EFFECTS OF VARIOUS INOCULUM AND F/M RATIO DURING BATCH AND CONTINUOUS ANAEROBIC DIGESTION OF FOOD WASTE

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Candidate's Declaration

I hereby declare that the work presented in this thesis is to the best of my knowledge, original, except as acknowledged in the text. This material has not been submitted, either in whole or in part, for degree at any University.

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This is to certify that the thesis entitled **"Effects of Various Inoculum and F/M Ratio During Batch and Continuous Anaerobic Digestion of Food Waste"** submitted by K. Dhamodharan (Registration No. 11610429) to the Indian Institute of Technology Guwahati for the degree of Doctor of Philosophy is a record of bonafide research work carried out by him under my supervision and guidance. The thesis work, in my opinion has reached the requisite standard fulfilling the requirement for award of the degree of Doctor of Philosophy. This work has not been submitted earlier for the award of any degree or diploma to the best of my knowledge and belief.

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ABSTRACT

Food waste (FW) as a highly decomposable feedstock can be utilized for the production of methane gas. Inoculum plays a major role in the process of anaerobic digestion by providing different kind of consortia. Animal dung is one of the best sources of inoculum for anaerobic digestion (AD) because of its digestive system. Animal dung has less biodegradable and more microbial consortia which can be well utilized for the purpose of inoculum in AD of FW. No study was carried to utilize various dungs to compare as inoculum in AD process of FW. The present study focuses on animal dungs as inoculum in different ratios to approach in novel way to enhance AD process and more methane production. In present study, mixture of FW from the Indian Institute of Technology Guwahati (IITG) hostels were used as substrate and digester sludge (DS), different livestock dungs such as poultry dung (PD), goat dung (GD), cow dung (CD), piggery dung (PGD) and rhinoceros dung (RD) were used inoculum. FW has high volatile solids percentage and it produces high volatile fatty acids during anaerobic digestion process so it needs high active methanogens and nitrogen content to increase the microbial growth rate for higher methane production. The results indicated that cow dung and piggery dung inoculum was more suitable for the anaerobic digestion of FW as compared to other dungs. Reactors inoculated with cow dung achieved higher methane production (227 mL/gVS degraded) and volatile solids degradation (54.6 %) at F/M ratio maintained at 2. The better adaptability of microbes in dungs during AD process had relationship with its higher activity and sufficient nutrients content. Soluble and carbohydrates portion of the food waste is easily degradable but lipids and fats are not easily degradable which increases the hydrolysis time. To overcome the lag in hydrolysis phase in anaerobic digestion, the effects of various pretreatments such as hot air oven, microwave, autoclave, alkali and electrohydrolysis on FW was studied. In the batch reactor study, four batch reactors with hot air oven pretreated, alkali pretreated, electrohydrolysis pretreated and untreated FW were studied for 40 days. The results revealed that for hot air oven pretreatment, the highest solubilisation of 1.4 times with respect to untreated FW was obtained at a temperature of 75°C and time of exposure of 90 min with the soluble chemical oxygen demand (COD) increasing from 740 g/kg of dry FW for control to 1027 g/kg of dry FW. In present research, the work was focused on reactor design for more methane production in less hydraulic retention time (HRT) with High organic loading rate (OLR) in a reactor which eradicates the problems stated by the

researchers on single and two stage reactor systems. Anaerobic bi-phased baffled reactor (ABBR) technology has been designed for the treatment of organic solid waste and to recover more biogas. It was shown that keeping only the solid fraction of digestate in the reactor enabled robust and stable operation over an extended period. Highest methane production was achieved with two different inocula (CD and DS) in lowest HRT in Laboratory scale ABBR. The HRT was varied from 8 to 14 days in both studies in that each HRT was maintained for 30 days. Results proved that the soluble COD decreased in higher percentage in cow dung as inoculum study and the gas production also attained 0.528 L/gVS/d in HRT 10 days compared to digested sludge reactor. ABBR provides the favorable conditions for both the acidogens and methanogens to achieve their best. Pilot scale ABBR (0.7 m^3) was fabricated and operated. At HRT 10 days the average biogas production (0.58 m^3/d) was higher. Pilot scale ABBR performed well at the environmental temperature varies from 22 to 34^oC. The COD reduction was less at the startup period, later it was increased to an average of 68-72% at HRT 10 days during first 30 days of the reactor.

Keywords: Food waste, anaerobic digestion, livestock dung, cow dung, biochemical methane potential test, pretreatment, anaerobic biphased baffled reactor.

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LIST OF ABBREVATIONS

FW	Food Waste
DS	Digested sludge
CD	Cow Dung
PD	Poultry Dung
PGD	Piggery Dung
GD	Goat Dung
RD	Rhinoceros Dung
MSW	Municipal Solid Waste
L	Litre
mg	Milligram
g	Gram
Kg	Kilogram
HRT	Hydraulic retention time
F/M	Food to microorganism ratio
TP	Total Phosphorous
C/N	Carbon/Nitrogen
TKN	Total Kjeldhal Nitrogen
NH_4-N	Ammoniacal Nitrogen
APHA	American Public Health Association
NH_3	Ammonia
NSW EPA	New South Wales Environmental Protection Agency
EBMUD	East Bay Municipal Utility District
h	Hour
VFA	Volatile Fatty Acids
VS	Volatile Solids
AAS	Atomic Absorption Spectrometer
N	Normality
Twh	Terawatt hour
kwh	Kilowatt hour
RPM	Round Per Minute
NaOH	Sodium Hydroxide
COD	Chemical Oxygen Demand

CHAPTER 1

INTRODUCTION

This chapter consists of brief discussion about food waste problems, anaerobic digestion of organic waste, food waste treatment through different anaerobic techniques, and energy recovery from food waste materials and role of different inoculum during anaerobic digestion process.

1.1 OVERVIEW

Food waste (FW) is one of the least recovered materials in the municipal solid waste (MSW) and is one of the most important materials to divert from landfill. FW that is disposed of in landfills decomposes to create methane a potent greenhouse gas that contributes to climate change. FW is a highly organic and moisture content (MC) material which need to be processed for better management or utilization before disposal. The dumping of FW on landfill sites releases methane due to organic matter degradation in anaerobic condition which is a greenhouse gas 25 times more potent than carbon dioxide. By utilizing every ton of FW which has been sent to landfill, 0.9 tons of CO_2-e can be saved (NSW EPA, Australia, 2012). FW generation always vary according to the places, seasons and culture of the peoples. Roughly one third of all food produced for human consumption is wasted yearly totaling 1.3 billion tones, as reported by the Food and Agriculture Organization of the United Nation (FAOUN, 2011). FW is managed in various methods such as reusing for cattle's feed, composting, anaerobic digestion (AD) (Chen et al., 2010) and landfill dumping (Zhang et al., 2007). Chang and Hsu (2008) and Chang and Chen (2010) stated that some of the characteristics of FW such as organic matter, carbon to nitrogen ratio, etc. are favorable for composting and some of the characteristics such as pH, moisture content are not favorable, so the combinations of food waste and bulking agent also affecting composting process. The higher MC and biodegradability of FW makes it more suitable for AD and biogas recovery. So, AD seems to be a more feasible technology in the treatment of food waste.

Anaerobic digestion is a complex biochemical reaction carried out in a number of steps by several types of microorganisms that require little or no oxygen to live. During this process, a gas that is mainly composed of methane (CH_4) and carbon dioxide (CO_2),

also referred to as biogas, is produced. The amount of gas produced varies with the amount of organic waste fed to the digester and temperature influences the rate of decomposition and gas production. When activated inoculum is kept in an anaerobic environment, specialized bacteria will develop that use the dead biomass as a source of organic matter for fermentative metabolic processes. The end products of the fermentation are mainly CH_4 and CO_2. The overall conversion process of complex organic matter into CH_4 and CO_2 can be divided into four steps: hydrolysis, acidification, acetogenesis and methanogenesis. Biological processes i.e., composting and AD provide advantage due to its natural treatment process over other technologies. AD has unique and integrative potential, simultaneously acting as waste treatment and resource process. AD also showed an excellent life cycle analysis performance when compared to other treatment technologies i.e., incineration as it can improve the energy balance. In addition, the residues are stable and hence a compost potential for agriculture (Mata Alvarez, 2003). There is a major concern in reducing and recycling waste as much as possible during the treatment process with respect to both energy and materials. AD is an ancient yet currently widely used technology that holds incredible promise for the future by producing energy and minimizing waste (Vavilin and Lokshina, 1996). In terms of global warming, which is often used as a reference value for ecological balance, AD scores much better than other treatment options (Baldasano and Soriano, 1999; Salminen et al., 2003). AD is preferentially suited for high water content or pasty waste materials. Although, this microbiological conversion has dual advantage of waste treatment and energy generation, these include increased residence time and the inability of microorganisms to completely ferment all the organics in the municipal solid wastes (Chandran et al., 2006).

Anaerobic digestion of solid organic waste has gained increased attention as a means for producing energy-rich biogas, destructing pathogenic organisms and reducing problems associated with the disposal of organic waste (Sonakya, 2001). Biodegradation tests carried out with semisolid and pasty proteins and lipids containing byproducts from slaughterhouses, pharmaceutical, food, beverage industries, distilleries and municipal bio-wastes showed a biogas yield ranging from 0.3 to 1.36 L/g of volatile solids (VS) added (Braun et al., 2003). In continuous fermentation test, variety of substrates such as animal fat, flotation sludge, stomach and gut contents, blood, food leftovers, etc., were added and hydraulic retention times (HRT) ranging between 12 and 60 days according to

the substrate added, fermentation temperature of 35°C, was maintained for stable operation and to attain maximum gas yield (Braun et al., 2003). When FW is digested anaerobically it has the potential to generate 367 m^3 of biogas per ton of dry waste at about 65% CH_4 with an energy content of 6.25 KWh/m^3 (EBMUD, 2008). This represents almost 5% of the total global electrical energy utilization of 20,181 TWh in 2008 (International Energy Statistics, 2011). In addition, transportation of FW to landfills and greenhouse gas emissions will be reduced by implementing AD.

AD requires optimum inoculum for better digestion and biogas recovery. Inoculums should contain active microbial communities, which are needed for AD. Another important parameter is the food to inoculum ratio (F/M) in high solid AD process carried out in batch mode. The use of a large inoculum amount in a batch process allows a successful digestion without pH adjustment, being a value of 1 (VS basis) used in the assessment of the biochemical methane potential (BMP) (Gunaseelan, 1997). In order for the AD process to operate properly there must be a balance between the food entering the biological system, and the microorganisms in the anaerobic reactor. A high F/M ratio means there is a greater quantity of food relative to the quantity of microorganisms available to consume that food. When the F/M ratio is high, the bacteria are active and dispersed and they multiply rapidly. But with a high F/M ratio the bacteria will not form a good microbial consortium. Operating the AD process with a high F/M ratio will typically result in a low production. A low F/M ratio means there are many microorganisms but there is a limited amount of food.it will results in substrate inhibition. So finding the best F/M ratio will enhance the process and improves the methane production. It varies according to the substrate, due to its amount of volatile fatty acids (VFA) and the ammonia produced by the hydrolysis of carbohydrates and proteins respectively to buffer the medium (Neves et al., 2004). The major process in a one-stage batch reactor is to prevent volatile fatty acids accumulation inside the “seed” particles beyond their assimilative methanogenic capacity. This accumulation can be prevented by increasing the amount of seed sludge, in order to overcome irreversible acidification during start-up (Veeken and Hamelers, 1999; Kalyuzhnyi et al., 2000). However, in the case of more recalcitrant wastes, the rate of methane production in BMP assays was optimized by decreasing the food to inoculum ratio to 0.5 g VS/g $VS_{inoculum}$ (Chynoweth et al., 1993). FW easily digest by itself, thus extra methane-producing bacteria are needed to utilize the volatile fatty acids (VFA) present at high concentration.

Mostly inoculum is preferred in a small amount because of the endogenous biogas production possibility to affect the target results (Lesteur et al., 2013). Studies were done on the basis of F/M ratio of different waste composition and different inoculums to attain the maximum gas production (Braun et al., 2003; Gerardi, 2003; Lesteur et al., 2013). A suitable inoculum can increase the degradation rate, enhance biogas production, shorten the starting time, and more stable digestion process (Lettinga et al., 1996). Gu et al. (2014) has tried various inoculum sources for the AD of rice straw and reported that digested manure produced the highest biogas amount compared to all other manures.

Pretreatment improves hydrolysis thus lowering the hydraulic retention time required. It was also found to improve the biogas yield. Pretreatment makes it possible to go for smaller reactor volumes thus making AD more economical. Ariunbataar et al. (2014) found that a thermal pretreatment of FW at 80°C for 1.5 h increased the methane gas production by 52%. Qiao et al. (2011) evaluated the biogas production from various biomass wastes with and without autoclave pretreatment. The work concluded that at a temperature of 170°C and an exposure time of 1 h the biogas yield from pig manure, cow manure, fruit/vegetable waste, and sewage sludge increased by 7.8, 13.3, 18.5 and 65.7% respectively. Using microwave pretreatment at 140°C and 30 min to meat processing wastewater an increased volatile solids (VS) solubilisation of 8.54% more than the untreated sample was achieved (Erden, 2012). This study also found that a combined microwave and alkali pretreatment gave a 43.5% higher solubilisation than the control. The biogas yield increased by 23.6% for microwave pretreated sample, while the combined alkaline and microwave pretreated sample showed an increase of 44.5%. Alkali pretreatment improved the soluble COD content of slaughterhouse solid waste to 1.94 fold higher than the untreated sample (Juarez et al., 2014). It was also found that the ratio of soluble COD and total COD increased from 0.3 to 0.7. Alkali pretreatment of pulp and paper sludge was found to improve the methane yield by 83% when compared to the untreated sample (Yunqin et al., 2009).

For treating solid waste from organic industries different kinds of digesters are in use. The retention time reported was varying from 13-22 days (Barker 2001). The applied solid content in association with the substrate loading rate is critical to the cost, performance and stability of anaerobic solid waste digesters (Lissens et al 2001; Alvarez and Liden 2008). However, the maximum CH_4 production rate decreased with increasing solid contents in the range of 1-10%. Heo et al., (2004) evaluated the biodegradability of

a traditional Korean FW consisting of boiled rice (10-15%), vegetables (65-70%), meat and eggs (15-20%) and showed a methane yield of 0.49 L/g VS at 35℃ after 40 days retention time. Zhang et al. (2007) analyzed the nutrient content of FW from a restaurant, showing that the FW contained appropriate nutrients for anaerobic microorganisms, as well as reported a methane yield of 0.4 L/g VS of FW in batch digestion test under thermophilic conditions (50℃) after 28 days. The concept of two/multi stages systems offers optimization of the digestion conditions by providing separate reactors for each step. The conditions in the first reactor are adjusted to favour the growth of organisms that are capable of breaking down biopolymers and releasing fatty acids (hydrolysis/acidification). The product of the first reactor is then passed to the second reactor, where methanogenesis occurs (Schober et al., 1999). The potential drawback of two/multi stages systems is the decrease in biogas yield due to solid particles removal from the feedstock in the second stage (Vandevivere et al., 2002). Although theoretically two/multi stage systems have the advantage of increase in both rate of conversion and extent of utilization of polymeric biomass material, the full scale application is very moderate. The industrialists prefer one-stage systems because they have simpler designs, suffer less frequent technical failures and have smaller investment costs. Moreover, for most organic waste, the biological performance of one stage systems is as high as that of two stage systems if the reactor is well designed and operating conditions are carefully chosen (Vandevivere et al., 2002). Hence, there are only few literatures available on the treatment of FW through different inoculum, pretreatment methods and waste combinations in anaerobic batch and continuous operation.

Therefore the objective of the present study was to treat the food waste using anaerobic digestion. Studies do conducted to find the optimum livestock inoculum to attain higher methane yield at different F/M ratio and the optimum pretreatment technique for food waste. In addition, studies have been conducted on design and optimization of continuous anaerobic reactor (0.7 m^3) for more efficiency in lesser days.

1.2 NEED OF THE STUDY

FW could not be disposed off in landfill sites due to high volatile solids content; the same has high potential for bio-energy generation. AD is the best option for the treatment of FW to recover biogas. FW can be hydrolysed quickly; methane-producing

bacteria are needed in abundant in inoculum which has been added to utilize the VFA present at high concentration (Lesteur et al., 2010). Even though many studies have been done in the field of AD of FW, very little research has been done in the field of source of inoculum. This study will enhance AD of FW from acidification of reactor and higher methane yield.

If FW contains more amounts of lipids and proteins such as cooking oil, fats, bones fibers, etc. thus making hydrolysis the rate limiting step. Pretreatment aims at improving the hydrolysis part of AD. It enhances the biogas as well as methane yield; it lowers the hydraulic retention time and reducing the required reactor volume. Hence, there is a need to study the design and operation of proper continuous reactor to enhance biomethanization process.

1.3 OBJECTIVE OF THE THESIS

The main objective of the study was to treat the food waste using anaerobic digestion. Studies do conducted to find the optimum livestock inoculum to attain higher methane yield at different F/M ratio and the optimum pretreatment technique for food waste. In addition, studies have been conducted on design and optimization of continuous anaerobic reactor (0.7 m^3) for more efficiency in lesser days. The scope of the thesis is limited to:

1. Biochemical methane potential (BMP) test of food waste for different F/M ratio with digester sludge and to study the effect of nitrogen addition on optimum F/M ratio.
2. Biochemical methane potential (BMP) test of food waste for different F/M ratio with different livestock dungs. Batch studies for optimum F/M ratio of digester sludge and different livestock dungs.
3. Pretreatment of food waste using hot air oven, microwave, autoclave, alkali and electrohydrolysis and batch studies for optimum pretreatment method using optimum livestock dungs as inoculum.
4. Design, fabrication and operation of lab scale continuous anaerobic reactor for AD of digester sludge and optimum livestock dung. Microbial diversity and kinetics studies for the lab scale continuous anaerobic reactor.
5. Fabrication and operation of pilot scale continuous reactor and its feasibility

studies.

1.4 SCOPE OF THE THESIS

The major scope of the work is to collect food waste, digester sludge and different livestock dung and to digest it anaerobically using different techniques. In the first attempt, the experimental set-up for the physico-chemical analysis and learning the protocols and operation of instruments was performed. Secondly, the performance of the BMP setup on the basis of volatile solids and different F/M ratio of FW and different inoculum was studied. This was followed by the batch studies for the optimum F/M ratio of different inoculum for the improvement of treatment efficiency. The scope also takes account of the pretreatment of FW using different methods to enhance the hydrolysis process for lower retention time. Comparison study of the efficiencies of batch reactors that run on substrates pretreated using optimum pretreatment. Thirdly, the optimum inoculum and optimum F/M ratio will be studied for the designed and fabricated continuous bi-phased baffled reactor studies and microbial succession using DNA extraction method. Finally, pilot reactor studies will be performed for optimum HRT days.

1.5 THESIS ORGANIZATION

The report has been organized in following chapters:

- Chapter 1 gives brief introduction of food waste generation and its problem in environment, anaerobic digestion of organic waste and objectives of thesis.
- Chapter 2 gives detail literature review of anaerobic digestion process, processing of food waste by different types of anaerobic process, optimum conditions required for anaerobes metabolic activity, different pretreatment techniques and different reactor studies for solid waste anaerobic digestion.
- Chapter 3 deals with collection and initial characterizations of raw materials such as food waste, digester sludge, poultry dung, goat dung, cow dung, piggery dung and rhinoceros dung. Experimental design of phase 1, 2, 3, 4, 5, 6 and 7 given in flow charts. The detail procedures for physico-chemical, biological analysis and molecular biology techniques are provided.
- Chapter 4 presents detail results and discussion during Biochemical methane potential study and batch study of food waste with different inoculum.

- Chapter 5 gives detail results and discussion during Pretreatment of food waste using hot air oven, microwave, autoclave, alkali and electrohydrolysis and Batch studies for optimum pretreatment method using optimum livestock dungs as inoculum.
- Chapter 6 contributes detail results and discussion during lab scale and pilot continuous reactor operation with optimum inoculum concluded from batch study and the microbial succession during lab scale continuous anaerobic digestion through 16s rRNA gene sequence method.
- Chapter 7 lists the conclusions and recommendations of the thesis.

CHAPTER 2

LITERATURE REVIEW

This chapter presents detail review on the research carried out in anaerobic digestion processes and problems associated in anaerobic digestion of food waste. Chapter also deals with detailed work in anaerobic reactors, optimum conditions for microbial activity and pretreatment methods for hydrolysis process.

2.1 ANAEROBIC DIGESTION PROCESS AND BIOCHEMICAL REACTIONS

When the anaerobic digester works properly, the conversion of the intermediate products (i.e. the products of the first three steps) is virtually complete, so that the concentrations of these are low at any time. In the hydrolysis process, macro molecules such as proteins, polysaccharides and fats that compose the cellular mass of the microorganisms are converted into smaller molecules that are soluble in water: peptides, saccharides and fatty acids. The hydrolysis or solubilization process is done by exo-enzymes excreted by fermentative bacteria. Hydrolysis is a relatively slow process and generally it limits the rate of the overall AD process. Polymers are transformed into soluble monomers through enzymatic hydrolysis.

$$nC_6H_{10}O_5 + nH_2O \xrightarrow{Hydrolysis} nC_6H_{12}O_6 \qquad (2.1)$$

The reaction 2.1 is catalyzed by extracellular microbial enzymes known as hydrolyses or lyses. Depending on the type of the reaction they catalyze, these hydrolyses can be esterase, glycosidase or peptidase. For example, lipases hydrolyze the ester bonds of lipids to produce fatty acids and glycerol. Lyses, on the other side, catalyze the non-hydrolytic removal of groups from substrates (Noike et al., 1985). The major class of anaerobic bacteria degrading cellulose include *Bacterioides succinogenes, Clostridium lochhadii, Clostridium cellobioporus, Ruminococcus flavefaciens, Ruminococcus albus, Butyrivibrio fibrosolvens, Clostridium thermocellum, Clostridium stercorarium* and *Micromonospora bispora.* The dungs of various animals such as cow, pig, etc. have been used as an inoculum in anaerobic digestion of food waste. The

anaerobes present in the dungs belong to the digestive system of the species. The predominant bacteria found to degrade the hemicelluloses in the rumen are *Bacterioides ruminicola. Bacteriodes fibrisolvens, Ruminococcus flavenfaciens* and *Ruminococcus albus* (Noike et al., 1985). The second step of the AD process is acidogenesis or acidification as given in reaction 2.2, a process that results in the conversion of the hydrolyzed products into simple molecules with a low molecular weight, such as VFA (e.g. acetic-, propionic- and butyric acid), alcohols, aldehydes and gases such as CO_2, H_2 and NH_3. Acidification is affected by a very diverse group of bacteria, the majority of which are strictly anaerobic, i.e. the presence of oxidants such as oxygen or nitrate is toxic.

The presence of oxygen utilizing bacteria is important to remove all oxygen that might be introduced into the system. The acidogenic bacteria are able to metabolize organic material down to a very low pH of around 4. Fig. 2.1 details about the anaerobic digestion of organic materials. The monomers results of first reaction become substrates for the microorganisms in the second stage where they are converted into organic acids by a group of bacteria. In the third step, acetogenesis, the products of the acidification are converted into acetic acids, hydrogen, and CO_2 by acetogenic bacteria. The first three steps of AD are often grouped together as acid fermentation. It is important to note that in the acid fermentation, no organic material is removed from the liquid phase: it is transformed into a form suitable as substrate for the subsequent process of methanogenesis.

$$nC_6H_{12}O_6 \xrightarrow{\text{Acid form bacteria}} 3nCH_3COOH \qquad (2.2)$$

In the final step of the AD process as in reaction 2.3, the products of the acid fermentation (mainly acetic acid) are converted into CO_2 and CH_4 by acetoclastic methanogens. Only then will organic material be removed, as the produced methane gas will largely desorbs from the liquid phase. In each of the four sequential steps, the catabolic reactions described above develop together with anabolic activity.

$$CH_3COOH \xrightarrow{\text{Methane forming bacteria}} CH_4 + CO_2 \qquad (2.3)$$

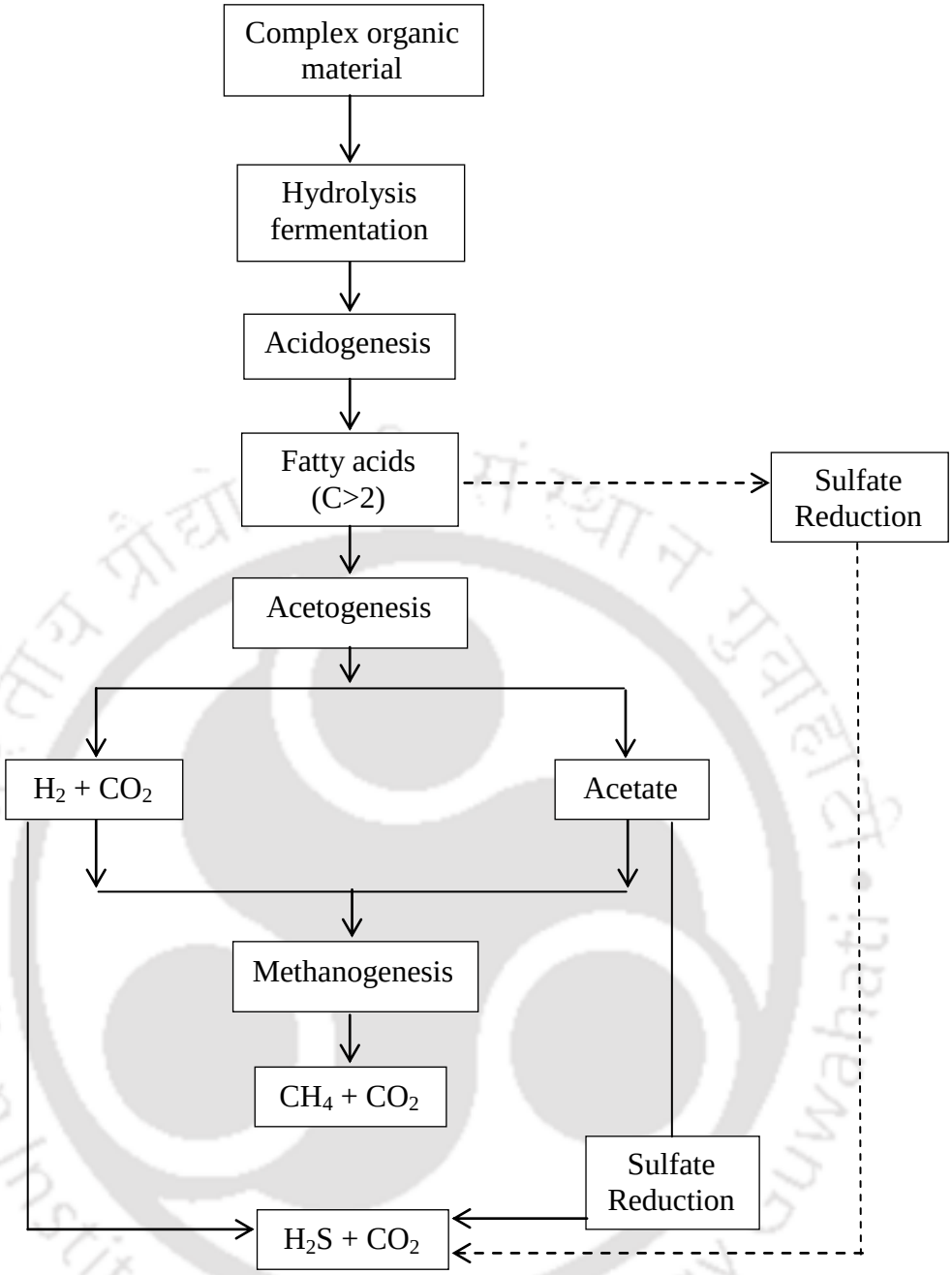


Fig. 2.1 Anaerobic digestion processes for recovery of biogas

The free energy exhausted from the reactions is partially utilized for synthesis of the anaerobes populations. Therefore, a large fraction of the digested organic matter is converted into biogas. These organic acids primarily acetic acid form the substrate for the third-stage. In the third step, CH_4 can be generated in two routes, fermenting acetic acid to CH_4 and CO_2 by acetoclastic methanogens and using CO_2 as a source of carbon and hydrogen as a reducing agent by hydrogenotrophic methanogens or formate generated by other bacterial species as given in reactions 2.4, 2.5 and 2.6 (Kouichi et al.,

2010). The most commonly found methanogens genera, in the biogas reactors are *Methanobacterium, Methanothermobacter* (formerly *Methanobacterium*), *Methanobrevibacter, Methanosarcina,* and *Methanosaeta* (formerly *Methanotrix*) (Sean et al., 2006). Similarly CO_2 can be hydrolysed to carbonic acid and to methane

$$CO_2 + 4H_2 \xrightarrow{\text{Reduction}} CH_4 + 3H_2O \quad (2.4)$$

$$CO_2 + H_2O \xrightarrow{\text{Hydrolysis}} H_3CO_3 \quad (2.5)$$

$$4H_2 + H_2CO_3 \xrightarrow{\text{Reduction}} CH_4 + 3H_2O \quad (2.6)$$

The CO_2 in the biogas are undesirable. They are removed for optimum performance of biogas as fuel. CO_2 is removed by passing the gas into lime water which turns milky due to formation of calcium carbonate. H_2S is also another undesirable in the biogas due to presence of sulphate in the substrate. But the percentage of H_2S present in biogas varies according to sulphate percentage.

For the simplest case, the conversion of carbohydrates, such as sugars (e.g., glucose, and starch or cellulose, an equal amount of CH_4 and CO_2 is produced (eq. 2.7).

$$C_nH_n\text{-}2O_{n\text{-}1} + nH_2O \longrightarrow ½\ nCH_4 + ½nCO_2 \quad (2.7)$$

In the case wastes containing proteins or fats, a larger amount of methane is produced, stoichiometrically from the complete degradation of the substrate. For proteins, the process is as eq. 2.8.

$$C_{10}H_{20}O_6N_2 + 3H_20 \longrightarrow 5.5\ CH_4 + 4.5\ CO_2 + 2NH_3 \quad (2.8)$$

This yields a CH_4:CO_2 ratio of 55:45; the exact biogas composition will depend on the individual substrate protein. For fats and vegetable oil (triglycerides), a typical CH_4:CO_2 ratio is 70:30.

$$C_{54}H_{106}O_6 + 28\ H_2O \longrightarrow 40\ CH_4 + 17\ CO_2 \quad (2.9)$$

2.2 FOOD WASTE AS A SUBSTRATE

The degradability rate of the substrate greatly depends on the composition of the input material considered for the digestion. It is also very difficult to estimate or measure the percentage of carbohydrates, lipids, and proteins in a heterogeneous substrate such as

FW due to the dynamic and sensitive nature of the biological process to the input composition. There are several techniques available to estimate the Biochemical methane potential (BMP) of the complex substrate such as FW including ultimate analysis (carbon, nitrogen and hydrogen), molecular formula (if known), computer simulation, and a literature review of experimentally determined biogas yields (Braun et al., 2003; FAOUN, 2011). As waste analysis is one of the most important steps in the AD process, knowing the general composition of the input material to the system is essential for calculating the amount and composition of the biogas produced as well as the amount of energy contained in the biogas.

Cho et al. (1995) did a laboratory experiment on BMP and solid state AD of korean FW (Boiled rice 73%, meat 6%, fried egg 9%, and rest of vegetables and sprouts). The study concluded that, the methane yield and anaerobic biodegradability of Korean mixed food waste are high: 472 mL CH_4/g VS added and 86%, respectively and to perform solid-state anaerobic digestion of Korean food wastes discharged at 15-30% TS successfully, the VFAs produced rapidly at the initial stage of fermentation need to be controlled using a two-phase digestion method.

Kim et al. (2006) evaluated the effect of temperature and hydraulic retention time on anaerobic digestion of food waste in three stage reactor in which *clostridium* species were isolated and again was inoculated to find the better performance. The results showed that the 12 d HRT in thermophilic condition produced maximum methane yield (223 L CH_4/kg soluble cod $_{degraded}$) and provides a best way for the anaerobic digestion of food waste with an elemental composition of C:47.8%, N:5.2%, O:40.9%, H:6.1%.

Liu et al. (2009) stated the effect of F/I ratio on biogas yields of food waste, green waste and their mixture in thermophilic and mesophilic conditions. The results showed a negative relation between biogas yield and the F/I ratios in the range of 1.6–5.0. At 50^{o}C and an F/I of 1.6, the biogas yields of 778, 631 and 716 mL/ g VS could be obtained, respectively, for food waste, green waste and their mixture after 25 d of digestion. The author concluded that the biogas yield was much better in thermophilic conditions compare to mesophilic conditions.

2.3 OPTIMUM CONDITIONS REQUIRED FOR ANAEROBES METABOLIC ACTIVITY

To maintain anaerobes with a high metabolic activity, it is necessary to have

controlled environmental conditions. The methanogens are very sensible towards adverse environmental conditions so it is very important to maintain optimal conditions for these microbes. The rate of biogas production depends: the nature of the substrate, total solids (TS), temperature, pH, toxicity, mixing, nutrients, slurry concentration, retention time, digester type, carbon to nitrogen ratio, alkalinity, initial feeding, volatile solids (VS), chemical oxygen demand (COD), etc. Anderson (1979) has stated that the concentration of water-soluble substances such as sugar, amino acid, proteins and minerals decrease with age of plants. This is because that non-water soluble substances such as lignin, cellulose, hemi cellulose and polyamides increase in content with the age of the plant. Therefore the vegetative matter from younger plants produces more biogas compared to those from the older plants. For waste products from animals, the type and age of animal, its feeding and living conditions, the age and storage of the waste product are factors affecting the quality and quantity of the gas produced. Generally finely ground waste products is said to produce more biogas due to large surface area of contact with microbes.

Table 2.1 Optimum conditions required for anaerobes metabolic activity

Parameters	**Optimum conditions**	**References**
Temperature	Mesophilic range (35 - 40^{o}C) Thermophilic range (50 - 65^{o}C)	Ljupka Arsova (2010)
pH	6.3 - 7.8	Van Haandel and Lettinga (1994)
Carbon-nitrogen ratio	25 - 30	Xiaojiao et al. (2012)
Volatile Fatty Acid	2000 - 3000 mg/ L	Ghosh and Pohland (1974)
Organic Loading rate (OLR) and Nutrient concentration	Varies according to the substrate and inoculum	--

2.3.1 TEMPERATURE

Anaerobic process is so sensible to temperature; change of acetic acid to methane depends mostly on temperature but conversion to acetic acid won't affect much by slight temperature variations. Speece (1996) has reported that the environmental temperature has a major influence on the anaerobic microbial systems, which affects the metabolic rate, ionization equilibria, substrate solubility and fats and bioavailability of iron. Higher temperature affects the activity of hydrogenotropic methanogens in the anaerobic process and enriches hydrogen producing bacteria and spore forming bacteria (Cecchi et al., 1989). The optimum temperature for anaerobic digestion is represented in Table 2.1.

