, 1.13, 2.04, 1.02 and 0.47 NL/g VFAs and attached growth reactor with biogas yield 0.25, 1.23, 2.31, 1.19 and 0.50 NL/g VFAs at 0.25, 0.5, 0.75, 1 and 1.25 g VFAs L^{-1} day^{-1} respectively. This study concludes that high biogas yield was at 6 OLR, high VFAs production at 7 OLR and high biogas yield at 0.75 VFAs loading rate g/L in attached growth reactor during VFAs loading rate.

CHAPTER

1

INTRODUCTION

There is no exaggeration and beyond the doubt that energy is substantial need of human being. Over the last century the energy utility has greatly evolved. The human activities like trade, agriculture, industry and transportation all depend on energy. To overcome the fundamental social needs such as hunger, illiteracy and disease the energy importance become crucial. It is clear that guaranteeing accessibility of enough, inexpensive, and nature friendly energy is the main issue faced in the twenty first century by the world. The energy plays a major role in socio economic growth but the demand in energy is increasing day by day. The population of the world is expected to reach 8.2 billion by the year 2025 with annual current 1% growth rate and it is expected that the energy demand worldwide will increase 58%. The population growth rate is higher in the developing countries and Pakistan is not an exception. The demand in energy in Asia is estimated to increase at rate of 3.7% per year (Ouda *et al*., 2016).

The human improvement has a strong relationship with energy success. With the human development the demand for energy is increasing. Since the start of the 20^{th} century the demand for food, clean water and energy become very high. Due to the increase in population the world will face challenges in food and literacy. The energy is the main requirement behind these issues. For example the electricity is vital in providing education, food and water supply. Electricity is very supportive in a broad range of income opportunities. The leading countries in the world in terms of population without access to electricity include Pakistan, India, and Bangladesh etc (Khan et al., 2012).

Due to increase in population of Pakistan the energy demand increases and it is assume that the electricity requirement will increase up to 45000 MW by the year 2030 (Rafique and Rehman., 2017). Currently in urban and rural areas of Pakistan the load shedding is 10-12 and 16-18 hour respectively (Zameer and Wang., 2018). There is not only the problem of electricity in Pakistan but there is also the problem of natural gas. During winter the gas shortages become sever. Also due to the conversion of vehicles to compressed natural gas (CNG) the demand for natural gas increased. The CNG program was started in 19982 at Karachi by the institute of hydrocarbon development Pakistan by launching the refilling stations of CNG at Karachi. In 1992 the CNG was commercialized by the Pakistan ministry of natural and petroleum resources. Pakistan stands 2^{nd} in the world in terms of CNG vehicles (Khan and Yasmin, 2014). The main problem with fossil fuels is their availability, cast and environmental issues. And it is considered that the oil and gas reservoirs will lost in about forty years (Pimentel, 2006).

In developing countries like Pakistan the wastes collection is 60% in major cities while in poor areas the situation become worst. To reduce the volume of waste they are dumped or incinerate in open areas (Sadef., 2016). Conventional landfilling with cattle manure causes environmental issues like methane release and leachate formation, as methane is 28 times more greenhouse gas than carbon dioxide and leachate cause ground water pollution (IPCC., 2013).

In this situation, the best alternate for fossil fuels and safe disposal of green grocery waste is the production of biogas by anaerobic digestion. This Technology has research focused worldwide, as it produces renewable, economical and ecofriendly energy with appropriate solid waste management. The energy produced through anaerobic digestion is more efficient than other waste to energy technologies including biological and thermochemical (Adekunle et al., 2015).

Anaerobic digestion is a series of biological process in which the organic material is degraded by the help of microorganisms in the absence of oxygen and to produce biogas as end product. The anaerobic degradation process consists of four different steps carried by bacteria and archaea. Each step is carried out by specific microorganisms, the product of each step act as a substrate for the other (Asam et al,. 2011). In the first step complex organic matter is hydrolyzed by the help of extra cellular enzymes secreted by microorganisms into soluble monomers (Schnurer and Jarvis 2009). The second step is the acidogenesis in which different kind of fatty acid acids (VFAs) are produced which are volatile in nature. During hydrolysis the simple monomers produced are then utilized and convert into different organic acids, such as acetic acid, butyric acid, propionic acid and lactic acid by different kind of obligate anaerobic and facultative anaerobic bacteria (Gerardi, 2003). The third step is the acetogenesis in which the products produced in the second step are consumed by acetogenic microorganisms and produce acetate, carbon dioxide and hydrogen. The fourth and last step of the anaerobic digestion is the methanogenesis in which carbon dioxide and hydrogen is converted into methane by hydrogenotrophic bacteria. The methanogenesis is the slowest step among all of the anaerobic digestion process (Siles et al., 2008).

There are many factors that affect anaerobic digestion process and biogas production like type of feedstock, total contents of solids, , carbon and nitrogen ratio uniform feeding, hydraulic retention time, loading rate, pH, temperature, VFAs, alkalinity and concentration of macro and micro nutrients (Jain et al., 2015). The temperature effect the process stability and methane production through changing biochemical pathways, shaping microbial community and composition, activity, diversity, and thermal stability of the biochemical process. The anaerobic digestion under thermophilic condition is functional under 55 to 60 degree centigrade (°C) with several advantages i-e the degradation rate is high, high reduction in waste and methane production. (Labatut et al., 2014) while drawbacks are the instability compare to mesophilic process and also they require more energy compare to mesophilic system. In thermal conditions the degradation rate is high as a result high amount of VFAs are produced that lower the pH and may inhibit the process that is the reason to prefer the mesophilic anaerobic digestion (Jang et al., 2014).

The optimum carbon to nitrogen ratio is 25 to 30 for anaerobic digestion. The carbon to nitrogen ratio shows the nutrient level of a substrate for anaerobic digestion. high level of carbon to nitrogen ratio result in high amount of ammonia which is toxic to microbial cells and hence process instability occur which results in failure of anaerobic digestion (Yenigün et al., 2013). The aerobic digestion require an average hydrolic retention time of 15 to 30 days. Hydraulic retention time is effected by feed composition and organic loading rate. Low retention time results in high VFAs accumulation.(Ziganshin et al., 2016)

The cattle manure is very important in initiating the anaerobic digestion because it is rich in methanogens and have the buffering capacity. But when manure is used as mono substrate it has lower bio methane potential as it has low carbon to nitrogen ratio. Anaerobic digestion found to be unstable due to high amount of ammonia accumulation which leads to the inhibition of methanogens (Tufaner and Avşar, 2016).

The cattle manure used as mono digestion it results in low stability and performance of anaerobicdigestion (Cook et al., 2017). The codigestion of cattle manure with green grocery waste may enhance the stability of anaerobic digesters due to better carbon to nitrogen ratio. The codigestion may alleviate the inhibitory effect of sulfide and high ammonia concentration. The hydrolysis of fruit and vegetable wastes is quick because of low amount of total solids and high volatile solids so the biogas production may be enhanced. Optimum carbon content has a positive effect on avoiding the access of ammonia because latest studies shown that volatile fatty acids could avoid the effect of ammonia by acting as buffer solution, result in higher biogas production. Adjustment of C/N rations needs for stable and long term anaerobic digestion process (Zhang et al., 2005). So therefore the anaerobic co digestion of cattle manure with green wastes attracts more attention for the production of biogas as well as for waste reduction (Bouallagui et al., 2005).

Normally anaerobic digestion is carried out in a single reactor with all the biochemical reactions i-e hydrolysis, acidogenesis and acetogenesis and methanogenesis all occur in the same reactor. The advantages are lower cost, simple in design and require less labor (Forster et al., 2008). In single stage the problems may occur if the acid phase is faster than the slow growing methanogens (Bouallagui et al., 2005). In one-stage system, combine acidogenesis and methanogenesis occur in the same vessel. The hydrogen formed by acidogenic metabolism in the same vessel creates high partial pressure of hydrogen which is inhibitory to acetogens. On increasing the feeding rate of the substrate, acidogenic activity, including mainly acetate, carbon dioxide, and hydrogen production, is increased, whereas the methanogenic population cannot increase its activity to the same extent. At higher organic loading rate, the hydrogen consuming reactions become saturated, accumulation of hydrogen partially inhibits its further formation and consequently more organic electron sink will be formed, causing imbalances and termination of methane production (Bouallagui et al., 2005). But the advantages of higher organic loading rate is that more waste can be treated in the same space and size and thus the total cost of the reactor may be reduced (Luste et al., 2010).

Generally the VFAs formed during AD are ultimately converted to CH4 and CO_2 by methanogenic bacteria. However the accumulation of VFAs may occur at higher organic loading rate. As a result the pH falls and even may result in failure of the AD (Palacio-Barco et al., 2010; Zhang et al., 2013). As mentioned above these VFAs are ultimately converting to methane so there is a need to utilize these VFAs.

To overcome the problems addressed earlier there is need of separate reactors for acidogenesis and methanogenesis to avoid the issue of pH and growth requirement. Therefore two stage anaerobic digestion system is required to attain quick digestion at higher organic loading rate and stable process (Aslanzadeh et al., 2014). In two stage process, the chances of toxic material accumulation are less by controlling the acidogenesis and protect the methanogens from overload and low pH shock. So that's why two stage processes can produce more biogas. But still at higher organic loading rate the efficiency of methanogens is become lower and are inhibited due to high volatile fatty acids accumulation. The pH is dropped from the range and thus the activity of methanogens is inhibited (Jabeen et al., 2015).

From the previous literature it is noted that in two stage anaerobic digestion process, increasing the loading rate the VFAs production increases with increase in biogas yield. But at higher organic loading rates the VFAs accumulation take place in second stage causes methanogen inhibition, while the VFAs production still can be increased in first stage by increasing the organic loading rate. So to avoid this inhibition and to stabilize the process the feeding was performed on the basis of volatile fatty acids loading rate in both suspended and attached growth methanogenic reactors and the process stability and biogas yield was compared. In order to attain maximum hydrolysis in first stage and high methane production in second stage. The study will help to improve the biogas technology by increase biogas production in two stage anaerobic digestion process with high waste treatment in same volume of the reactor. This will make the waste to biogas technology more attractive and will leads to commercialize the biogas production in Pakistan.

Aim:

Aim of the study was, Effect of organic loading rate on the performance of acidogenic reactor and methanogenic reactor during two stage anaerobic codigestion of cattle manure with green grocery waste.

Objectives include:

1. To study the effect of organic loading rate on VFAs production in acidogenic reactor.
2. To study the effect of organic loading rate on biogas yield in methanogenic Reactor.
3. To study the effect of VFAs loading rate on biogas yield in methanogenic Reactor.
4. To study the effect of increased VFAs loading rate on biofilm and methane yield in attached growth methanogenic reactor.

CHAPTER 2

LITERATURE REVIEW

The anaerobic decomposition is the process by which complex organic matter is decomposed by the symbiotic action of microorganisms in the absence of oxygen into biogas, additional cell matter and nutrients, refractory organic matter and leaving salts. The biogas has methane content round about 60%, 39% CO_2 while small amount of H_2S and vapors of water. The bio Methane is odorless and colorless gas that burns with clear blue flame similar to that of LPG gas. Microorganisms from two biological kingdoms, the Bacteria and the Archaea carry out this process in strict anaerobic conditions (Christy et al., 2014). Naturally this process occur in environment like in marshes, swamps, ponds, paddy fields, landfills and animal intestinal tracks (Issazadeh *et al.*, 2013). The anaerobic digestion stabilizes the organic matter by reducing the volatile solids in the organic matter and converting some fraction of the volatile fatty acids into biogas. This process reduced the pathogens and the odor of the wastes. Nutrients can be obtained by this process in the form of organic fertilizer (Christy *et al.*, 2014).