2.3.2 CARBON-NITROGEN RATIO

Nitrogen is the major nutrient for the growth of microbes. The amount of nitrogen uptake by the aerobic and anaerobic microbes varies according to their nature. The review stated that bacteria in anaerobic digestion use the carbon 25-35 times higher than nitrogen. For better digestion, the ratio of the carbon to nitrogen should be about 25-30:1 in the substrate. Nitrogen is one of the major limiting nutrient in the treatment of MSW, due to that other sources such as manure, clean sewage-sludge (bio solids), and urea can be used as a supplementary source. If the nitrogen content is low, microbial populations remain less and it might take longer duration to digest the available carbon. Excess nitrogen, may also cause problem of ammonia formation which affects the anaerobic process. For solid wastes with a high C/N ratio, the ammonia inhibition effect can be controlled by dilution with water; it decreases the concentration of ammonia toxicity. The concentrations of carbon and nitrogen indicate the performance of the anaerobic digestion process. Whereas carbon acts as the energy source of the microorganisms and nitrogen plays a role in enhancing microbial population. Nitrogen (Nitrate) can be utilized in two ways by the microbes in anaerobic digestion such as assimilatory (nitrate used as electron acceptor converted to nitrogen gas also called as denitrification) and dissimilatory (nitrate converted to ammonia also called as ammonification). More nitrate addition leads to ammonification and less leads to nitrogen deficiency. By this way nitrogen controls the microbial population.

2.3.3 VOLATILE FATTY ACID (VFA)

The different concentrations of VFA (equivalent to acetic acid) in an anaerobic batch system were shown to have a derivative effect on each phase of the hydrolysis, acidogenesis and biogas production related to the AD process. Apart from pH, VFA of

the reactor inhibits the cellulolytic activity at concentrations ≥2 g/L. Therefore, the rate of cellulose hydrolysis and the glucose fermentation also gets affected above 4 g/L thereby having inhibitory effect on the production of biogas. In addition CH_4 to CO_2 ratio gives much difference above 6 g/L VFA if cellulose and glucose is used as major source (Siegert and Banks, 2005).

In the co-digestion of cellulose waste material with paper as primary substrate, biogas production was reduced more than a half due to 1 g/L initial VFA, showing inhibition of the hydrolysis process. In case of glucose as primary substrate biogas production was more than halved when VFA was above 8 g/L which indicated that the digestion process was less sensitive to inhibition by VFA (Komisar et al., 1998). The accumulation of VFA in specific place results in deranged microbial consortia, which leads to failure of the anaerobic digestion process operation (Labatut et al., 2011; Vijayaraghavan et al., 2012).

2.3.4 ORGANIC LOADING RATE (OLR) AND SOLID RETENTION TIME (SRT)

The relative residence time needed for full degradation and the limit of anaerobic microbes to degrade the substrates can be carried out by BMP assay (Yebo et al., 2011). The investigation on the effects of stepwise increase in OLR and SRT on integrated two-stage process stated that at steady state, the optimal OLR and SRT were found to be 22.65 kg vs/m^3 d (160 h) for hydrogen fermentation reactor and 4.61 kg vs/m^3 d (26.67 d) for methane fermentation reactor, respectively (Norio et al., 2012). Stable operation of single stage wet AD of FW at an OLR of 9.2 kg VS (15.0 kg COD)/m^3/d was achieved with a high VS reduction (91.8%) and high methane yield (455 mL g/VS/d). Norio et al. (2012) stated that the cell density increased in the periods without organic loading, and reached to 10.9×10^{10} cells m/L after 187 d, which was around 15 times higher than that of the seed sludge.

2.3.5 NUTRIENT CONCENTRATION

Trace inorganic elements such as iron, nickel, cobalt, and zinc have to be in desired concentrations to initiate the digestion process. Wastes are different in their nutrients concentration, so for the proper function of AD an appropriate amount of inorganic elements have to be added. Finding the nutrient requirements can be tough and it depends on characteristics of the waste, nutrients availability, the reactor design and other parameters (Raposo et al., 2011).

2.4 BIOCHEMICAL METHANE POTENTIAL (BMP)

BMP is the important and valuable assay for the interpretation of AD. In recent years, BMP tests have increased which is evident from the broad band of research carried out with BMP assays (Ruihong et al., 2009). The BMP assay results of Korean mixed FW digestion in comparison to other individual FW such as boiled rice, fresh cabbage and cooked meat digestion with cellulose as control has shown higher methane production (472 mL/g VS) with 86% reduction of total VS (Cho et al., 1995). From the analysis of FW collected in the city of San Francisco, California it was stated that the nutrient contents were well balanced for the growth of anaerobic microorganisms for the production of methane and the methane yields of 348 and 435 mL/g VS after 10 and 28 d digestion (Ruihong et al., 2009). The AD of Fruits and Vegetable Solid Waste produced methane with a minimum of 0.3 L/g VS added in every sample which shows the commercial value in AD (Nallathambi, 2004).

In the AD of Bermuda grass, the conversion of cellulose to simpler organic was higher than hemicellulose by the supply of external nitrogen both in mesophilic and thermophilic conditions and it requires least amount of enzymes and energy (Ghosh and Henry, 1985). From the co- digestion of FW with piggery wastewater suggested the addition of trace elements increased the biogas production in FW AD and results a high methane yield of 0.396 m^3/kg VS added and 75.6% VS destruction (Lei et al., 2011). BMP experiment was conducted with four different food waste (meatball, chicken, cranberry and ice cream processing wastes) for co-digestion with flushed dairy manure at a ratio of 3.2% food waste and 96.8% manure (by volume), which equated to 14.7% (ice-cream) to 80.7% (chicken) of the VS being attributed to the food waste to find the suitability for anaerobic digestion. All experiments showed increase in methane production from 67.0% (ice cream waste) to 29.4% (chicken processing waste) compared to digesting manure as single substrate. It proves high methane potential for food waste additions compared to low methane production potential of the flushed dairy manure (Maria and Stephanie, 2013).

2.5 BIOGAS COMPOSITION AND SPECIFIC GAS PRODUCTION

Sankar et al. (2007) reported that the Upflow anaerobic filters attached with solid feed anaerobic produced 2 m^3 of biogas/m^3 of reactor volume per day, whereas the conventional biogas digester produced 0.1 - 0.2 m^3/m^3 volume of digester volume per

day. Chea et al. (2009) investigated the AD of organic fractions of MSW in two phase reactor with continuous operation. During the reactor's startup period, the process was stable and there was no occurrence of inhibition as methane composition leveled off at 66% with higher rate of biogas production and the reactor was fed in continuous mode and methane content of the biogas reduced to 30-40%. Ghangrekar et al. (2005) evaluated the effect of different biogas production rates on UASB reactor performance and on the characteristics of the sludge developed and observed that biogas yield higher than 0.7 m^3/m^3vs .d was sufficient to carry out natural mixing inside the reactor. However, very high biogas yield, greater than 2.3 m^3/m^3 VS.d was observed to be unfavorable for determining the requisite sludge age and necessary strength of granules.

2.6 PRETREATMENT PROCESS

AD is now practiced widely for the volume reduction of sludge and energy recovery. Several advantages such as low energy requirement, low sludge production, low nutrient requirements are advantages of AD. Also AD does not disturb the stabilized heavy metals in the sludge and hence can be safely disposed into landfill sites. Different pretreatments can be utilized for solid waste such as mechanical (i.e. ultrasound, high pressure and lysis), thermal, chemical (i.e. ozonation, alkali) and biological pretreatments (Xing and You-cai, 2009; Carrere et al., 2010). The sludge pretreatment is possible to reduce the necessary retention time of sludge for digestion, the final quantity of the sludge and also enhances biogas production (Valo et al., 2004, Yebo et al., 2011).

The effects on biodegradability of substrate vary depending on its characteristics for example lignocellulose substrates such as agricultural waste, Paper and pulp waste requires more time for its hydrolysis which affects biodegradability however in case of easily available organics such as food waste, glucose takes less time to degrade in compare. Pretreatment effects for waste from food industry are mainly correlated with the organic wastes of slaughterhouse because of its organic composition (Cozzolino et al., 1992; Baldasano and Soriano, 1999; Salminen et al., 2003; Hejnfelt and Angelidaki, 2009; Luste et al., 2009) or it's also correlated with waste from the dairy industry (Palmowski et al., 2006; Battimelli et al., 2009). Some of the pretreatments have been in application are thermal and chemical, followed by ultrasonic and microwave pretreatments. Particle-size reduction of waste from the food industry is induced by

chemical and ultrasonic pretreatments, while solubilisation results from all pretreatment types applied.

Effects from the pretreatment of waste from the food industry are varying and highly dependent on both the pretreatment mechanism and the substrate composition. Thermal pretreatment (70 and 133^{o}C) and chemical (alkali) pretreatments for slaughterhouse waste was not found effective, because of its higher biodegradable nature (Baldasano and Soriano, 1999, Hejnfelt and Angelidaki, 2009). In contrast, Luste et al. (2009) found a significant increase in soluble COD from chemical (acid and alkali) as well as ultrasonic and low temperature thermal (70^{o}C) pretreatment of different substrates from the meat processing industry. The solubilisations due to pretreatment leads enhanced biodegradability in few cases, but in some cases combinations of pretreatment and substrate the biodegradability gets decreased due to inhibitory intermediate formation (Luste et al., 2009). Formation of toxic compounds was also reported from the high temperature thermal pretreatment (133^{o}C, >3 bar) (Cozzolino et al., 1992).

2.6.1 THERMAL PRETREATMENT

Bougrier et al. (2007) reported the impacts of thermal pretreatment on the AD of waste activated sludge. It was found that thermal treatment at 190^{o}C gave better degradation then at 135^{o}C in terms of total COD, lipids, carbohydrates and protein removals and methane production. However, treatment at 190^{o}C produced refractory soluble COD. In every case, with or without pretreatments, lipids degradation yield (67% without pretreatment and 84% with 190^{o}C treatments) was higher than carbohydrates (56% without pretreatment and 82% with 190^{o}C treatments) and proteins (35% without pretreatment and 46% with 190^{o}C treatments) degradation yields. Methane production was seen to increase by 25% after the 190^{o}C treatment.

Thermal pretreatment was initially used for sludge dewaterability and allows the degradation of gel structure (Carrere et al., 2008). It also leads to the solubilization of complex sludge particulates and enhances AD (Haug et al., 1978; Bhattacharya et al., 1996; Borja et al., 2003). Hydrolysis which is the rate limiting step can be enhanced and the retention time for AD could be reduced by thermal pretreatment. An optimum temperature of 160^{o}C-180^{o}C and treatment duration of 30-60 minutes was reported for sewage sludge (Van Haandel and Lettinga, 1994; Tanaka et al., 1997; Tiehm et al., 1997). Pretreatment at moderate temperature of 70^{o}C has also been reported but it takes a longer pretreatment time (Gavala et al., 2003; Ferrer et al., 2008). The enhancement in

biogas production by thermal pretreatment is related to COD solubilization (Bougrie et al., 2007). Salminen and Rintala (2002) reviewed many pretreatment techniques to increase methane production from feather by combined thermal and enzymatic treatments and reported an increase in methane yield of 37-51% whereas thermal (70^{o}C to 120ºC), chemical (NaOH: 2-10g/ L, 2-24 h) and enzymatic treatments were not much efficient in yielding methane which increased only in the range of 5-32%.

- **Hot air oven**

Ariunbataar et al. (2014) studied on the thermal pretreatment of food waste using a simple oven. At first, the methane yield after thermal pretreatment for 1 h at various temperatures such as 70, 80, 100, 120 and 140°C were analysed. Then after selecting the optimum temperature based on the results, thermal pretreatment was given at the best temperature for different times of exposure such as 1, 1.5, 4 and 8 h. This paper concluded that a temperature of 80°C and a time of exposure of 1.5 h was optimum for thermal pretreatment using a hot air oven.

- **Microwave oven**

Wei et al. (2009) worked on the anaerobic digestion of microwave pretreated sludge. The parameters considered for the study were volatile suspended solids and soluble COD. The study was done at various temperatures such as 80, 120, 150 and 170°C with time of exposures of 1, 5, 10, 20 and 30 min. It was observed that the highest VSS solubilisation was observed at 170°C with a time of microwaving of 30 min, whereas the highest COD dissolution was observed at 170°C with a time of exposure of 10 min.

Shahriari et al. (2011) investigated the effects of pretreatment on the anaerobic digestion of organic fraction of municipal solid waste. In this study, the samples were microwaved at 115, 145 and 175°C with a constant ramp time of 40 min and a 1 min of holding time after attaining the required temperature. The soluble COD was highest for the sample pretreated at 175°C but it was observed that the biogas yield actually decreased for 175°C pretreated sample. This was attributed to the formation of refractory compounds called melanoidins which decrease the biodegradability of COD. It was also found that the sample pretreated at 145°C had the highest cumulative biogas production.

Erden (2012) studied the effect of microwave and combined alkaline with microwave pretreatment on the anaerobic digestion of meat processing wastewater sludge using RSM (Response surface methodology). An optimum temperature and time of exposure of 140°C and 30 min were used for the pretreatment. The results showed

that the solubilisation of volatile solids was 8.5% higher than untreated for microwave pretreatment and 42.5% higher for combined alkaline and microwave pretreatment. The biogas yield increased by 23.6% for microwave pretreated sample, while the combined alkaline and microwave pretreated sample showed an increase of 44.5%.

- **Autoclave**

Li and Noike (1992) investigated the enhancement of anaerobic digestion of waste activated sludge using autoclave pretreatment. This study selected temperatures in the range of 62 to 175°C and time periods of 15, 30, 60, 90 and 120 min. It was found that the optimum temperature and time period for pretreament was 170°C and 60 min respectively.

Tampio et al. (2013) worked on the effect of pretreatment using autoclave on food waste and its comparison to an untreated sample. The autoclave pretreatment was given at a temperature of 160°C and pressure of 6.2 Bar. It was observed that methane yield from the autoclaved food waste sample was lesser than the untreated one. Even though the soluble COD of the autoclaved sample higher than that of the untreated one, this didn't reflect in the biogas yield. It was concluded that at 160°C, refractory compounds called Maillard compounds were formed which are not readily decomposable.

2.6.2 MECHANICAL PRETREATMENT

Comminution of organic materials through a combination of grinding, and/or milling can be applied to reduce cellulose crystallinity. The size of the materials is usually 10-30 mm after chipping and 0.2-2 mm after milling or grinding. Kouichi et al. (2010) has reported that AD batch experiments revealed that particle size has been reduced by bead milling at 1000 rpm increased the methane yield by 28% in comparison with other disposer treatment. FW depicted the mean particle size of substrates ground decreased from 0.84 to 0.39 mm by the results of bead mill pretreatment for particle size reduction and solubilization increased approximately 40% of the total COD by bead milling. In order to increase the solubilization of organic matter and reduce the size of the fleshings. Various pretreatment methods were reported to increase the biogas recovery and to reduce retention time in the reactor. Mincing the fleshings with cutter and feeding it into conventional anaerobic digester is one of the studies reported by Shanmugam and Horan (2009). Pretreatment of the substrate by mechanical disintegration (size reduction of particles) had positive effects on the anaerobic biodegradability of the substrate. The

obvious reason is the increase of the available specific surface to the medium (Hejnfelt and Angelidaki, 2009).

2.6.3 ULTRASONIC PRETREATMENT

Ultrasonic treatment works similar to mechanical pretreatment which disrupts the cell structure and flocs. Two mechanisms linked with ultrasonic treatment; cavitation, which happens at low frequencies, and chemical reactions due to the release of $OH^{\bullet}$, $HO_2^{\bullet}$, $H^{\bullet}$ radicals at high frequencies. In sludge treatment, low frequencies between 20 to 40 kHz were most effective (Carrere et al., 2008). Ultrasound pretreatment of sewage sludge at 31 kHz for 64 seconds has reduced the retention time of AD from 22 to 8 days and a VS removal of 44% as reported by Tiehm et al. (1997). This method has been considered for sludge pretreatment but the energy needed is high. Comparative study of the effect of ultrasonication on the anaerobic biodegradability of food waste in single and two-stage systems was conducted by Elsayed and Nakhla (2011). The results showed the ultrasonication in first stage hydrogen reactor followed by methane reactor yeilds more hydrogen (4.8 L $H_2/L_{reactor}$) and methane production (3.2 L $CH_4/L_{reactor}$) (Elsayed and Nakhla, 2011).

2.6.4 OZONATION

Ozonation can be carried out as a pretreatment for sludge prior to AD (Yeom et al., 2002). Weemaes and Verstraete (1998) reported Ozonation pretreatment for mixed sludge which led to an increase of CH_4 production from 110 to 220 mL/g COD_{in} with a retention time of 30 days at a temperature of 33^{o}C. Ozonation pretreatment of sewage sludge led to an increase of methane production from 82 to 173 mL/g COD_{in} with a retention time of 30 d (Yeom et al., 2002). Ozonation pretreatment was not carried out for the FW due to readily degradable organic waste and it's not necessary in case of FW.

2.6.5 ALKALI PRETREATMENT

Alkali treatment is one of the best practice for complex matter solubilization, with the efficiency in order NaOH > KOH > $Mg(OH)_2$ and $Ca(OH)_2$ (Kim et al., 2003). But too high concentrations of Na^+ or K^+ may cause subsequent inhibition of AD (Neves et al., 2006). Dilute NaOH treatment of organic materials has been found to cause swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure. Alkali pretreatment has also been performed with combinations of thermal or ultrasonic pretreatment. The

studies by Yiying et al. (2008) report the combination of alkali and ultrasonic pretreatment for waste activated sludge. The solubilization of COD levels was higher than those with ultrasonic or alkaline pretreatment alone. Yunqin et al. (2009) studied the effect of alkali pretreatment using NaOH on the anaerobic digestion of pulp and paper sludge. From the dosage study, the optimum dosage was found to be 8 g NaOH/100 g TS_{sludge}. At this dosage it was observed that the soluble COD increased by 83% and the volatile fatty acid concentration attained a peak value of 1040 mg acetic acid/L. The gas measurements revealed that the methane yield of pretreated sample increased up to 1.8 fold of the control. This paper indicated that alkali pretreatment can improve the methane yield from the anaerobic digestion of pulp and paper sludge.

Zhu et al., 2010 investigated the enhancement of solid-state anaerobic digestion of corn stover using alkaline pretreatment. NaOH was selected as the alkali and dosage studies were conducted at various dosages of 1, 2.5, 5 and 7.5% (w/w). Optimum dosage was found to be 5% at which the biogas yield increased by 37% to reach 372 L/kg VS. At higher NaOH loadings, the volatile fatty acid production hastened inhibiting the methanogenesis and ultimately lesser biogas production.

Florez-Juarez et al. (2014) worked on the alkali pretreatment of slaughterhouse solid waste to increase the efficiency of anaerobic digestion. The study was conducted at two temperature ranges, psychrophilic and mesophilic. Alkali pretreatment using NaOH was carried out at various dosages ranging from 0.1 to 0.7 g/g VS. The time of exposure for the pretreatment was kept at 24 hours. The results revealed that the optimum dosage was 0.6 g/g VS, at which the soluble COD increased to 1.94 fold of the control. It was also observed that the ratio of soluble COD to total COD increased from 0.3 to 0.7 at this dosage. At the mesophilic range, a volatile solids removal efficiency of 47.2% was observed.

2.6.6 BIOLOGICAL PRETREATMENT

Biological treatment using various types of rot fungi, a safe and environmentally friendly method, is increasingly being advocated as a process that does not require high energy for lignin removal from a lignocellulosic biomass, despite extensive lignin degradation. Hee and Song (2011) investigated the effect of enzymatic solubilization of FW and methane production in upflow anaerobic sludge blanket reactor. The optimum conditions of FW hydrolysis were enzyme mixture ratio of 1:2:1 with carbohydrase: protease: lipase, respectively, 0.2% (w/w FW) of mixture dose, and 10 hours hydrolysis

reactions. Upto 95% of soluble COD removal efficiency with an observed methane gas yield of 350 mL-CH_4/g-Soluble COD and 9.1 g-Soluble COD/L/d of OLR. Raynal et al. (1998) have evaluated that hydrolysis rate from the COD removal in the anaerobic digester per unit time and expressed in g COD/L.d. However, few solid wastes have a low biodegradability in spite of the high COD content and, so further studies needs to look in the enhancement of biomethenization process of such wastes (Neves et al., 2006).

2.6.7 ELECTROHYDROLYSIS

Electrohydrolysis pretreatment can have serious effects on the structure of organic matter. When a high-intensity, external electric field is applied, a critical electric potential is induced across the cell membrane, which leads to rapid electrical breakdown and local structural changes of the carbon molecules. Tartakovsky et al. (2011) studied on the effect of electrolysis on the anaerobic digestion of wastewater. The electrodes were placed in the sludge bed of the reactor. A voltage between 2.8 and 3.5 V was continuously applied to the reactor. This provided continuous supply of oxygen and hydrogen into the reactor. The oxygen created micro aerobic conditions which helped in the hydrolysis of wastewater whereas one part of the hydrogen produced was converted to methane by methanogens and the other part improved the combustibility of biogas produced. It was observed that the methane yield had improved by 10 to 25% when compared to the control and also stability of the reactor had also significantly improved.

Zhen et al., 2014 investigated the effect of combined electrical-alkali pretreatment to enhance the hydrolysis of waste activated sludge during anaerobic digestion. The parameters considered for this study were degree of disintegration of soluble COD, total and volatile suspended solids removal, release of protein and polysaccharides. The voltages considered for this study were 5, 10, 15 and 20 V combined with samples with no pH adjustment, 9.2, 10.2, 11 and 12.2 respectively. This paper found that the optimum voltage and pH for combined electrical- alkali pretreatment was 5 V at a pH of 9.2. This condition had the highest methane yield with 20.3% improvement over the control after 42 days.

2.6.8 CO-DIGESTION OF WASTES

For the co-digestion of a mixture of 70% manure, 20% FW and 10% sewage sludge (TS concentration around 4%) at 36^oC, for an OLR of 1.2 g VS/L d the maximum value obtained was 603 L CH_4/kg VS feed (Maranon et al., 2012). Different pretreatment

methods have been discussed above in order to enhance its biodegradability. Co-digestion is another concept employed in AD in which the sludge is mixed with another waste substrate with high organic content and requires disposal (Braun at al., 2003). Carrere et al. (2010) reported that co treatment of sludge with other organic substrate can be done to enhance the AD. The co-digestion of fruit and vegetable waste and waste activated sludge in acidogenic Continuous stirred tank reactors and methanogenic inclined tubular digesters operated at 30°C were used. Optimized AD was reached in a two-stage system at an OLR of 5.7 kg VS/m^3/d, overall HRT of 13 days (3 days acidogenic HRT, 10 days methanogenic HRT), with 40% VS reduction and a system biogas yield of 0.37 m^3/kgVS added (Dinsdale et al., 2000).

2.7ANAEROBIC REACTORS

In past two decades much efforts has been committed towards the performance of AD especially in Organic solid waste treatment. In the treatment of solid waste problem was confronted in hydrolysis process particularly in complex polymeric substances (Eastman and Ferguson, 1981; Noike et al., 1985; Raposo et al., 2011). The applied solid content in association with the substrate loading rate is critical to the cost, performance and stability of anaerobic solid waste digesters (Riggle, 1998; Lissens, 2001). In continuous AD of slaughterhouse or co-digestion of slaughterhouse waste with manure, fruit and vegetable waste the specific CH_4 yield was found to be 351-381 mL CH_4/g VS.d such as 270 – 350 mL CH_4/g total VS in batch. The CH_4 production at 10% solids was less in comparison to 5% solids; it is due to excess substrate or less buffering capacity (Dinsdale et al., 2000). The researchers used many reactors such as single and two stage reactor, Upflow anaerobic solid state (UASS) reactor, semi dry anaerobic digester, solid state anaerobic digester and hybrid reactors for the treatment of organic waste which is well suitable for food waste treatment has been discussed below.

2.7.1 SINGLE AND TWO-STAGE ANAEROBIC REACTORS

In single stage reactor, all the four process hydrolysis, acidogenesis, acetogenesis and methanogenesis has taken place in the same reactor i.e. conversion of polymeric organic compounds to CH_4, H_2S, NH_3 and CO_2 are observed to occur in the same reactor. But in two-stage reactor, the hydrolysis and acidogenesis take place in initial reactor and the utilization of those acids by methanogenesis is anticipated to take place in the final

reactor. The separation of digesters as hydrolysers and methanizers changes the process dynamics of the digesters by allowing the individual bacterial species separately to perform the hydrolysis and methanogenesis. It has been found that the performance of two-stage anaerobic digesters is providing more efficient than the single stage digesters (Mata Alverez, 1987, Lissens et al. 2001). Instead of that many other researchers Viturtia et al. (1995), Bhattacharya et al. (1996), Vavilin and Lokshina (1996), Ong et al. (2000) and Veeken et al. (2000) reported that single stage are much better than two stage digesters particularly for proteinaceous waste. The amount NH_3 produced need to be sufficient for neutralizing the accumulated VFA and then it forms ammonium acetate or ammonium bicarbonate maintain favorable pH. More ammonia production must affect the process of methanogens by neutralizing all the VFA and affects CH_4 content in biogas.

Trouque and Forster (2000) has reported that VS reduction in three dual digestion configurations were similar, but it was more effective than single stage and two-stage with VS reduction of 60%, however with single stage it was low. The production of methane by hydrogenotropic methanogen using hydrogen produced during hydrolysis process, this process was disturbed in two stage reactors i.e. syntrophic relation gets affected between hydrogen formers and consumers which lead to reactor performance reduction (Stephen and Pohland, 1986). Costello et al. (1991a,b) observed that the two-stage reactors maintains pH for proper functioning of both hydrolytic acidogenic and methanogenic bacteria at pH 6 and pH 7 respectively. Kim et al. (2002) explained that two phase reactor has much better performance than the single phase reactor because it would not affect the slow growing methanogens by changes in environmental conditions. The authors have denoted that methane forming process was disturbed by lower temperature which might be due to the unbalanced reaction rate between acetogens and methanogens and confirmed two-phase was more beneficial than the single-phase reactor; it also proved in VS reduction efficiency.

Held et al. (2002) stated that segregation of organic waste in two common fractions and two stage anaerobic process of liquid portion in an Upflow Anaerobic Filter is a betting technology to present technology. It was expressed that, CH_4 content was higher in the second stage than the first stage. Ince et al. (1985) and Sosnowski et al. (2002) reported that the single stage system requires 37 d HRT whereas the two-stage requires 30 d HRT for maximum COD removal (80%). Mesophilic condition is recommended if

two-phase system is used for proteineous waste such as fish and meat and reported that NH_3 toxicity was observed to be the rate-limiting step even at low HRT continuous stirred tank reactor both in mesophilic and thermophilic condition (Guerrero et al., 1999). Hansen et al. (1998) reported that single phase system is more advantageous than the two phase system as denitrification and methanogenesis in a single reactor would be optimum for the neutralizing capacity of VFA and further it prevents from the drop in pH and VFA toxicity.

Two-phase system can be considered to be more advantageous over the single stage under different climatic conditions i.e. low summer and high winter. Furthermore, it can be stated that the hydrolysis of protein is the rate-limiting step for methogenesis. The hydrolysis of lipids and carbohydrates increased with increase in HRT, whereas protein hydrolysis occurred only in methanogenic condition and further revealed that hydrolysis was the rate limiting step for conversion of carbohydrates, while both hydrolysis and acidification were the rate limiting step for conversion of protein, hence two phase system was suggested for the protein enriched wastes (Miron et al., 2000). Dinsdale et al. (2000) have studied the suitability of two-phase system for fruit and vegetable waste and the acetogenic and methanogenic reactor was expected to operate successfully. The researchers have further reported that the stable two-phase system was achieved for vegetable and fruit waste with HRT of 3 d for acetogenic reactor and 10d for methanogenic digestion, which is again supporting the hydrolyser to methaniser.

Yu et al. (2002) reported that the efficient degradation of organic matter is dependent on coordinate metabolism of acid forming and CH_4 forming bacteria. It was emphasized that separating the optimum condition for each bacterial group can increase the anaerobic process stability and overall degradation rate and hence the two-phase system was considered more efficient than single-phase system. Kuba et al. (1990) has reported that the methanogenic phase outlet gives better quality in two phases than in the single phase AD for synthetic waste, which was also supported by Nozhevnikova et al. (1999). Shimizu et al. (1993) that the overall gas production rate of two phase process was four times greater than single phase process.

Kaul and Nandy (1992) have reported that CH_4 production rate from two-phase digesters was 7 times higher than that of the conventional single stage digester. Parkin and Owen (1986) stated that the phase separation of digester would only be feasible for the substrate where hydrolysis step is clearly the overall rate-limiting step which was

also confirmed by Miron et al. (2000) who stated that hydrolysis of lipids and carbohydrates increased with increasing SRT, whereas protein hydrolysis only occurred under methanogenic conditions. Under methanogenic conditions, hydrolysis was the rate-limiting step in the whole digestion process. Tembhurkar and Mhaisalkar (2008) experimented biomethanation of kitchen waste suitably adopting two phase anaerobic treatment using anaerobic fixed film fixed bed reactor. The acidogenesis is process (first phase) suitably incorporated a new approach wherein the kitchen waste was appropriately kept in submerged conditions in the acidogenesis reactor to obtain leachates.

2.7.2 SEMI-DRY AD PROCESS OF ORGANIC SOLID WASTE

Based on semi-dry (20% dry matter) anaerobic process, two full-scale industrial plants have reported to be installed in Italy under thermophilic condition. From the experiments conducted, it is reported that the plant is able to produce nearly 2.5 m^3/d of biogas and 39 tons/d of digested sludge (10% dry matter) from 500 tons of municipal solid waste. The dewatered digested sludge is then co-composted with parts of the fresh organic fraction to produce rich composts (55% dry matter) (Cozzolino et al., 1992).

2.7.3 UPFLOW ANAEROBIC SOLID STATE REACTOR (UASS)

The experimental results of the UASS concept equipped with liquid recirculation proved its feasibility in the digestion of solid waste to methane gas. It had been shown that the UASS reactor's hydrolytic and methenogenic performance is amongst the highest reported for the digestion of solid biomass. When connected to an anaerobic filter, the methane production of the UASS reactor can be stabilized at OLRs as high as 17 gVS/L/d (Jan et al., 2010) and potential drawback of this was the restricted use for colloidal substances such as starch as they can lead to compaction and clogging of the solid state bed.

2.7.4 SOLID STATE ANAEROBIC DIGESTION (SS-AD)

SS-AD has been more advantageous over liquid AD in various aspects including smaller reactor volume, lower energy requirements for heating, minimal material handling, and lower total parasitic energy loss. Biogas production from SS-AD is more or less equal to liquid AD. SS-AD generally occurs at solid concentrations higher than 15%. In contrast, liquid AD handles feedstocks with solid concentrations between 0.5

and 15%. Animal manure, sewage sludge, and food waste are generally treated by liquid AD, while Organic fraction of MSW and lignocellulosic biomass such as crop residues and energy crops can be processed through SS-AD (Yebo et al., 2011).

Table 2.2 Anaerobic digestion of Food waste and its reactor configurations

Sl. no.	Waste type	Reactor configuration	Biogas yield	Conditions maintained	Reference
1	Food waste	Batch solid state AD	472 mL CH_4/gVS added	Mesophilic condition	Cho et al. (1995)
2	Food waste	Three stage anaerobic reactor	223 mL CH_4/g soluble cod $_{degraded}$	Thermophilic conditions and varies HRT	Kim et al. (2006)
3	Food and green waste	Batch reactor	716 mL/ g VS of mixture	Thermophilic condition	Liu et al. (2009)
4	Dairy manure and food waste	Hybrid anaerobic solid-liquid bioreactor	302 mL/ g VS of fine materials	Mesophilic condition	El-Mashad and Zhang (2010)
5	Food waste	Single stage wet AD	455 mL/ g-VS	Mesophilic condition and 10.5 kg VS OLR	Norio et al. (2012)
6	Food waste	Leached bed reactor	_	Mesophilic condition	Xu et al. (2012)

2.7.5 HYBRID BIOREACTORS

Several different designs of hybrid reactors have been proposed. The majority of the laboratory and full scale examples of hybrid reactors have been realized following a simpler design, where the filter is located in the upper part of the reactor without any gas, solid, and liquid separation device. Studies have been undertaken on AD of the solid fraction of kitchen waste using this type of reactor (Lou et al., 2002). The anaerobic

digestion of food waste done by different researchers was given in Table 2.2.

2.7.6. Microbial diversity

Numerous studies have been conducted to gain a better understanding of the microbiomes present in AD reactors and their influence on the efficiency and stability of the AD processes (Chen et al., 2008). While initial studies employed traditional cultivation-based methods, the primary methods in current use are DNA-based molecular biology methods such as cloning and sequencing of either functional or 16S ribosomal RNA (rRNA) genes, FISH, DGGE, single-stranded conformation polymorphisms (SSCP), and quantitative PCR (Malin and Illmer, 2008; Sousa et al., 2007). Because it allows for identification of potential known and unknown microbes present in AD reactors, cloning and sequencing of 16S rRNA genes has been generally favored over other methods. Most studies to date, however, have focused on a single specific AD system (e.g. upflow anaerobic sludge bed (UASB) reactors or continuous stirred tank reactors (CSTRs) processing a single waste stream (e.g. municipal sewage, brewery wastewater). Many of the datasets published contain a small numbers of cloned sequences (generally <100), thus revealing only a small portion of the full diversity present in anaerobic digesters (Lefebvre et al., 2007). Some of these studies were further limited by a narrow focus on one particular microbial group such as the Archaea or a particular phylum (Hori et al., 2006). Additionally, many sequences recovered from AD systems were deposited into GenBank but have not yet been reported in the literature, contributing little to no additional information on the microbial diversity and its function. As a result, the understanding of the microbiomes involved in AD is fragmented and likely biased, exemplified by these microbiomes still being regarded as a ‘‘black box’’. This knowledge gap limits the understanding of how these complex microbiomes either hamper or enhance the efficiency and stability of AD systems.

A few studies have examined the microbial diversity of anaerobic digesters using relatively large (>200 sequences) 16S rRNA clone libraries (Chouari et al., 2005; Riviere et al., 2009). Additionally, a few studies have used 454-pyrosequencing, either alone or in combination with the Sanger sequencing technology, to analyze the microbiomes in anaerobic digesters, producing large datasets of short, difficult to classify sequence reads (Krause et al., 2008; Krober et al., 2009).

2.8 CONCLUDING REMARKS

Anaerobic Digestion is the process by which microorganism's breakdown the biodegradable organic matter in the absence of oxygen. It consists of 4 steps occurring simultaneously; they are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Main end products of anaerobic digestion are methane and carbon dioxide. Benefits of anaerobic digestion process are 1) It produces methane which can be used as a cooking fuel or for generating electricity 2) Sludge production is very less in anaerobic digestion when compared to aerobic processes making sludge handling very easy 3) The digestate from anaerobic digestion is rich in nitrogen and phosphorous, thus making it good compost 4) Since anaerobic digestion takes place in the absence of oxygen, it doesn't need aeration thus reducing a lot of electricity consumption.

Anaerobic digestion of Indian FW has many challenges. The high spice, salt and oil content in Indian cuisine make it difficult for bacteria to digest it. Green chilly that are used commonly in Indian food are also a deterrent for bacteria. So even when food waste is already cooked and easily degradable substrate, the above mentioned contents in it make anaerobic digestion challenging. Even though anaerobic digestion seems to be a very useful process it is not without shortcomings. Anaerobic digestion of food waste is a problematic process because of its highly volatile and soluble nature which leads to souring effect inside the reactor. So to control this high active methanogens required to convert the fatty acids produced. There is no study on activity of different livestock dungs as inoculum in anaerobic digestion of FW and reactor design to control the acidification effect on methanogens.

Anaerobic digestion suffers from higher hydraulic retention time when compared to aerobic processes. It needs a minimum of 30 to 40 days of hydraulic retention time for achieving the desired treatment efficiency. For anaerobic digestion of organic solids containing lipids and proteins, hydrolysis is the rate limiting step. So improving hydrolysis can in turn lower the hydraulic retention time. Pretreatment aims at improving hydrolysis step of anaerobic digestion. Many studies have been done on the topic of anaerobic digestion; there are still many areas in it which require more research. There has been no comparative study between hot air oven, microwave, autoclave, alkali and electrohydrolysis pretreatment. The number of studies done on the effects of pretreatment on food waste is very limited.

Therefore, the objective of this study is to treat the FW using anaerobic digestion.

Studies do conducted to find the optimum livestock inoculum to attain higher methane yield at different F/M ratio and the optimum pretreatment technique for FW. In addition, studies have been conducted on design and optimization of continuous anaerobic reactor (0.7 m^3) for more efficiency in lesser days.

CHAPTER 3

MATERIALS AND METHODS

Different experiments were performed to accomplish the stipulated objectives. The research was carried out into different phases using different livestock dungs and digester sludge as inoculum. Chapter presents the different methods to achieve optimum inoculum and optimum retention time to achieve higher biogas production.

3.1 INOCULUM AND SUBSTRATE

The digested sludge (DS) was obtained from an anaerobic digestion tank designed for conversion of cattle manure to biogas from Amingoan village, Assam. Poultry dung (PD), goat dung (GD), cow dung (CD) and piggery dung (PGD) was obtained from poultry farm, goat farm, cattle farm and piggery farm respectively in Amingoan village, Guwahati, Assam and rhinoceros dung (RD) was collected from Pobitora wildlife century Morigoan district Assam, India (Fig. 3.1). The food waste (FW) was collected from a hostel on the campus of Indian Institute of Technology Guwahati, India. The composition of FW varied according to the daily menu of the hostel mess. Therefore, the FW were separated and remixed. The collected FW was ground into a uniform fine particle using an electrically operated grinder.

3.1.1 CHARACTERIZATION OF FOOD WASTE

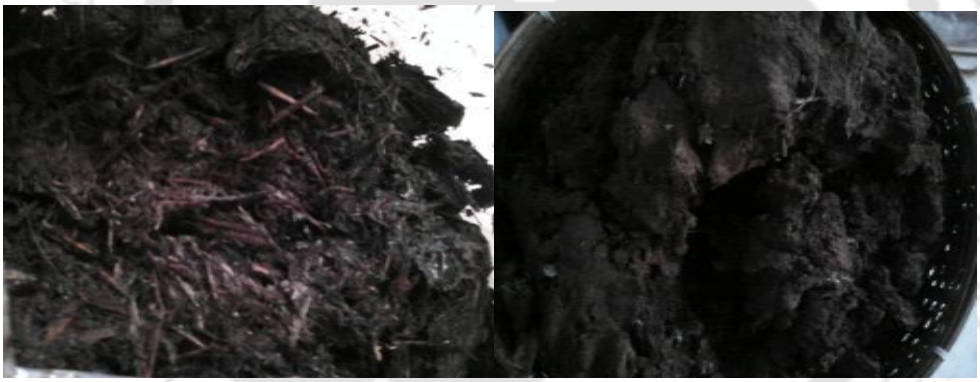
Initially 20 g of FW were taken in a conical flask with 100 mL of distilled water and agitated for 2 h at 150 rpm in a mechanical shaker and it was used to measure pH using a digital pH meter. Moisture content (MC), TS and VS were analyzed for the food samples using standard procedures according to APHA (1998). The FW was dried in hot air oven until all the moisture is removed, then powdered and sieved in 0.2 mm and chemical oxygen demand (COD) and total kjeldahl nitrogen (TKN) was analyzed. TKN was measured after Kjeldahl nitrogen digestion using a digestion mixture of H_2SO_4, K_2SO_4, and $CuSO_4$ (APHA 1998).

3.1.2 INOCULUM ACTIVITY TEST

The VS content of the inoculum was determined and its activity test was done. The

inoculum was taken in serum bottles such that there was 1.5 g VS content and acetic acid with mineral media was added as the substrate. The organic loading rate was 1.0 g COD/g VS_{added}. The bottles were filled up to 500 mL using de-aerated water using nitrogen gas. The reactors were connected to aspirator bottles filled with 6% NaOH. After closing the reactors, nitrogen gas was purged to remove the oxygen inside the reactor. The amount of gas produced is measured by collecting and quantifying the amount of NaOH released due to accumulation of biogas.

Poultry Dung (PD) Goat Dung (GD)



Rhinocerous dung (RD) Cow dung (CD)

Piggery dung (PGD)

Fig. 3.1 Different livestock animal dungs used as inoculum

3.2 EXPERIMENTAL DESIGN

In order to accomplish the objectives, the proposed research work was carried out in eight different phases (Fig. 3.2). In phase I and III, experiments were conducted on the effects of different F/M ratios of different inoculum during biochemical methane potential studies. In phase II and IV, batch experiments of optimum F/M ratio of different inoculum was conducted to find hydraulic retention time (HRT). In Phase V, the effects of different pretreatment methods on hydrolysis and its batch experiments were performed. In phase VI and VII, lab scale continuous reactor study for optimum inoculum, HRT and microbial diversity of best optimum inoculums were performed. In phase VIII, pilot scale reactor (0.7 m^3) was operated for best optimum inoculum.

3.2.1 ANAEROBIC BMP SETUP WITH DIGESTER SLUDGE IN DIFFERENT F/M RATIO

The batch reactor set up was arranged using 1 L glass bottles closed with rubber corks. In sum of 15 batch reactors, 3 were used as a control with 200 mL of digester sludge, macro and micro nutrients which was made upto 500 mL (Fig. 3.3). Rest of the 12 reactors were fed with different quantity of FW to make the different F/M ratio of 1.0, 1.5, 2.0 and 2.5 with essential macro and micro nutrients in addition of 200 mL digester sludge and made upto 500 mL as triplicates. To maintain anaerobic condition nitrogen gas was purged inside all the 15 reactors. After that, the 15 reactors were connected to aspirator bottles having 6% NaOH as mentioned above in sludge activity test and gas produced was measured. The experiment was conducted for 30 days.

- **Effect of nitrogen addition in anaerobic bmp setup with digester sludge**

The batch reactor set up was arranged using 1 L glass bottles closed with rubber corks. In sum of 15 batch reactors, 3 were used as a control with 200 mL of digester sludge, macro and micro nutrients which was made upto 500 mL. Rest of the 12 reactors were fed with 90 g of FW with essential macro and micro nutrients in addition of 200 mL digester sludge and made upto 500 mL. The amount of digester sludge and FW taken was based on optimization studies of F/M ratio. The experiment for four F/M ratio 1.0, 1.5, 2.0 and 2.5 based on VS basis was studied and F/M 2.0 was observed as best. To study the effect of nitrogen concentration in the constant F/M ratio, in 12 reactors four different nitrogen concentration were added as 250, 500, 750 and 1000 mg/L in triplicates. To maintain anaerobic condition nitrogen gas was purged inside all the 15

reactors. After that, the 15 reactors were connected to aspirator bottles having 6% NaOH as mentioned above in sludge activity test and gas produced was measured. The experiment was conducted for 30 days.

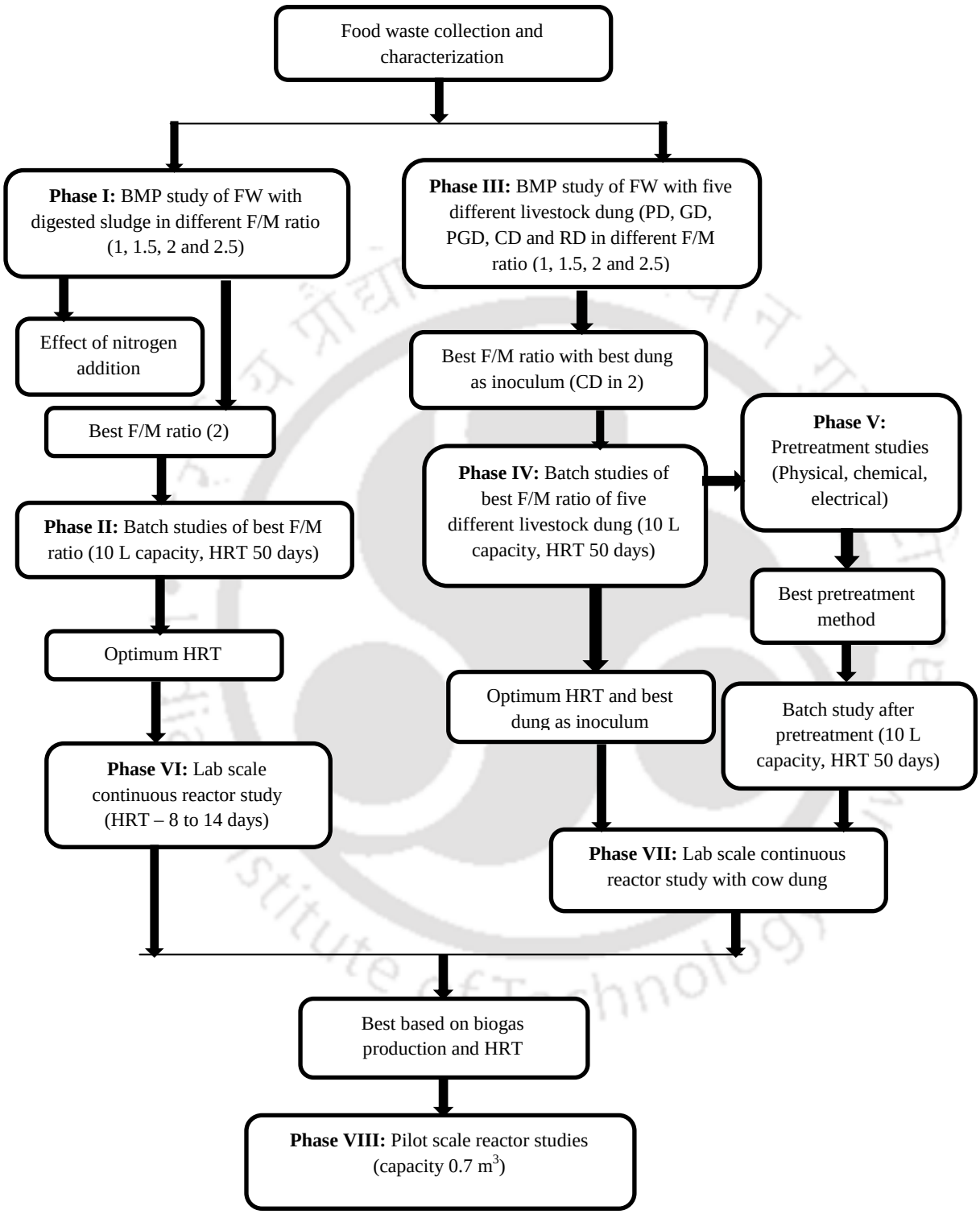


Fig. 3.2 Experimental design of the research work

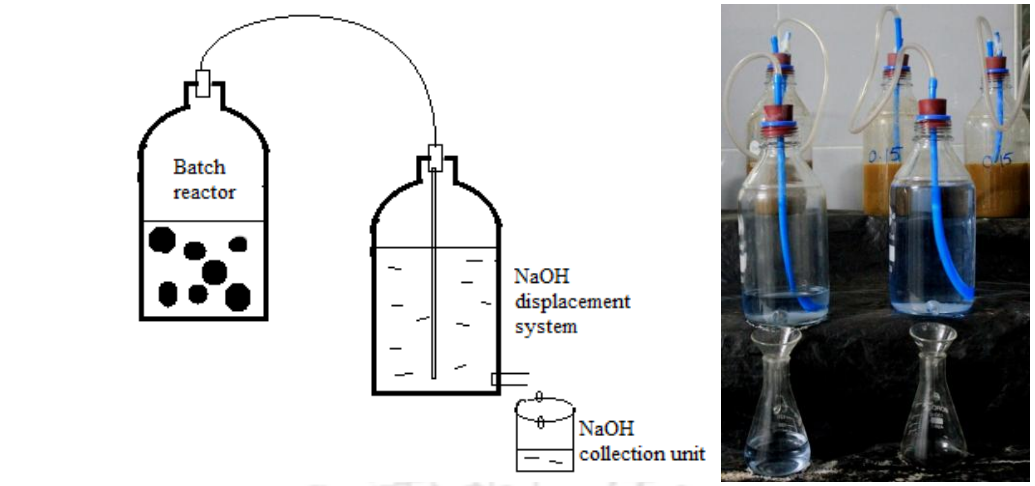


Fig. 3.3 Schematic diagram and Anaerobic batch setup with different F/M ratios

3.2.2 ANAEROBIC BMP SETUP WITH FIVE DIFFERENT LIVESTOCK DUNGS

In order to accomplish the objectives, the research is proposed to be carried out in different phases as summarized in Fig. 3.4. The batch reactor was prepared using 1 L reagent glass bottles with rubber corks for closing. In each inoculum study, 15 batch reactors were used, 12 reactors were fed with 90 g of FW with essential macro and micro nutrients in addition of 200 mL of inoculum. Rest of the 3 was used as a control with only 200 mL of inoculum, macro and micro nutrients. Finally all the 15 reactors were made upto 500 mL using distilled water. The reactors were maintained at 30^{o}C. The amount of different livestock inoculum and FW taken was based on optimization studies of F/M ratio. Four F/M ratio 1.0, 1.5, 2.0 and 2.5 based on VS basis was studied. All the parameters should be monitored properly such as temperature, pH, physical and chemical characteristics of substrates, food/microorganisms (F/M) ratio that affects BMP test (Browne and Murphy, 2013). To maintain anaerobic condition nitrogen gas was

purged inside all the 15 reactors. After that, the 15 reactors were connected to aspirator bottles having 6% NaOH as mentioned above in sludge activity test and gas produced was measured (Elliott and Mahmood, 2007). The experiment was conducted for 30 days.

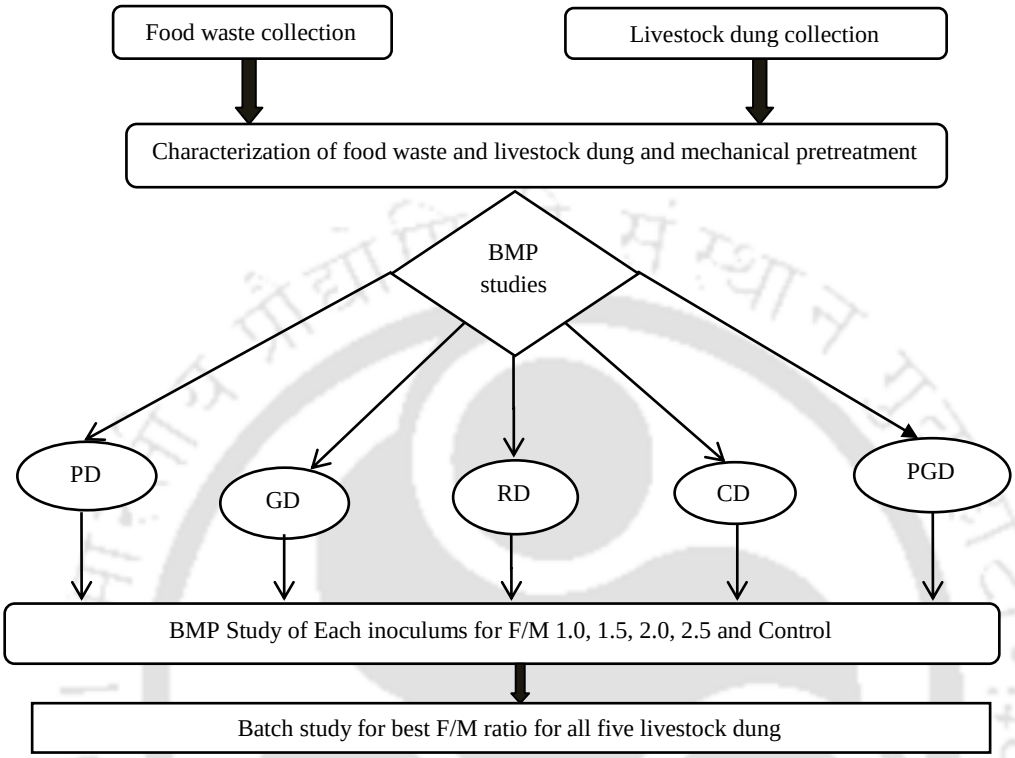


Fig. 3.4 Experimental design of phase III and phase IV

3.2.3 BATCH SETUP FOR BEST F/M RATIO OF DIFFERENT INOCULUM

AD batch study is a large scale of BMP study. It was an extended study of BMP study to find maximum methane production time and retention time. HRT for continuous reactor was fixed based on the diminishing day of maximum methane yield rate in batch reactor. Batch study was experimented using 20L plastic can as reactor. Working volume of 10 L was carried out (20 times of BMP study). Batch experiment was studied for best F/M ratio of all livestock dungs and digester sludge used for BMP study (Fig 3.5). Micro/Macro nutrients [$FeCl_3$ (40 mg/L), $ZnCl_2$ (0.5 mg/L), $CaCl_2$ (50 mg/L), $MgSO_4$ (400 mg/L), $CoCl_2$ (10 mg/L) and $NiCl_2$ (0.5 mg/L)] and phosphorous buffer solution (80 mg/L) were added (Demirer et al., 2000). Nitrogen purging was done on stating of reactor after that bottle was properly closed and connected to gas collection system. The

experiment was conducted for 50 days. The samples were collected periodically for analysis and gas collection was measured daily.

Fig. 3.5 Batch reactor setup with NaOH displacement system

3.3 PRETREATMENT METHODS

For sample preparation, 100 mL of distilled water was added to 100 g of FW and grinded into a liquid consistency. The instruments used for pretreatment studies have been shown in Fig 3.6.

3.3.1 THERMAL PRETREATMENT

- **Hot air oven pretreatment**

In this study, pretreatment of the FW sample was done using a hot air oven. This study was divided into two parts; temperature study and temporal study. In the temperature study, the best pretreatment temperature was selected from 60, 70, 75, 80, 85 and 90°C for 60 min based on pH, volatile solids, soluble COD and volatile fatty acids. A control sample was kept without giving any pretreatment. In the temporal study, the sample was kept at the best pretreatment temperature obtained from the temperature study at different times of exposure such as 30, 60, 90 and 120 min.

- **Microwave pretreatment**

Microwave oven has been used for pretreatment of prepared FW. Samples were kept at 100,140, 160 and 180°C for 2+1 min (2 min. exposures +1 min. stand time) and 4+1 min. An untreated sample was kept as control.

- **Autoclave pretreatment**

Pretreatment of the FW sample was done using an autoclave. This study had two parts; temperature study and temporal study. In temperature study, samples were exposed to 60, 80, 100 and 120°C for 40 min. An untreated sample was kept as control. The best temperature was selected based on parameters such as pH, volatile solids, volatile fatty acids and soluble COD. After the optimum temperature for pretreatment was selected, temporal study was conducted at various times of exposures such as 20, 40, 60 and 80 min.

Hot air oven Microwave Oven Autoclave

Electrohydrolysis setup

Fig. 3.6 Instruments used for pretreatment

3.3.2 ALKALI PRETREATMENT

For the pretreatment of FW NaOH solution was added. The study was divided into two parts; dosage study and temporal study. In the dosage study, different dosages of NaOH such as 0.05, 0.1, 0.2, 0.5, 1, 2.5, 5 and 7.5% (w/w) of TS were added to the samples and exposed for 24 h. A sample without any NaOH addition was kept as

control. The best dosage was selected based on parameters such as pH, volatile solids, volatile fatty acids and soluble COD. After selecting the optimum dosage, temporal study was conducted at the optimum dosage and different time of exposures such as 0, 1.5, 3, 6, 12 and 24 h.

3.3.3 ELECTROHYDROLYSIS PRETREATMENT

In this study, pretreatment of FW was done by passing DC current through samples containing a grinded mixture of 100 g FW and 100 mL water. Graphite Electrode was used for this study and they were placed at a distance of 10 cm (Fig 3.6). The samples were continuously stirred at 300 rpm with the help of an insulated flash mixer to keep the mixture in suspension. In the voltage study, different voltages such as 10, 15, 20, 25, 30, 35 and 40 V were applied to samples for an exposure time of 1 h.

3.4 DESIGN OF CONTINUOUS ANAEROBIC DIGESTER

The design of a digester is based on two important factors such us, based on the amount of waste available and the gas produced based on the wastes and based on the needs. Most of the digesters are based on the second objective since it is easy to adjust the feed available than to have insufficient gas. Design characteristics based on the size as follows:

- **Raw material availability:** The gas production is proportional to the amount of raw material digested.
- **Type of material:** C/N ratio of the raw material should be in the optimum range for better digestion. If the raw material is an easily digestible one, the size of the digester can be reduced proportionally.
- **Size of raw materials:** The feed material should be cut into pieces so that the surface area for the reaction is the maximum. Also, the slurry produced should flow smoothly. The scum produced should be minimized.
- **Heating requirements:** If the digester is situated in cold areas, sufficient heating arrangements should be provided to keep the digestion temperature within the optimum range. Burying the digester under the ground helps to minimize the temperature fluctuations of the ambient around the digester.
- **Mixing requirements:** Providing a mechanism of mixing the feed inside the digester helps to ensure the easy availability of feed to the bacteria for the reactions. Also it provides proper slurry flow inside the digester and avoids the

formation of scum.

The lab scale reactor was designed based on the batch studies and from the review of literature and by the amount of feed need to be feed daily. Amount of slurry fed is 1 L/d (1:1 ratio = 0.5+0.5 L/d of grinded FW: water). The density of slurry is assumed to be 1.0 kg/m^3. so the volume of the feed is 0.001 m^3/d and Retention time was maintained for 14 days obtained from initial batch studies.

So, Volume of the digester required = 0.001×14 = 0.014 m^3

Using a ratio of 1 to 1.4 for height to diameter ratio,

Diameter of the reactor = 0.25 m

Height of the reactor = 0.30 m

The gas produced was stored in gas pack, even though the dome is designed for 40 to 60% of the total gas produced. Dome bottom diameter = 0.25 m (same as digester diameter).As a rule, height of dome is taken as 0.2D to 0.25D, Height of dome = 0.05 to 0.0625 m.

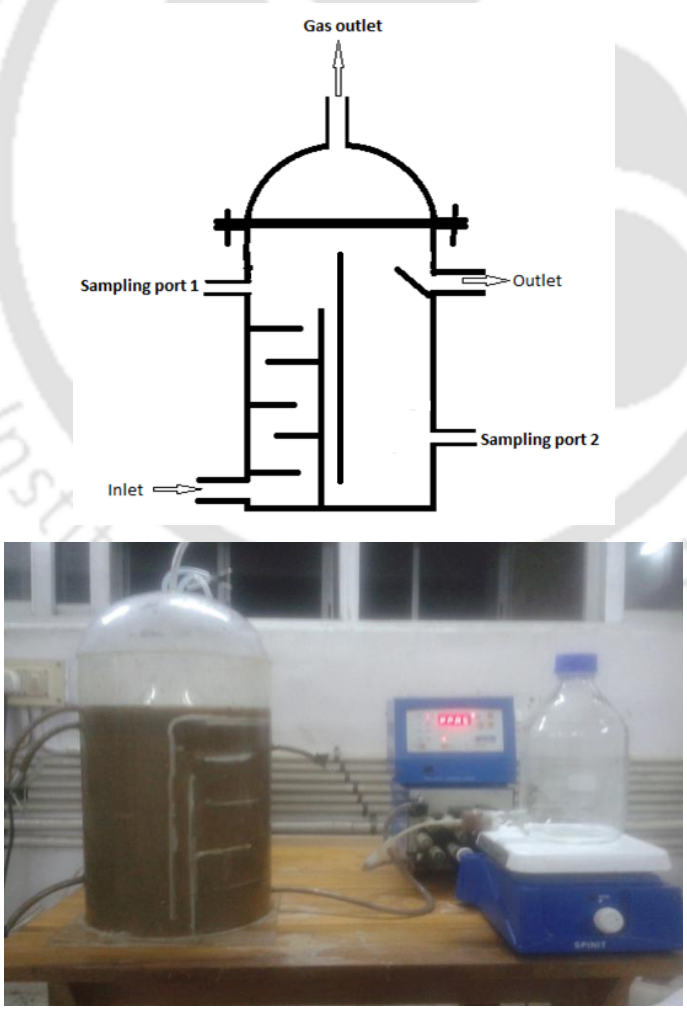


Fig. 3.7 Schematic and pictorial view of continuous anaerobic digester

The ABBR systems divided into two segments such as phase 1 and phase 2 and also provide plurality of baffles in the phase 1. More specifically in this process, fermentation (acidogenic bacteria and acetogenic bacteria) and methanogenesis (methanogenic bacteria) are performed in the above said phase 1 and phase 2 of the reactor system. Further, the horizontal baffle configuration can improve retention times and perform hydrolysis, acidogenesis and acetogenesis in the phase 1(first segment). Acetogenesis and methanogenesis occur in the phase 2 (second segment). This horizontal baffle arrangement in first phase can bring maximum control over the microorganism and assist to intensify fermentation thereby obtaining optimum bio gas generation.

Further, the intermediate products produced during digestion process in phase 1 have been flowed to bottom portion of phase 2 through separator. Phase 2 have a sludge bed at the bottom which consist of higher microbial consortia. The headspace was provided to hold the biogas. In previous literatures, single stage reactor showed the problem of acidification and restriction methane production while in the two stage reactor synergistic work of microbial consortia has been restricted by separating the hydrolysis and acidogenesis with acetogenesis and methanogenesis. In present study, the design has been proposed to solve both the problems. The present technology is economically feasible in treating the organic solid waste and by recovering energy it have commercial value. The problem focused on organic solid waste treatment has been solved by bi-phasing the reactor to avoid acidification and maintaining the synergism effect of acido, aceto and methanogens.

3.5 OPERATION OF LAB SCALE ANAEROBIC BIPHASED BAFFLED REACTOR (ABBR)

A 20 L ABBR (14 L of working volume) without biomass recycling was used (Fig. 3.7). The reactor was maintained in room temperature (25^{o}C). The reactor was equipped with a peristaltic pump to maintain the continuous flow and several input/output ports located at the bottom and top of the reactor for biogas outlet, sampling and feed inlet. In this type of reactor, each HRT has been calculated by a fixed inflow rate. In this sense, a decreasing sequence of HRTs, from 14 days to 8 days, was imposed to evaluate the influence of this parameter on the organic matter degradation, the biogas daily generation and the volatile fatty acids production. Each HRT was maintained for 30 days in order to reach stable operation. As known, the organic loading rate (OLR) depends on both HRT and the concentration of organic matter in the feeding. However, it can be

pointed out that, in this work, the characteristics of the feeding used have remained fairly constant so that the analysis could be done either on the basis of OLR or HRT.

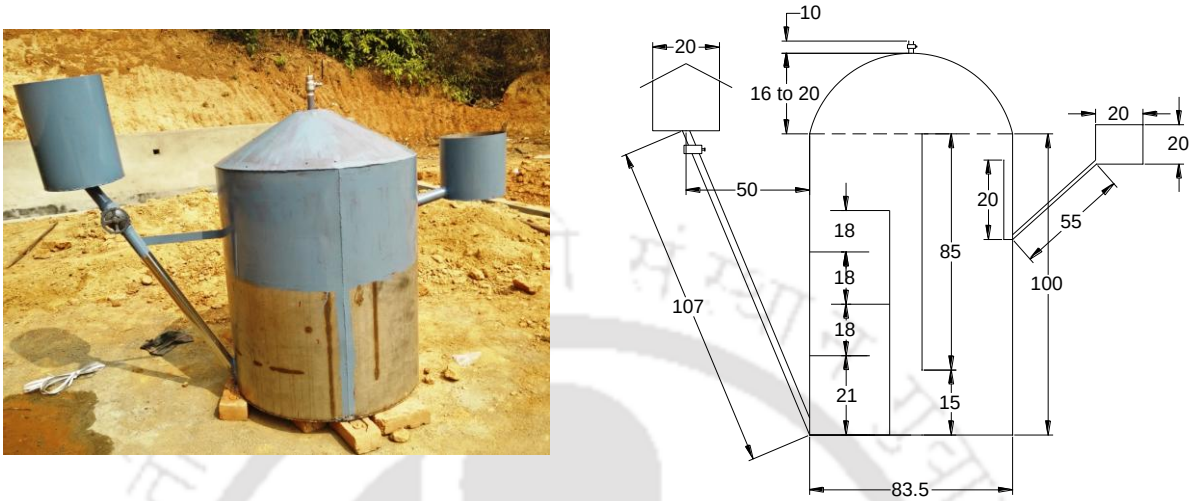


Fig. 3.8 Pictorial view and schematic view of Pilot scale anaerobic digester
(All measurements are in 'cm')

3.6 OPERATION OF PILOT SCALE ABBR

A 700 L ABBR (500 L of working volume) without biomass recycling was fabricated and operated (Fig. 3.8). The reactor was installed in open environment. The reactor flow was controlled with a gate value to maintain the continuous feed. In this reactor, HRT was fixed constant at 10 days to evaluate the influence of this parameter on the organic matter degradation, the biogas daily generation and the volatile fatty acids production. The pilot scale reactor was maintained for 200 days including acclimatization in order to reach stable operation.

3.7 ANALYSIS OF SAMPLES

Different experimental methods are required in the study to accomplish the stipulated objectives. Physico-chemical analysis of the waste samples was carried out in Environmental Engineering laboratory, Department of Civil Engineering, Indian Institute of Technology Guwahati, Guwahati, India.

3.7.1 PHYSICO-CHEMICAL ANALYSIS

Different experimental methods were used in the study to accomplish the stipulated objectives. Experimental procedures of physico-chemical parameters are explained below.

Temperature was monitored using a digital thermometer throughout the anaerobic digestion period. The pH was measured in filtered supernatant (BIS: 10158-1982). Volatile solid (VS) and ash content were also measured according to BIS, 10158-1982. Initial weight of the crucible was taken as W_1 g. Weigh (10 ± 0.1g) of ground sample (screened through 0.22 mm sieve) in crucible and kept it in a muffle furnace operating at a temperature of 550-600^{o}C for 2 h. After 2 h crucible was taken out off the muffle furnace and kept in desiccator for ½ h for cooling and then final weight of crucible with sample was taken as W_2 g. Volatile solids content of the sample was calculated as

$$VS\ (\%) = \frac{(5 - (W2 - W1)}{5} x\ 100$$

Total Kjeldahl nitrogen (TKN) was analyzed using the Kjeldahl method and NH_4-N using KCl extraction (Tiquia and Tam, 2000). For TN analysis 0.2 g of sample (passed through 0.22 mm sieve) was taken and catalyst mixture (potassium sulphate and cupric sulphate, 5:1) of 3 g was added, and digested with 10 mL conc. H_2SO_4 using digestion equipments at 400^{o}C for 2 h (end color of digested sample was green).

After digestion, make the digested sample 100 mL. 10 mL of diluted sample distillate using distillation unit (Pelican Equipments, Chennai, India) with 40% NaOH and distilled water, distillate was collected in 25 mL boric acid with mixed indicator. Collected distillate (clear green color) and titrate with 0.02 N H_2SO_4 at end point purple color. The TN was calculated as follow:

$$TN\ (\%) = \frac{14x\ (S - B)x\ N}{wt.}$$

Where, S = mL of standard sulfuric acid used for sample, B = mL of standard sulfuric acid used for blank, N = Normality of standard sulfuric acid, Wt. = Weight of sample in g.

For the analysis of NH_3-N, 5 ml sample was taken in a reagent bottle and shaken with add 50 mL of 2 M KCl in a horizontal shaker for 2 h. After shaking sample was filtered and supernatant was taken for NH_3-N analysis using Phenate method of Standard methods (APHA, 2005).

The Flame photometer (Systronic 128) was used for analysis of Na, K and Ca concentration, and Mg concentration was measured by atomic absorption spectrometer (AAS) (Varian Spectra 55B) after the digestion of 0.2 g sample with 10 mL of H_2SO_4 and $HClO_4$ (5:1) mixture in block digestion system (Pelican equipments, Chennai, India) for 2 h at 300^oC.

Volatile fatty acids (VFA) was analysed by titration method on basis of pH. Titrate 50 mL of the sample in a 100 mL beaker to pH 4.0 with the appropriate strength sulfuric acid (depends on alkalinity), note acid used, and continue to pH 3.5 to 3. A magnetic mixer is extremely useful for this titration. Carefully buffer pH meter at 4 while lightly boiling the sample a minimum of 3 min. Cool in cold water bath to original temperature. Titrate sample with standard 0.05 N sodium hydroxide up to pH 4.00, and note burette reading, and complete the titration at pH 7. (If this titration consistently takes more than 10 ml of the standard hydroxide, use 0.100 N NaOH.). Calculate volatile acid alkalinity (alkalinity between pH 4 and 7). mL 0.05 N NaOH × 2,500 Volatile acid alkalinity = ml sample. For a 50 mL sample the volatile acid alkalinity = 50 × ml 0.05 N NaOH, or 100 × mL 0.100 N NaOH. Calculate volatile acids. Case 1. > 180 mg/L volatile acid alkalinity: Volatile acids = volatile acid alkalinity × 1.50, Case 2. < 180 mg/L volatile acid alkalinity: Volatile acids = volatile acid alkalinity × 1.00 (Dillalo and Albertson, 1961).

VFA was also measured using high performance liquid chromatograph (HPLC, LC-20AD, Shimadzu, Japan) equipped with a UV Detector (SPD-20A), refractive index (RID-10A) detector and Rezex ROA column (300 7.8 mm, Phenomenex, USA). Organic acids were detected in UV detector at 210 nm whereas substrates and solvents were quantified in RI detector at 37°C. The mobile phase 0.005 N H_2SO_4 was used at a flow rate of 0.5 mL /min and the column oven temperature was kept at 28°C. Samples were filtered with 0.2 µm PVDF syringe filter and 10 µL of sample was used for analysis.

Gas samples were measured using gas chromatography (GC, Dhruva, Chromatograph and Instruments Company, India) equipped with molecular sieve 5A column and TCD detector. 1 mL of sample was used for analysis. The nitrogen gas was used as a carrier gas.

3.7.2 CULTURE-INDEPENDENT ANALYSIS (MOLECULAR METHOD)

Genomic DNA was extracted from samples with beat-beating method, using a DNA extraction Kit (Hi PurA™ Soil DNA kit) as per manufacturer's instruction. Extracted yield DNA was measured through spectrophotometer. The kit was designed in such a way that apart from soil DNA, it was efficient to isolate DNA from other sources such as sludge, soils etc. In addition, lysis time period was elongated, as a pre-treatment for DNA extraction process. The integrity and purity of the DNA was checked by horizontal gel electrophoresis in 0.7% agarose gel.

3.7.3 INSTRUMENTS USED

Table 3.1 shows the different instruments that were used during the investigation and experimental analysis.

3.8 KINETIC STUDY FOR BMP AND BATCH STUDIES

In order to characterize each experiment and to evaluate the effect of nitrogen addition in BMP on the methane yield, kinetic analysis was done. The cumulative methane gas production of the best reactors was fitted with Gompetrz Eq. 3.1.

$$Y = P \times \exp\left\{-\exp\left[\frac{R_m \times e}{P}(\lambda - 1) + 1\right]\right\} \qquad (3.1)$$

Where Y represent the volume of methane gas accumulated (L) with respect to time t (d), P is the methane gas production potential (L CH_4), R_m is the maximum methane production rate (L CH_4/d), λ is the lag phase time (d) and *e* is constant equal to 2.71. The adjustment by non-linear regression of the pairs of experimental data (Y, t) using Matlab R2013b allows the calculation of the variables P, R_m and λ.