2.1 Anaerobic digestion process

Anaerobic digestion occur in four major stages i-e hydrolysis, acidogenesis and acetogenesis and the last one is the methanogenesis.

2.1.1 Hydrolysis

In first step the organic matter is depolymerized. During this stage the complex organic matter is hydrolyzed into simple and smaller units by the action of hydrolytic microorganisms *like micrococci, butyrivibrio, clostridia, selenmonas, fusobacterium* and *streptococcus*. They secrete different kind of enzymes like cellulose, amylase, protease, xylanase and lipase (Christy *et al.*, 2014).

The hydrolytic reactions occur in two steps, the first is the action of extra cellular enzymes released by strict anaerobes and facultative anaerobic microorganisms. In this step the colonization of bacteria takes place and cover the surface of solids. Where they release extracellular enzymes that converts the complex polymers into monomers which are then utilized by these hydrolytic bacteria. In the next step of hydrolysis the bacteria will degrade these particles at constant depth per unit time (Vavilin *et al.*, 1996).

2.1.2 Acidogenesis

In second step the monomers produced in the first step are consumed and these acidogens cornvert them into volatile fatty acids i-e acetic acid, butyric acid, lactic acid, propionic acid and other short chain fatty acids. The microorganisms involved in this step are *streptococcus, bacillus, lactobacillus, salmonella and E.coli* etc (Kalyuzhnyi *et al.*, 2000). The hydrolytic and acidogenic microorganisms are about ten times faster growing compare with methanogens (Mosey and Fernandes, 1989).

2.1.3 Acetogenesis

In third step the volatile fatty acids produced are utilized by the acetogens and produce carbon dioxide, hydrogen and acetate. The optimum pH value for acetogens is 6. They are strictly anaerobic and they are sensitive to oxygen because they contain acetyl coA enzyme which is extremely sensitive to oxygen (Wood and Ljungdahl, 1991). They are sensitive to variation in organic loading rate and environmental changes. They require more time to adjust to the new environment. These are slow growing. They are sensitive to increase hydrogen concentration (Xing *et al.*, 1997). There is syntrophic association between acetogens and hydrogen utilizing methanogens because they can't function at high hydrogen concentration. The microorganisms involved in acetogenesis are *Clostridium aceticum* and *Acetobacterium woodii* (Weiland., 2010).

2.1.4 Methanogenesis

This is the last and fourth step of in which by the action of methanogens in anoxic condition and produce methane. The optimum pH value is 6.6– 7.6 (Solera et al., 2002). Methane production occurs in two ways either directly from acetate or from hydrogen and carbon. Six orders of methanogens namely *Methanobacteriales, Methanocellales, Methanococcales, Methanomicrobiales, Methanopyrales* and *Methanosarcinales* are involved in methane production (Yun et al.,2017). Co enzymes unique to methane producing bacteria are coenzyme M and coenzyme F420 and F430 containing nickel. The co enzyme M reduce carbon dioxide to methane while the coenzyme containing nickel is hydrogen carrier in methane producing (Hallam., et al 2003

2.1.4.1 Hydrogenotrophic Methanogens

They utilize carbon dioxide and hydrogen to produce methane. They help to keep low partial pressure of hydrogen which is required for the acetogenic activity (Demirel and Scherer, 2008).

2.1.4.2 Acetotrophic methanogens

This group of methanogens split the acetate into carbon dioxide and methane. This carbon dioxide may be then converted to methane by hydrogenotrophs. Acetotrophic methanogens are reproduce more slowly than hydrogenotrophs. The accumulation of hydrogen negatively affect the growth of these acetoclastic methanogens. At relatively high partial pressure of hydrogen the methane production is reduced (Demirel and Scherer, 2008).

2.1.4.3 Methylotrophic Methanogens

The methylotrophic methanogens are able to consume methylated compounds. They are not using carbon dioxide instead they directly convert the methylated groups into methane. These methanogens convert the methylated compounds into cognate corrinoid protein which is then convertd to CoM. the methyl-CoM then enters the methanogenic pathway and reduce to methane. Methyl-CoM subsequently enters the methanogenesis pathway and is reduced to methane (Liu and Whitman, 2008).

2.2 Factors that affect anaerobic digestion

A numbers of factors that affect the anaerobic digestion process like feedstock, total contents of solids, carbon and nitrogen ratio, uniform feeding, hydraulic retention time, loading rate, pH, temperature, volatile fatty acids, alkalinity and concentration of macro and micro nutrients.

2.2.1 Feed stock

Anaerobic digestion was used to treat the animal manure and sewage sludge in past. But due to increase environmental issues there was need of new strategies of waste management. So in the recent past the field of anaerobic digestion is becoming vast to treat a variety of municipal, agriculture and industrial waste. When the feed stock having more lignin then the degradation become difficult (Jain *et al.*, 2015).

For anaerobic digestion the feed stock composition varies significantly. In concentrated animal farming the dung collected in the form of slurry which has total solids of 3-12% (Sutaryo *et al.*, 2012; Zhang et al., 2014). While the chicken manure having total solids up to 30% (Wielen et al., 2002). The dry matter of agriculture waste varies extensively. The waste of agro industries contains total solids fever than 1%. The process of anaerobic digestion is affected by the type of components such as wood, straw, inorganic matter like glass and plastic etc. these undesirable components often cause failure of anaerobic digestion process. Initially the VFA varies with kind of feedstock slurry, storage condition, and handling of these wastes. The cow manure has fever VFA concentration than pig manure. Normally the VFA contents of animal slurry have no inhibitory effects. The agroindustrial waste the fast degradation of carbohydrates, protiens and lipids can produce high concentration of VFA that may cause inhibition of the reactor (Jain *et al.*, 2015).

Food waste usually having high amount of soluble organic matter that are easily degradable and can produce high amount of VFA in the early stages of digestion process that may cause sudden drop in the pH and may cause the inhibition of methanogenic process or low production of methane. In order to decrease this inhibition by increase production of VFA at the early stages of digestion process, the co-digestion with feedstock having high buffering capacity are added. This would help in better stability in the process of anaerobic digestion (Lu *et al.*, 2007).

2.2.2 Total solid contents

The optimized SSAD reactor of the same size can treat more waste than liquid anaerobic digestion reactor with respect to dry matter. Initially the substrate concentration effects the anaerobic digestion of municipal solid waste during mesophilic batch conditions. When the concetration of total solids increased from 20-30% Fernandez et al reported that the COD removal was reduced from 80.69-69.05%. At 30% total solids contents the methane yield was 17% which was lower when the total solid contents were 20% (Fernández *et al.*, 2008).

2.2.3 Carbon to nitrogen ratio

In addition to carbon the amount of nitrogen in the waste is also a critical factor in the biogas production. The nitrogen is required by the microbial cell for the synthesis of their proteins in biological perspectives. The carbon is consumed for energy. In literature it is mentioned that the best range for C/N ratio is 25/1-30/1. Inapproptiate C/N ratio may cause inhibition of the process because at high C/N ratio the VFAs accumulation occur while at low C/N ratio the amount of ammonia production increases. The amount of ammonia and VFAs in the reactor are key intermediates. The high concentration of VFAs and ammonia in the reactor can negatively affect the activity of methanogens and potentially can cause the inhibition of the AD process (Parkin and Owen, 1986). Different feed stock having different optimal C/N ratios. The high concentration of nitrogen in the algal biomass were optimized with the addition of waste paper for the production of biogas (Yen and Brune, 2007). The co-digestion of digested sludge and onion the C/N ratio kept 15 (Romano and Zhang, 2008).

2.2.4 Uniform feeding

In order to obtain better digestion there is a need of uniform feeding so that the microorganism are relatively retained in constant contact with waste. So therefore the feeding must be uniform in terms of quality and quantity and also the feeding must be done after same time of interval (Wang et al., 2012; Giuliano et al., 2013).

2.2.5 Retention time

The period required for biogas generation from the waste inside the reactor is known as the retention time. The time is depended on the kind of substrate and temperature. The doubling time for methanogenic bacteria is from 2 to 4 days. So therefore the retention time must not be less than 4 days, otherwise the bacteria will wash out in the effluent and will affect the whole biogas process (Kwietniewska and Tys, 2014; Guendouz et al., 2008).

2.2.6 Loading rate

The quantity of waste added to the reactor/digester per unit volume per day is known is loading rate. Normally municipal waste treatment plants are operated at organic loading rate from 0.5 to

1.6 kg VS/m^3/day. The higher loading rate is possible but the optimum conditions are compromised. If the reactor is loaded with too much waste at a time the accumulation of acid will occur and the inhibition of the reactor may occur. The advantages of higher organic loading rate is that more waste can be treated in the same space and size and thus the total cost of the reactor may be reduced (Luste and Luostarinen, 2010).

2.2.7 Temperature

The process of anaerobic digestion has two major temperature zones. In literature it has been mentioned that there are thermophilic and mesophilic microorganisms which are responsible for anaerobic digestion at these two temperature ranges. The optimum thermophilic range is about 55°C while mesophilic range is around 35°C. In temperate climates waste water digestion plants are heated up to 35 °C as to decrease the time required for the digestion. (Hartmann and Ahring, 2006; Kuo and Lu 2004).

The thermophilic temperature range is not applied because due to high temperature it is not cost effective. The temperature is very lethal as it directly affects the activity of microorganisms. Any deviance from standard temperature will result in poor performance of the digester (Jain *et al.*, 2015).

The startup performance was poor during mesophilic conditions in SSAD. Later on thermophilic operation was developed for SSAD and it has been recognized as accepted and reliable mode as at thermophilic temperature 55°C can speed up the process of AD and also the high temperature help in pathogen reduction of SSAD (Hartmann and Ahring, 2005; Mashad et al., 2004).

2.2.8 pH value

During various stages of anaerobic digestion the pH changes. Initially at the stage of acid formation the pH value is around or less than 6. After 2-3 weeks the pH increases because the volatile acids are digested and methane is produced. To sustain constant gas production, the suitable pH range in the reactor is necessary (Palasti et al., 2009; Sutaryo et al., 2012).

Usually the digester is buffered in order to maintain the pH in between 6.6 to 7.6 (Solera et al., 2002). The microorganisms are active and efficient digestion occur in this range. Beyond this range the methanogens are detrimental. It should be noted that there must not be any abrupt change in pH by the addition any material which is expected to cause imbalance in the population of microorganisms (Sutaryo et al 2012).

2.2.9 Stirring

Subsequently the bacteria in reactor having limited reach to food. It is required that digestate in the reactor is mixed properly in order to get their food supply. It has been found that by little mixing the digestion process is improved. However it has been found that vigorous shaking may retard the AD (Jain *et al*., 2015).

2.2.10 Volatile fatty acids

The organic acids or VFAs mainly contain acetic acids, butyric acids, propionic acids and valeric acid. These are the key intermediates products of organic waste during AD (Palacio-Barco et al., 2010). Generally the VFAs formed during AD are ultimately converted into CH4 and carbon dioxide by acetogenic and methanogenic bacteria. However the accumulation of VFAs may occur at higher organic loading rate. As a result the pH falls and even may result in failure of the AD (Palacio-Barco et al., 2010; Zhang et al., 2013). Among the above mentioned acids the propionic and acetic acid having a major role in the production of biogas. Previous research confirmed that the acetic acid concentration higher than o.8 g/L cause failure of AD (Buyukkamaci and Filibeli, 2004; Pullammanappallil et al., 2001). Previous research showed that propionic to acetic acid ratio must not exceed 1.4 otherwise it will lead to the failure of AD. This ratio of propionic to acetic acid ratio might therefore be used as indicator in imbalance of AD (Buyukkamaci and Filibeli, 2004).

Numerous conventional methods are used for the determination of VFAs, such as HPLC, Gas chromatography and ion exchange with simple pretreatment are used. Usually these methods are material demanding, time consuming analysis are not authentic to be applied in fieldwork. To overcome the limitations in traditional methods, recently online methods based on GC, back titration and on titration methods and were confirmed to be obtainable, (Palacio-Barco et al., 2010; Krapf et al., 2013)

The pH is determined by VFAs which another important parameter affecting the AD. The pH range for acidogenesis is 5.5-6.5(Kim et al., 2003) while for methanogens the pH range is 6.6-7.6 (Solera, 2002). The main VFAs present at low pH are butyric and acetic acids, while at higher or around neutral pH propionic and acetic acids are present (Appels et al., 2011). Both type of bacteria producing acid and the number of bacteria could be maintained by the control of pH (Horiuchi et al., 2002).

2.2.11 Ammonia

The ammonia is produced during the degradation of proteins and other organic substrates rich in nitrogen. It is mainly present in the form of ammonium ions and free ammonia (Yenigün and Demirel, 2013; Whelan et al., 2010). it can be utilized as necessary nutrient for the microbial growth but also can be toxic at high concentrations (Walker et al., 2011). The ammonia plays a vital role in stabilizing the C/N ratio. Which significantly might affect the AD performance (Wang et al., 2012). The amount of ammonia is low when the C//N ratio beyond 30 which result in lower methane yield and AD performance. Previous research confirmed that ammonia play a vital role in increase the buffering capacity of AD as ammonia neutralize the effect of VFAs produced during AD (Zhang et al., 2013; Wang et al., 2012)

Lower concentration of ammonia is required for bacterial growth while higher concentration result in the inhibition of bacteria (Lauterböck et al., 2012; Appels et al., 2011). Chen et al pointed out that when the concentration of ammonia is more than 1.7- 1.4g/L the methane yield is reduced 50 %. The digestion is inhibited when the free ammonia (NH_3) concentration is 1.7-1.8 g/L and it is specifically confirmed that the inhibition is due to the presence of (NH_3). The vast range of ammonia concentrations causing failure is depend on the difference in feedstock, environmental conditions, inoculums and adaptation periods. The high concentration of ammonia not only causes the inhibition of AD but also as a result ammonia emissions from effluent occur (Zhang and Jahng, 2010).

2.2.12 Macro and micro nutrients

For the survival and efficient growth of microorganism several micro and macro nutrients are needed. Macro nutrients are carbon, nitrogen, sulfur and phosphorus. The sufficient ratio of nutrients is C:N:P:S is 600:15:5:1. The micro nutrients are like, iron, cobalt, nickel, molybdenum, selenium and tungsten are vital and should be added in order to improve the growth rate of microorganisms. For the biogas production when energy crops are used alone the micronutrients are needed (Abdoun et al., 2009). There is a need of nickel for all the methanogens because nickel is required for the synthesis of the co- factor F430, which is involved in the formation of methane. The cell needed cobalt to synthesize the co- containing corrinide factor III. The function of molybdenum, tungsten and selenium is not clear. Only a few methanogenic bacteria growth depend on these trace elements. The concentration range for these micronutrients is low and in between the range of 0.05 und to 0.06 mg/L. The iron is the only micronutrient required in higher concentration from 1-10 mg/L. For the fermentation of mono substrate the addition of these micronutrients become necessary to attain stable process and higher loadings (Weiland, 2010).

2.3 Types of anaerobic reactors

There are two types of AD based on the number of stages or reactors involved.

2.3.1 Single stage AD

In a single stage all the biochemical reactions i-e hydrolysis, acido and acetogenesis and methanogenesis all occur in the same reactor. The advantages are lower cost, simple in design and require less labor (Forster-Carneiro et al,. 2008). In single stage the problems occur if the acid phase is faster than the slow growing methanogens. In one-stage system, combining acidogens and methanogens in one vessel hydrogen formed by acidogenic metabolism while high partial pressure of hydrogen is inhibitory to acetogens. On increasing the feeding rate of the substrate, acidogenic activity, including mainly acetate, carbon dioxide, and hydrogen production, is increased, whereas the methanogenic population cannot increase its activity to the same extent. At higher organic loading rate, the hydrogen consuming reactions become saturated, accumulation of hydrogen partially inhibits its further formation and consequently more organic electron sink will be formed, causing imbalances and termination of methane production (Bouallagui et al., 2005). But the advantages of higher organic loading rate is that more waste can be treated in the same space and size and thus the total cost of the reactor may be reduced (Luste and Luostarinen, 2010)

Generally the VFAs formed during AD are ultimately converted into CH_4 and carbon dioxide by acetogenic and methanogenic bacteria. However the accumulation of VFAs may occur at higher organic loading rate. As a result the pH falls and even may result in failure of the AD. As these VFAs are ultimately converting to methane so there is a need to utilize these VFAs (Palacio-Barco et al., 2010; Zhang et al., 2013)..

2.3.2 Two stage AD

In two stage process acidogenesis and methanogensis are performed in separate reactors and hence the chances of toxic material accumulation are less by controlling the acidogenesis and protects the methanogens from overload and low pH shock. So that's why two stage process can produce more biogas. But still at higher organic loading rate the efficiency of methanogens is become lower and after they are inhibited due to high volatile fatty acids accumulation. The pH is dropped from the range and thus the activity of methanogens is inhibited (Jabeen et al., 2015).

Form the previous literature it is note that in two stage anaerobic digestion process increasing the loading rate the VFA production increasing with increase in biogas yield. But at high organic loading rates the VFA accumulation take place in second stage causes methanogeic inhibition, while the VFAs production still can be increased in first stage by increasing the loading rate.

2.4 Biogas applications

2.4.1 Direct combustion

The biogas is used for several purposes. Normally the biogas produce on small scale is used for the purpose of cooking (Gautam et al., 2009). Generally the biogas use for cooking in the household is from 30-45 $m^{3/month.}$ The biogas lift in the reactor may be used for space and water heating (Axaopoulos and Panagakis, 2003). The biogas can be directly burned for commercial purposes although its calorific value is less. The minimum pressure biogas supplied is 10 mbars, while the pressure of natural gas used for domestic cooking is 20 mbars (Grima-Olmedo et al., 2014). The recommended ratio for air fuel is 4:1 required for biogas burner (Noor et al., 2014). The biogas efficiency for heating it is near 55% (Kadam and Panwar, 2017).

2.4.2 Electricity generation

Now in several developed countries the biogas is burned to produce mechanical and electrical energy by the help of biogas combustion in controlled system (Kadam and Panwar, 2017). The biogas can be utilized in direct injection, single cylinder and compression combustion engines. These engines are modified to operate on duel fuels cell condition in order to generate electricity (Tippayawong et al., 2007). This study was carried out to evaluate the economic viability of electricity generated from biogas. the price of the electricity was fixed at 0.07 € per kWh with repayment duration of five years and five months(Pipatmanomai et al., 2009). In Germany the biogas power in 2001 were 1050 which are increased to 6000 in 2010. This increase is about 20% (Budzianowski and Chasiak., 2011.

The biogas has the capability to produce electrical energy on demand. Worldwide nine GW of electricity is produced using biomass. Pakistan stand fifth in the world in production of sugarcane with fifty million tons of cane with ten million tons bagasse. There are about eighty sugar mills in Pakistan having the capability to produce about three thousand MW of electricity by the help of biogas production. But presently only generate 700 MW of electricity (Amjid et al., 2011).

2.4.3 Compressed bio-methane

As the CNG (compressed natural gas) is used instead of petrol and diesel and Pakistan is in the top three countries with highest number of CNG vehicles. CNG may be collected from sewage treatment plant and landfills. Biogas can be modified to CNG by compressing and purifying to remove the impurities present in biogas. The purified and compressed gas is filled in the cylinders which can be used as bio-methane or bio-CNG (Amjid et al., 2011).

2.4.4 Bio Fertilizer

The useful nutrients present in the digestate coming out from a biogas plant could serve as an alternative for artificial fertilizer which possess significant energy for agricultural purposes as well as it is much economical as compared to the use of artificial fertilizers. Artificial fertilizers contains (N, P_2O_5, K_2O) and its energy consumption is 3.5 GJ/ ha, which also results in the emission of CO_2 as a greenhouse emission gases, which in turn shows that the natural gas was utilized in the manufacture of artificial fertilizers and energy for the transport and meaningful application of fertilizers will be provided by conventional diesel. Which clearly shows that the use of bio-fertilizer is much more economical than artificial fertilizers and costs for about 54 €/ ha. (Carvajal-Muñoz & Carmona-Garcia., 2012).

Analytical results confirmed that the digestate application is advantageous. In comparison with animal manure is the higher C/N ration and the uptake efficiency of nitrogen is higher. The nitrogen is directly available for plants because during anaerobic digestion the organic nitrogen is converted to ammonium. similarly, during biogas production organic waste are easily degraded Into biogas, whereas, complex compound (lignin) degradation is difficult and which Is lift behind. Therefore digestate enhancing the soil properties. It was also analyzed that macromolecule such as (P, Na, Ca, K, Mg) are higher for digestate. The nitrogen and phosphorus ratio for the digestate is 4 times greater than animal manure. (Mg ,Ca, OC) increase the soil structure and properties, (Vaneeckhaute et al., 2013). It is a bright prospect for farmers to use manure for anaerobic digestion and use the digestate as alternative for synthetic fertilizers. (Uddin et al., 2016)

2.4.5 Environment

The wood is used for household cooking in the developing countries which results in deforestation and also in the emission of greenhouse gases. (Strassburg et al.,2009). By using biogas for burning purpose will not only help in the reduction of deforestation in the developing world but also will decrease the greenhouse gas emission (Katuwal and Bohara, 2009).

2.5 Future of biogas technology

In order to use biogas for transportation the biogas must be upgraded. There are impurities like carbon dioxide vapors of water and H_2S that should be removed. There is several techniques membrane separation, pressure rotation adsorption and water scrubbing that can be used to upgrade biogas. Currently the biogas is injected directly into the methane gas grid. The gas is then directly delivers to the consumers. The biogas may be utilized for the generation of electricity onsite. One waste water treatment reactor in United Kingdom generates about 14000 m^3day^{-1} of biogas which is used for dual fuel engine of 520 KW. So it is suitable to use the dual fuel engines instead of biogas engines. Biogas could be compressed into cylinders for the operation of vehicles. In Sweden the biogas is used to operate buses and trains with refilling stations near the streets. The biogas may be compressed and upgraded up to 150 kg/cm^3 pressure to drive the six cylinders truck. By the help of two phase pressure reduction method it can travel a distance of up to 96 km. Stafford. The market of biogas will determined the future of biogas. In the near future there will be plenty of vehicles that will be running on biogas (Kadam and Panwar, 2017).

2.6 Biogas technology and Pakistan

In order to overcome the present energy crisis in Pakistan the best alternative source is the biogas. The first biogas plant in Pakistan was installed in Sindh in 1959 (Ghimire, 2007). In 1974 special attention was given by the government of Pakistan to biogas plants and across the country 21 biogas plants were installed by the Pakistan council of Appropriate Technology. In 1986 a project was launched with the aim to start 4000 biogas reactors by the director general of renewable energy resources. The biogas support programe in the 2000 was launched with the aim to install 1200 biogas reactors. Later on 10000 biogas reactors were expected to be start in the year 2006. The Pakistan dairy development company also took its part in the installation of biogas plants and under this programme 556 biogas plants were installed in the year 2009 (Sreekrishnan et al., 2004). In the year 2009 with total budget of Rs 356 million 14000 biogas plants were installed by rural support program network. The Pakistan council of renewable energy technology installed 4016 biogas reactor across the country since 2002. The biogas production of these reactors is 20545 m^3/ day (Uddin et al., 2015). In Pakistan the biogas potential is 14.25 million m^{3}day^{-1} (Sheikh, 2010). The installation of these reactors were in two stages, in the first stage 1596 reactors were installed across the country, while in the second stage 2513 reactors were installed. Most the reactors are used for the purpose of cooking. The (PCRET) submitted a project with the amount 481.66 million for the year 2014-15. The objectives are to install 10100 biogas reactors all over Pakistan with the aim to provide inexpensive energy. As the biogas provide green energy and has no or less contribution to greenhouse gas emissions. Due to the current energy scenario it is become necessary to utilize the biogas potential to overcome the energy requirement of Pakistan. Literature shows that the biogas has positive impact on the environment and economical in terms of cost. The pretreatment methods should be adopted in order to improve the efficiency of biogas. The biogas reactors should be installed on industrial scale to produce electricity and bio-fertilizer in order to overcome the current energy crisis in Pakistan (Uddin et al., 2016).