3.9 SIMULATION STUDIES

ADM1 model suits best because of its wide applicability and thorough characterization of parameters to define the process. The model includes three overall biochemical steps-acidogenesis, acidogenesis and methanogenesis as well as extracellular disintegration step and extracellular hydrolysis step. They have a number of parallel reactions. Authors have stated that ADM1 possess good predictive capabilities for different configuration (Blumensaat and Kellar, 2005). The intent of ADM1 was to model steady state processes but it has been shown that a dynamic loading regime in

terms of varying masses of waste is not a great difficulty (Ozkan-yucel and Gökcay, 2010). Literatures using ADM1 model during co-digesting FW with another substrate and found that the model at the very best predicts average trend of transient processes (Derbal et al., 2009). Other authors achieve a very close fit to data (Zaher et al., 2009a). Literatures containing ADM1 simulations with anaerobic digestion of FW has not been found for present study. The ADM1 was implemented using Aquasim 2.0. Table 3.2 shows the estimated constant value of ADM1 model for AD of FW and Table 3.3 represents the abbreviations of the ADM1 constants.

Table 3.1 Different instrument used for experiments

Parameter Analysis	Instrument	Company
Sieving	Sieve	Unique Drawing & Survey emporium
Moisture content	Class- II High Accuracy	Satwik Scale Industries
pH	µ pH system 361	Systronics
Volatile solids	Muffle Furnace	International Commercial Traders
COD	COD Digester	HACH
PCR machine	96 Universal gradient	peqSTAR
Agarose gel electrophoresis	-	Slash Lab
Micro centrifuge	-	ThermiSci
VFA	HPLC	Shimadzu
Biogas	GC	Chromatograph and Instrument Company

Table 3.2 Estimated values with trial and error for ADM1

Parameters	**Default value as per ADM1**	**Estimated value**
k_{dec_xaa}	0.02	0.0797
k_{dec_xac}	0.02	1
k_{dec_xc4}	0.02	0.1
k_{dec_xfa}	0.02	0.9
k_{dec_xpro}	0.02	0
k_{dec_xsu}	0.02	0.3823
k_{dis}	0.5	9.3632
k_{hyd_ch}	10	18.067
k_{hyd_Li}	10	90.3881
k_{hyd_pr}	10	98.089
K_{la}	200	185.537
k_{m_aa}	50	0
k_{m_ac}	8	0.1
k_{m_c4}	20	0.02
k_{m_fa}	6	0.1
k_{m_pro}	13	0.1
k_{m_su}	30	0.1
k_{s_ac}	0.15	0.01
k_{s_c4}	0.2	0.01
k_{s_fa}	0.4	9.99
k_{s_pro}	0.1	7.883
k_{s_su}	0.5	1.01

Table 3.3 Abbreviation table for ADM1 parameters

Abbreviation	Parameter
k_{dec_x}	decay rate for degrading organisms
k_{dis}	complex particulate disintegration first order constant
k_{hyd}	hydrolysis first order constant
K_{la}	Apparent mass-flux coefficient
k_m	maximum uptake rate degrading organisms
k_s	half saturation constant for degradation
k_i	inhibitory concentration for C4 degrading organisms
Aa	Amino acids
Ac	Acetate
C_4	Butyrate and valerate
Fa	Long chain fatty acids
Pro	Propionate
Su	Monosaccharide
Ch	Carbohydrate
Li	Lipids
Pr	Proteins

CHAPTER 4

BIOCHEMICAL METHANE POTENTIAL AND BATCH STUDY OF FOOD WASTE WITH DIFFERENT INOCULUM

This chapter deals with the results and discussion of initial characterization of food waste and all inoculums. The chapter do explains evaluation of biogas production and organic matter degradation in Biochemical methane potential (BMP) study and batch study of food waste with different inoculum.

- **Characterization of food waste**

The moisture content (MC), volatile solids (VS), and volatile solids to total solids ratio (VS/TS) of different days food waste (FW) sample are given in Fig. 4.1. All the values for MC and VS are reported on wet weight basis. The MC varies from 69 to 81% and VS/TS percentage varies 76 to 86%. The values of pH, MC, TS, VS, chemical oxygen demand (COD) and total kjeldahl nitrogen (TKN) are reported in Table 4.1. The characteristics of the FW varied in good range due to its composition of boiled rice contents with spices, extract of vegetables and amount of oil percentage during collection time. The percentage of VS/TS of FW assures that it's having a very high utilizable organic content. The pH was slightly lesser than the neutral range, which needed in the case of anaerobic digestion. The COD concentration confirms combined with the VS/TS ratio, for high methane production.

- **Inoculum activity test**

The digester sludge used as the seed sludge was characterized and the parameters are given in Table 4.1. The characterized parameters are compared with the reported values (Elliott and Mahmood, 2007; Lopez and Espinosa, 2008; Eskicioglu et al., 2009) and observed that it was within the range of reported values and hence used as inoculum in batch studies. The observations for biomethanation potential of seed sludge are given in Fig. 4.2. The gas production was monitored continuously upto 120 h. Neutralized acetic acid was fed at every decline phase of gas production. After every feeding the increase in production rate was observed. The amount of gas production depicts the degradation of

neutralized acetic acid added. Increase in the gas production rate from every previous feeds shown the good quality of anaerobic sludge.

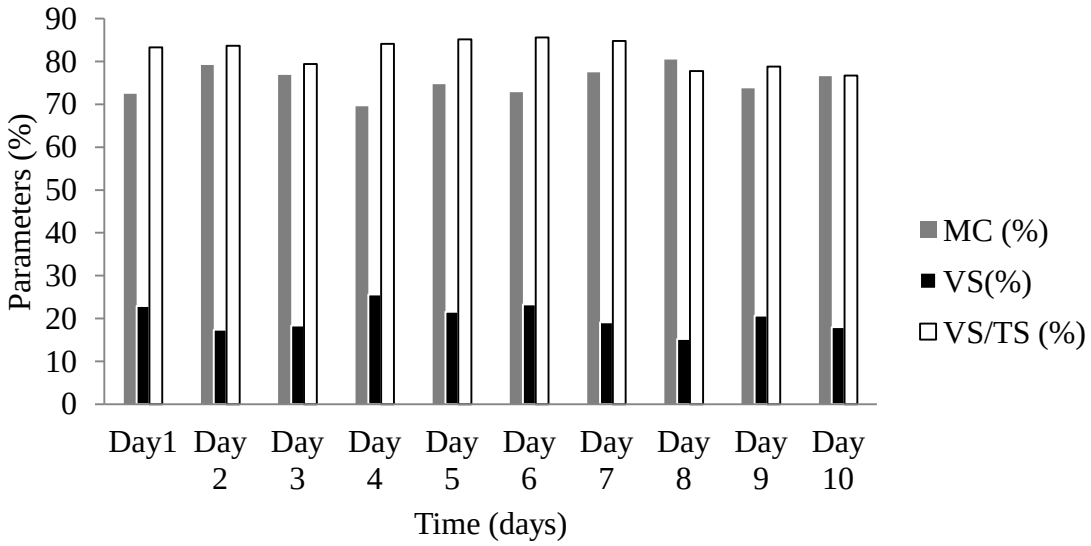


Fig. 4.1 Percentage of MC, VS and VS/TS of different day's food sample in wet basis

Table 4.1 Characteristics of FW and seed sludge

Parameters	Units	FW	Seed sludge
pH		4.4-6.0	6.72-6.84
MC	%	75.4±3.4	95.0±0.26
TS	%	24.6±3.4	4.9±0.14
VS	%	20.3±3.2	3.2±0.17
FS	%	4.4±0.8	1.8±0.11
VS/TS	%	81.9±3.4	63.2±1.2
TCOD	g/L	132±6	43±0.2
TKN	g/L	3.0±0.4	0.8±0.2

4.1 PHASE I: BMP SETUP WITH DIGESTED SLUDGE AS INOCULUM IN DIFFERENT F/M RATIO

The food to micro-organism ratio (F/M) is an important characteristic in batch anaerobic digestion processes and also in the degradation of the organic solid particles. Microorganisms has its own way of degradation rate with the substrate according to that

the specific F/M ratio need to be maintain. F/M ratio depending on the substrate nature, amount, and its VFA and the ammonia production from the hydrolysis of carbohydrates and proteins of substrate respectively. Mostly inoculum is used in fewer amounts due to its endogenous biogas production possibility to affect the final result (Lestuer et al., 2010). The more F/M ratio affects the reactor due to higher acid production and its accumulation in the reactor (Neves et al., 2006). In this study, four F/M ratio was performed i.e. 1, 1.5, 2, 2.5 and one control.

- **Methane production rate and VS**

The highest VS reduction of 56% was observed in F/M 2 from the F/M ratio studies of food waste and digester sludge. Next to F/M 2, higher VS reduction of 51% was there in F/M 1.5 reactor. Fig. 4.3 showed the VS reduction percentage in different F/M ratio maintained reactors. The C/N ratio of the F/M ratio studies was around 47±1.2 in all the reactors. Fig. 4.4 represents the cumulative bio-methane production in all the reactors including control. It clearly depicts that F/M 2 reactor has highest gas production than all other reactors and also obtained maximum gas production of 364 mL/g VS degraded. The amount of biogas produced is directly proportional to the amount of VS degraded. From this result it is found that F/M 2 is better.

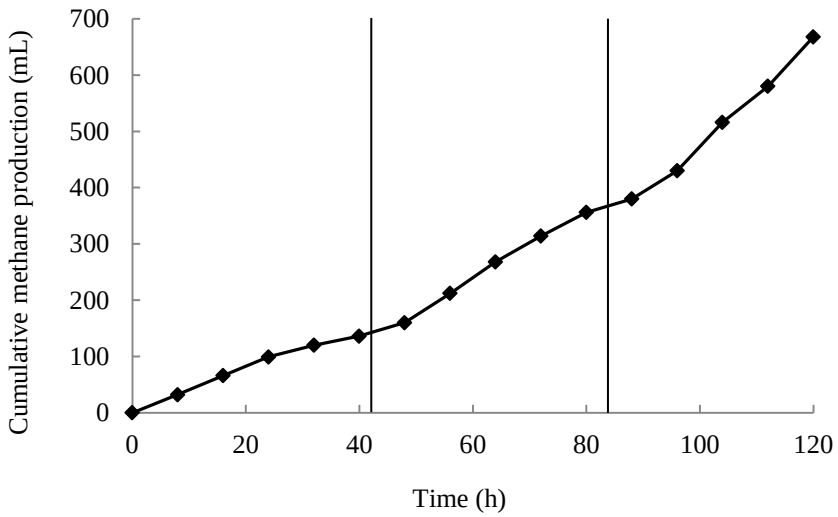


Fig. 4.2 Methane production in digester sludge activity test

➢ **Volatile fatty acids (VFA)**

When FW, highly soluble organic material is utilized as a raw material for the anaerobic reactor, the pH of the reactor decreased in very earlier days due to conversion of free usable organics to fatty acids. It severely inhibits the methanogenic process, which requires a neutral pH range (Misi and Forster, 2002; Bouallagui et al., 2009). During the biological degradation of fruit-vegetable waste and FW, ammonia can be produced from the nitrogenous compounds like proteins, phospholipids, nitrogenous lipids and nucleic acids (Kayhanian, 1999). The pH values were drops at initial time of the reactors, later it gradually increased to neutral and it maintained till the last day of digestion. The pH drop may be due to the production and accumulation of VFA and its recovery may due to increased methanogenic activity and the acids were utilized. The VFA profile for different reactors was given in Fig. 4.5. FW contains high nitrogen content due to proteins phospholipids, nitrogenous lipids and nucleic acids which produces ammonia. While degrading it get mixed with the reactor solution to form ammonium bicarbonate and helps in buffering the pH of the digester (Murto et al., 2004). When FW was fed in different concentration into digester, the fast degradation process resulted in very high acid accumulation in F/M 2.5 reactor, and disturbed the methanogenesis similarly decreased the biogas production.

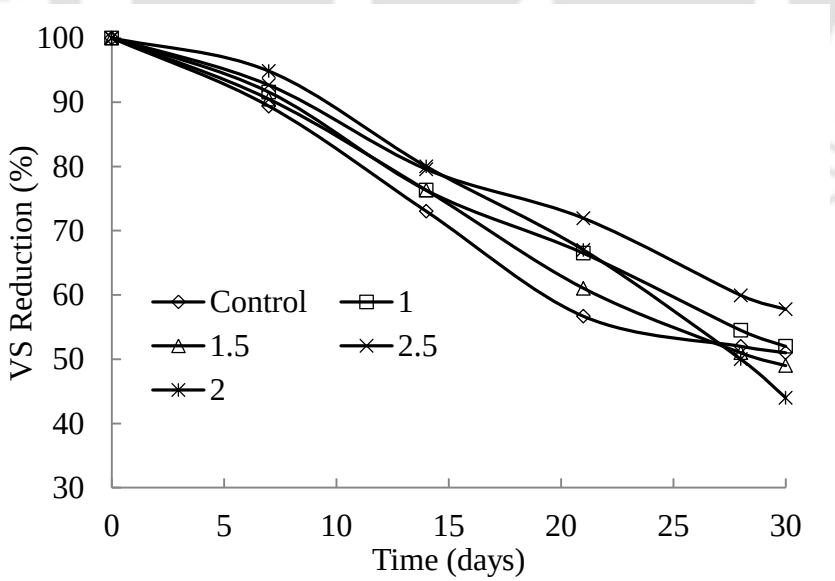


Fig. 4.3 VS reduction in BMP of FW with digester sludge different F/M ratio

A high COD removal was achieved in the present study, in which 56% was obtained for the F/M 2 anaerobic batch reactor. The cumulative methane gas production is higher in F/M 2 reactor then others matching to the proportionality trend of VS reduction. The percentage of VS reduction was lower in F/M 2.5 reactor than the control. It may because of high acid accumulation and inhibition in methanogenesis process. This reduces the amount of methane gas production lower than the control reactor. If VFA concentration is more, the methanogenic process will get affected which results a sharp drop of pH value (Cho et al., 1995). Ammonia nitrogen can be produced as a by-product of AD, principally from the mineralization of organic nitrogen during the deamination of proteins and amino acids. An excessively high C/N ratio increases the acid formation which retards methanogenesis activity (Ghasimi et al., 2009).

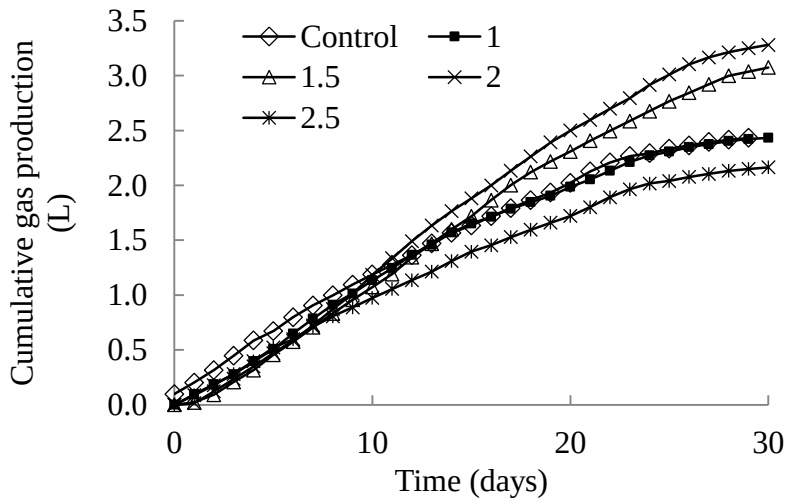


Fig. 4.4 Methane production in BMP of FW with digester sludge for different F/M ratio

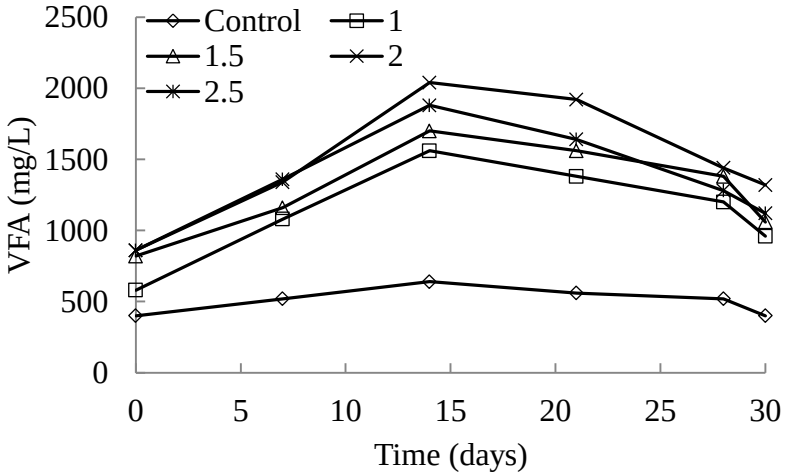


Fig. 4.5 VFA profile in BMP of FW with digester sludge for different F/M ratio

- **Kinetic study**

The cumulative methane gas production values were fitted with Gompertz equation curve to find the improvement in efficiency of nitrogen addition reactors (Lee et al., 2013). In the curve fitting R^2 was higher than 0.9. The kinetic parameters for F/M 2 reactor were calculated as being P = 3.112 L CH_4, R_m = 0.1216 L CH_4 /d, λ =1.738 d and Y = 2.968 L CH_4 respectively. F/M 2.5 ratio maintained reactor had lower P and Rm.

4.1.1 EFFECTS OF NITROGEN ADDITION ON BMP SETUP

The highest VS reduction of 56% was observed in F/M ratio equal to 2 from the F/M ratio studies of food waste and digester sludge. The C/N ratio of the F/M ratio studies was around 47±1.2 in all the reactors. In the present study to reduce the C/N ratio, nitrogen was added in the form of nitrate (Akunna et al., 1992). The C/N ratio of different reactors after the addition of nitrogen is 29.9, 21.84, 17.19 and 14.18 for 250, 500, 750 and 1000 mg/L respectively. Fig. 4.6 shows the percentage of VS reduction is higher from AD of FW with nitrogen addition as compared to AD of the FW alone. It clearly shown that 500 mg/L nitrogen added reactor has highest VS reduction of 68% than all other reactors and also obtained a maximum gas production of 410 mL/gVS degraded. Nitrate can be utilized in two ways in AD process such as assimilatory (nitrate used as electron acceptor converted to nitrogen gas which also called as denitrification) and dissimilatory (nitrate converted to ammonia also called as ammonification). More nitrate addition leads to ammonification and less leads to nitrogen deficiency which reduces the VS reduction. In 500 mg/L nitrogen addition reactor the ratio of carbon to nitrogen is optimum compare to others, which increase the microbial growth and degradation. Next to 500 mg/L, higher VS reduction of 60% was there in 750 mg/L nitrogen added reactor. The amount of biogas produced is directly proportional to the amount of VS degraded. Fig. 4.7 represents the cumulative biomethane production in all the reactors including control. To increase the methane production for inoculums with low background concentrations of trace metals in AD of food waste supplementation of trace metals mixture is required (Facchin et al., 2013). When FW is used as a main substrate for the anaerobic reactor, the pH of the reactor decreased drastically due to conversion of easily available organics to organic acids. It affects the methanogenic activity, which needs a neutral pH (Misi and Forster, 2002; Bouallagui et al., 2009).

During the biological degradation of fruit-vegetable waste and FW, ammonia can be produced from the nitrogenous compounds like proteins, phospholipids, nitrogenous lipids and nucleic acids (Kayhanian, 1999). From the present analysis, it was found that the amount of total kjeldhal nitrogen content in FW is less for the proper degradation of organic matter and also for the production of ammonia to maintain the pH. Micro-organisms also need some amount of ammonia to form cellular protoplasm for growth and reproduction.

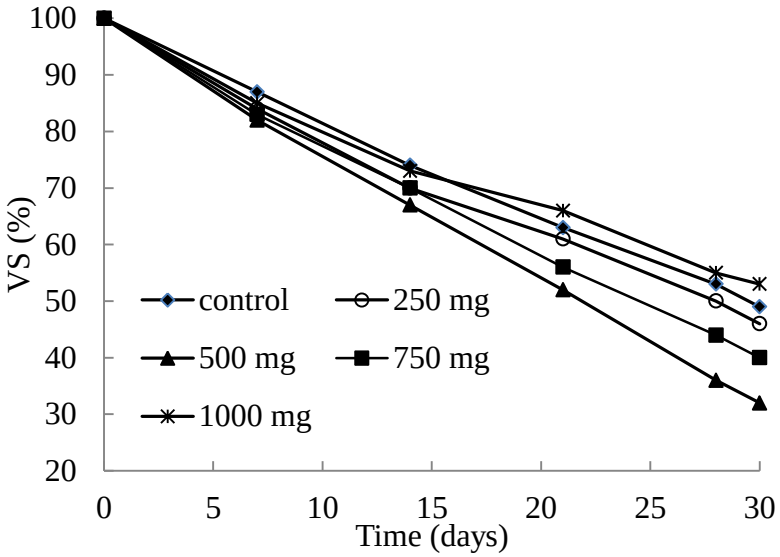


Fig. 4.6 Percentage of VS reduction BMP of FW in different nitrogen added reactors

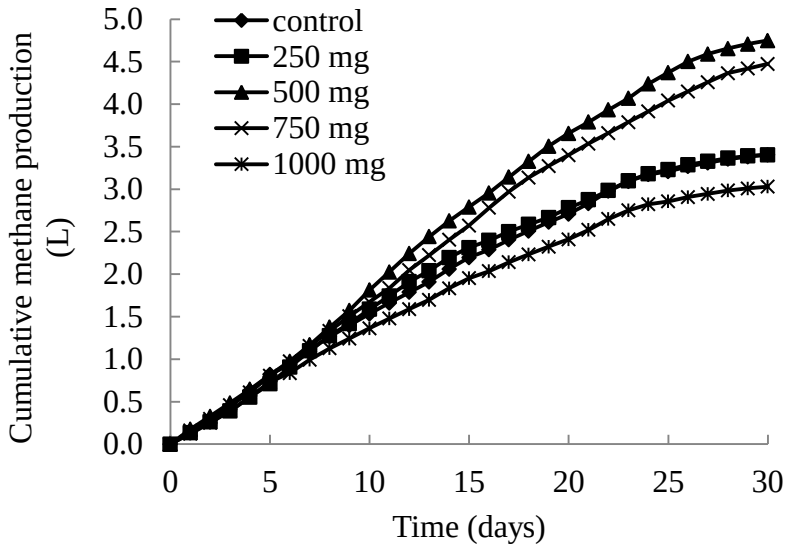


Fig. 4.7 Methane production in BMP of FW in different nitrogen added reactors

The pH values were initially dropped upto day 5 in all the reactors after it gradually increased to neutral and it was maintained till the last day of digestion. In 1000 mg/L nitrogen added reactor, pH initially decreased then it raised to neutral after it again reaches acidic condition. The pH decrease was due to the production of VFA and its accumulation, after day 5 the methanogenic activity increased and the acids were utilized. The VFA profile for different reactors was given in Fig. 4.8. FW contains high nitrogen content due to proteins which produces ammonia. While degrading it get mixed with the reactor solution to form ammonium bicarbonate and helps in buffering the pH of the digester (Murto et al., 2004). From the characterization, FW used in this experiment had low nitrogen content. The results showed that the addition of nitrogen with FW in a proper ratio can improve pH buffering capacity in digester, which improves the methanogenic activity compared to control reactor which was slightly disturbed by its acidic condition. From the results for TKN, it is confirmed that the percentage of nitrogen were decreased slightly in all the reactors and percentage reduction is lesser in high nitrogen added reactor. When FW was fed alone into digester, the fast degradation process resulted in high acid accumulation and nitrogen deficiency which disturbed the methanogenesis similarly decreased the biogas production.

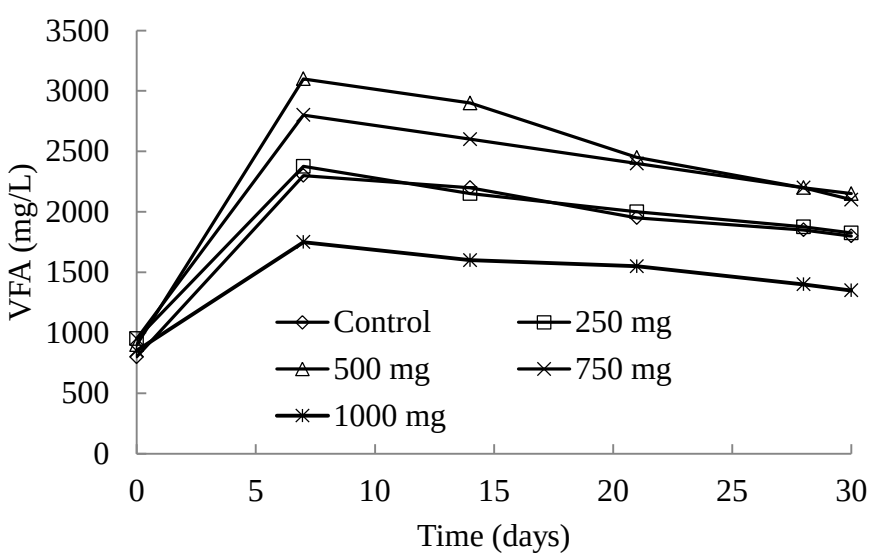


Fig. 4.8 VFA profile in BMP of FW in different nitrogen added reactors

A high COD removal was achieved in the present study, in which 68% was obtained for the 500 mg nitrogen added anaerobic batch reactor. The cumulative methane gas

production is higher in 500 mg nitrogen added reactor then others matching to the proportionality trend of VS reduction. The percentage of VS reduction was lower in 1000 mg addition of nitrogen than the control. It may because of high ammonia production due to over nitrogen source. Because of that the amount of methane gas production was also reduced than the control reactor. If ammonia concentration is more, the methanogenic process will get affected which results a sharp drop of pH value. Ammonia nitrogen can be produced as a by-product of AD, principally from the mineralization of organic nitrogen during the deamination of proteins and amino acids. An excessively high C/N ratio increases the acid formation which retards methanogenesis activity (Ghasimi et al., 2009). The pH increases by the increase of NH_3-N concentration. Ammonia toxicity can be avoided if pH of the reactor is maintained within the optimum range of 6.8-7.5, so proper percentage of nitrogen is must for the better AD of FW.

- **Kinetic study**

The cumulative methane gas production values were fitted with Gompertz equation curve to find the improvement in efficiency of nitrogen addition reactors (Lee et al., 2013). In the curve fitting R^2 was higher than 0.9. The kinetic parameters for 500 mg added reactor were calculated as being P = 3.8804 L CH_4, R_m = 0.1538 L CH_4/d, and λ =1.8217 d, respectively (Table 4.2). 1000 mg added reactor had lower P and Rm. Table 4.2 lists the P, R_m and λ values with Y values for each case studied.

Table 4.2 Kinetics values for nitrogen addition reactors which fits non-linear regression

Experiments	P (L CH_4)	R_m (L CH_4 /d)	λ (d)	R^2	Y (L CH_4)
Control	2.0486	0.1081	0.0837	0.94	1.973736
250 mg nitrogen addition	2.5925	0.1226	1.0272	0.97	2.427864
500 mg nitrogen addition	3.8804	0.1536	1.8217	0.99	3.403551
750 mg nitrogen addition	3.6646	0.1407	1.6571	0.99	3.182106
1000 mg nitrogen addition	1.8235	0.0962	0.0829	0.94	1.756805

- **Conclusion**

In Anaerobic digestion of FW, the highest of 59% VS reduction was observed in F/M ratio 2 maintained reactors with higher methane yield. In the F/M ratio 1.5 reactors, the VS reduction was less due to deficiency of microbial count compare to F/M ratio 2.

In F/M 2.5 reactor the VS reduction is lesser than other reactors and control it may be due to high acid accumulation. The results concluded that only the requisite amount of food is mandatory for microorganisms to get better anaerobic digestion of FW neither more nor less to achieve higher methane yield.

In AD of FW, highest of 68% VS reduction was observed in 500 mg/L nitrogen added reactor with higher methane yield. In the 250 mg/L nitrogen added reactor, the VS reduction was less due to nitrogen deficiency. In 1000 mg/L nitrogen addition reactor the VS reduction is lesser than the entire nitrogen added reactor and control it may be due to ammonium toxicity. The kinetics studies revealed the perfect curve fit with high R^2 value in all the reactors. The results concluded that only the requisite amount of nitrogen is mandatory for better AD of FW neither more nor less to achieve higher methane yield, although F/M ratio was optimized.

4.2 PHASE II: BATCH STUDIES OF BEST F/M RATIO OF DIGESTER SLUDGE

Batch study has been conducted for the confirmation of organic matter degradation pattern and to finalize the hydraulic retention time. The batch reactor was 20 L capacity with a working volume of 10 L. Best F/M ratio of 2 was found from BMP studies has been accommodated for this study.

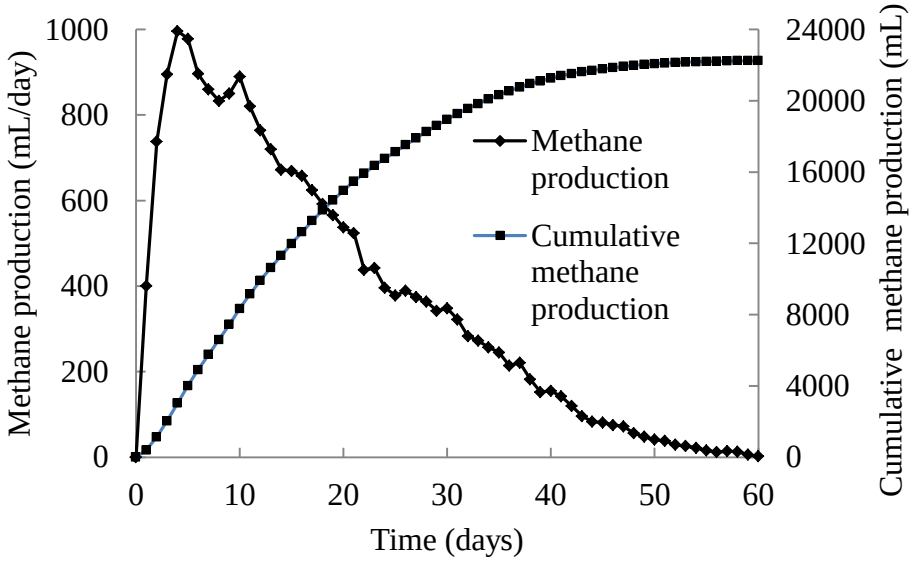


Fig. 4.9 Cumulative and methane rate production in batch reactor with digester sludge

- **Cumulative methane production and methane production rate**

Fig. 4.9 shows the methane production rate (Q) of batch reactor maintained at F/M ratio 2 at room temperature. The maximum Q value of 996 mL/d was achieved in the reactor because of highly available free organics with sufficient microbial community to utilize. The minimum Q value of 6 and 2 mL/d was observed on the 59 and 60th day of the reactor respectively. The methane production rate was higher at initial 10 days and started to decrease later. The cumulative methane production was achieved to 22.25 L CH_4. The rate limiting step of anaerobic digestion of organic wastes is the first step of hydrolysis or solubilization, where the cell wall is broken down allowing the organic matter inside the cell to be available for biological degradation (Noike et al., 1985, Wang et al., 1997).

- **pH and VFA profile**

The pH decrease was due to the production of VFA and its accumulation, after day 4 the methanogenic activity increased and the acids were utilized. The VFA profile for different reactors was given in Fig. 4.10. The pH was decreased at initial phase of reactor and it was maintained 7.0 using $NaHCO_3$. The maximum total VFA was observed at initial day of analysis later it was in decline phase due to methane producing archeae and its methane production.

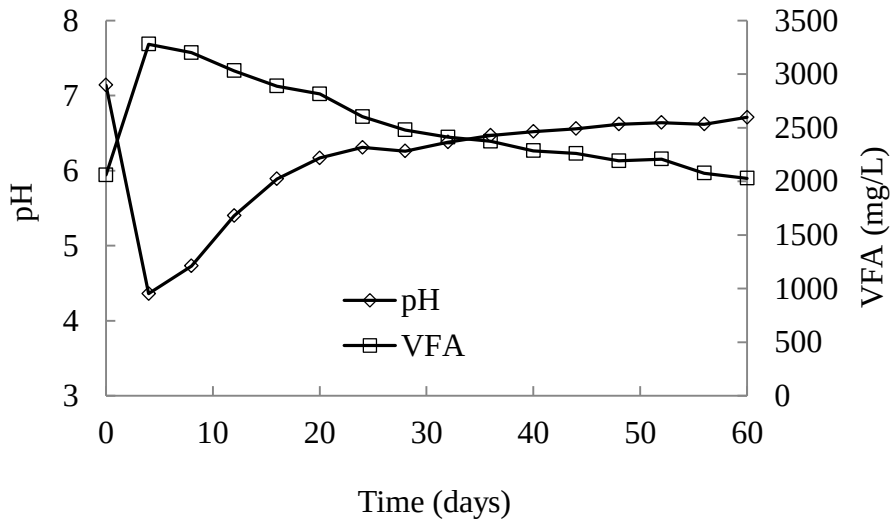


Fig. 4.10 pH and VFA production in batch reactor with digester sludge

- **Conclusion**

In batch studies, percentage of VS reduction followed the same trend as the best F/M ratio 2 in BMP of food waste. Maximum of 996 mL/d methane production rate was achieved in this study. The VFA productio was high at initial days later it was stabilized by the methane producers.

4.3 PHASE III: BMP STUDY OF FW WITH FIVE DIFFERENT LIVESTOCK DUNG (PD, GD, PGD, CD AND RD) IN DIFFERENT F/M RATIO'S

In this phase IV, five different livestock dung was utilized as an inoculum during anaerobic digestion of food waste. Each inoculum study was performed in four different F/M ratios i.e. 1, 1.5, 2, 2.5 and control.

- **DIFFERENT LIVESTOCK DUNG ACTIVITY TEST**

The different livestock dungs such as poultry dung (PD), piggery dung (PGD), goat dung (GD), cow dung (CD) and rhinoceros dung (RD) used as the seed sludge was characterized and the parameters are given in Table 4.3. The characterized parameters are compared with the reported values (Elliott and Mahmood, 2007; Torres and Llorens, 2008; Eskicioglua et al., 2010) and observed that it was within the range of reported values and hence used as inoculum in batch studies. The biogas production was monitored for 42 h, following that second feeding was given and the biogas production was monitored upto 84 h. Finally, third feeding was given and the test was continued upto 120 h and the biogas was monitored. The amount of biogas production depicts the degradation of neutralized acetic acid added. Increase in the biogas production rate from every previous feeds showed the good quality of anaerobic sludge.

- **Methane production rate**

Methane gas production is one of the main parameter which need to analyses and optimize for biogas reactor. It should be analyze on the basis of total production, rate of methane production, production on the basis of per unit VS used, time when its production become virtually seized and time when it achieved almost its maximum amount. In case of PD as per shown in Fig. 4.11(A), although the very first day the rate of methane production was high for F/M ratio 2 but it was couldn't able to sustain for

long time and get succeed by F/M 1.5 on 3rd day itself. It is clearly observed by graph that F/M 1.5 is best ratio followed by F/M 1.0.