CHAPTER 3

MATERIALS AND METHODS

Materials and Methods

The current research study was conducted in the Bioenergy and Biorefinery Lab (BBL), Department of Microbiology, Quaid-i-Azam University, Islamabad. The whole research was in accordance to Standard Microbiological procedures.

3.1 Substrate for anaerobic co-digestion

The current research work was conducted on co digestion of fruit and vegetable with cattle manure waste on the basis of VS. The most significant ratio of fruit and vegetable (FVW) with cattle manure was (1:1) according to previous research work done in our lab by Waris Khan . Mixture of Cow and buffalo manure round about 25 kg was collected nearby dairy form. After that debris was separated from cattle manure and store at 4°C till use. Approximately, 41.5 kg of (FVW) was collected from market of Barakahu Murree road Islamabad and transported to microbiology research lab within 1 hour. To make fine particle the fruit and vegetable was manually grinded. After grinding the homogeneous mixture was then stored at -4°C for further analysis. Fruits and vegetable amount is shown in (table 3.1).

Table 3.1 Composition of green grocery waste.

Fruit and vegetable waste	English name	Quantity
Fruit waste	Banana	3 kg
	Apple	2.5 kg
	Melon	3 kg
	Guava	2.5 kg
Vegetable waste	Apple gourd	4 kg
	Bottle gourd	4 kg
	Carrot	3.5 kg
	Turnip	4 kg
	Pumpkin	4 kg
	Radish	3 kg
	Potato	8 kg
Total quantity of fruit and vegetable waste		41.5 kg

3.2 Determination of total solid (TS) and volatile solid (VS)

The grinded fruit and vegetable were mixed homogeneously and the total solids and volatile solids were found out. Similarly the cattle manure total solids and volatile solids were determined, reported by Sluiter in 2008.

3.2.1 Determination of Total solids

- Crucible with organic matter were incinerate in the muffle furnace at 550°C for 2.5 hours.
- After that the crucibles were stored at room temperature till cool down, and weighted at the electric balance.
- Afterward the sample was added in same crucible, were again weighted. The weight was estimated by the given equation
- Sample weight = (weight of crucibles together with sample) - (weight of crucibles).
- In order to remove the moisture content the crucibles having sample were transferred to oven at 105°C for overnight. The dry sample in the crucibles was weighted again at electric balance and the weight was determined by given formula. Dry weight of sample = (weight of dry sample in the crucibles- weight of crucibles without sample)

  The TS (%) was estimated by the given equation

$$\text{TS (\%) of the sample} = \frac{\text{weight of dried sample}}{\text{Weight of initial sample}} * 100$$

3.2.2 Determination of volatile solids

To investigate the volatile solids the sample was incinerated for 2.5hour at 550°C muffle furnace. At same temperature and incubation time the organic materials were volatilized while inorganic materials were remaining as ash. Afterward the crucibles ware kept at room temperature to cool down completely and then weighted at the electrical balance. Weight was determined by the given formula:

Ash weight = (crucible with ash) – (crucible weight)

Sample VS was also calculated by the given formula

$$VS\ (\%)\ of\ TS = \frac{(weight\ of\ dried\ sample - weight\ of\ ash}{Weight\ of\ dried\ sample} * 100$$

3.3 Determination of volatile solid reduction

Two stage anaerobic co-digestion of volatile solids was determined by the given formula:

$$VS\ reduction = \frac{VS\ of\ influent - VS\ of\ effluent}{VS\ of\ influent}$$

3.4 Reactor design for experiment

Steel reactors were used for two stage continuous anaerobic co-digestion. Each reactor consists of 10-liter total volume whereas the working volume was 5-liters. The remaining space was left for biogas production as well as for mixing the liquid during shaking in a reactor.

3.5 Reactor design for two stage anaerobic co-digestion

Two interrelated reactors were used for anaerobic co-digestion, one reactor for methanogenesis while another one for acidification. For inoculum filling upper end was used for opening which is enclosed with lid. Methanogenesis reactor consist of three valves one at bottom end while two at opening end similarly acidification reactor has two valves one at bottom and another on the top. The acidification and methanogenesis were connected through pipe of top valves. For feeding transfer both the reactors were connected at bottom end. Bottom valves of methanogenesis and acidification reactors used for N_2 removal as well as for feeding inlet and digestate respectively as shown in the figure 3.1.

3.6 Procedure for inoculum development

Already established inoculum was used for biogas production. The prepared inoculum was added to 10-liter reactor and incubated for one week at 37C. for removal of oxygen gas nitrogen flushing was used as a result microorganism actively grow and started biogas production.

3.7 Anaerobic digestion experiment

Determination of biogas production and stability during two stage anaerobic co-digestion of manure with fruit and vegetable was analyzed under controlled temperature (37C). All the digestion was performed in the succeeding mode with regular feeding and the FVW: cattle manure was measured at 1:1 on the basis of VS.

3.8 Two stage anaerobic co-digestion

To initiate the experiment of anaerobic co-digestion both the reactor were filled with 3- liter inoculum, after that permitted with nitrogen gas to remove the dissolved oxygen to exhibited anaerobic environment. After that feeding was started up to 5-liters volume in the acidification reactor up to 96 hours without removal of digestate. After 96 hours 0.5-liter of sample was taken from same reactor and transferred to methanogens reactor and then transferred to acidification reactor. Same procedure was repeated up to 8 days without any removal of digestate from methanogenenic reactor until the working volume up to 5-liters. O.5-liter digestated was removed on daily basis from methane producing reactor secondly the effluent which recovered from acidogenic reactor was transfer to methanogenic reactor and then acidogenic reactor was feed. The hydraulic retention time for two stage anaerobic co-digestion was 10 days, the experiment was carried out for more than twenty days each having ten days retention time. After study state CO_2 from biogas was removed by potassium hydroxide (KOH) 3 molar, and calculated CH_4 content for biogas. on daily basis pH for both reactors were determined while alkalinity, VFA's and volatile solids reduction were analyzed after 3 days. Organic loading was performed at concentration of (5.5, 6, 6.5 and 7 g VS L^{-1} day^{-1}).

3.9 Biogas measurement

In methanogenic reactor the gas outlet was tightly coupled with bag (2.5-liter) volume in which accumulation of biogas occurred gas was measured by syringe on daily basis and unfilled the bag for subsequent day. The biogas was measured on daily basis with the helped of syringe.

3.10 Operational parameter

In operational parameter four different parameters were analyzed such as hydraulic retention time, flow rate organic loading rate and VFAs loading rate.

3.10.1 Hydraulic retention time

Anaerobic co-digestion the hydraulic retention time was 10 days and the experiment was performed for two retention time mean 20 days. This time was more and above as compared to replicating time for methanogenesis and degradation of organic waste.

3.10.2 Organic loading rate

Four different organic loading rates were analyzed for two stage anaerobic co-digestion that consists of 5.5, 6, 6.5 and 7g VS L^{-1} day^{-1}.

3.10.3 Flow rate

Flow rate was calculating by the given equation:

$$\text{Flow rate} = \frac{\text{Reactor working volume}}{\text{Hydraulic retention time}}$$

Working volume= 5 L

Hydraulic retention time= 10 days

Flow rate= 5 L/ 10 days= 0.5 L/day

3.10.3.1 Concentration

Following equation was used for concentration calculation

OLR (g VS L^{-1} day^{-1}) = concentration (gvs/L) * Flow rate (L/day)/ volume of reactor (L)

There for

Concentration = OLR*(V/FR)

3.10.3.2 Concentration for 5.5 organic loading rate:

OLR = 5.5

FR = O.5 L

V = 05 L

So

Concentration = OLR* (V/FR)

Concentration = 5.5* (5/0.5)

Concentration = 5.5*10

Concentration = 30 g VS L^{-1}

For 0.5 L total Concentration = 27 gVS

Anaerobic co-digestion ratio for both fruit and vegetable with manure was 1:1 there for both concentration was equal such as 13.75 gVS at 3 OLR.

Manure weight at 5.5 OLR= concentration * 100/VS

= 13.75*100/14.98

= 91.789 g

Substrate weight at 5.5 OLR = concentration* 100/VS

= 13.75*100/9.69

= 141.898 g

Table 3.2 Concentration for slurry for anaerobic digestion at different OLR

OLR (g VS/L/day)	5.5	6	6.5	7
Concentration (gVS/L)	55	60	65	70

3.10.3.3 VFAs loading rate

After studying the effect of higher organic loading rate the feeding was started on the basis of VFAs loading rate. For acidogenic reactor the organic loading rate was kept 7 g VS/L/day for the whole experiment but for methanogenic reactor the feeding was started on the basis of VFAs loading rate. The VFAs loading rate was started from 0.25 g VFAs/ L/day and was kept increase to o.5, 0.75, 1 and 1.25 VFAs g/L/day. In order to start the VFAs loading rate the VFAs produced in acidogenic reactor were determined and then 0.25 g VFAs /L were fed in methanogenic reactor. In order to maintain 0.5 flow rate, water was added with the effluent. The same procedure was adopted for 0.5b, 0.75, 1 and 1.25 g VFAs/L/day for the higher VFAs loading rate in methanogenic reactor.

3.11 Determination of Alkalinity and volatile fatty acids

Alkalinity and volatile fatty acids for anaerobic digestion was analyzed accordance to standard procedure of APHA, 20^{th} ed., p. 2-27, method 2320B (1998) given in the following steps.

- 10 ml sample was taken from methanogenesis and acidogenic reactors in 50 ml beaker.
- After that pH was determined
- With the help of burette 0.1 N H_2SO_4 was added into sample to bring the pH to 4.3 and alkalinity was determined
- By adding of more 0.1 N H_2SO_4 pH was bring down into 3.5 and VFA's was determined in a sample.
- The sample was then boiled for 2-3 minutes and then cooled down to 60 C.
- With the help of burette 0.1 N NaOH was added into sample to bring the pH to 7 and VFA's was determined.

3.12 Calculations for Volatile fatty acids and alkalinity

VFA's (mg/L) = (V ml of alkali expended * Normally the alkali used * 50000) / (V ml of sample taken).

Alkalinity (mg/L) = (V ml of acid expended * Normally the acid used * 50000) / (V ml of sample taken).

CHAPTER 4

RESULTS

4.1 Feed stock

The present study was designed to evaluate the effect of organic loading rate of on performance of acidogenic and methanogenic reactor during two stage anaerobic co digestion of cattle manure and green grocery waste. The feed stock used for the production of biogas was fruit and vegetable waste with cattle manure. For the FVW and cattle manure, the total solids and volatile solids were determined. The total solids and volatile solids were and respectively and that of manure was respectively.

Table 4.1 VS and TS of feed stock.

Biomass	TS (%)	VS of TS (%)	VS of Sample (%)	Moisture (%)
Fruit and vegetable waste	9.01	89.33	8.05	90.99
Cattle Manure	17.99	83.3	14.98	82.01

4.2 Effect of organic loading rate on biogas yield

The ratio for mixing the FVW and cattle manure was 1:1 on VS basis. The co-digestion was performed under controlled temperature 37 °C. To investigate the higher organic loading rate the feeding was started from 5.5 OLR g VS L^{-1} day^{-1}, and then kept increases the OLR.

4.2.1 Biogas yield at 5.5 OLR:

The experiment was started with the OLR 5.5g $VS_{added}L^{-1}day^{-1}$ in two stage AD. During the acclimatization stage the production was not constant and keep fluctuate. After the acclimatization stage the experiment gained Steady State. During the steady state the biogas yield was 0.242 NL g^{-1} VS. The pH of the acidogenic reactor was from 4.85 to 4.93, while that of methanogenic reactor the pH was in the range of 7.13 to7.25 during the steady state of the experiment NL (Fig 4.1). The biogas production during this stage was 6.70.

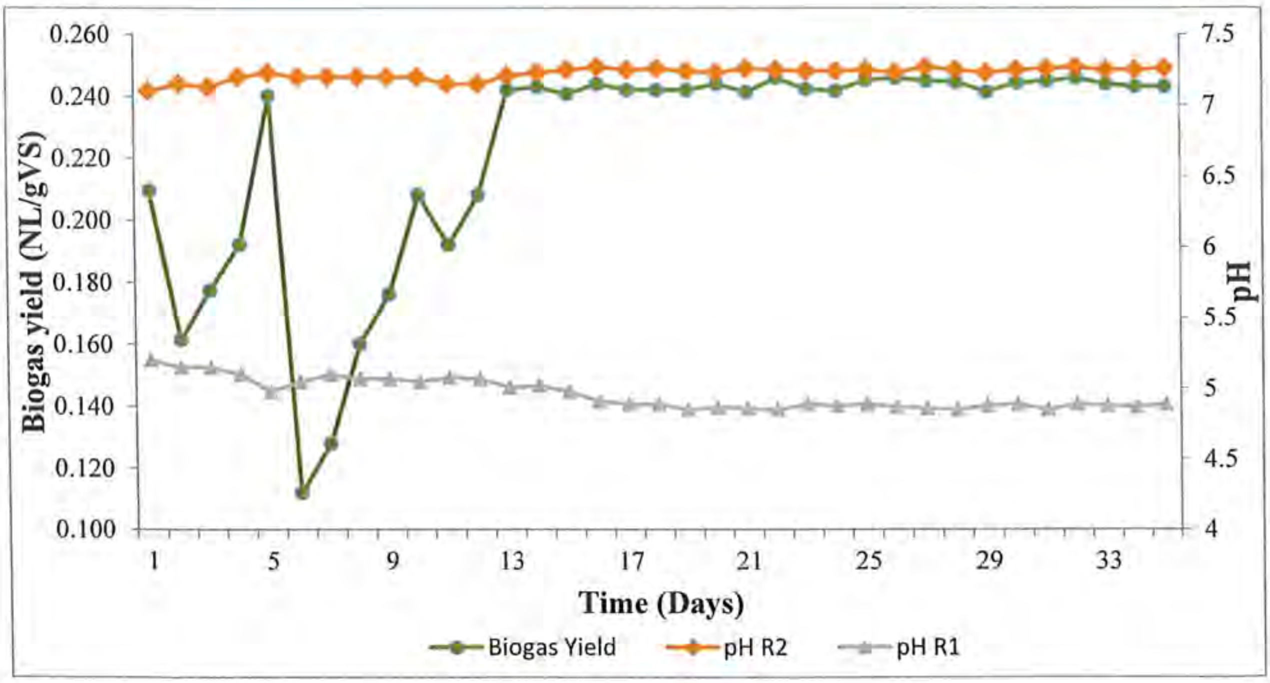

Figure 4.1 Biogas yield and pH of acidogenic (R1) and methanogenic (R2) reactor at organic loading rate 5.5g VS $L^{-1}day^{-1}$.

4.2.2 Biogas yield at 6 OLR:

The OLR was increased after more than two retention time of the previous experiment at steady state and the feeding was started at OLR 6 gVS $L^{-1}day^{-1}$. Initially the biogas production was low but after day five the experiment gained the steady state. During the steady state the biogas yield was 0.292 NL/g VS added that was high as compare to OLR 5.5g VS $L^{-1}day^{-1}$. The pH of the acidogenic reactor was decreased to 4.57, while the pH of methanogenic reactor was 6.97-7.05 still in optimum range during the steady state of the experiment (Fig 4.2). The biogas production during this stage was 8.6 NL.

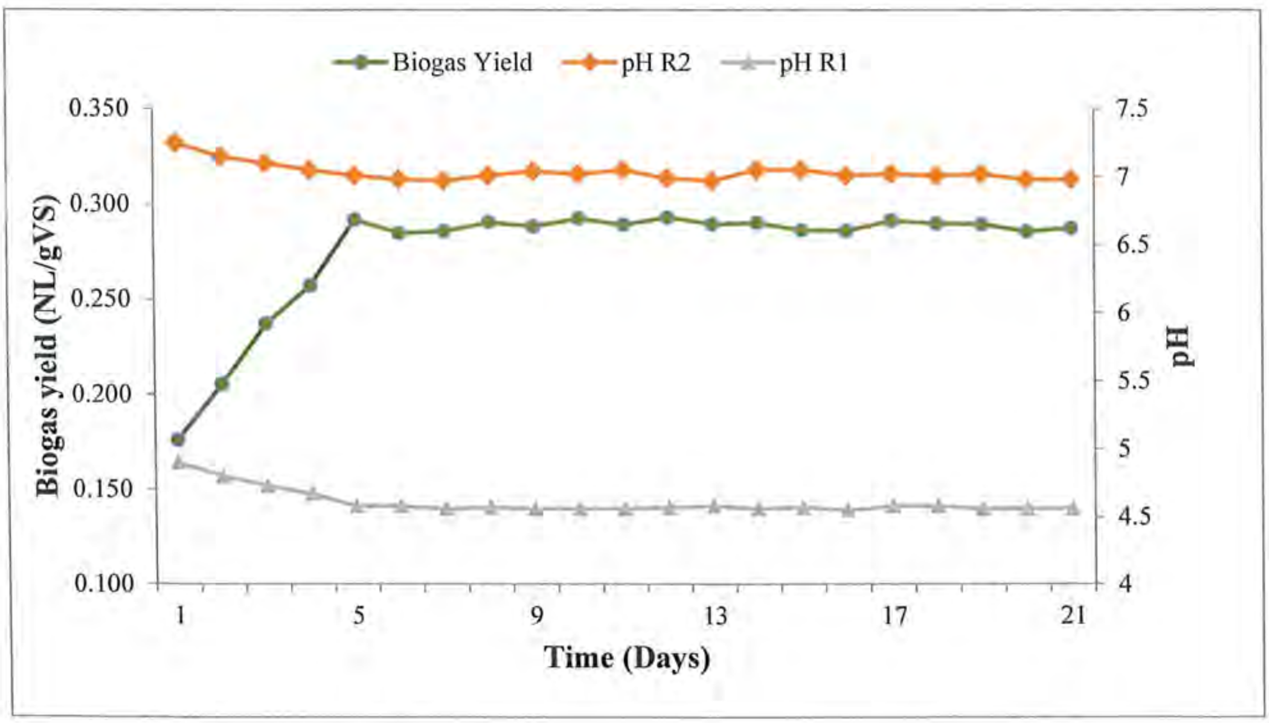

Figure 4.2 Biogas yield and pH of acidogenic (R1) and methanogenic (R2) reactor at organic loading rate 6g VS $L^{-1}day^{-1}$.

4.2.3 Biogas yield at 6.5 OLR:

The OLR was further increased up to 6.5g VS $L^{-1}day^{-1}$. During the early days at this higher OLR the biogas production was low but after few days the production increases and gains the steady state. During the steady state the biogas yield was 0.21 NL g^{-1} VS. The pH of the acidogenic reactor was fluctuated in the range of 4.45 to 4.49, while that of methanogenic reactor the pH fluctuated between 6.75-6.81 during the steady state of the experiment (Fig 4.3). The biogas production during this stage was 6.7 NL.

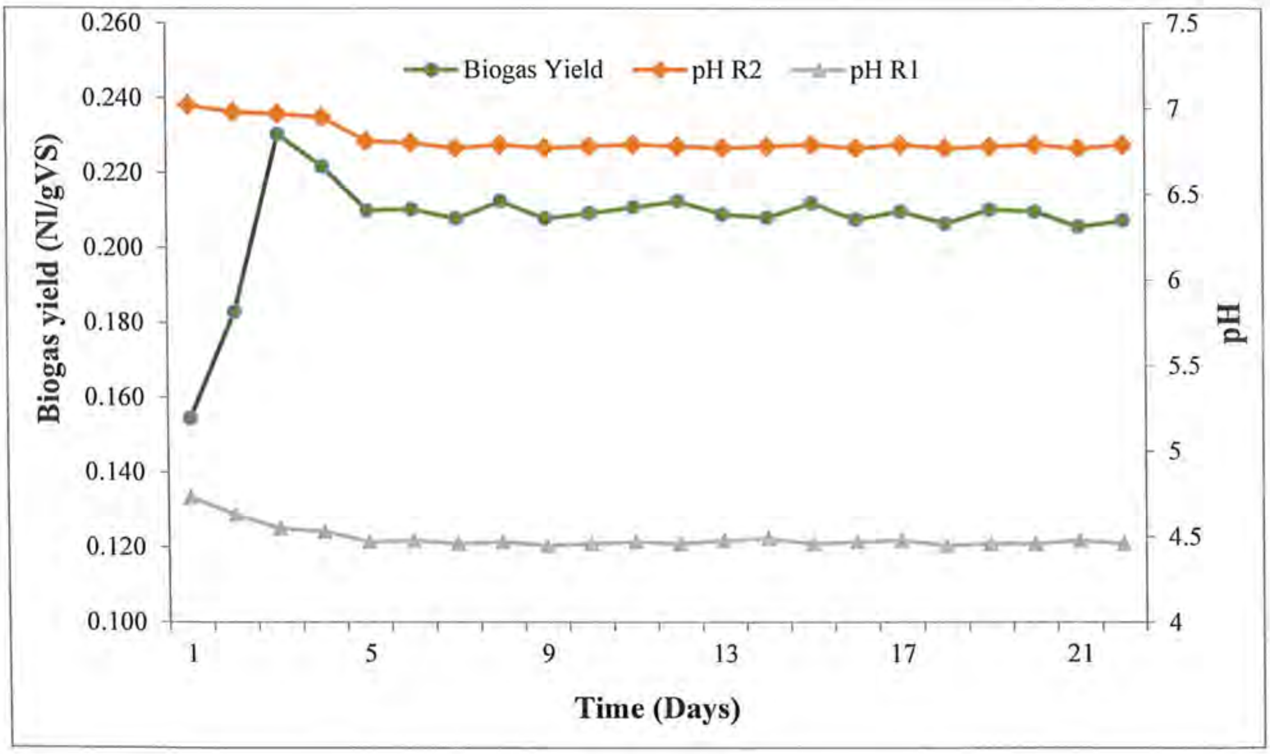

Figure 4.3 Biogas yield and pH of acidogenic (R1) and methanogenic (R2) reactor at organic loading rate 6.5g VS $L^{-1}day^{-1}$.

4.2.4 Biogas yield at 7 OLR

After two retention times OLR was further increased to 7g VS L^{-1} day^{-1}. During the early days at 7 OLR the biogas production was keep fluctuate but after some days the production was in steady state. During the steady state the biogas yield was 0.168 NL g^{-1} VS. The pH of the acidogenic reactor was flucktuated between 4.41 to 4.4, while that of methanogenic reactor the pH was droped to 6.56 that was below optimum level during the steady state of the experiment (Fig 4.4). The biogas production during this stage was 5.9 NL.