Table 4.3 Initial characteristics of FW and Different livestock inoculums

Parameters	Livestock inoculum					
	Food Waste	**Goat Dung**	**Poultry Dung**	**Cow Dung**	**Rhinoceros Dung**	**Piggery Dung**
Moisture Content (%)	75.4±3.4	45.7±0.6	78.42±0.8	79.8±2.3	80.29±0.4	72.23±1.6
Total solids (%)	24.6±3.6	55.1±1.5	21.6±0.9	20.19±1.4	19.7±1.2	26.7±1.8
Volatile solid (%)	20.3±3.2	39.2±0.9	16.2±0.5	15.25±1.1	15.62±0.8	22.18±1.3
pH	5.02-6.64	7.35-7.51	6.53-6.63	7.05-7.25	6.60-6.74	6.52-6.94

Methane production in PD reactor on 10th day for F/M 1.5 was about 2.3 L which was 74% of total gas production i.e. 3.04 L on 28th day. F/M ratio 2.0 found poor for methane generation followed by F/M 2.5. Although the total methane production for F/M 1.5 and 2.5 was almost same but time taken for attending peak was more for F/M 2.5 which was 17-18 days as compare to F/M 1.5 which was 8-9 days. It was also observed that the rate of gas production (Calculated on 7 day average basis) was get reduced after 25 days as slope of graph approach towards zero hence it indicated that anaerobic digestion almost completed for the reactor. Abubakar and Ismail (2012) reported the cumulative methane production in anaerobic digestion of cow dung was found to be maximum 0.25 L/gVS added for F/M ratio 1.0, in present study the maximum methane production was found to be 0.362 L/gVS reduction for PD with F/M 1.5 which is quite higher. Methane production in case of goat dung was found to be lesser although the VS content of dung was very high, suggested that it does not good as inoculums without acclimatization. It was observed that initially the methane gas production was very low for GD rate was almost zero till 7-8 days, except in case of F/M ratio 1, after 8th day a sudden increase in rate of methane production was observed for F/M 1.5, 2, 2.5 including control (Fig. 4.11 B). Similar result also found by Thaniya et al. (2012) during the study of anaerobic digestion of decanter cake with different type of

wastewater in their experiment the methane production get started after 20th day of starting . For goat dung, time took to attend the peak rate of methane production was 8-10 days which is more than usual time i.e. 4-6 days. So it can be conclude that goat waste is not good for AD directly, until it is not activated properly. It was also observed that the methane production did not cease even after 28th day and there was significant rate of methane production even on 28th d of BMP run. Hence, it may conclude that to utilize the substrate fully the rector should be run more than 28 days.

For RD showed maximum gas production for F/M 1.5 was 2.32 L follow by F/M 2.0 with 2.19 L nearly same as F/M 1.0 (Fig 4.11). Minimum methane production shows for control 525 mL. Initial gas production rate (Calculated on 7 day average basis) led by F/M 1.0 with 171 mL/d follow by F/M 1.0 with 150 mL/d till 7th day. F/M 1.5 leads the rate of gas production after 7th day with 112 mL/d between 7-14 days, 55 mL/d between 14-21 days and 15 mL/d between 21-28 days. Gas production for RD observed to be lowest among all the dung studies here. Methane gas production has been found to be maximum for CD F/M 2.0. Gas production for F/M ratio 2.0 shows 3.43 L the maximum one follow by F/M ratio 1.5 which is 2.64 L. For CD Control showing minimum gas production of 715 mL followed by F/M ratio 2.5 which is 2.05 L. For CD the best F/M ratio i.e. 2.0 leads the methane gas production from first day with a significant gap unlike the other inocula where best F/M ratio also shows nearly same methane production in initial days. The rate of gas production for CD (Fig 4.12 D) shows same for F/M 1, 1.5.and 2.5 in initial days approximately 89 mL/d, led by F/M ratio 2 which shows 156 mL/d for first 7 days average value, which is less than that observed for 8-21 days i.e. 233 mL/d which further reduce to 70 mL/d for last 21-28 days.

Methane gas production result for PGD as inoculums shows maximum value 3.34 L for F/M 1.5 next to 3.09 L for F/M 2.5. Minimum methane production observed for control value with 939 mL. Methane gas production rate shows maximum for F/M 1.5 with value of 231 mL/d between 7-14 days which further reduces to 151 mL/d, 44 mL/d and 53 mL/d between 7-14, 14-21 and 21-28 days respectively. Methane production of 0.301 L /gm VS reduction was observed for best F/M ration of 1.5. The order of best inoculum for AD of FW based on their methane gas production is CD >PGD > PD > GD >RD for F/M 2.0, 1.5, 1.5, 2.0 and 1.5 respectively. It was observed that although the initial methane production rate was very high for poultry and piggery dung 210 and 238

mL/d (7 day average value) compare to other inoculums but it doesn't sustain for long time and it decreased to 40 mL/d after 14th day.

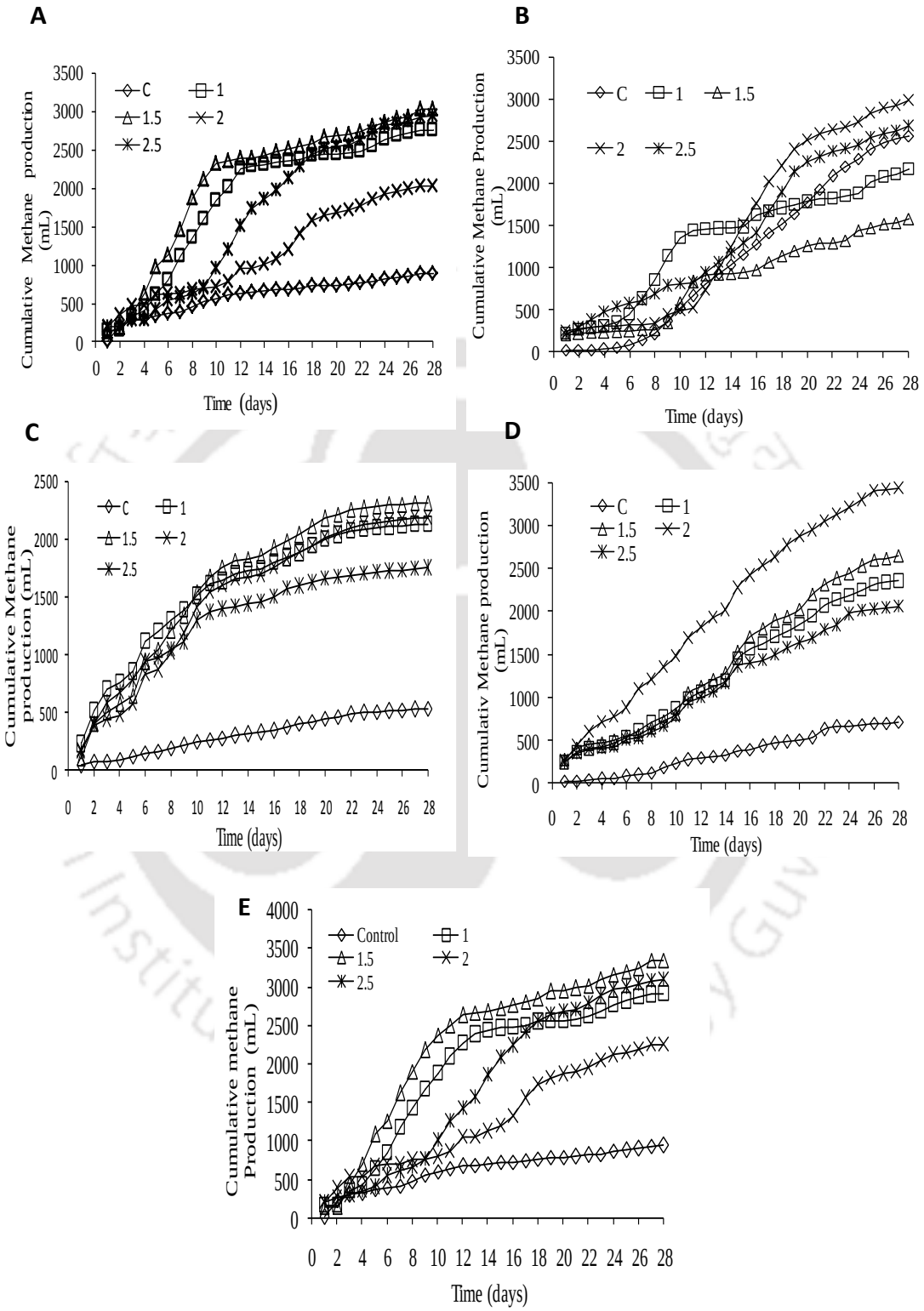


Fig. 4.11 Variation of cumulative methane during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD)

Whereas for cow dung initial methane production rate observed to be very high 245 mL/d and it was consistent till 21st day around 130-150 mL/d. Initial methane production rate was very low for goat dung 45 mL/d which further increase with time maximum to 192 mL/d between 14-21 days, and drop to 58 mL/d at end of reaction. Methane gas production for control was very high for Goat dung which predicts that it contains large percentage of biodegradable organic material than microbes as inoculum source. Inoculum plays a major role in AD of FW because of its easily available VFA which affects the pH. The CD inoculum and next PGD inoculum gave maximum gas production because of its digestive system adopts the FW digestion compare to other livestock dungs. The best performance for food waste biodegradation and methane generation was found in the reactor with 20% of total solid and 30% of inoculum which gave growth to an acclimation stage with acidogenic/acetogenic activity between 20 and 60 days and methane yield of 0.49 L CH_4/g VS (Forster et al., 2008).

- **Methane production per gram VS added**

Fig. 4.12 showed the cumulative methane production per gram initial total volatile solid added. Fig. 4.12 shows PD and CD produced maximum methane production per gram VS with 75 mL each. PGD produced 60.4 mL/VS added whereas GD and RD observed 25.4 and 29.8 mLCH_4/VS added. The chronicle order as per CH_4/g VS added are PD=~CD>PGD>RD>GD. It need to mention here this order significantly differ with total cumulative methane production order according to which CD>PGD>PD>GD>RD. Table 4.4 provides the comparison of ultimate methane production from different waste.

- **pH**

pH is the one of the most important criteria for proper functioning of BMP reactor. Methane production microbes function effectively between pH range of 6.5 and 8.2, with an optimum near pH 7.0 (Speece, 1996; Eckenfelder, 1999). It has been also observed that maximum biogas yield in AD at pH range 6.5-7.5 (Liu and Fang, 2007). A drastic pH drop will inhibit the initiation of methane fermentation with no sufficient buffering capacity (Kang and Jewell, 1990). Thus, pH was maintained on every 4th and 7th day in the reactor using 3 M $NaHCO_3$ solution. pH was maintained 6.5 to 7.5 on 0th d after that it was suddenly dropped in pH was observed on 4th day ranged 4-5, therefore, it was maintained to neutral range (Fig. 4.13). It can be concluded that there was sudden fall in pH due to acidogenesis and formation of volatile fatty acid and other form of acid during

initial day of starting of reactor where as its consumption in next step of methanogenesis is very less hence a net accumulation of volatile acids took place.

Table 4.4 Comparison of ultimate methane yields

Substrate	Inoculum	Ultimate methane yield (m^3/kgVS added)	Reference
Food Waste	Poultry dung	0.362	This study
Food Waste	Goat dung	0.144	
Food Waste	Rhinoceros dung	0.174	
Food Waste	Cow dung	0.305	
Food Waste	Piggery dung	0.302	
Boiled rice	Anaerobic digestion Sludge	0.294	Cho et al. (1995)
Cooked meat		0.482	
Fresh cabbage		0.277	
Cellulose		0.356	
Mixed Food Waste		0.472	
MSW	Residue from sequential batch anaerobic composting (SEBAC)	0.186-0.222	Owen and Chynoweth (1992)
Yard waste		0.134-0.209	
Paper		0.084-0.349	
Food packaging		0.318-0.349	
Straw	Horse dung	0.17	Kusch et al., (2007)
Coarse-cut fodder maize	Digester sludge from a municipal wastewater treatment plant	0.211± 0.006	Raposo et al., (2006)

*Calculated for 28 days cumulative methane production per gram of volatile solid reduction.

Low pH condition was unfavorable for methanogenesis resulting less methane production was observed during these days, but as soon pH was maintained to neutral range the methanogenic bacteria became active and rate of methane production increased.

This could also be justified by comparing with control data where neither too much variation in pH was observed nor too much variation in methane production was found. For each set of trial pH of reactor was maintained between 6.5-7.5 ranges on 0^{th} day. Drop in pH was observed on 4^{th} day for all F/M ratios i.e. 1, 1.5, 2, 2.5 and each inoculum within range 4.0-5.5, however not much drop in pH value was observed for control in most of dung except in case of RD whose min value of pH for control on 4^{th} d was 5.56 (Fig. 4.13). pH of reactors again maintained to neutral range on 4^{th} d as a result not much drop was observed on 7^{th} day. pH provide information about acidogenesis stage pH also shows information about the extent of degradation. Observing the pH profile of different inoculum trial, it was found that the maximum drop in pH value on 4^{th} day of reactor found for CD 3.24 and 3.41 for F/M ratio 2.5 and 1.5 respectively, while minimum drop was observed for RD with value 4.18 and 4.71 for F/M 2.5 and 1.0 respectively. Comparing the pH profile of best F/M for all trial Fig 4.13(F) it was observed that minimum pH value on 4^{th} day found for GD and CD with 3.70 and 3.68 respectively and maximum pH on 4^{th} day found for PGD and RD with value 4.81 and 4.80 respectively. It was also observed that there was very little variation in pH value was observed alter 7^{th} day for most of the trial except in case of GD and RD where a significant change in pH value was observed till the end of the reactor period.

- **Volatile solids**

The volatile solids (VS) in organic wastes are measured as total solids minus the ash content, as obtained by complete combustion of the feed wastes. VS comprise the biodegradable volatile solids (BVS) fraction and the refractory volatile solids (RVS). Kayhanian (1995) showed that knowledge of the BVS fraction of MSW helps in better estimation of the biodegradability of waste, biogas generation, organic loading rate and C/N ratio. It is observed for PD the initial VS concentration ranged 80-90% of dry weight. for all F/M which get reduced with reactor time proceeds, as the maximum reduction in VS was observed in between 0 to 7 days for all F/M ratios except for F/M 2.5 for which it was between 7 to 14 days (Fig. 4.14). Maximum reduction in VS was observed for F/M 1.5 with 41.9% follow F/M ratio 2.0 with 39.7% calculated by taking

initial VS as 100%. For GD the VS reduction was observed maximum between 7 to 14 days which was also period for maximum methane production rate in case of GD the

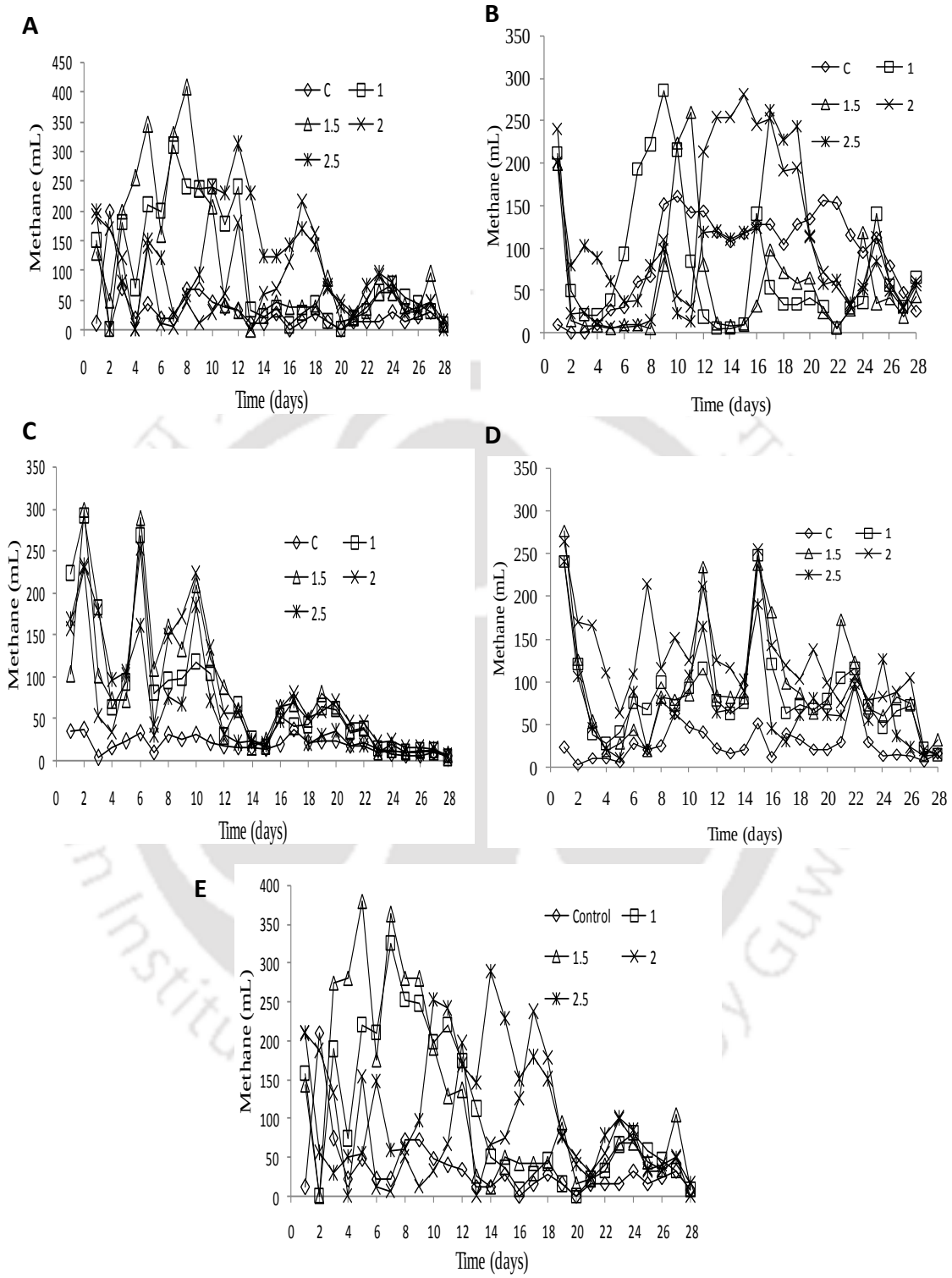


Fig. 4.12 Variation of methane production rate during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD)

maximum reduction in VS was observed 33.91% for control follow by F/M ratio 2 and 2.5 with value 22.33 and 17.55% respectively.

Maximum VS reduction for control can be justified by methane production for control was 2.58 L which was maximum for all five trial control value. For RD, the VS reduction was minimum for control with 11.2% and maximum VS reduction was observed for F/M ratio 1 with 42.8% and F/M 2.5 with 36.1% respectively. For RD most of VS reduction took place between 0 to 7 days. In case of CD Fig 4.14 D shows the VS reduction in 0 to 7 days was very less the destruction of VS started after 7^{th} day and continue till end of reactor period. VS reduction control was 26.21% and for F/M ratio 2 and 2.5 was 54.58 and 44.16% respectively. For PGD, pH reduction was observed throughout the reactor period with significant amount VS destruction was more faster between 0 to 7 days reduction in VS for control was 38.82% and maximum for F/M 1 and 1.5 with 45.71 and 45.51% respectively. Comparing the best F/M ratios, the maximum reduction in VS was observed for CD with 54.58% next to PGD with 45.51% .Minimum reduction in VS for GD with 22.33%. Based on reduction in VS the following inocula are arrange in following order CD>PGD>PD>RD>GD. Szikriszt et al. (1988) reported that 41% VS reduction for pretreated MSW and Sharma et al. (1988) reported 45% VS reduction for Mirabilis jalapa leaves (0.088 mm size). Chynoweth et al., (1985) reported 32.3% reduction for cotton wood anaerobic digestion and it was reported 56% reduction of AD treatment of water hyacinth with primary sludge (3:1) (Owen and Chynoweth, 1992).

- **Volatile fatty acid**

Volatile acid is one of important parameter verify the extent of degradation i.e. conversion of long chain complex molecules to simpler forms. Formation of VFA is an indicator of hydrolysis process of reactor. The dominant VFA produced are acetic acid, propionic acid and butyric acid. VFA concentrations can increase above 6,000 mg/L without any loss of methane production (Owen and Chynoweth, 1992).Volatile acids represent the control of AD process. Initially, with hydrolytic and acetogenic activities, VFA is supposed to decrease. After organic matters were transformed to acids, the removal can be interpreted as gas production. Another aspect is, that they can indicate process stability overload and any form of inhibition (Mussoline et al., 2013). In this study it was observed that the initial VFA concentration for CD was found higher value for all F/M maximum 5813 mg/L for F/M ratio 2.5.There after continuous increase in

VFA was observed till 14th day of sampling almost for all samples except there was small decrease observed for F/M 2.0 at 7th day then after it followed the same trend (Fig. 4.15 A).

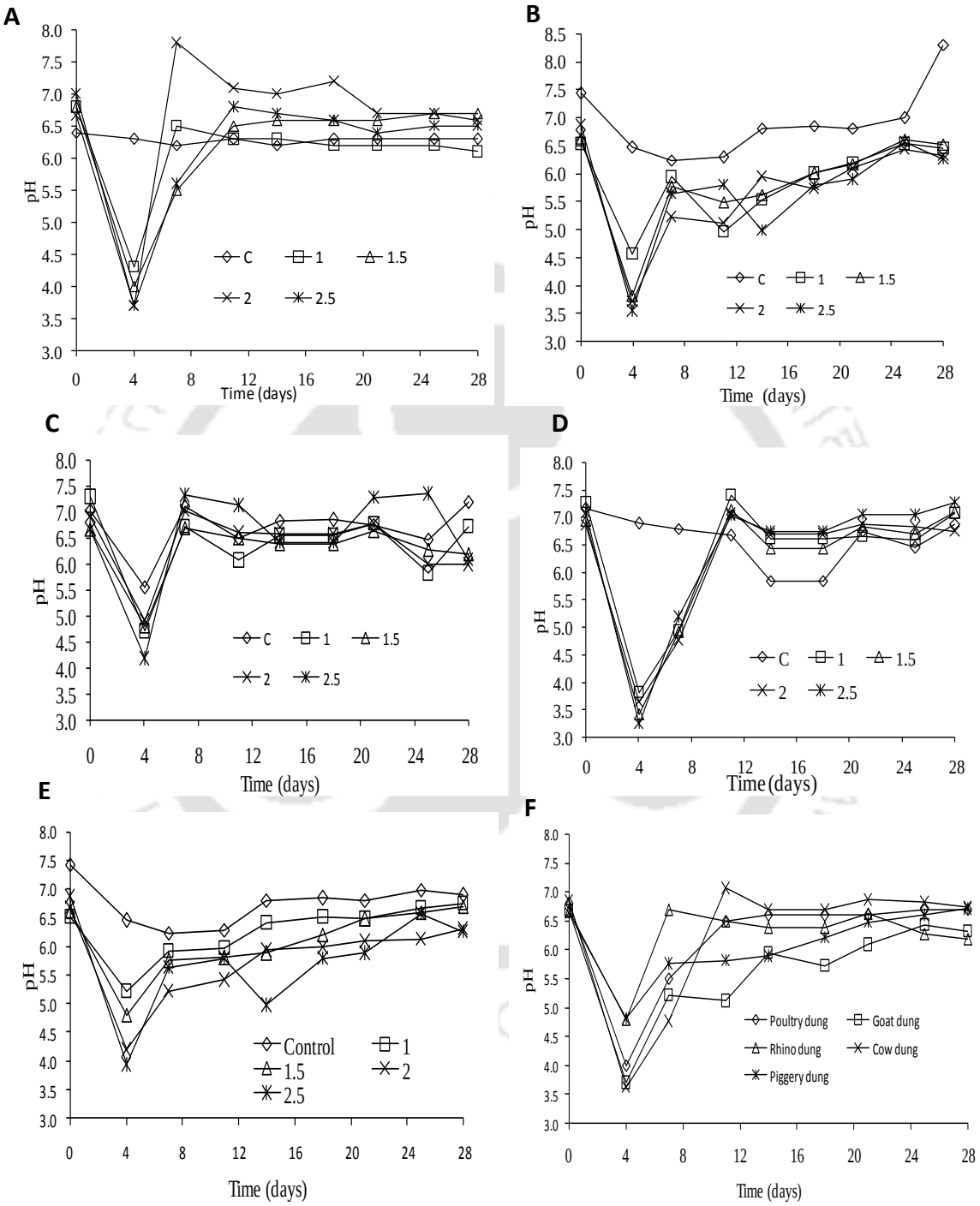


Fig. 4.13 Variation of pH during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD; F: Best F/M of all five inocula)

It needs to be mention here while VFA concentration in reactor was high at same time rate of methane production was also observed to be high. Since VFA was observed to be increasing in 7 to 14 days period although the there was considerable methane production also observed during this period. Hence, it could be concluded that hydrolysis process was faster than that of methanogenesis could be due to accumulation of VFA inside reactor resulting its concentration increased which further get decreased for period 14 to 21 days due to its utilization in further methanogenesis. In case of GD, not much variation in VFA concentration was observed for all F/M ratios till 7th day including control. Thereafter there was an ample increment in VFA concentration was observed after 7th day from starting of reactor almost for all set of F/M ratios except in case of control for which not much change observed throughout reactor period. The VFA concentration became stable after 14 days and not much increase in VFA was observed on 21st day. The increment of 10-20% in VFA concentration was observed on 21 to 28 days excluding control.

Final day concentration of VFA was found to be very high compare to initial concentration (except F/M 1.0 and control) indicated that further possibility of degradation and more methane gas production was possible if the reaction time extended for more than 28 days. High VFA concentration also shows that hydrolysis process was dominated by methanogenic during whole reactor period and final VFA concentration was found to be high and increasing trend. In case of RD, different trend was observed from other inoculums, initial VFA concentration for RD observed to be very high with range 5000–6500 mg/L for all F/M ratio except control with 750 mg/L. Thereafter significant Drop in VFA value observed on 7th day after small increase was observed from 7 to 14 days which further increase till 21st day with maximum value of 7625 mg/L for F/M ratio 2.5. Variation in VFA was observed between 0 to 7 days and 14 to 21 days was higher than 7 to 14 and 21 to 28 days. Not much variation in VFA was observed after 21st d till end. VFA variation for CD shows different pattern than last three inoculums result. Change in VFA shows very less till 14th day of reactor period with maximum value 2625 mg/L for both F/M 2.5 and 2.0. Increase in VFA value was observed from 14th day maximum to 21st day with 5438 mg/L for F/M 2.5. VFA value for control was observed to be maximum on 28th day with 2063 mg/L. In case of PGD, the VFA concentration found to be increasing at faster rate till 7th day with maximum and minimum value 5824 mg/L for F/M 2.5 and 2100 mg/L for control respectively. Not

much change in VFA was observed between 7 to 14 days of reactor. Slight decrease in VFA was observed at end of reactor period. Similar observation also reported by (Raposo et al., 2006) during anaerobic digestion of maize with digested sludge found maximum total VFA 6000 g COD/L on 7th day of reactor for I/S ratio 1.0.

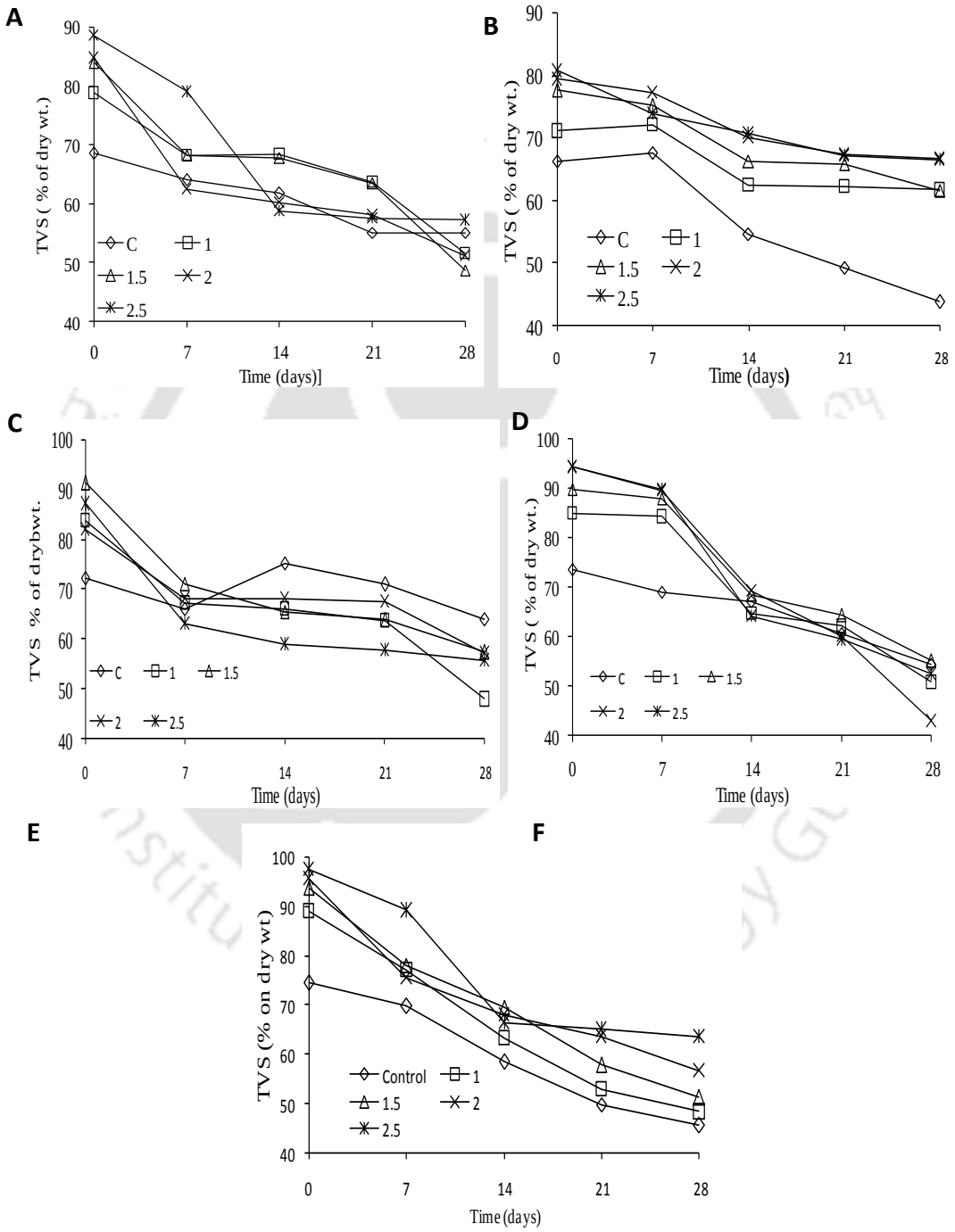


Fig. 4.14 Variation of volatile solids during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD)

Comparing the best F/M ratios for different inoculum, two set of trends was observed in VFA profile for poultry and cow dung. In CD reactor, the VFA value got increased to maximum at initial days of the reaction and then started decreasing gradually. In case of GD and RD, it decreased till 7^{th} day later it starts to increase with variation in rate and the trend continues till 28^{th} day of reaction. VFA for PD was maximum on 14^{th} day with value 9313 mg/L. Maximum VFA was observed for GD i.e., 10542 mg/L on 28^{th} day whereas minimum value was in case of cow dung with 750 mg/L on 0^{th} day. In the mixture, each consortium is acting synergistically for cellulose degradation, transforming these sugars in VFA and finally into methane. The VFA profile for different reactors was given in Fig 4.15. FW contains high nitrogen content due to proteins which produces ammonia. While degrading it get mixed with the reactor solution to form ammonium bicarbonate and helps in buffering the pH of the digester (Murto et al., 2004). VFA formation is second stage of anaerobic reaction resultant due to acidogenesis process. VFA concentration also effects methanogenesis stage of anaerobic process which reduces biogas production. Readily available VFA facilitate the methanogens process and so VFA formation is supposed to be one of limiting step for biogas production. Increasing VFA concentration was absorbed during initial days due to production of acid at faster rate than methanogenesis steps (consume fatty acids) which leads to accumulation of fatty acid inside system consequently increases the VFA concentration.

- **Sulfate (SO_4^{2-})**

Under anaerobic conditions, sulphate is used as an electron acceptor and hence reduced to sulphide by sulphate reducing bacteria (SRB) (Boe, 2006; Chen et al., 2008). Two groups of SRB are responsible for the reduction, the incomplete and the complete oxidisers. The first group oxidises compounds like lactate to acetate and CO_2, whereas the second one converts acetate to CO_2 and HCO_3 (Chen et al., 2008). SRB can metabolise a number of substrates, such as alcohols, organic acids, aromatic compounds and long-chain fatty acids. Normally, inhibition through competition does not occur in the first stage of digestion since the SRB are not capable of degrading biopolymers (Foster et al., 2008). Sulfate rich substrate with small retention time help to dominate SRB over methane producing bacteria consequently H_2S formation took place instead of CH_4 in last phase of AD process (Foster et al., 2008).

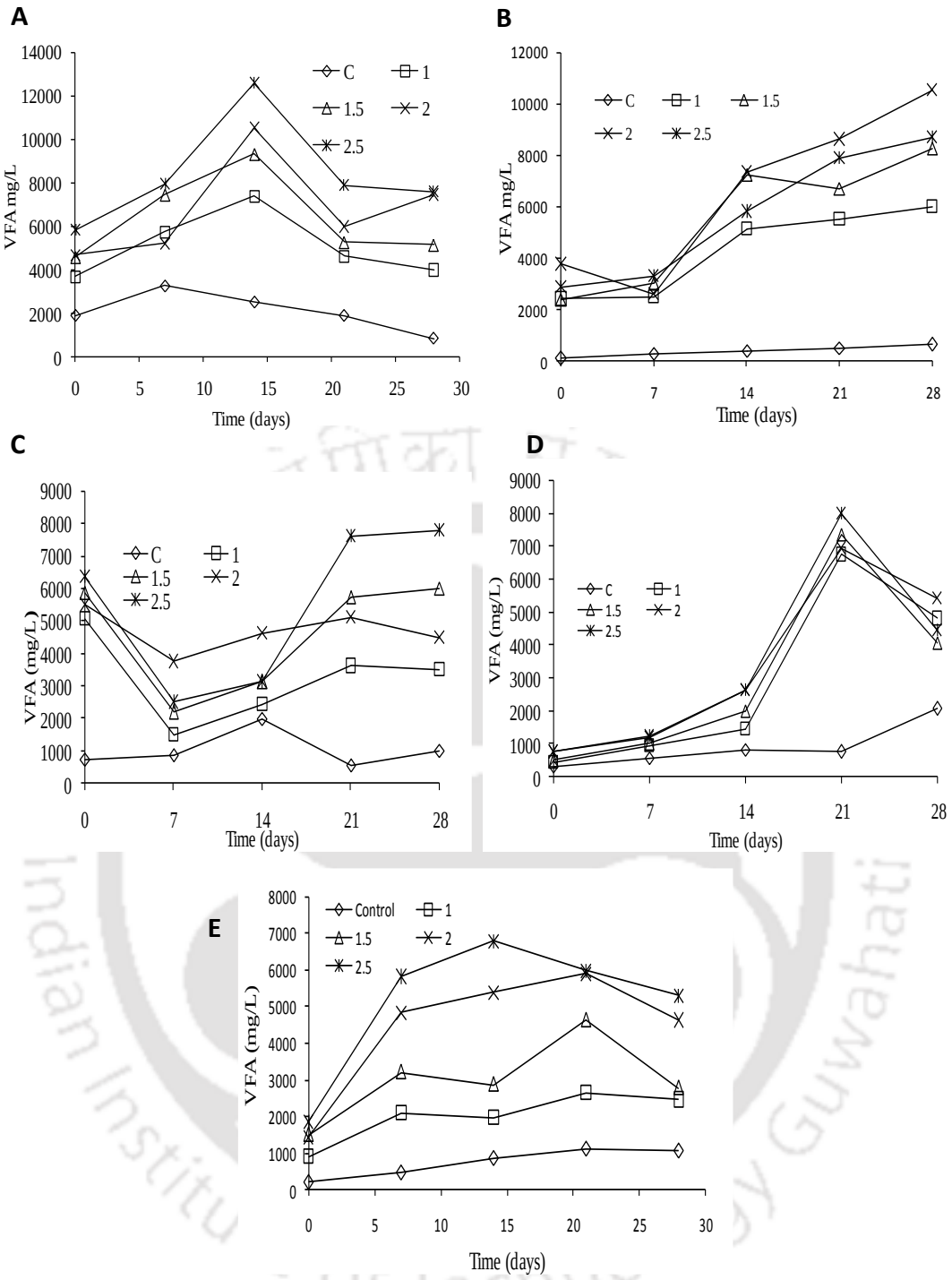


Fig. 4.15 Variation of total volatile fatty acids during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD)

In present study there was not any regular pattern was observed for sulfate variation in different set of experiment. In case of PD, less change in sulfate concentration was observed during 0 to 7 days for all F/M ranging 3000-5000 mg/L after that a sudden drop in sulfate concentration was observed on 14th day with minimum value of 1237 mg/L for control and 1408 mg/L for F/M 1.0 (Fig 4.16), as reported by Li et al., (1995). H_2S

formation was more for higher COD/S ratio (60) compare with intermediate (15-1.5) and lower (0.75), this might be one of reason for more drop in sulfate concentration for F/M 2.0.

After 14th day there was a sharp increase in VFA was observed on 21st day that end with a small decreased except in case of F/M ratio 2.5 where not much change was observed between 21 to 28 days. In case of GD, Variation of sulfate was observed in irregular pattern, initial sulfate concentration ranging 2500–4000 mg/L for all F/M except for control which is 1481 mg/L on 0th day. On 7th day small change in sulfate concentration, slight increase for F/M 1.5 and 2.5 whereas slight decrease for F/M 2.0, 1.0 and control. Small decrease was observed on 21st day also ended with slight increase except control which decreased on 28th day. For RD, sulfate concentration for F/M 2.5 was varying drastically during whole reactor period (Fig 4.16). Initial concentration was found to be high 5676 mg/L after that a steep drop was observed on 7th day with 2112 mg/L concentration and 2137 mg/L on 14th day which further increase sharply with 5708 mg/L on 21st day and end with a slight drop with 4972 mg/L. Variation for other F/M ratio not much and lies within range of 2900–4000 mg/L. Sulfate variation in case of CD and PGD followed approximately same trend with maximum value for F/M 2.0 on 0th day with 4338 and 4437 mg/L respectively (Fig 4.16) . Decrease in concentration range 500-1000 mg/L was observed at end for all F/M ratios in both trials. Comparing the best F/M ratio of all the trial Fig 4.16(F) it was observed that not much changes in sulfate profile for all best trials except in PD, where a sharp minimum of 1408 mg/L concentration was observed on 14th day final sulfate concentration for all trial lies in range of 3000 – 4000 mg/L.

- **Total kjeldahl nitrogen (TKN), total phosphate (TP), ammonia**

The initial and final TKN concentration was monitored in every reactors and it is shown in Fig 4.17. In case of PD, RD and PGD final concentration was increased and in GD initial concentration was 4-6 g/L later it increased to 5-10 g/L at final. In control and F/M 2.5, decrease of TKN was observed in final concentration. Initial ammonia concentration for best F/M found to be maximum for PD with 10.1 mg/L and maximum final concentration for GD with 7.3 mg/L (Fig 4.18). Final ammonia concentration increases for GD, CD and PGD, whereas it decreased for PD and RD. Total phosphorous concentration was reduced for all best F/M ratios, initial range was observed to be 90-

110 mg/L and it increased to final concentration of 70-90 mg/L. In case of PGD, reduction of TP concentration from 116 to 42.9 mg/L was observed (Fig. 4.19F).

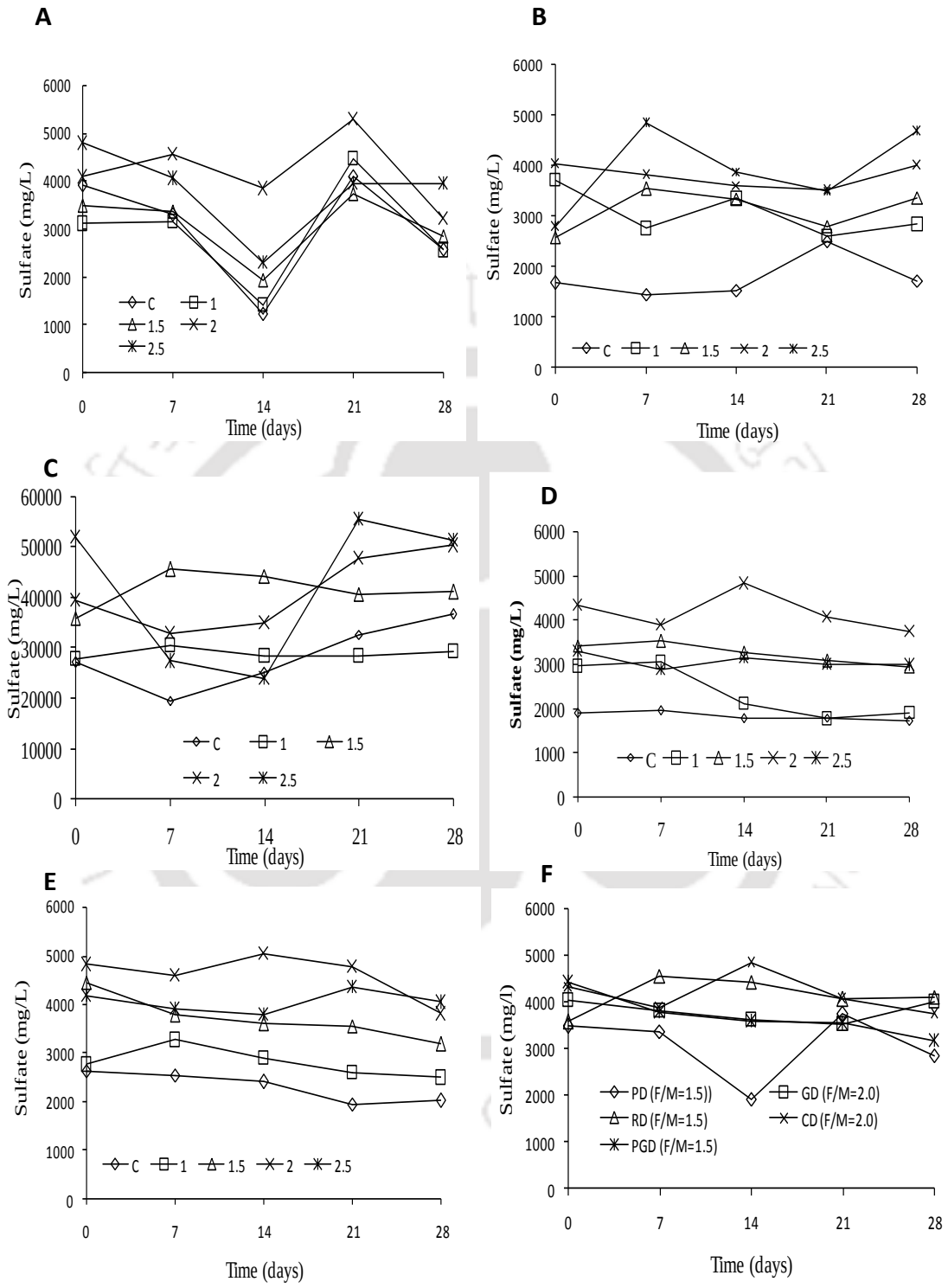


Fig. 4.16 Variation of sulfate during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD; F: Best F/M of all five inocula)

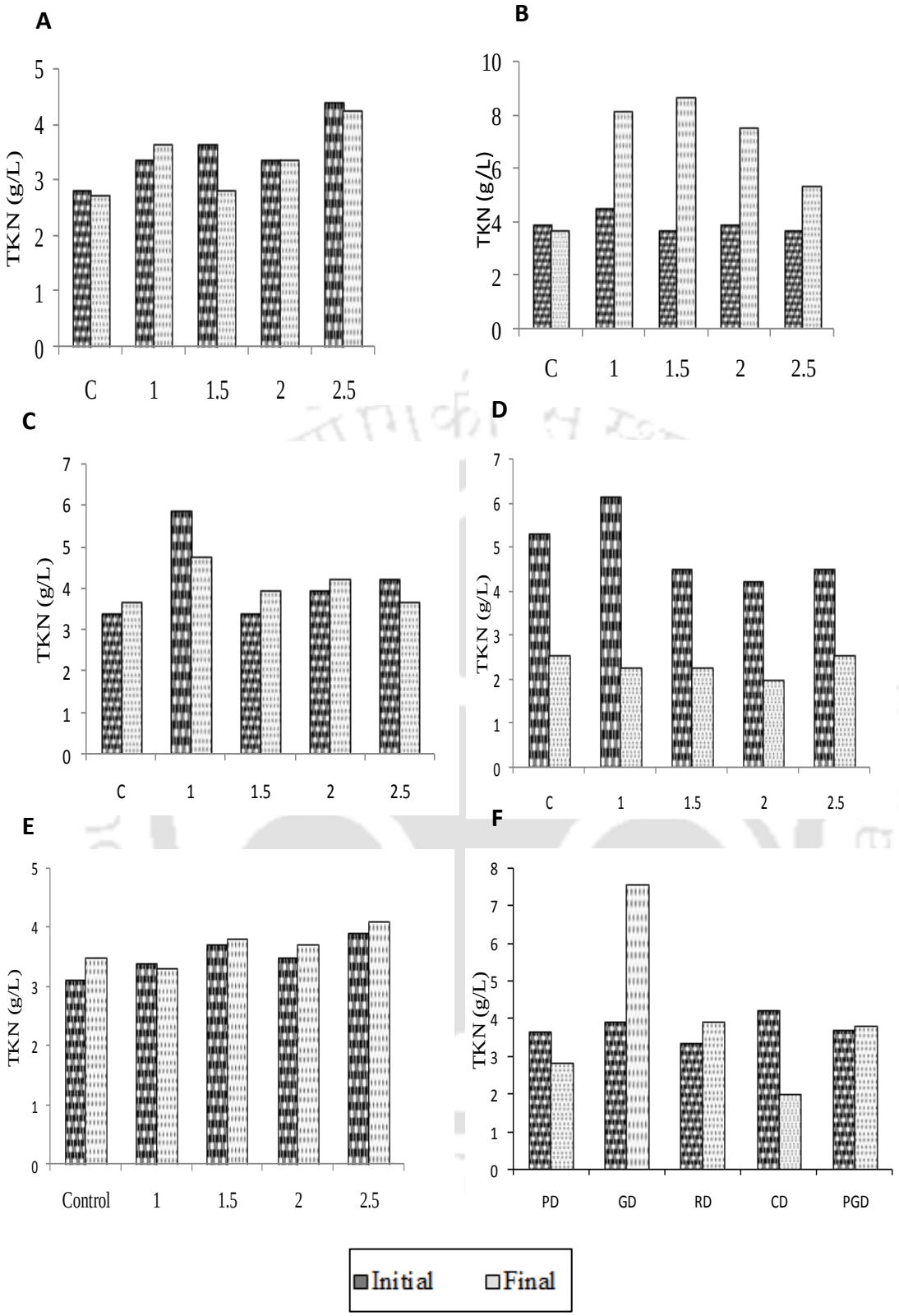


Fig. 4.17 Variation of TKN during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD; F: Best F/M of all five inocula)

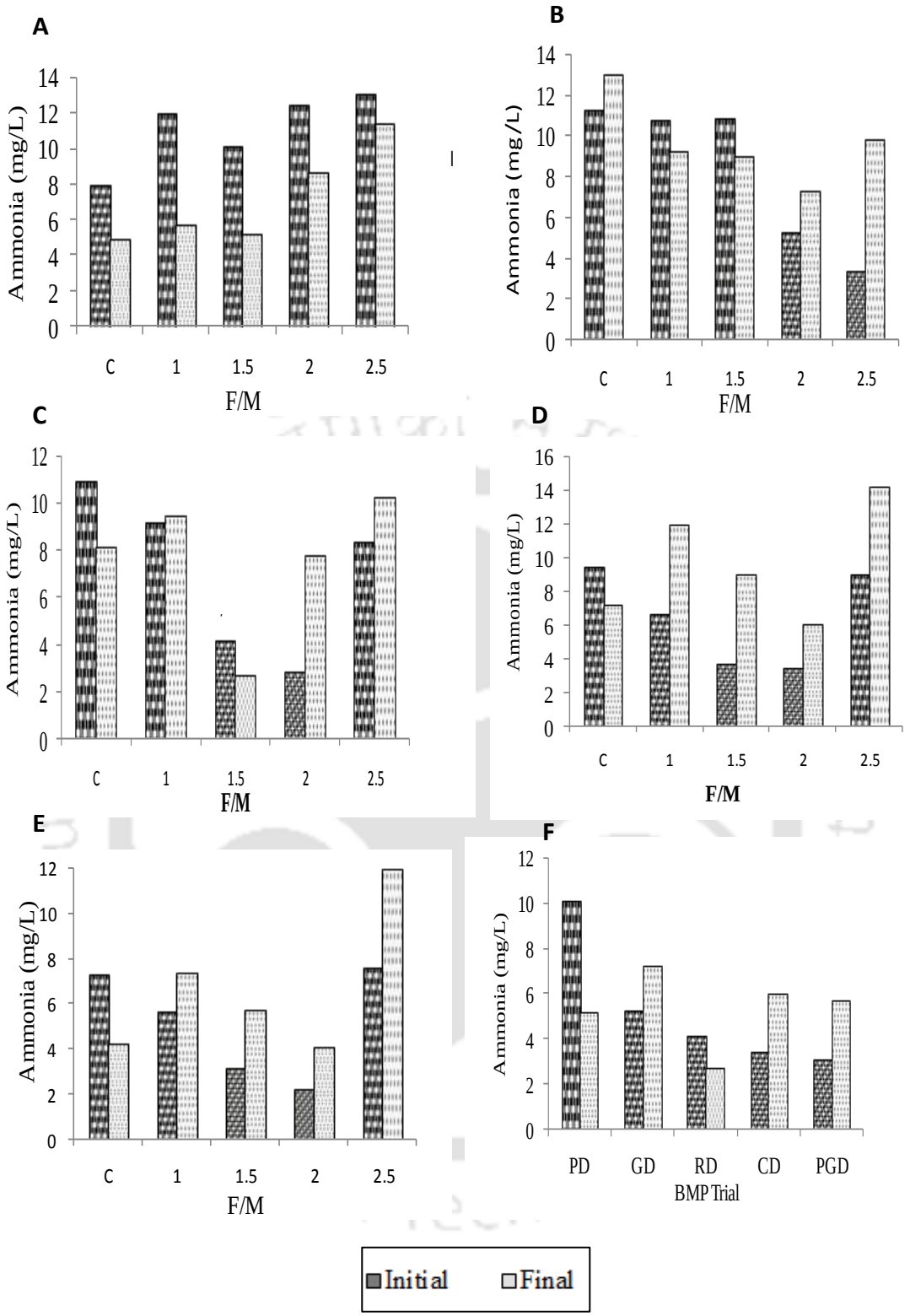


Fig. 4.18 Variation of ammonia during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD; F: Best F/M of all five inocula)

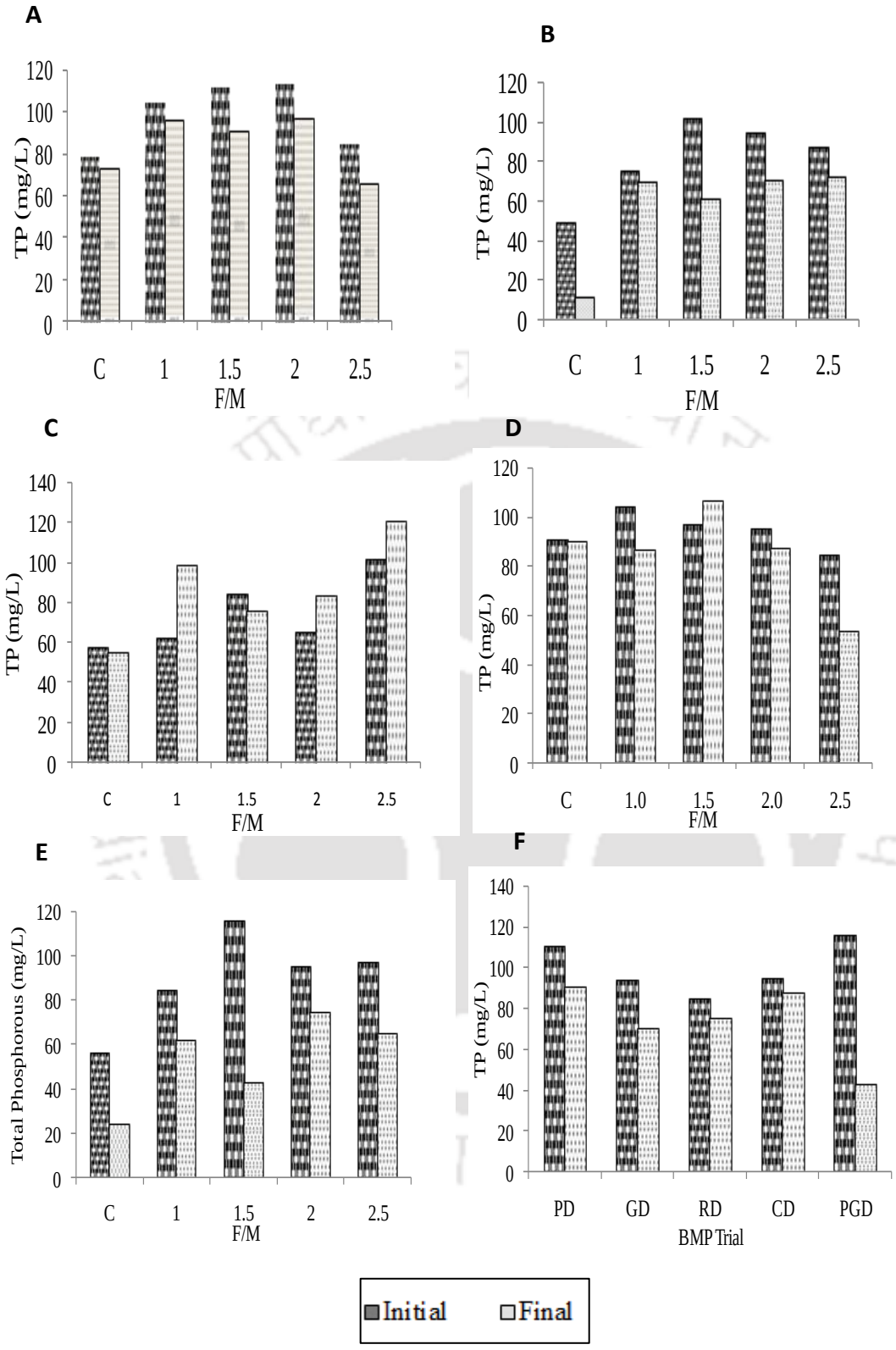


Fig. 4.19 Variation of total phosphorous (TP) during different livestock dung BMP experiments (A: PD; B: GD; C: RD; D: CD; E: PGD; F: Best F/M of all five inocula)

4.3.1 COMPARISION OF BEST F/M OF DIFFERENT LIVESTOCK DUNG BMP REACTORS

➢ **ANAEROBIC BMP SETUP**

Five different BMP setups were tested during a period of 30 days to evaluate the anaerobic digestion of FW at five different livestock inoculums with different F/M ratio varied from 1.0 to 2.5. Chudoba et al. (1992) reported that Food to microorganism ratio (F/M) is one of the most important parameter influencing anaerobic biodegradation by their experiment done for activated sludge batch testing. Inoculum plays an important role in anaerobic reactor startup by balancing the populations of syntropho bacteria and methanogens. This population balance helps in syntrophic metabolism that is thermodynamically feasible in anaerobic digestion (Pandey et al., 2011). Theoretically, the methane yield should be independent of the F/M and it should only effects the kinetics of the process, but it is observed experimentally that it neither only influence the rate of AD process but also extent of reaction. Most methanogenic microorganisms have an optimum pH of between 7 and 8, while the acid-forming bacteria often have a lower optimum pH (Angelidaki and Sanders, 2004).

- **Methane production**

Methane gas production is most important parameter that needs to be optimized in anaerobic digestion process. The methane production varies with many factors such as inoculum, VS, VFA, and temperature. It is always measured in two forms one is on the basis of cumulative production and another is per day methane production rate. Higher biodegradation of food waste and maximum methane production was found in reactor with 20% of TS and 30% of inoculum: give rise to an acclimation stage with acidogenic/acetogenic activity between 20 and 60 days and methane yield of 0.49 L CH_4/g VS (Forster et al., 2008).

In this study it was observed that AD of FW with cow dung used as an inoculum provided maximum methane production of 3.47 L at F/M ratio maintained at 2.0, followed by PGD which with 3.36 L at F/M ratio maintained at 2.0. Next to that, GD and PD were observed with similar cumulative methane production on 30th day of reactor with 3.09 and 3.05 L respectively. Fig. 4.20 and 4.21 showed methane production rate and cumulative methane production from different livestock inoculum added AD reactors. The order of best inoculum for AD of FW based on their methane gas

production is CD > PGD > GD > PD > RD. As shown in Table 4.6 and Fig. 4.25, it was observed that initial methane production rate was very high for PD and PGD 210 and 231 mL/d respectively (7 days average) compared to other inoculums but it doesn't sustain for long time and it decreased to 40 mL/d after 14 days. Whereas for cow dung it was more or less consistent till 21st day around 130-150 mL/d. Initial methane production rate was very low for goat dung (45 mL/d), which further increased with time to a maximum of 192 mL/d between 14-21 days, and then drops to 58 mL/d at end of reaction. Methane gas production for control was very high for GD which predicts that large percentage of GD is organic material than microbial inoculum source. Inoculum plays a major role in AD of FW because of its easily available VFA which affects the pH. The CD inoculum and next PGD inoculum gave maximum gas production because of its digestive system adopts the FW digestion compared to other livestock dungs.

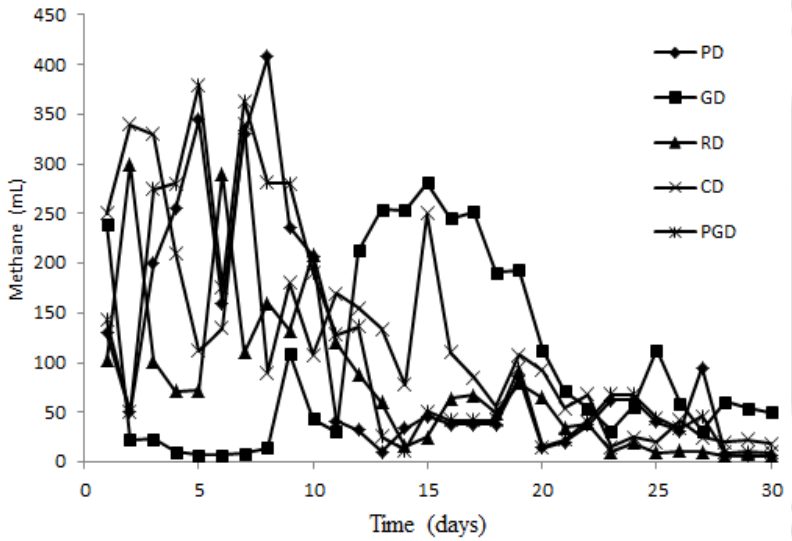


Fig. 4.20 Methane production rate of best of different livestock inoculum reactors

- **Volatile solids**

Decrease in VS indicates the mass loss from the AD system; it can be correlated with biogas production. Higher degradation of VS produces more biogas in controlled AD process. The VS reduction is majorly depends on the inoculum activity and adoptability of the inoculum towards substrate. In the present study maximum VS reduction was observed in case of CD which is 54.6% followed by PGD with 45.5%, which produces the cumulative bio-methane gas of 3.43 and 3.34 L respectively. The results revealed that the activity of CD and PGD is more than other livestock dungs. The

digestive system of the cows and pigs in this locality are well adapted to the food waste because of its daily feeding system. Fig. 4.21 shows the VS reduction in AD of FW with different inoculums. In present study the VS reduction order was CD > PGD > PD > RD > GD.

Table 4.5 Best F/M ratio of different inoculums based of cumulative methane production

Inoculum	**Best F/M Ratio**	**Total Methane production (L)**
Poultry dung	1.5	3.04
Goat dung	2	2.98
Rhinoceros dung	1.5	2.32
Cow dung	2	3.43
Piggery dung	1.5	3.35

The VS in GD used AD reactors showed less reduction compare to gas production it might be due to its dryness which restricts inoculum activity compare to other livestock inoculum. GD inoculum added reactor at F/M ratio maintained 2 showed VS reduction of 33.6% while rest of the livestock inoculums gave more than 40% VS reduction. More loss in volatile solids analogy was observed with more mass loss from reactors in form of gas production.

- **Volatile fatty acid**

Different pattern in VFA profile was observed with poultry and cow dung inoculum as compared to other livestock inoculums. The VFA value increased initially and then started decreasing at the final stage, but in case of GD and RD it decreased till 7^{th} day after that it started to increase. VFA for PD was found maximum on 14^{th} day with value of 9.3 g/L. Maximum VFA was observed for GD with 9.8 g/L on 28^{th} day whereas minimum value was in the case of CD with 0.75 g/L on 7^{th} day. In the mixture, each consortium was considered to act synergistically for cellulose degradation, transforming the sugars in VFA and finally into methane. The VFA profile for different reactors is detailed in Fig. 4.21. FW contains high nitrogen content due to proteins which produces ammonia. During degradation it got mixed with the reactor solution to form ammonium bicarbonate and helps in buffering the pH of the digester (Murto et al., 2004).

VFA formation is second stage of anaerobic reaction resultant due to acidogenesis process. VFA concentration also effects methanogenesis stage of anaerobic process

which reduces biogas production. Readily available VFA facilitate the methanogens process and so VFA formation is supposed to be one of limiting step for biogas production. Increasing VFA concentration was absorbed during initial days due to production of acid at faster rate than methanogenesis steps (consume fatty acids) which led to accumulation of fatty acid inside system consequently increased the VFA concentration.

Table 4.6 Methane production rate of FW on 7 days average with different livestock inoculums

Time (d)	Methane production rate (mL/d)				
	Poultry dung	**Goat dung**	**Rhinoceros dung**	**Cow dung**	**Piggery dung**
0-7	210	45	149	155	231
7-14	137	131	112	132	150
14-21	40	192	60	131	44
21-28	47	58	14	70	66

- **Kinetic study**

The cumulative methane gas production values were fitted with Gompertz equation curve to find the improvement in efficiency of nitrogen addition reactors (Lee et al., 2013). In the curve fitting R^2 was higher than 0.9. The kinetic parameters for cow dung added reactor were calculated as being P = 3.8804 L CH_4, R_m = 0.1538 L CH_4 /d, and λ =1.8217 d, respectively Rhinoceros dung added reactor had lower P and Rm.

- **Conclusion**

The results of this study demonstrated that there were significant differences between different inoculums. The reactors inoculated with CD had shorter initial time and achieved higher biogas production than reactors inoculated with other inoculums. Livestock inoculum such as GD and RD failed to create a favourable condition for FW digestion. Methane production was unsteady and retained in a low level in reactors inoculated with GD and RD in 30 days period of AD. CD followed by PGD with the

highest activity and most suitable nutrient content, achieved the highest methane production and showed the best degradation among all livestock inoculums.

4.4 PHASE IV: ANAEROBIC BATCH REACTOR WITH BEST F/M RATIO OF DIFFERENT LIVESTOCK DUNG

Five different dungs batch reactors were tested during a period of 50 days to evaluate the anaerobic digestion of FW for methane production. Chudoba et al. (1992) reported that Food to microorganism ratio (F/M) is one of the most important parameter influencing anaerobic biodegradation by their experiment done for activated sludge batch testing. Microbial balances made syntrophic metabolism thermodynamically feasible in anaerobic digestion (Pandey et al., 2011).

- **Methane production**

Fig. 4.22 shows the cumulative methane gas production for different inoculums batch study. The maximum methane production was obtained from CD added reactor with 52.54 L on 50th day followed by PGD with 50.44 L. Methane productions from RD observed to be 42.27 L on 50th day which is the lowest among all five inoculums. The production of methane followed similar pattern in all different inoculums except GD. At initial phase of the experiment, GD performed less because of its less activity and moisture content. Fig. 4.23 shows rate of methane production. Initial week the gas production was nearly same for all reactors 1.5 to 1.8 L/d on an average except for GD which is lowest rate of 0.82 L/d. Maximum gas production was observed for CD at initial days with 1.8 L/d next to PGD with 1.72 L/d. The methane production from RD was quite good till 16th day with 1.48 L/d for 0-7 days and 2.25 L/d for 7th-14th day on an average, but after 16th day a drop in methane production rate was observed. Methane production rate at final days was observed to in range of 0.05 to 0.1 L/d for all the trial. On the basis of total methane production the inoculums could be arrange in following order CD > PGD > PD > GD > RD.

- **Volatile solids**

The reduction of volatile solids is shown in Fig. 4.24. It was observed that maximum reduction of volatile solids for CD with 38.4% follow by CD with reduction of 38.6%. Minimum reduction of VS was observed for RD with 29.8%.VS reduction for PGD was observed to be 35.1% which was quite less than reduction from PD reactor (36.1%).

Based on VS reduction result all four inoculums can arrange in following Chronological order. Based on VS reduction the order was observed as CD>PD>PGD>GD>RD.

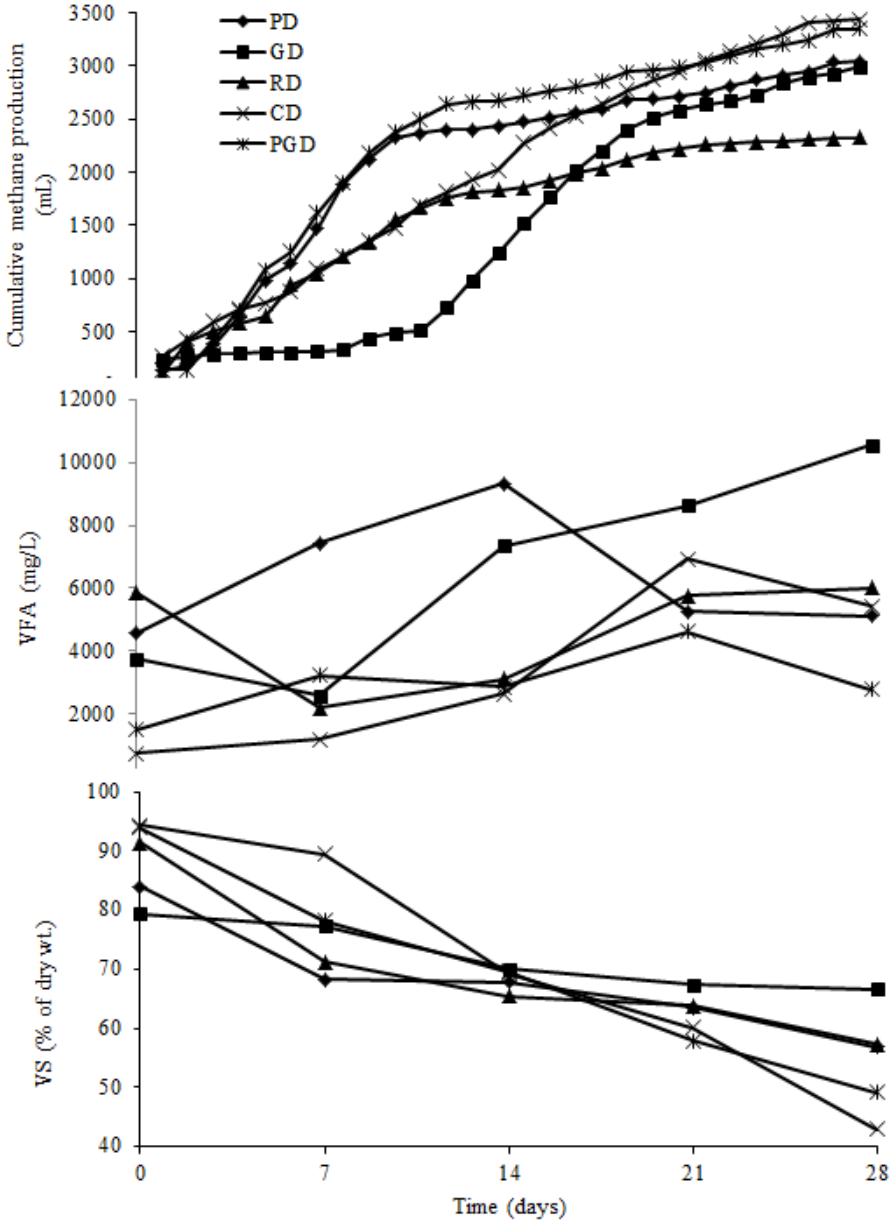


Fig. 4.21 Cumulative methane production, VFA and VS of best of different livestock inoculum added reactors

- **pH**

When FW is used as a main substrate for the anaerobic reactor, the pH of the reactor decreased drastically due to conversion of easily available organics to organic acids. It affects the methanogenic activity, which needs a neutral. Fig. 4.25 shows the pH range of all the reactors.

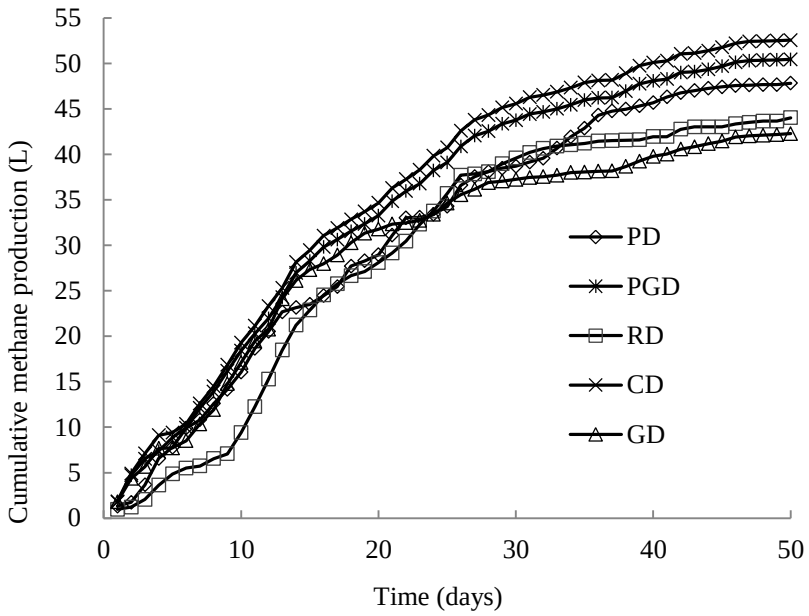


Fig. 4.22 Cumulative methane production of different livestock dung in batch reactor

Initial pH was in the range of 5.5-6.5, and it was maintained in neutral range using sodium bicarbonate solution. Drop in pH was observed much in initial days for all reactors in which minimum for PD and maximum for RD with 5.31 and 4.25 respectively. Minimum pH value was observed on 4th day, 4.01 and 4.02 for GD and PD respectively. pH adjustment was done for initial days, after 8th day of reactor pH adjustment was not required except a slight adjustment on 20 and 32nd day for CD and RD batch reactors.

- **Volatile fatty acids**

Each substrate has its optimum F/I ratio, considering the potential amount of volatile fatty acids (VFA) produced and its capacity to buffer the medium due to the ammonium produced by the hydrolysis of proteins (Lesteur et al., 2010). VFA production during the batch process of five different inoculum is shown in Fig. 4.26. It was observed that, the VFA production was increasing in initial days and maintained a level till end of the reactor except PD and GD which started to decrease at the end. Maximum VFA value was observed at starting for PD with 4500 mg/L and minimum value for CD with 925 mg/L for CD.