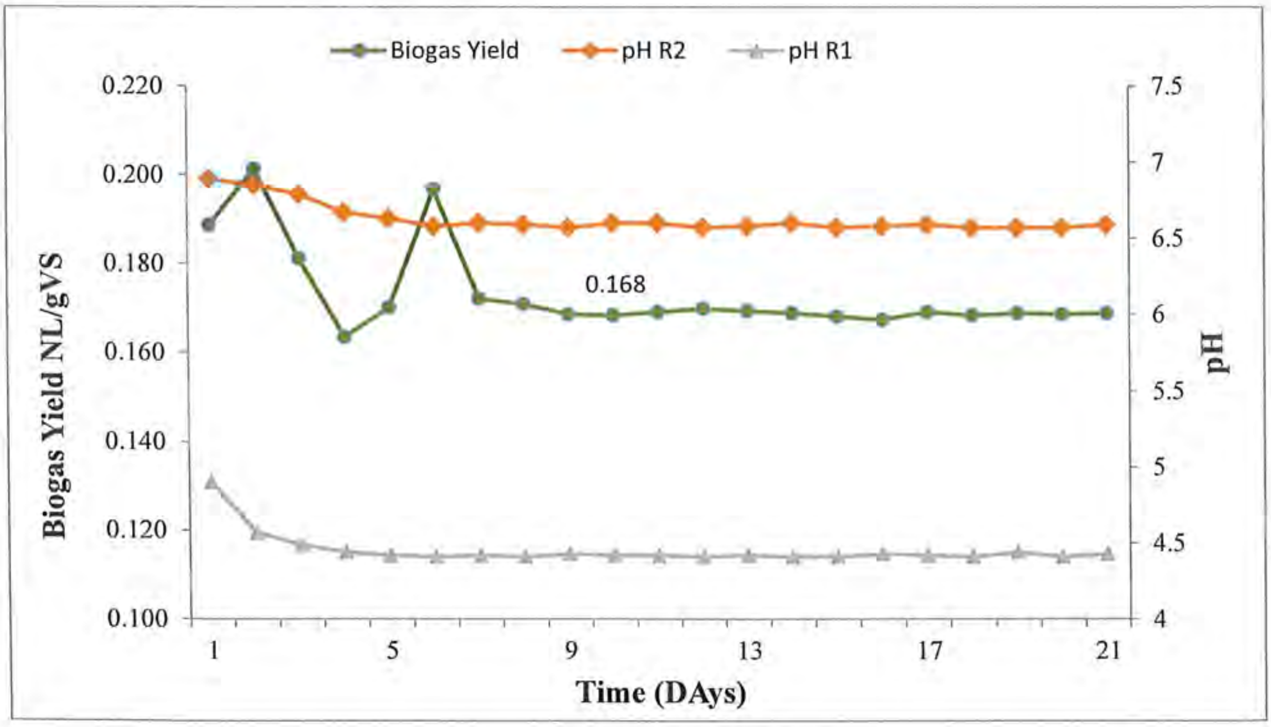

Figure 4.4 Biogas yield and pH of acidogenic (R1) and methanogenic (R2) reactor at organic loading rate 7g VS $L^{-1}day^{-1}$.

4.2.5 Biogas production and biogas yield

At the start of the experiment at OLR 5.5 g VS L^{-1} day^{-1} the biogas production was 6.70 NL with the biogas yield of 0.242 NL/g VS when the organic loading rate was increased to 6 the biogas production and yield was increased to 8.6 NL and 0.292 NL/g VS respectively. But with further increase in loading rates both at 6.5 and 7 OLR the biogas production was 6.7NL and 5.9 NL and the yield was decreased to 0.201 NL and 0.16 NL/g VS respectively (Fig 4.5a and 4.5b).

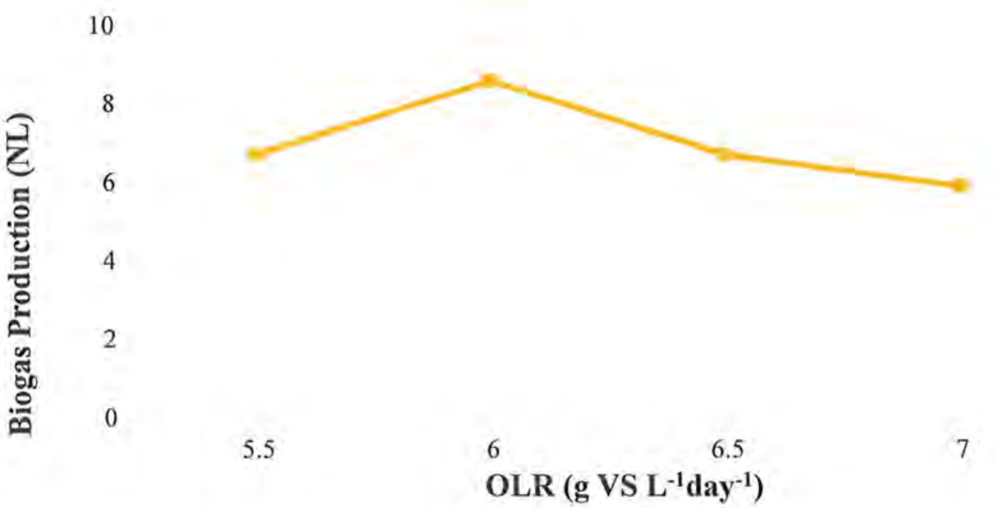

Figure 4.5a biogas production in normalized Liters at different organic loading rates

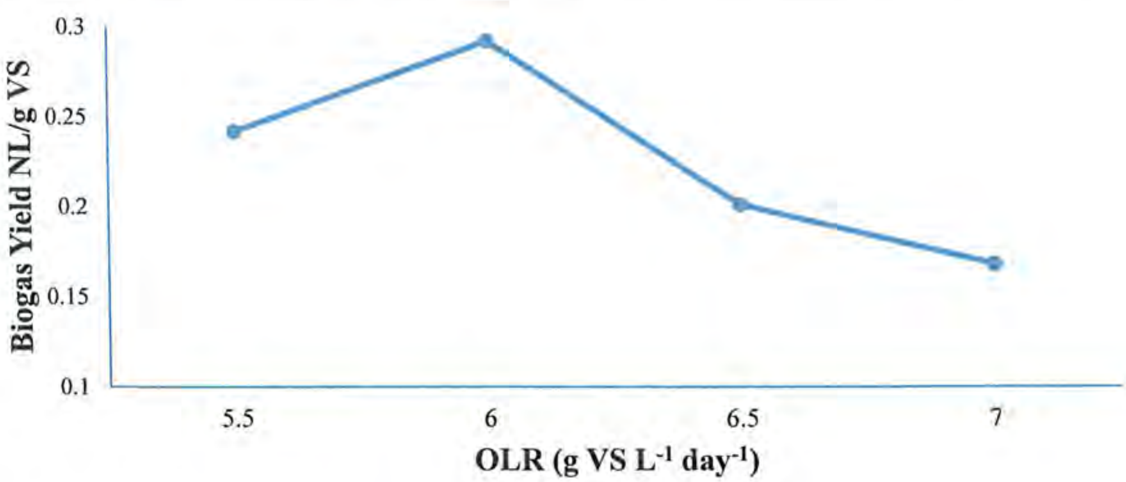

Figure 4.5b Biogas yield and production at different OLR

4.2.6 VFA formation in R1 and accumulation in R2

During the 5.5 OLRgVS $L^{-1}day^{-1}$ the VFAs formation was 5.6g L^{-1} in R1 while the VFAs accumulation in R2 was 1.300 gL^{-1}. At 6 OLR g $VSL^{-1}day^{-1}$ the VFAs production in R1 was 6.3g L^{-1} and the VFAs accumulation in R2 was 1.45g L^{-1}. The VFAs formation in R1 during 6.5 OLR was 9.85g L^{-1} while the accumulation was increased up to 5.4g L^{-1} in the R2. The highest OLR was 7 and the VFAs production in R1 was 13.3g L^{-1} while the accumulation in R2 was 6.7g L^{-1} (Fig 4.6).

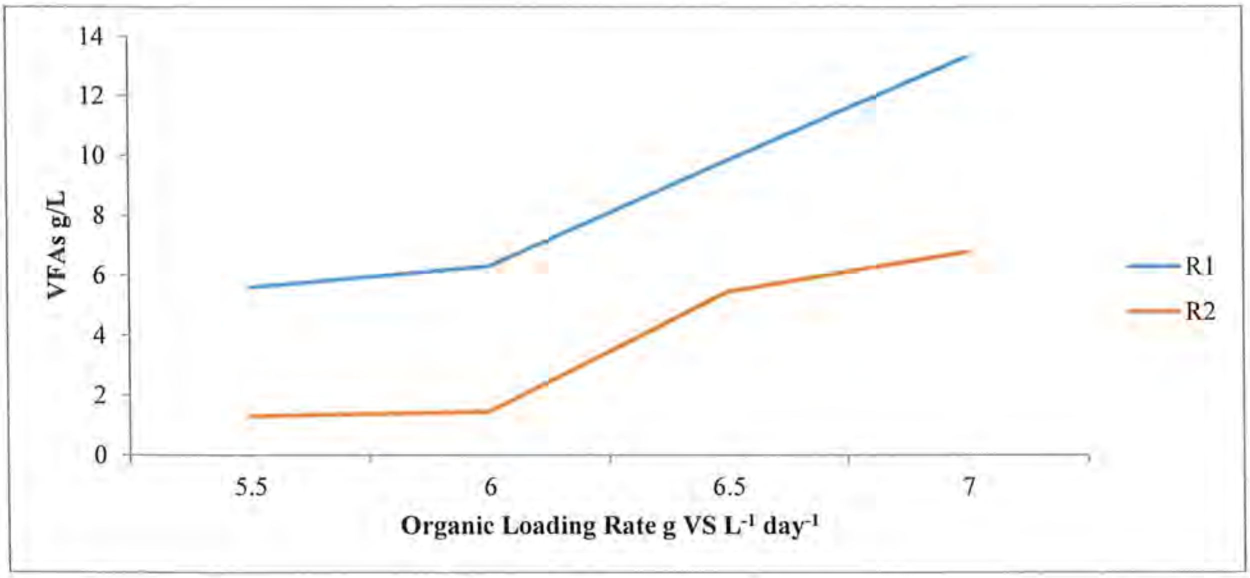

Figure 4.6 VFAs formation in acidogenic (R1) and accumulation methanogenic (R2) reactor at different OLR.

4.2.7 Alkalinity of R1 and R2

The alkalinity of the R1 was 2g L^{-1} and that of R2 was 4.53 g L^{-1} at 5.5 OLR. The alkalinity of R1 and R2 was 1.34 and 3.77 gL^{-1} respectively during 6 OLR. At 6.5 OLR the alkalinity of R1 was 3.32 and that of R2 was 5.46g L^{-1}. During 7 OLR the alkalinity of R1 was 3.71 while that of R2 was 5.87g L^{-1} (Fig 4.7).

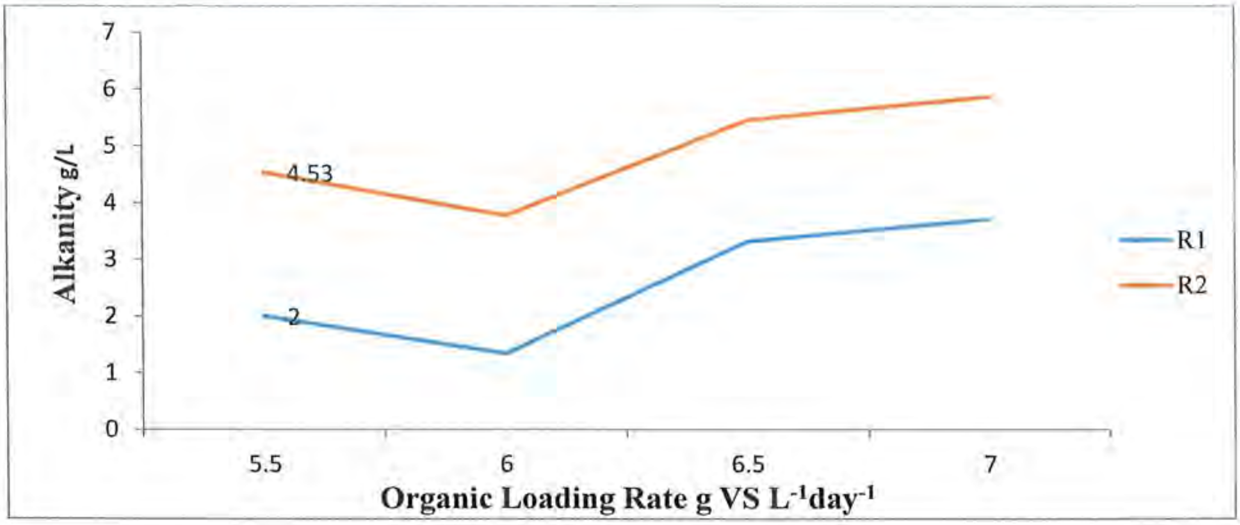

Figure 4.7 Alkalinity of acidogenic (R1) and methanogenic (R2) reactor at different OLR.

4.3 Effect of VFAs on biogas yield in suspended and attached growth methanogenic reactors

After stydydying the organic loading rate the feeding was started on VFA bases in both suspended and attached growth methanogenic reactors.

4.3.1 Effect of VFAs loading rate on biogas yield in suspended growth reactor

After studying the higher OLR up to 7 g VS L^{-1} day^{-1} the feeding was started on the basis of VFAs in R2 in both the suspended and attached growth methanogenic reactors. The OLR was kept the same for R1.in R2 the feeding was on the basis of VFAs. The VFAs loading was started from 0.25 g VFAs added L^{-1}, and then kept increase the loading rate to 0.5, 0.75,1, and 1.25 g VFAs L^{-1} day^{-1}.

4.3.1.1 VFAs loading rate 0.25

In order to load 0.25 g VFAs L^{-1} concentration the VFAs were diluted by adding water with the effluent from R1. The biogas yield during the steady state was 1.559 NL/g VFA. The pH of R2 was 7.2 (Fig 4.7).

4.3.1.2 VFAs loading rate0.50

Then after fifteen days the VFAs loading rate was increased to 0.5 g VFAs $L^{-1}day^{-1}$ and operated for fifteen days. The biogas yield was 1.805 NL/g VFA at steady state. And the pH of R2 was around 7.09 (Fig 4.8).

4.3.1.3 VFAs loading rate 0.75

The loading of VFAs was increased to 0.75 g VFAs $L^{-1}day^{-1}$ and the experiment was run for fifteen days. The biogas yield was 2.925 NL/g VFA and the pH of R2 was 6.97 during the steady state of the experiment (Fig 4.8).

4.3.1.4VFAs Loading Rate 1 g VFAs $L^{-1}day^{-1}$

Further increase the biogas yield was 1.02 NL^{g-1} VFAs, the pH of the reactor was decreased to 6.85 during steady state of the experiment. The experiment was run for two retention times (fig 4.8).

4.3.1.5 VFAs Loading Rate 1.25 g VFAs $L^{-1}day^{-1}$

After this the VFAs loading rate was further increased to 1.25 g VFAs $L^{-1}day^{-1}$. This experiment was also run for two retention times. During the steady state the biogas yield was further decrease to 0.47 NL^{g-1} VFAs as shown (fig 4.8).

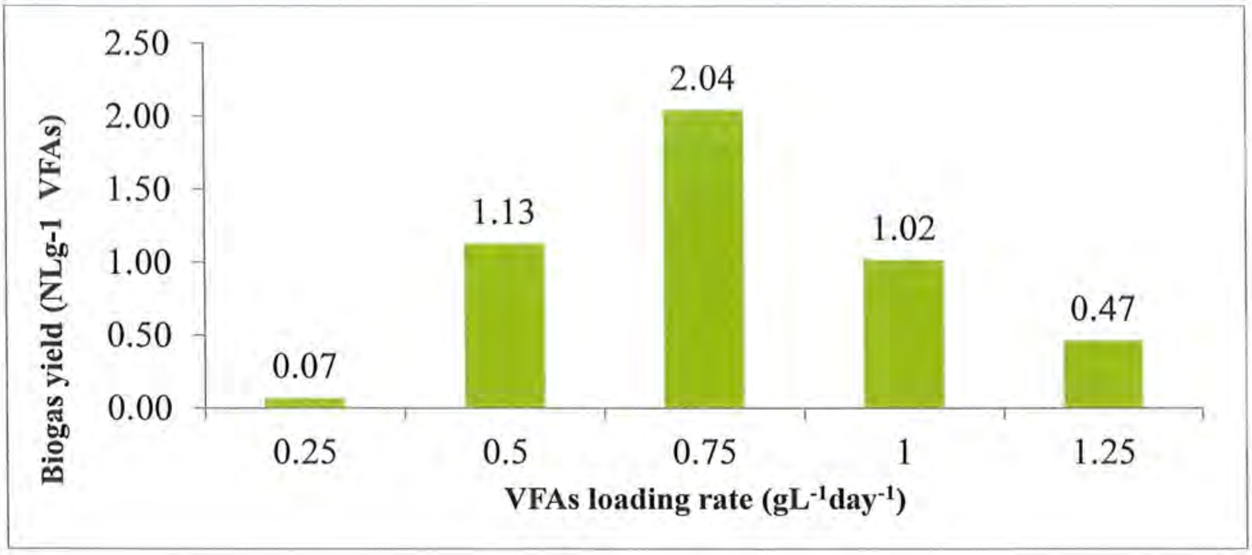

Fig 4.8 Biogas yield at different VFAs loading rate during suspended growth reactor

4.3.1.6 VFAs accumulation in suspended growth Reactor

During VFAs loading rate the VFAs accumulation was high at 0.25 g VFAs L^{-1} but after further increase the VFAs accumulation decrease and at 0.75 g VFAs loading rate lowest VFAs accumulation was recorded but further increase in VFAs loading rate the VFAs accumulation in methanogenic reactor was increased. The highest VFAs accumulation was at 1.25 $gL^{-1}day^{-1}$, at which the VFAs accumulation was 4180 mg/L of VFAs as shown in the fig 4.9.

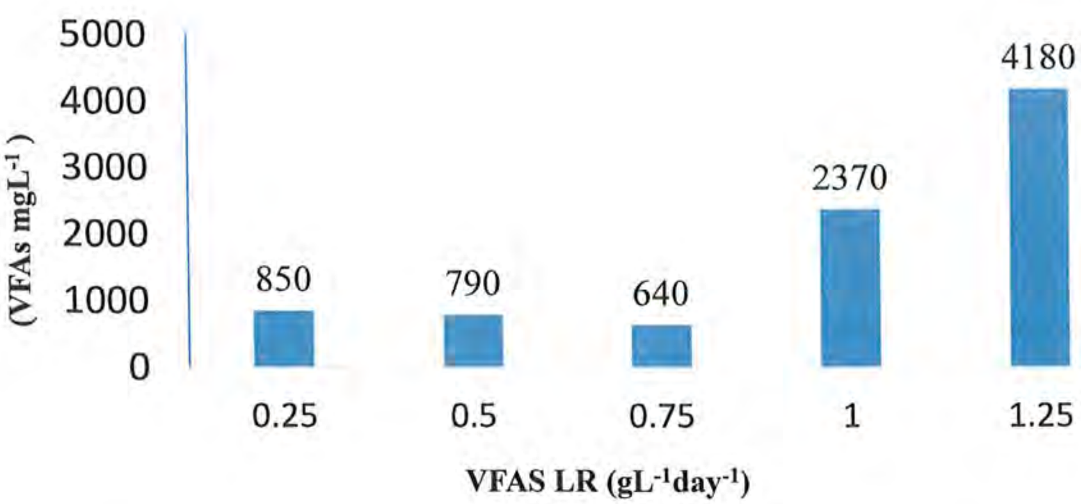

Fig 4.9 VFAs accumulation during suspended growth methanogenic reactor

4.3.2 Effect of biofilm on biogas yield during VFAs loading rate

The biogas yield was then determined in attached growth reactor.

4.3.2.1 VFAs loading rate 0.25

To study the effect of VFAs and biogas yield on biofilm the same experiment was performed in attached growth R2. The VFAs loading was started from 0.25 and then operated on 0.5 and 0.75 g VFAs $L^{-1}day^{-1}$. At 0.25 g VFAs $L^{-1}day^{-1}$ the biogas yield was 2.845 NL/g VFAs and the pH was 7.21 during the steady state as shown in the fig 4.10.

4.3.2.2 VFAs Loading rate 0.5

After stable process at 0.25 g VFAs loading rate the VFAs loading was increased to 0.5 and 0.75 g VFAs $L^{-1}day^{-1}$. The production was low in the initial days of the experiment. At steady state the production was 2.604 NL/g VFAs and the pH was around 7.10 as shown in fig 4.10.

4.3.2.3 VFAs Loading rate 0.75

The VFA loading was increased to 0.75 g VFAs $L^{-1}day^{-1}$ and the experiment was operated for fifteen days. Initially the production was low but at steady state the biogas yield was improved to 3.385 NL/g VFA and the pH was 7 (Fig 4.10) .

4.3.2.4 VFAs Loading Rate 1 g VFAs $L^{-1}day^{-1}$

Further increase the biogas yield was 1.19 NL^{g-1} VFAs, the pH of the reactor was decreased to 6.89 during steady state of the experiment. The experiment was run for two retention times (fig 4.10).

4.3.2.5 VFAs Loading Rate 1.25 g VFAs $L^{-1}day^{-1}$

After this the VFAs loading rate was further increased to 1.25 g VFAs $L^{-1}day^{-1}$. This experiment was also run for two retention times. During the steady state the biogas yield was further decrease to 0.5 NL^{g-1} VFAs while the pH of the reactor further decrease to 6.77 (fig 4.10)

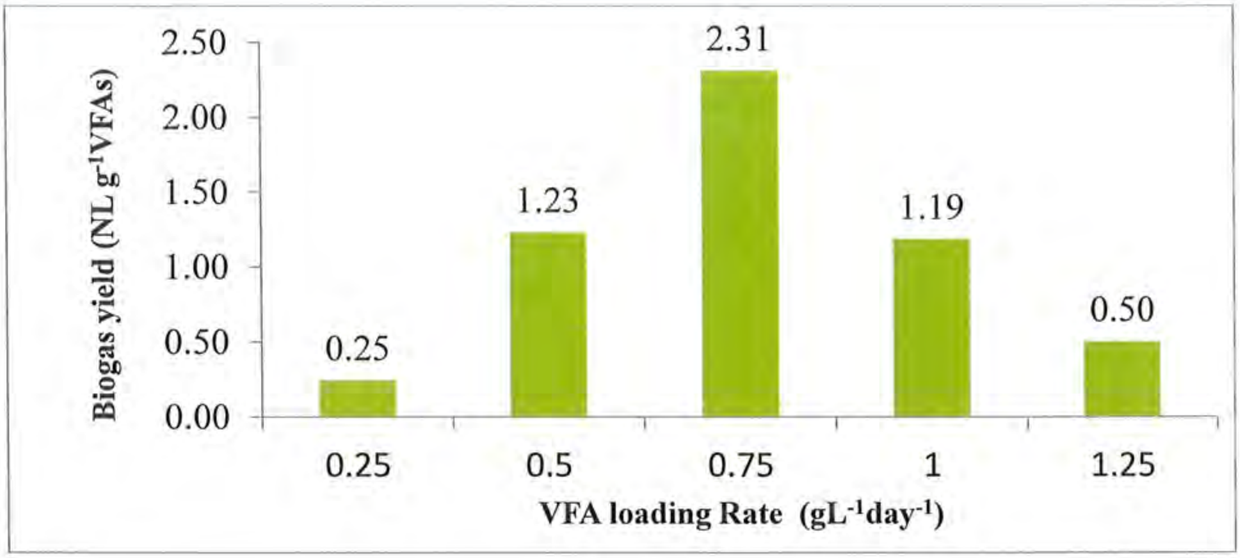

Fig 4.10 Biogas yield at different VFAs loading rate

4.3.2.6 VFAs accumulation during attached growth reactor

During attached growth reactor the VFAs accumulation was decreased with increase in VFAs loading rate but after 0.75 g VFAs $L^{-1}day^{-1}$ but after further increase in VFAs loading rate the VFAs accumulation was increased with increase in VFAs loading rate. At 1.25 g VFAs loading rate $L^{-1}day^{-1}$ the maximum VFAs accumulation was there in the effluent (fig 4.11).