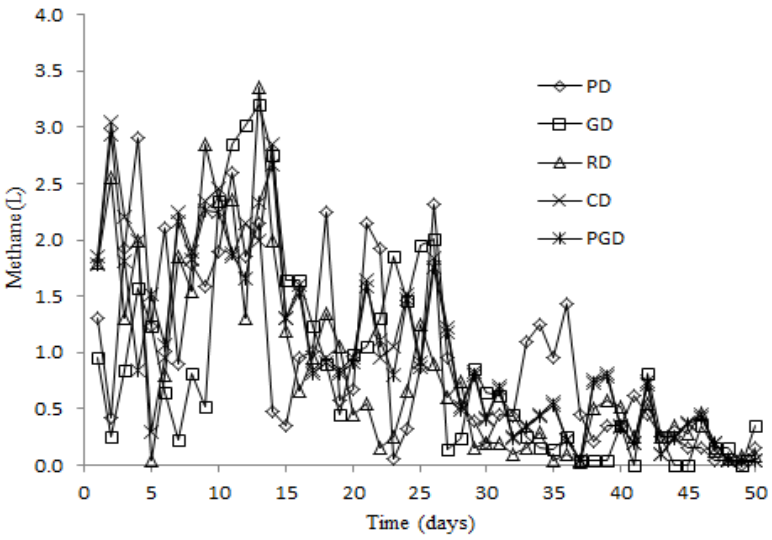


Fig. 4.23 Methane production rate of different livestock dung in batch reactor

Maximum VFA value during the experimental period was observed for PD on 4th day and for GD on 12th d with value of 8000 and 8250 mg/L respectively. For RD and CD the VFA value was observed maximum on 36 and 40th day with 8000 and 8125 mg/L respectively. For PGD the value was high at 24th day with 7836 mg/L.

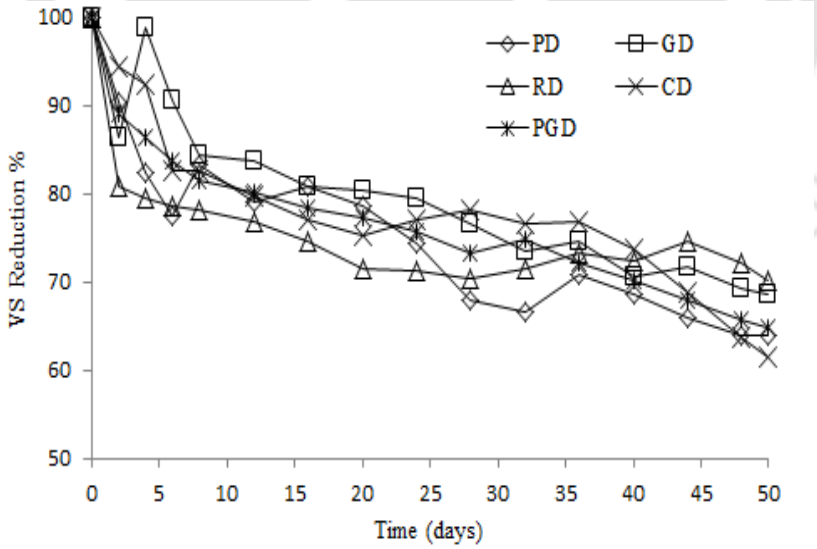


Fig. 4.24 Volatile solids reduction of different livestock dung in batch reactor

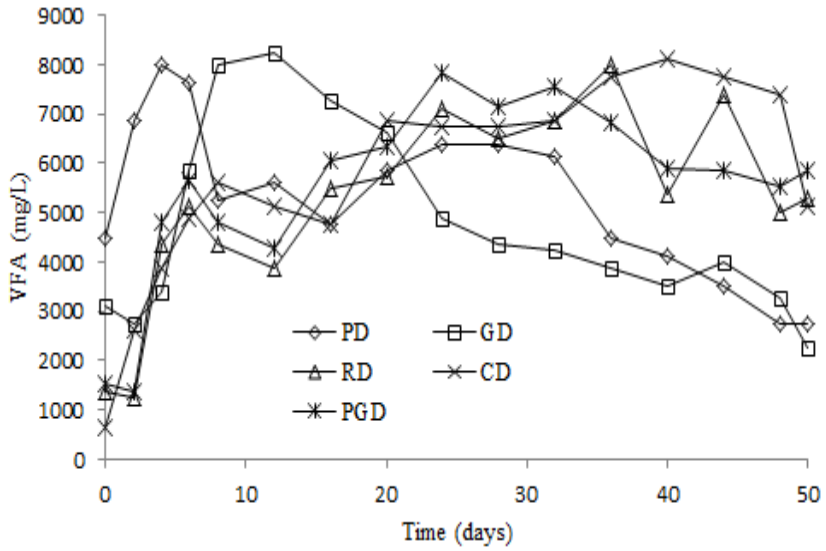


Fig. 4.25 VFA profile of different livestock dung in batch reactor

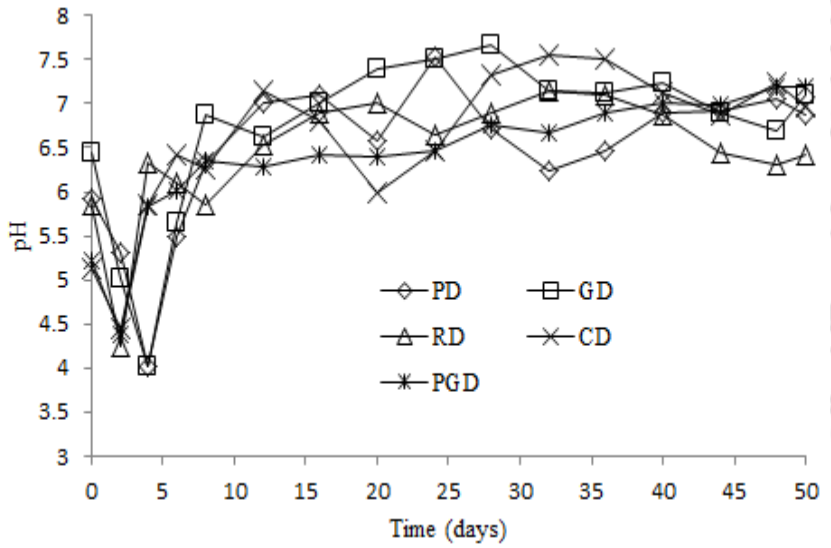


Fig. 4.26 pH profile of different livestock dung in batch reactor

- **Ammonia, Soluble Iron, Magnesium and Calcium**

Fig. 4.27 showed the initial and final concentration of ammonia in every reactor. The ammonium concentration was higher for PD with 10.1 mg/L at initial and maximum final concentration for GD with 7.3 mg/L. Final ammonia concentration was increased for GD, CD and PGD whereas decreasing for PD and RD. Many study suggests that the anaerobic reaction effected by lots of light and heavy metals. Combination of Na^+ and K^+ or Na^+ and Mg^{2+} resulted in around 10% increase in methane yield compared to that

produced by Na^+ alone (Kugelman and McCarty, 1964). Moderate concentrations of light metals ions ((Na, K, Mg and Ca) stimulate microbial growth, excessive amounts slowly down the growth, and even higher concentrations can cause severe inhibition or toxicity (Soto et al., 1993). The optimal Mg^{2+} concentration was reported to be 720 mg/L for the anaerobic bacterium *Methanosarcina thermophila TM1* and a Methanosarcinae-dominated UASB reactor (Ahring et al., 1991). Heavy metals also play a significant role in AD many heavy metals are part of the essential enzymes that drive numerous anaerobic reactions. Analysis of ten methanogenic strains showed the following order of heavy metal composition in the cell: Fe>>Zn>>Ni>Co=Mo>Cu (Takashima and Speece, 1989). In present study Fe, Mg and Ca were analysed as shown in Fig. 4.28. The initial soluble iron concentration was very less for all the trial which increases with time and observed to be maximum at the end of reactor. At 0^{th} day highest soluble Fe concentration was observed to be maximum for CD 191 mg/L and minimum for RD with 8.5 mg/L, whereas Fe concentration for PD found to be highest throughout the reactor period. End day maximum concentration found to be maximum for PGD with 808 mg/L follow by GD with 770 mg/L, lowest for CD with 231 mg/L. Profile for soluble Mg found to be similar to Fe which was increased with reactor time 0^{th} day maximum soluble Mg concentration found for GD with 0.818 mg/L and minimum for PD with 0.494 mg/L. At final day Mg concentration increases for all inoculum CD and GD with 2.494 and 2.115 mg/L respectively.

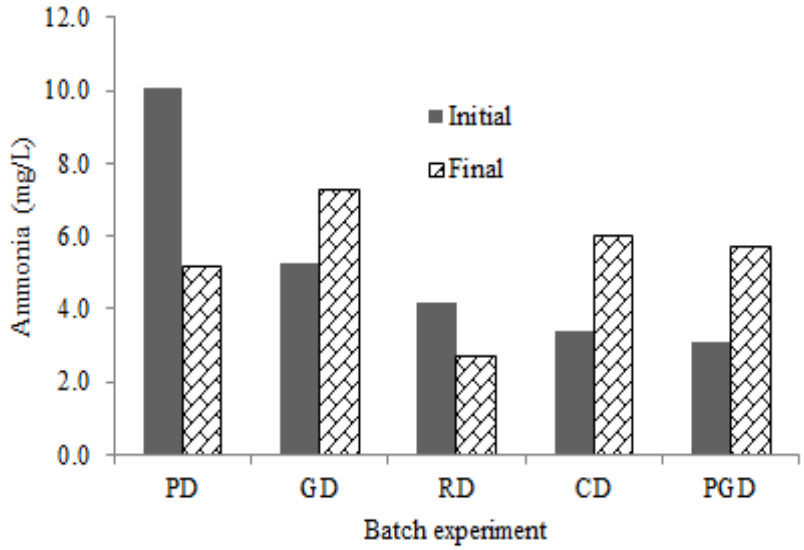


Fig. 4.27 Concentration on ammonia on different livestock dung in batch reactor

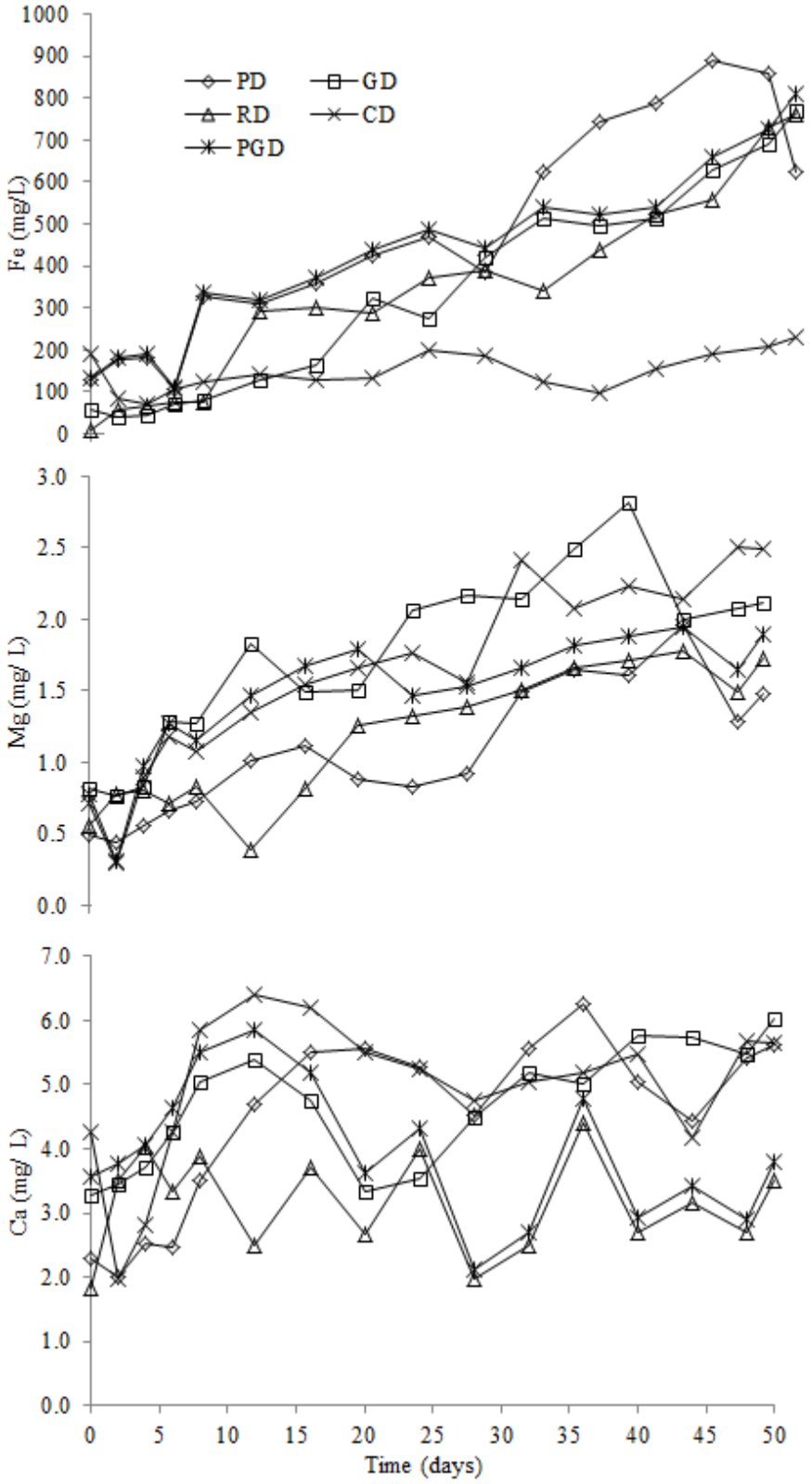


Fig. 4.28 Fe, Mg and Ca concentrations on different livestock dung in batch reactor

Increase in concentration of soluble Fe and Mg with time could be degradation of complex organic matter and availability of bounded metal to readily soluble form. Variation in Calcium profile was observed to be different from other two elements. Initially concentration of Ca deceases for CD and PD then it further increases till 12th day after that it showed irregular pattern with final concentration maximum for CD and GD with 5.64 and 6.02 mg/L respectively and minimum for RD with 3.52 mg/L.

➢ **Kinetic studies**

The cumulative methane gas production values were fitted with Gompertz equation curve to find the improvement in efficiency of nitrogen addition reactors. In the curve fitting R^2 was higher than 0.9. The kinetic parameters for CD reactor were calculated as being P = 53.04 L CH_4, Rm = 1.826 L CH_4/d, and λ =1.463 d, respectively. Rhinoceros dung added reactor had lower P and Rm than others.

• **Conclusion**

The results of present study validated that there were substantial differences between different inoculums. The reactors inoculated with CD achieved higher biogas production than reactors inoculated with PGD, PD, RD and GD. PD and PGD produced higher ultimate methane yield compare to others. Sludge such as RD failed to create a suitable environmental for FW digestion. Biogas productions was unstable at initial and performed well at final days in reactors inoculated with RD and GD. CD, with the highest volatile solids reduction and most suitable nutrients content, achieved the highest biogas production and showed the best adaptability among all inoculums.

CHAPTER 5

PRETREATMENT STUDIES

This chapter deals with the effect of different pretreatment methods such as thermal, chemical and electrohydrolysis on solubilization of food waste to decrease the retention time during anaerobic digestion of food waste. Anaerobic digestion of food waste in batch scale after pretreatment using best pretreatment method from the pretreatment study.

5.1 PHASE V: PRETREATMENT STUDY

In pretreatment study, the optimum temperature, time of exposure for hot air oven, microwave and autoclave pretreatment were selected based on four parameters i.e., pH, volatile solids, soluble COD and volatile fatty acids.

5.1.1 THERMAL PRETREATMENT

5.1.1.1 Hot air oven pretreatment

Hot air oven study was conducted in two stages:

- Temperature Study
- Temporal Study
- **Temperature study**

In Temperature study, seven samples were prepared and six of those samples were exposed to a different temperature for 1 h and the remaining sample was kept as a control. The temperatures used for this study were 60, 70, 75, 80, 85 and 90°C. After pretreatment, the samples were analysed for pH, volatile solids, soluble COD and volatile fatty acids.

- **pH**

The pH of the control was found to be 3.78. This pH is highly acidic for acidogens as well as methanogens. For acidogens the optimum pH range is 5.5-6.5 whereas for methanogens it is 6.5 to 8.2 (Speece, 1996). If an influent with pH of 3.78 is fed into the digester at high OLR the digestion can be inhibited due to the accumulation of VFA. Inhibition of the methanogens does not happen below a pH of 5.5 (Liu et al., 2006). The current study showed that the pH of the pretreated sample has marginally increased, but

more or less the pH values are around a pH of 4 (Fig. 4.1). Inside an anaerobic digester, the pH of the system depends on the concentration of volatile fatty acids, bicarbonates, alkalinity and the carbon dioxide concentration.

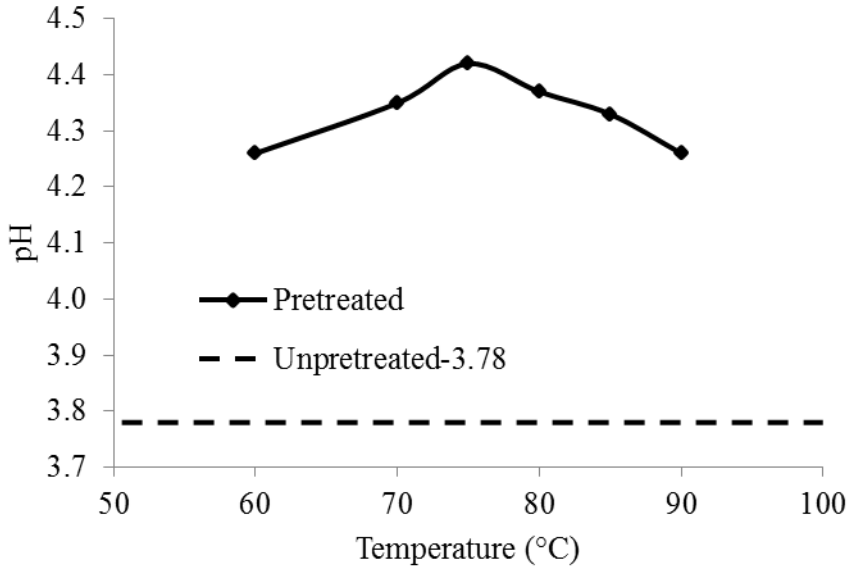


Fig. 5.1 Variation of pH with hot air oven pretreatment temperature

- **Volatile solids (VS)**

Volatile solids represent the organic matter in a sample. It comprises of biodegradable volatile solids and refractory volatile solids. High biodegradable volatile solids content with a low refractory content is suitable for anaerobic digestion. The VS of the untreated sample was found to be 92.1% of total solids percentage. Not much variation was found in the VS content of pretreated samples within themselves and also in comparison with the control (Fig. 5.2).

- **Soluble COD**

From the study it was found that the highest soluble COD with 800 g/kg of dry food waste was observed for the sample pretreated at 75°C (Fig. 5.3). Ariunbataar and Panico (2014) had concluded from their study that the optimum temperature for hot air oven pretreatment is 80°C. The temperatures considered in their study were 70, 80,100, 120, 140 and 150°C. But their study didn't focus on 75°C which is why their work gives 80°C as the optimum pretreatment temperature. The ratio of specific methane production of the sample pretreated at 80°C to the untreated sample was found to be 1.27 (Ariunbataar et. al., 2014). Interestingly, the ratio of soluble COD of pretreated sample to control at 80°C was also 1.27 (Fig. 5.4). Whereas the ratio of soluble COD of pretreated to control

at 75°C was found to be 1.32 by this study. Hence from the soluble COD point of view a pretreatment temperature of 75°C will be optimum for hot air oven pretreatment.

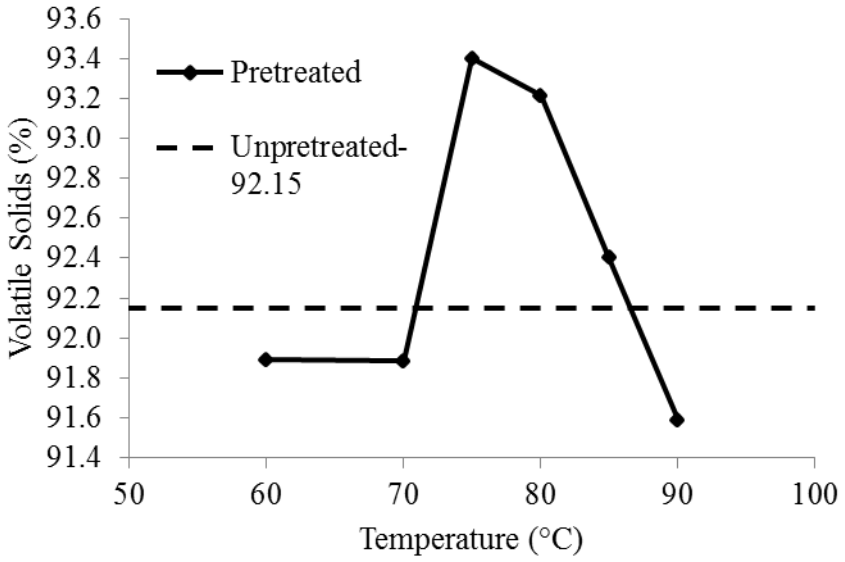


Fig. 5.2 Variation of volatile solids with hot air oven pretreatment temperature

- **Volatile fatty acids (VFA)**

Volatile fatty acids have a significant impact on the pH of an anaerobic system. Accumulation of VFA can cause the pH of the system to drop causing depletion of methanogens. Initial volatile fatty acids can also add up in the biogas yield. So, higher initial volatile fatty acids are preferable for higher biogas yield. But the digester should be monitored to prevent VFA accumulation which will be harmful to methanogens. In the current study it was found that the highest VFA concentration with 12000 mg/L was achieved by the sample pretreated at 70°C followed by the sample pretreated at 75°C with 11700 mg/L (Fig. 4.5). Since soluble COD plays a more vital role in the final methane yield, it can be deduced that 75°C will be the optimum temperature for hot air oven pretreatment. The decrease in VFA after 75°C might be due to vaporisation losses during pretreatment which became more prominent at higher temperatures. It was reported that the for autoclave pretreatment of waste activated sludge, VFA concentration was increasing with the increase in pretreatment temperature until at 175°C the concentration suddenly drops (Li and Noike, 1992). This discrepancy maybe due to the fact that the current hot air oven pretreatment study employs pretreatment temperatures up to 90°C only as against the high temperatures used in Li and Noike (1992).

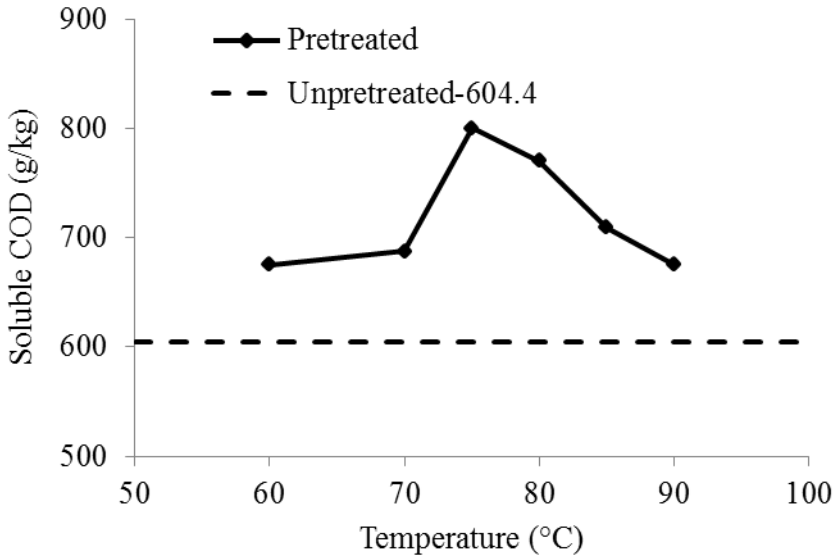


Fig. 5.3 Variation of soluble COD with hot air oven pretreatment temperature

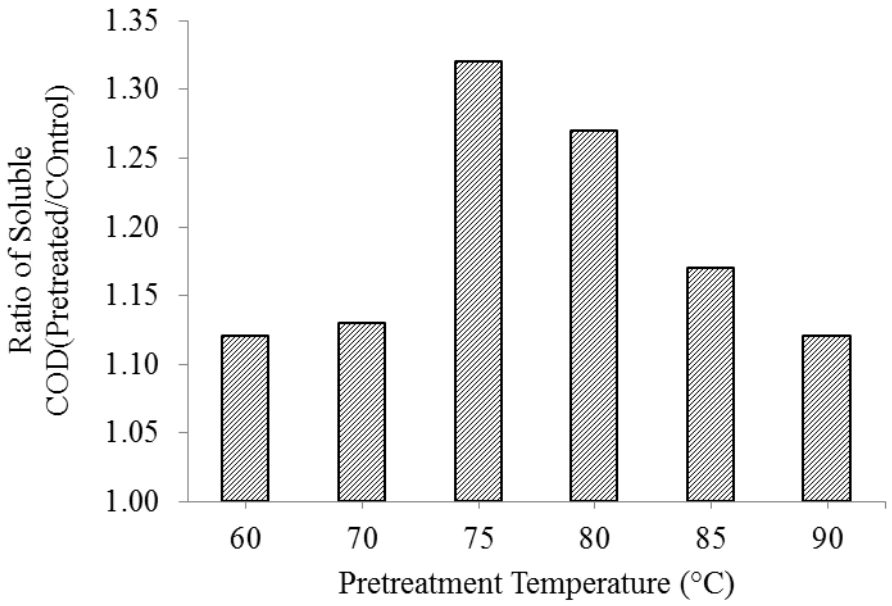


Fig. 5.4 Variation of ratio of soluble COD of pretreated and control with hot air oven pretreatment temperature

Temporal study

In Temporal study, optimum temperature obtained from the temperature study (75°C) was chosen and samples were exposed to different time periods at the same temperature. In total five samples were prepared and four of those samples were exposed for different temperatures such as 30 min, 60 min, 90 min and 120 min while the remaining sample was kept as control. The samples were then analysed for pH, VS, soluble COD and VFA.

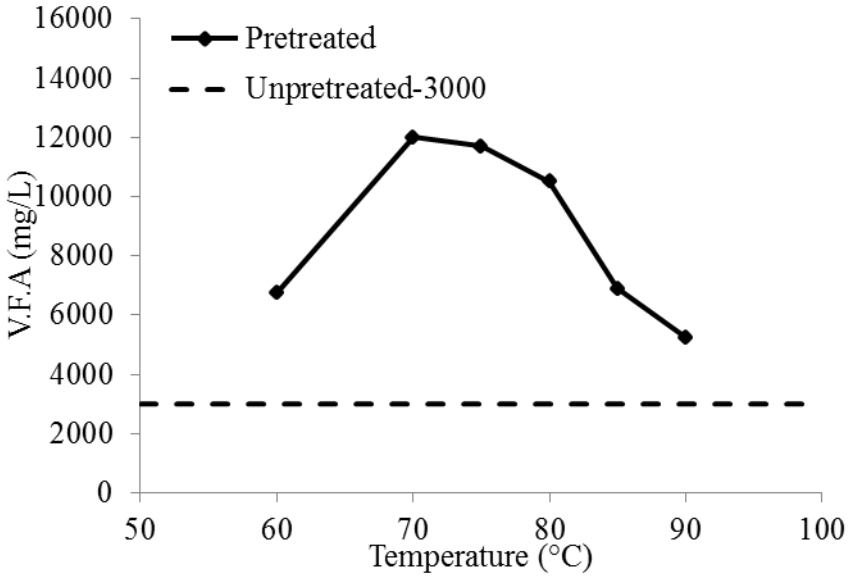


Fig. 5.5 Variation of VFA with hot air oven pretreatment temperature

- **pH**

From the analysis it was found that the pH is increasing with the time of exposure till 90 min with a value of 5.9 but at 120 min it is marginally dropping (Fig. 5.6). All pretreated samples have higher pH than the control indicating solubilisation of proteins. Since 5.9 is within the optimum pH range of 5.5-6.5 which is favourable for acidogens it can be deduced that hot air oven pretreatment is beneficial for pretreating food waste (Speece 1996). So as per the results obtained 90 min is the optimum time of exposure for hot air oven pretreatment of food waste. A previous study has concluded 90 min to be the optimum time of exposure for pretreating food waste (Ariunbataar et al., 2014).

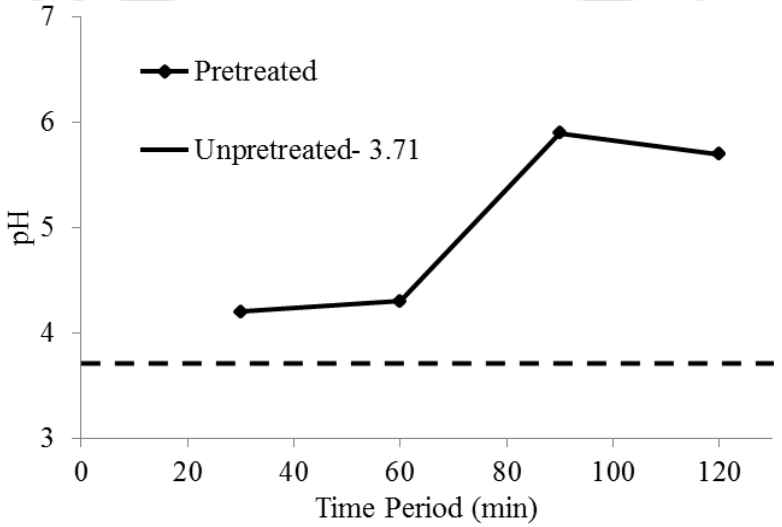


Fig. 5.6 Variation of pH with hot air oven time of exposure

- **Volatile solids (VS)**

The current study did not find any significant variation in the volatile solids content of pretreated samples as well as the control (Fig. 5.7). So it can be deduced that the time of exposure does not have any significant impact on the volatile solids content. A study done on the microwave pretreatment of sludge for anaerobic digestion analysed the volatile suspended solids (VSS) dissolution ratio and found that at 30 min exposure time the highest value of 36.4% was obtained (Wei et al., 2009).

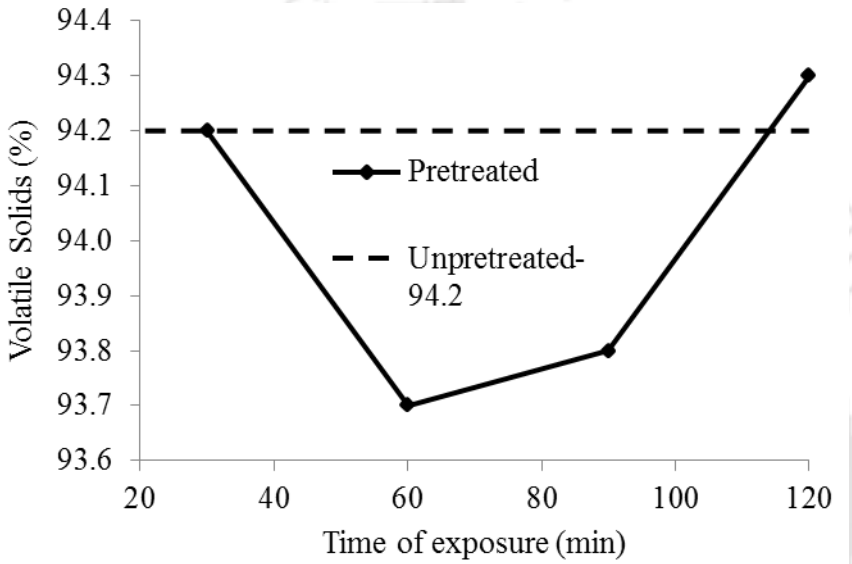


Fig. 5.7 Variation of volatile solids with hot air oven time of exposure

- **Soluble COD**

From the data from the temporal study, it can be seen that soluble COD is highest at 90 min of exposure with a value of 1027 g/kg of dry food waste after that the increase in soluble COD is not considerable (Fig. 5.8). So it can be concluded that 90 min is the optimum temperature for hot air oven pretreatment. . Previous studies have (Ariunbataar et al., 2014) also arrived at the same result.

The time periods they considered were 60 min, 90 min, 2 h and 4 h. But the parameter considered in that work was specific methane production unlike soluble COD, pH, VFA and volatile solids considered in this study. From Fig. 5.8 it can be seen that the soluble COD of the sample pretreated at 75°C and 90 min has improved 1.39 times than the control (Fig. 5.9). During the temperature study the sample pretreated at 75°C and 1 h improved only by 1.32 again confirming that 90 min of exposure is best for hot air oven pretreatment of food waste.

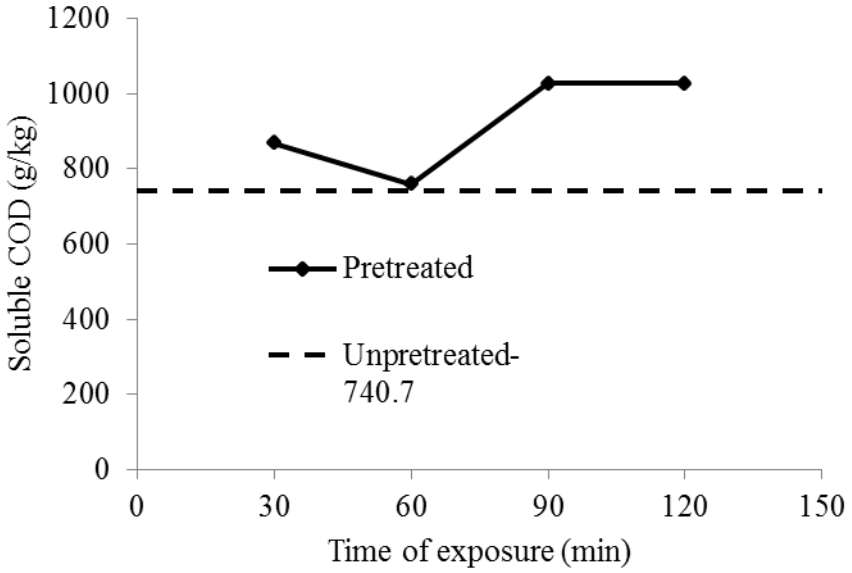


Fig. 5.8 Variation of soluble COD with hot air oven time of exposure

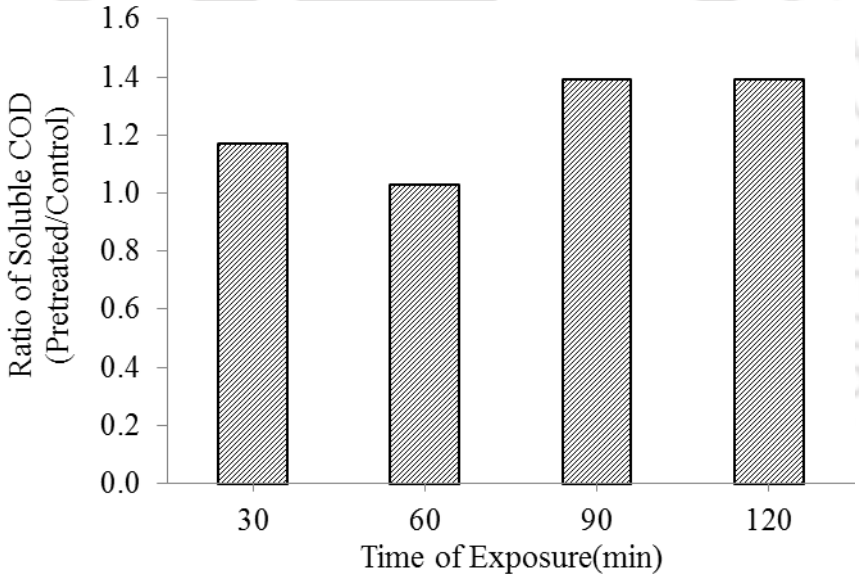


Fig. 5.9 Variation of ratio of soluble COD of pretreated and control with hot air oven time of exposure

- **Volatile fatty acids (VFA)**

The VFA content of the pretreated sample is increasing towards 90 min and at 120 min the VFA content is dropping (Fig. 5.10). Since the highest VFA content is for the sample pretreated for 90 min with a value of 9500 mg/L, it can be deduced that 90 min is the optimum time of exposure with respect to VFA. It was reported that the for autoclave pretreatment of waste activated sludge, VFA concentration was increasing with the increase in time of exposure (Li and Noike, 1992).