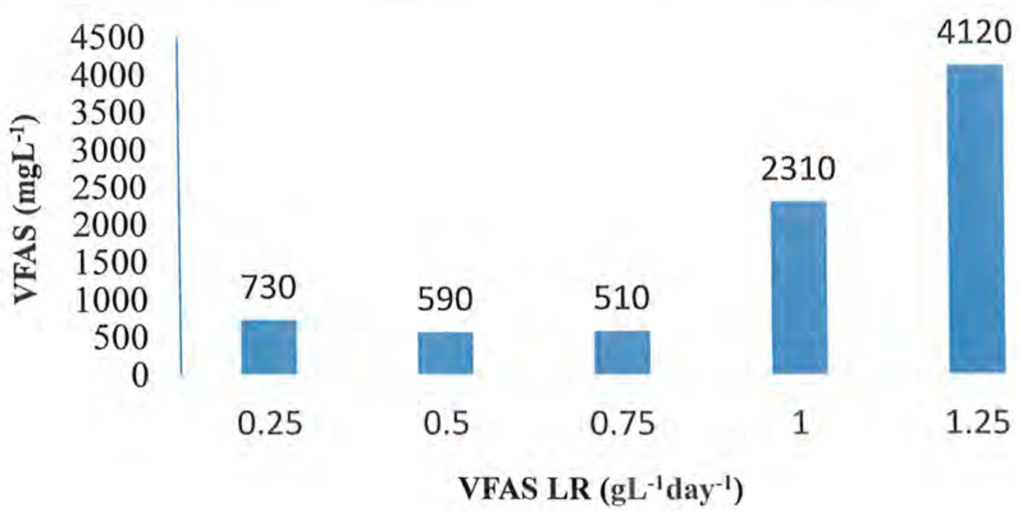

Fig 4.11 VFAs accumulation during attached growth methanogenic reactor

CHAPTER 5

DISCUSSION

In the present study effect of organic loading rate on VFAs formation in R1 and biogas yield in R2 was evaluated. The feed stock for anaerobic digestion was cattle manure and green grocery waste. The ratio was kept 1:1 in order to attain the optimum C/N ratio as for stable and high biogas yield the C/N must be 30:1 (Yenigun et al., 2013). The cattle manure have low C/N ratio while the green grocery waste have high C/N ratio (Tufaner andAvsar., 2016). The VS of manure was 14.98 % and VS of TS was 83.3 %. The green grocery waste used have 9.01% VS and 89.33% VS of TS in literature it is showed that the green grocery waste has high amount of water, low total solids and high volatile solids (bouallagui et al., 2005).

To study the effect of organic loading rate the reactor was fed at 5.5g $VSL^{-1}day^{-1}$ at steady state the biogas production was 6.70 NL with biogas yield of 0.242 NL/g VS while pH of R1 and R2 was 4.9 and 7.2 respectively. The optimum reported pH for methanogenic activity is 6.6-7.6 (Solera et al., 2002) while it have been reported that high acidogenic activity occurs at lower pH values. The pH for the acidogenic reactor must be in the range of 4.3-6.5 (kim *et al.*, 2003). When the loading rate was increased to 6g VS $L^{-1}day^{-1}$ the biogas production increased to 8.6 NL with the biogas yield of 0.292 NL/g VS with further increased in loading rate to 6.5g VS L^{-1} day^{-1} biogas production decreased to 6.7 NL with biogas yield of 0.21 NL/g VS while at 7g VS L^{-1} day^{-1} the biogas production was further decreased to 5.9 NL with the biogas yield of 0.168 NL/g VS. It has been noted that increasing loading rate up to 6 the biogas yield increases while further increase in loading rate biogas yield decreases. It has been reported in the literature that increasing the organic loading rate to a specific range the biogas yield increases but beyond the specific range further increase of the organic loading rate results in the decrease of biogas yield. The decrease in the biogas yield is because of VFAs accumulation (Demirer et al., 2004). The VFAs of methanogenic reactor was 1.3 and 1.5 g/L at OLR 5.5 and 6 respectivly while the VFAs accumulation increased to 5.46 and 6.78 g/L in case of 6.5 and 7 OLR. The biogas reduction is due to the higher VFAs accumulation and high acidity in R2. It has been reported that the optimum pH for methanogenic activity is 6.8 to 7.2 (Rajeshwari et al., 2000), in order to remain the reactor pH stable the feeding range must be optimized. The VFAs production effect the pH of the reactor as for high biogas production the amount of VFAs must be lower than 2 g/L (Jain and Mattiasson., 1998).

At the start of the experiment the pH of both reactors were at neutral but with continuous feeding the pH of reactor 1 become acidic it was 4.9 with further increasing the loading rate the pH of reactor 1 show decrease, it was 4.56, 4.46 and 4.42 at 6, 6,5 and 7 OLR respectively. While it has been reported that high acidogenic activity occurs at lower pH values. The pH for the acidogenic reactor must be in the range of 4.3-6.5 (kim et al., 2003). The pH of reactor 2 was 7.26, 6.98, 6.75 and 6.57 at 5.5, 6, 6.5 and 7 OLR respectively. With increasing the loading rate the pH of reactor 2 decreased that is due to VFAs accumulation. It has been reported that high methanogenic activity is attained in pH range of 6.8-7.2 (Rajeshwari et al., 2000). The low biogas yield may be due to low pH of reactor 2 at 7 OLR.

The preference of reactor 1 is determined by high amount of VFAs production, while the high VFAs accumulation in reactor 2 leads to process instability or failure. At 5.5 and 6 OLR the VFAs production was 5.6 and 6.3 g/L while increasing the OLR the VFAs production increased to 9.85 and 13.3 g/L at 6.5 and 7 OLR. The VFAs produced in R1 determine the pH of the reactor. At low pH the VFAs are produced in high amount. At high VFAs production the pH of the reactor become low. the VFAs accumulation at different organic loading rate was 1.3, 1.45,5.46 and 6.78 g/L at 5.5, 6, 6.5 7 OLR respectively. The results confirmed that with increasing the OLR the VFAs accumulation increases in R2. The VFAs production affect the pH of the reactor as for high biogas production the amount of VFAs must be lower than 2 g/L. The optimum pH range for R2 is 6.8-7.4 but increasing the organic loading rate the VFAs accumulation increases and hence the pH of R2 decreases (Mehta et al., 2002).

The alkalinity of R1 at 5.5, 6, 6.6 and 7 OLR was 1.95 , 1.3, 3.32 and 3.71 g/L respectively and that of R2 was 4.53, 3.77, 5.46 and 5.87 g/L respectively. Overall the alkalinity was increased with increasing organic loading rate in case of reactor 1 and 2 but at 6 organic loading rate there was slight decrease. In literature it has been showed that with increasing the organic loading rate the alkalinity increases (Najafpour et al., 2006)

From the above results it was established that at OLR 7 the VFAs production in reactor 1high but the problem was in reactor 2 the pH become low than optimum value and high VFAs accumulation leads to process instability and low biogas yield. The VFAs produced in reactor 1 not only affect the reactor 2 but also lost in the effluent and the fact is that it has high biogas potential. The present study was designed to optimize the VFAs loading rate for reactor 2 in order to get high biogas yield. For this purpose the reactor 2 feeding was started with 0.25, 0.5 and 0.75g VFA $L^{-1}day^{-1}$. Initially the biogas yield per g of VFAs was high that may be due to the accumulation of VFAs but with the time biogas yield decreased and reached to steady sate with the yield of 0.07 NL/g VFA. When the VFAs loading rate was increased to 0.5 and 0.75 g VFA $L^{-1}day^{-1}$ the biogas yield increased to 1.13, 2.04 but after further increase in VFAs loading rate to 1 and 1.25 there was decrease in biogas yield which was 1.02 and 0.47 NL/g VFA.

The attached growth reactor for methanogenic activity was used in order to study the effect of biofilm on biogas yield. The biogas yield at VFAs loading rate 0.25, 0.50, 0.75, 1 and 1.25 g VFAs $L^{-1}day^{-1}$ in attached growth reactor was 0.25, 1.23, 2.31,1.19 and 0.50 NL/g VFA. The biogas was yield was higher in attached growth in comparison with suspended growth reactor (Heijnen et al., 1989).

In the attached growth reactor the biofilm not only help in the biogas yield. Due to the presence of biofilm in the attached growth reactor the washing out of methanogens in the effluent was not only overcome but they also help in the high biogas yield. It has been reported that the support material will have thin biofilm in case of low feeding rate while at high feeding the biofilm was thick (Heijnen et al.,1989). In literature it has been reported that the biofilm help in higher biogas yield and also help in remove VS and TS more than suspended growth reactors. (Gong et al., 2011).

CHAPTER 6

CONCLUSION, RECOMMENDATIONS AND FUTURE PERSPECTIVES

Conclusion

The current research was conducted to study the effect of organic loading rate on the performance of acidogenic reactor and methanogenic reactor during two stage anaerobic codigestion of cattle manure with green grocery waste. The experiment was carried out at controlled temperature 37^0C. It is concluded that increasing the organic loading rate from 5.5 to 6 Organic loading rate the biogas yield increases but further increase in organic loading rate the yield decreases. The volatile fatty acids formation in acidogenic reactor was increased with increase in organic loading rate. During 7 organic loading rate the pH and the biogas yield was decreased. At this organic loading rate the process condition were not optimum. During the VFAs loading rate the biogas yield and the performance of the methanogenic reactor was stable with increase in VFAs loading rate. The VFAs accumulation in methanogenic reactor was low when the feeding in methanogenic reactor was kept on the basis of VFAs loading rate

Recommendations

On the basis of current research it is recommended that reactor 1 should be operated at higher organic loading rate in order to produce high amount of VFAs and the reactor 2 must be operated at optimum VFAs loading rate of 0.75 g VFA/L/day. The VFAs loading rate will not only help to enhance the biogas production and yield but will also help to reduce the environmental consequences caused due to the discharge of effluent containing high VFA during operation of reactors on higher organic loading rates.

Future prospects

The future prospects are given below:

1. The higher VFAs loading rate can be studied in reactor 2 in order to enhance the biogas yield.
2. Effect of different types of VFAs can be studied and their effect on biogas production.
3. Effect of organic loading rate and VFA loading rate on biogas yield should be studied using substrate other than green grocery waste.
4. Effect of organic loading rate on methanogenic population should be studied.

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