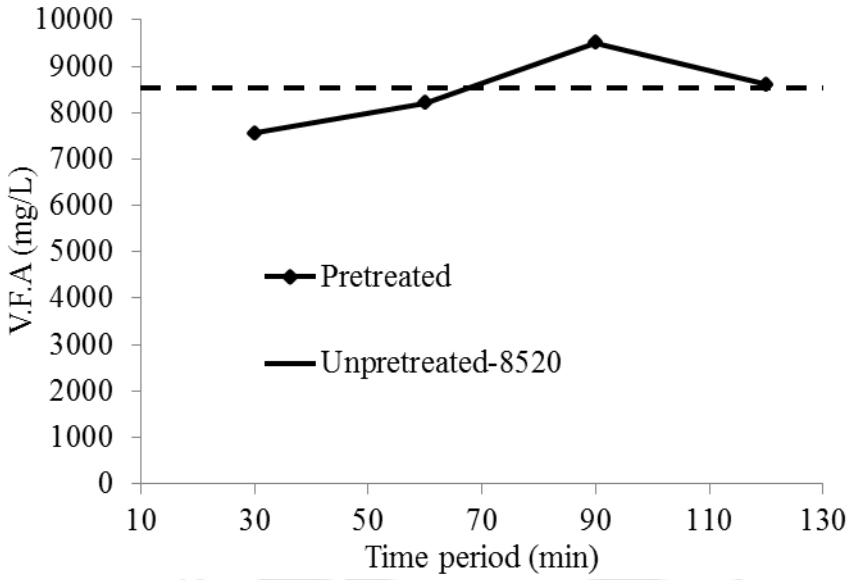


Fig. 5.10 Variation of VFA with hot air oven time of exposure

5.1.1.2 Microwave pretreatment

Microwave pretreatment study was done in two parts.

- 2 min exposure+1 min stand time
- 4 min exposure+1 min stand time

2 min exposure+1 min stand time

In this study, every sample was exposed to a different temperature for the same time duration of 2 min microwaving and 1 min of stand time. The temperatures studied were 100,140,160 and 180°C. An untreated sample was kept as control.

- **pH**

The variation in pH with the pretreatment temperature was minimal (Fig. 5.11). All the pH values were around 4.5 including the control. Inside an anaerobic digester, the pH of the system depends on the concentration of volatile fatty acids, bicarbonates, alkalinity and the carbon dioxide concentration.

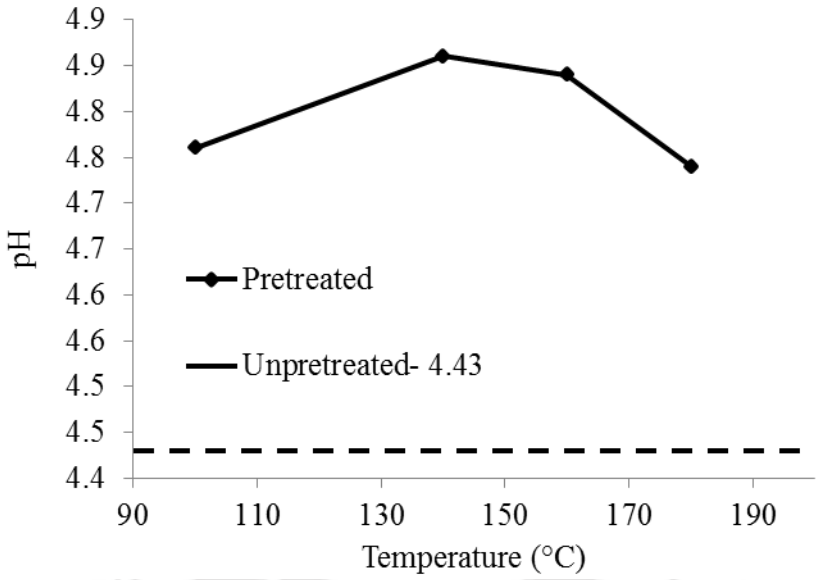


Fig. 5.11 Variation of pH with microwave pretreatment temperature (2 min)

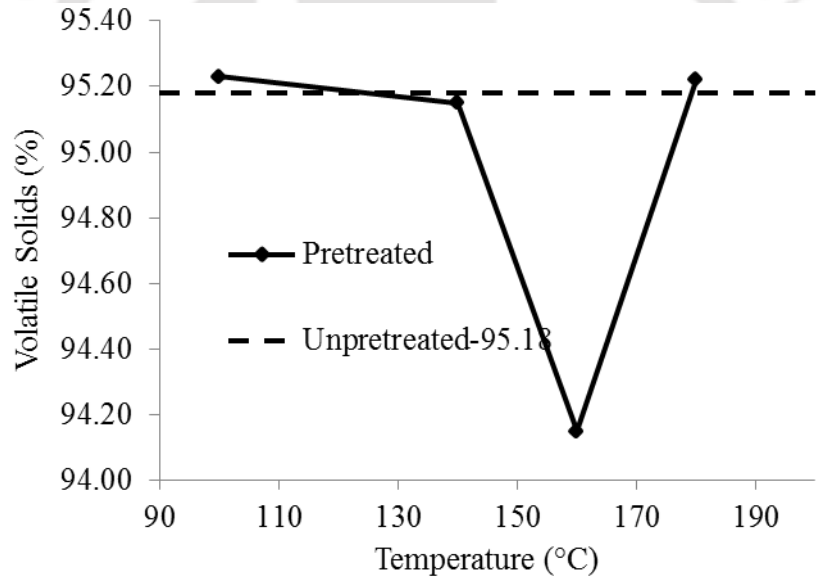


Fig. 5.12 Variation of volatile solids with microwave pretreatment temperature (2 min)

- **Volatile solids (VS)**

The microwave pretreatment did not have any significant impacts on the volatile solids concentration (Fig. 5.12). A study done on the microwave pretreatment of sludge for anaerobic digestion analysed the VS dissolution ratio and found that at 170°C the highest value of 36.4% was obtained (Wei et al., 2009). VS comprises of biodegradable volatile solids and refractory volatile solids. High biodegradable volatile solids content with a low refractory content is suitable for anaerobic digestion. An optimum pretreatment temperature cannot be deduced from the obtained data.

- **Soluble COD**

The soluble COD is decreasing with the increase in pretreatment temperature till 160°C and then rises at 180°C. The highest soluble COD was at 100°C with a value of 852 g/kg of dry food waste (Fig. 5.13). The increase in soluble COD at 180°C was attributed to the formation of melanoidins caused by Maillard reactions that happens between proteins and sugars at elevated temperatures (Shahriari et al., 2011). Even though soluble COD is increasing due to the formation of melanoidins, it was found that the biogas production actually decreases because melanoidins are refractory compounds which are difficult to degrade (Shahriari et al., 2011). Since the soluble COD content is highest for the sample pretreated at 100°C and there is formation of melanoidins at 180°C, the optimum temperature for microwave pretreatment can be fixed at 100°C.

- **Volatile fatty acids (VFA)**

The VFA of the sample is increasing with increase in pretreatment temperature (Fig. 5.14). But the obtained values have VFA content less than the control. This might be due to the loss of volatile compounds during pretreatment. The VFA of the pretreated sample was increasing with the increase in pretreatment temperature with 180°C with the highest VFA content.

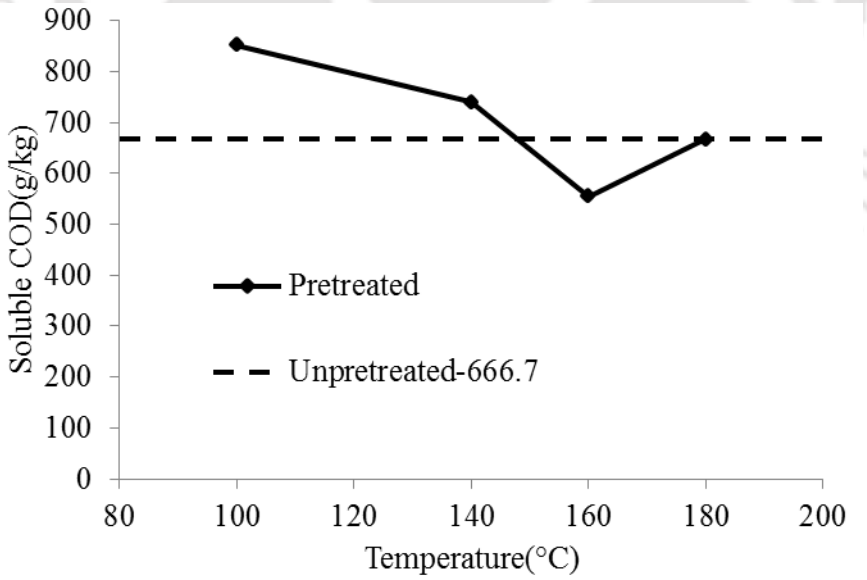


Fig. 5.13 Variation of soluble COD with microwave pretreatment temperature (2 min)

4 min exposure+1 min stand time

In this study, every sample was exposed to a different temperature for same time duration of 4 min microwaving and 1 min of stand time. The temperatures studied were 100,140,160 and 180°C, while an untreated sample was kept as control.

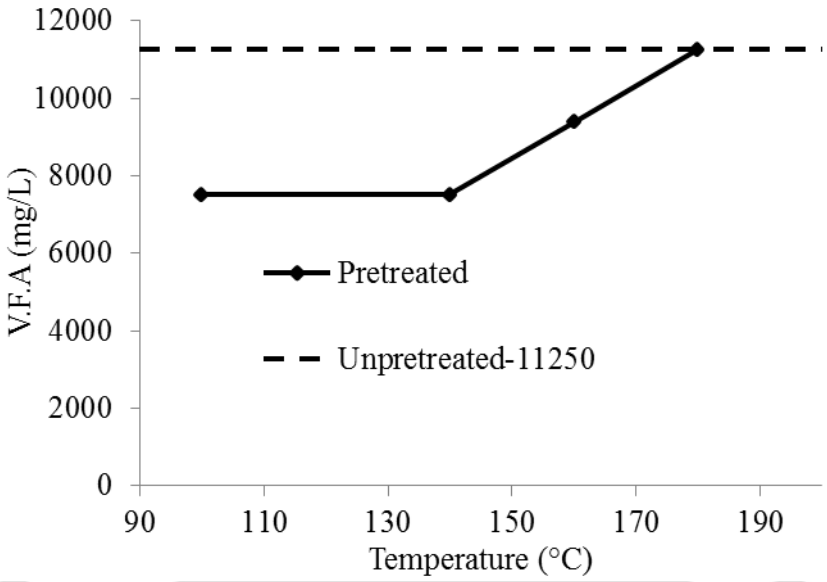


Fig. 5.14 Variation of VFA with microwave pretreatment temperature (2 min)

- **pH**

The pH of the pretreated samples had significantly improved when compared to the control (Fig. 5.15). Highest pH was obtained for the sample pretreated at 140°C with a value of 5.57. The pH of the pretreated samples was almost similar without any considerable difference. Inside an anaerobic digester, the pH of the system depends on the concentration of volatile fatty acids, bicarbonates, alkalinity and the carbon dioxide concentration.

- **Volatile solids (VS)**

The variation in volatile solids concentration with pretreatment temperature was minimal (Fig. 5.16). The values of all the samples including the control had similar VS concentrations. Hence an optimum pretreatment temperature cannot be deduced from the results. A study done on the microwave pretreatment of sludge for anaerobic digestion analysed the volatile suspended solids (VSS) dissolution ratio and found that at 170°C the highest value of 36.4% was obtained (Wei et al., 2009).

- **Soluble COD**

The soluble COD for the pretreated samples were less than the control (Fig. 5.17). But it can be observed that the change of soluble COD with pretreatment temperature follows the same trend. The decrease in soluble COD of pretreated samples with respect to the control can be attributed to the loss of volatile organic matter through evaporation during the pretreatment process. The soluble COD is decreasing with the increase in pretreatment temperature till 160°C and then rises at 180°C. The highest soluble COD among the pretreated samples was obtained at 100°C with a value of 632 g/kg of dry food waste.

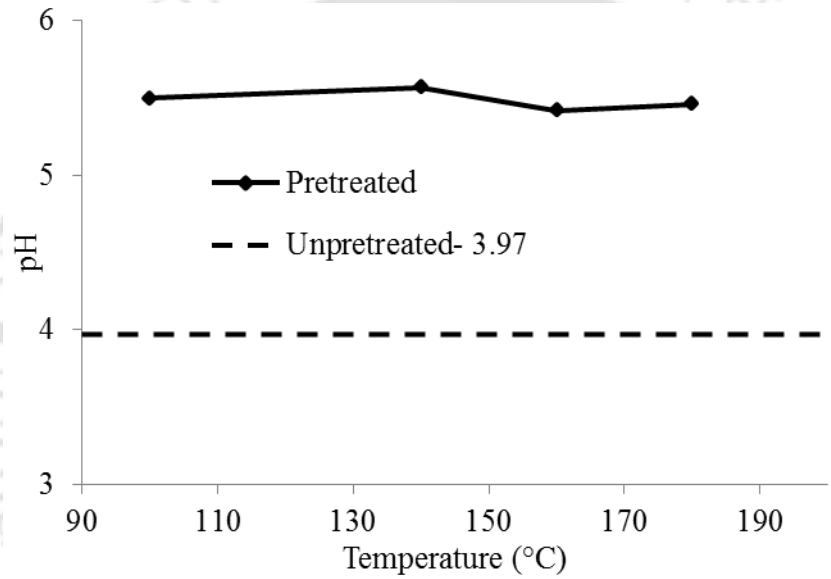


Fig. 5.15 Variation of pH with microwave pretreatment temperature (4 min)

The increase in soluble COD at 180°C was attributed to the formation of melanoidins caused by Maillard reactions that happens between proteins and sugars at elevated temperatures (Shahriari et al., 2011). Even though soluble COD is increasing due to the formation of melanoidins, it was found that the biogas production actually decreases because melanoidins are refractory compounds which are difficult to degrade (Shahriari et al., 2011). From these it can be concluded that 100°C will be the optimum temperature for microwave pretreatment of food waste.

- **Volatile fatty acids (VFA)**

From the data it is observed that the volatile fatty acids concentration follows a rogue pattern which strengthens the possibility of evaporation losses at an exposure time of 4

min +1 min stand time (Fig. 5.18). Highest VFA was shown by the control with a value of 11250 mg/L. Obtaining an optimum pretreatment temperature from the above data is not feasible. But it can be concluded that 2min +1 min stand time is superior to 4 min+ 1 min of exposure time.

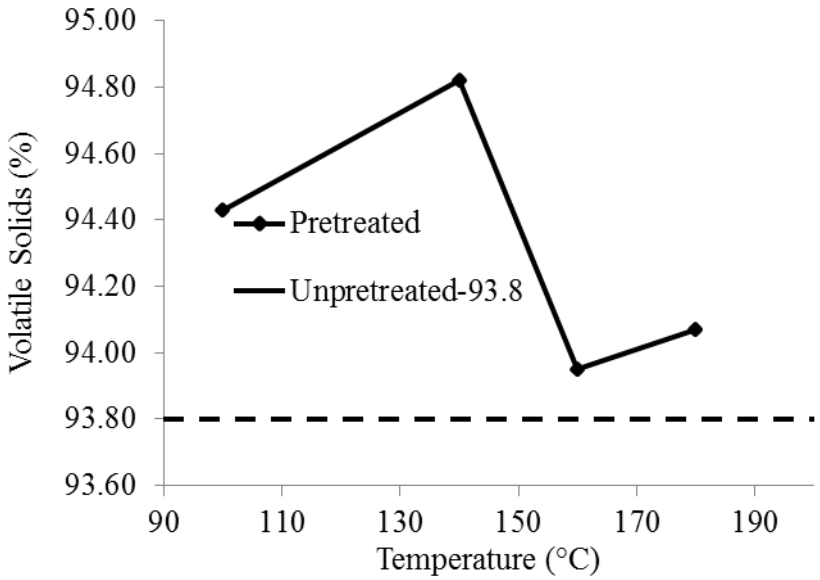


Fig. 5.16 Variation of volatile solids with microwave pretreatment temperature (4 min)

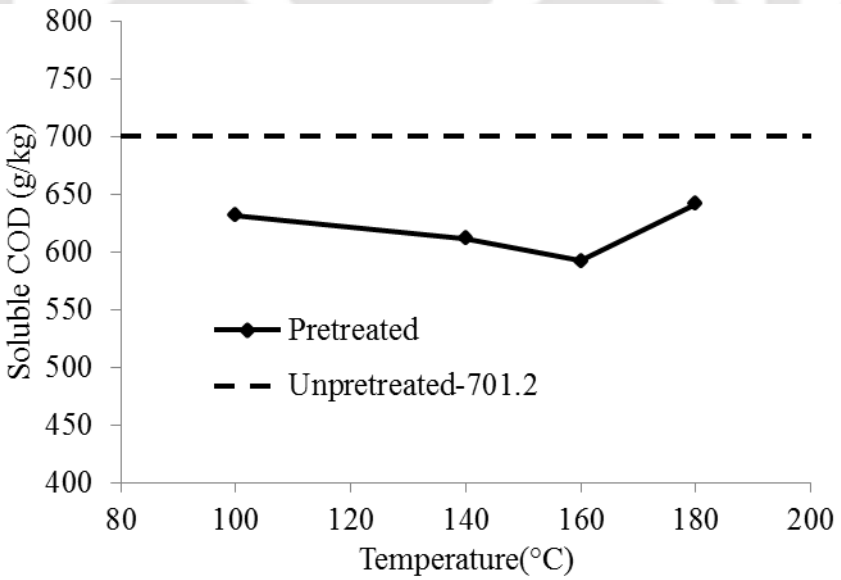


Fig. 5.17 Variation of soluble COD with microwave pretreatment temperature (4 min)

5.1.1.3 Autoclave Pretreatment

Autoclave pretreatment was also carried out in two stages. The two stages are:

- Temperature study

• Temporal study

Temperature study

In this study, 5 samples were taken and 4 of them were exposed to 60, 80,100, and 120°C respectively for time duration of 40 min. The remaining one sample was kept as a control.

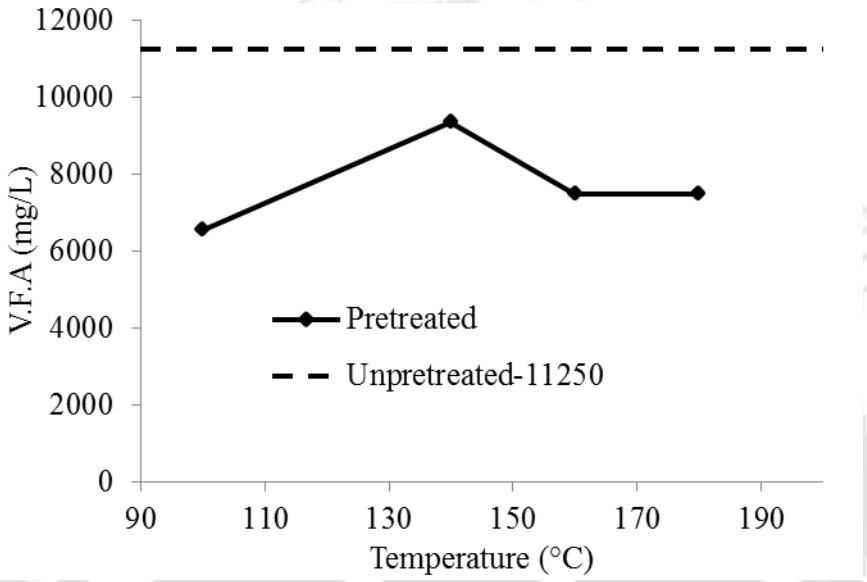


Fig. 5.18 Variation of VFA with microwave pretreatment temperature (4 min)

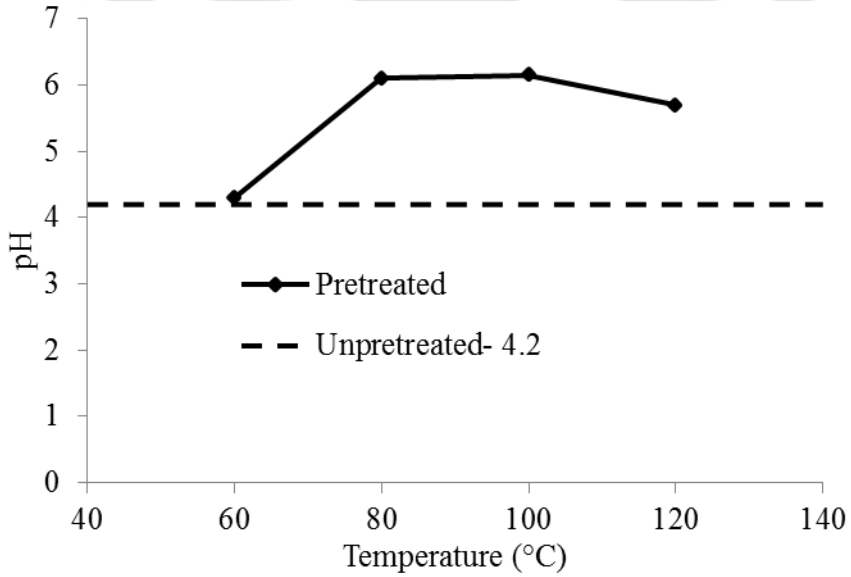


Fig. 5.19 Variation of pH with autoclave pretreatment temperature

- **pH**

The pH is increasing from 4.3 for the sample pretreated at 60°C to 6.15 for the sample pretreated at 100°C and then decreasing again at 120°C (Fig. 5.19). The highest pH value was for the sample pretreated at 100°C. The value of 6.15 brings the pH close to the optimum range of 6.5 to 7.5 suitable for anaerobic digestion (Liu and Fang, 2007).The optimum pretreatment temperature for autoclave can be concluded as 100°C with respect to pH.

- **Volatile solids (VS)**

The analysis of the VS values indicates that pretreatment temperatures do not have any significant impact on the volatile solids concentration (Fig. 5.20). All the values obtained including the control had similar VS concentration. Hence an optimum pretreatment temperature cannot be deduced from the obtained values.

- **Soluble COD**

The soluble COD value is initially decreasing towards 80°C and then suddenly increasing at 100°C to a value of 711 g/kg of dry food waste and then flattens out to 120°C (Fig. 5.21).

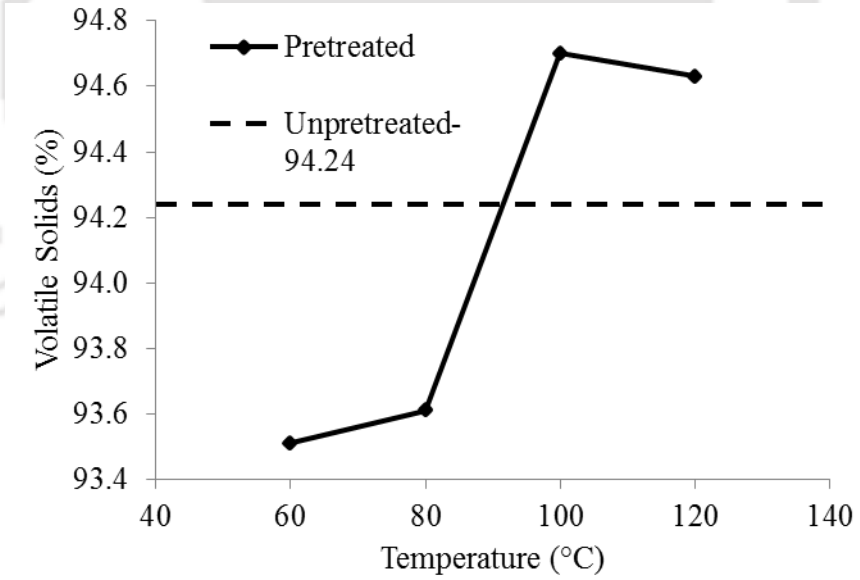


Fig. 5.20 Variation of volatile solids with autoclave pretreatment temperature

From the graph it can be deduced that 100°C is the optimum pretreatment temperature for autoclave pretreatment as far as soluble COD is concerned. For waste activated sludge it was found that a temperature range of 160 to 170°C was the optimum for high methane production (Li and Noike, 1992). Tampio et al., 2013 used 160°C to

pretreat food waste using autoclave following the work by Li and Noike (1992). They found increased solubilisation of COD but decrease in methane yields. This decrease in methane yield was attributed to Maillard reactions. But this huge difference between the optimum pretreatment temperature for waste activated sludge and food waste maybe due to the fact that food waste had already undergone thermal treatment during cooking which makes it comparatively easier for solubilisation.

- **Volatile fatty acids (VFA)**

The VFA values were similar to control at pretreatment temperatures of 60 and 80°C, then at 100°C it drops drastically to 3750 mg/L (Fig. 5.22). This sudden decrease in VFA may be due to the loss of volatile organic acids at high temperatures due to vaporisation. No pretreated sample had higher VFA than the control. It was reported similarly in the studies by Tampio et al. (2014) that VFA concentration was lower for autoclaved food waste when compared to the control.

Temporal study

In temporal study, samples were exposed to the optimum temperature of autoclave pretreatment found from the temperature study (100°C). Each sample was exposed for a different time period such as 20, 40, 60, and 80 min respectively.

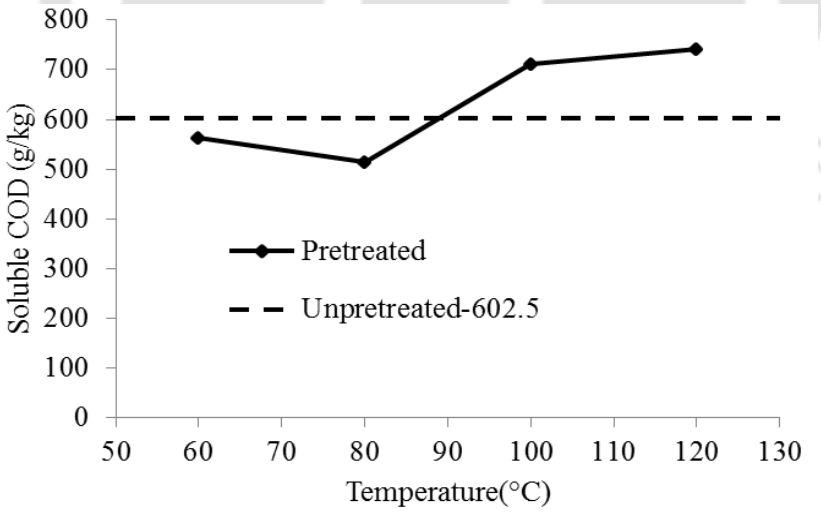


Fig. 5.21 Variation of soluble COD with autoclave pretreatment temperature

- **pH**

pH of all the pretreated samples were well above the control indicating solubilisation of proteins (Fig. 5.23). All the values obtained were within the optimum range of 5.5 to

6.5 favourable for acidogens (Speece, 1996). From the data obtained the sample pretreated for 40 min and 60 min is the best in terms of pH.

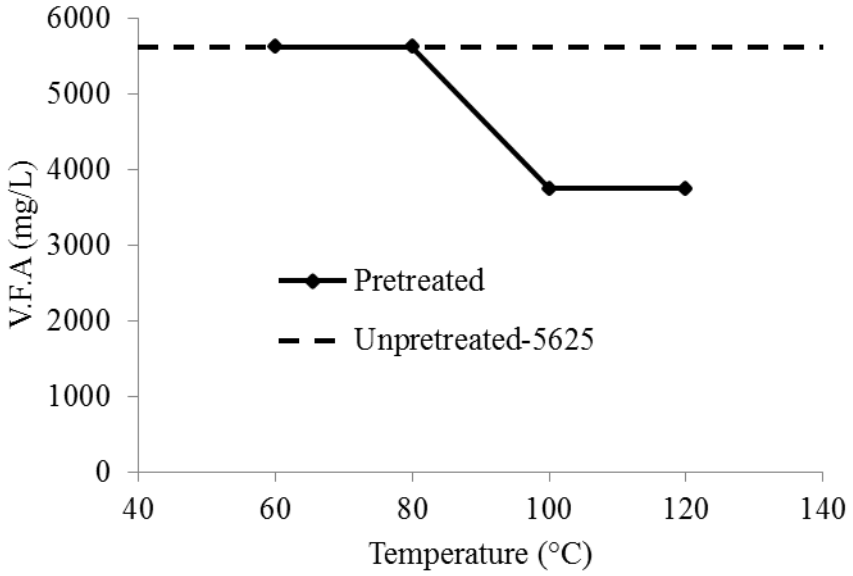


Fig. 5.22 Variation of VFA with autoclave pretreatment temperature

- **Volatile solids (VS)**

The results of VS indicate that autoclave pretreatment does not have any significant impacts on the VS concentrations (Fig. 5.24). No significant change in VS concentration was observed even between pretreated and control samples. Hence an optimum time of exposure cannot be deduced from these results.

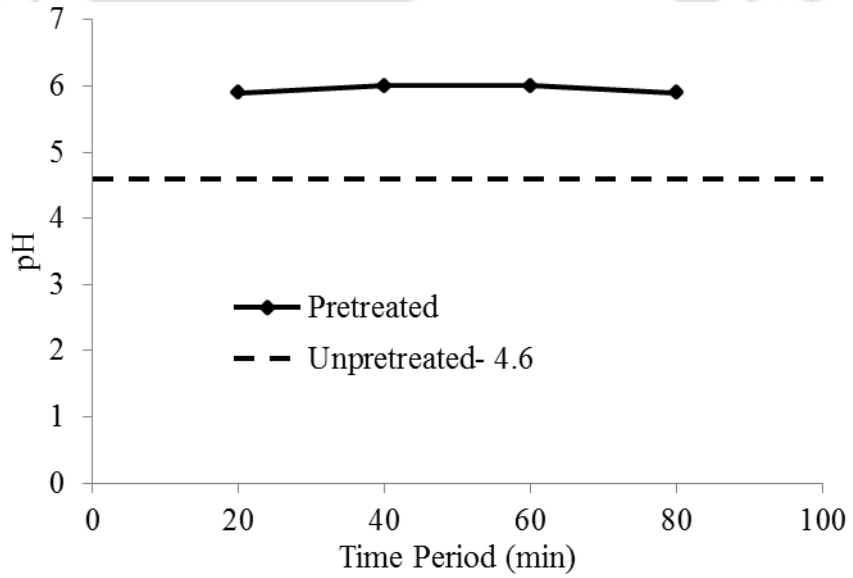


Fig. 5.23 Variation of pH with autoclave time of exposure

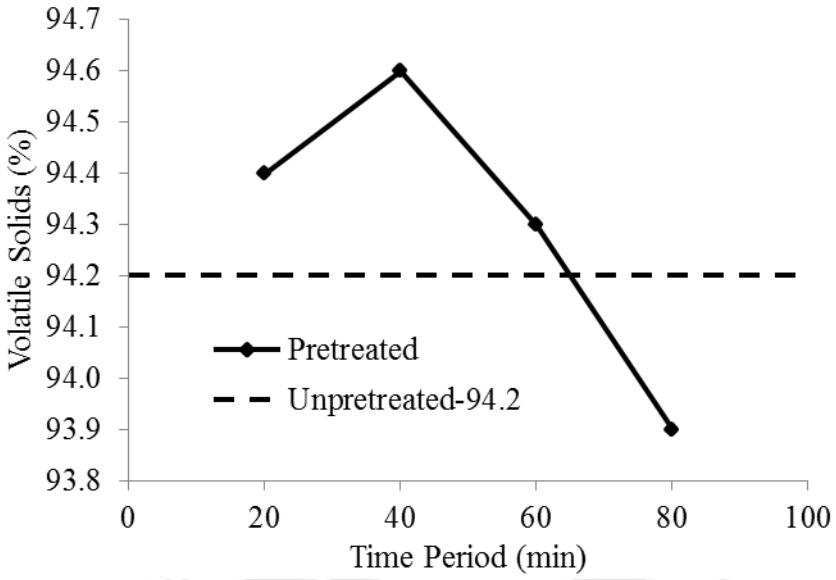


Fig. 5.24 Variation of volatile solids with autoclave time of exposure

- **Soluble COD**

The soluble COD is increasing from 20 min to 40 min, then decreasing at 60 min and then again increasing at 80 min (Fig. 5.25). Even though the highest soluble COD was at 80 min, at just the half time of exposure, that is, at 40 min an almost similar soluble COD was observed. So it can be deduced that 40 min is the optimum time period of exposure for autoclave pretreatment of food waste. Li and Noike (1992) reported that the optimum time of exposure for waste activated sludge was 60 min. Tampio et al., (2013) also used 60 min as the time period of autoclave pretreatment of food waste following the results by Li and Noike (1992). But the current study has shown that as food waste is already a thermally treated organic matter such a high time duration of pretreatment is not required.

- **Volatile fatty acids (VFA)**

The VFA concentration is increasing as the time of exposure increases with the highest VFA value at 60 min and then suddenly decreasing at 80 min (Fig. 5.26). This drop at 80 min may be due to the vaporisation of some of the volatile fatty acids due to longer time of exposures to autoclaving. From these results it can be deduced that 40 min and 60 min are the optimum time of exposures as far as VFA is concerned. Since the VFA values between 40 min and 60 min is minimal and the highest soluble COD was observed at 40 min, it can be concluded that 40 min is the optimum time of exposure for autoclave pretreatment.

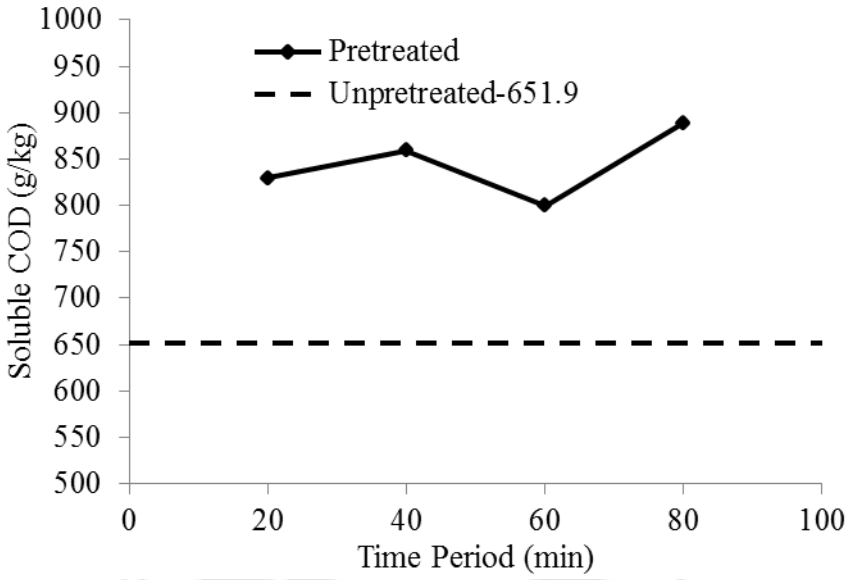


Fig. 5.25 Variation of soluble COD with autoclave time of exposure

5.1.2 Alkali Pretreatment

Alkali pretreatment study was carried out in two stages. They are

- Dosage study
- Temporal Study

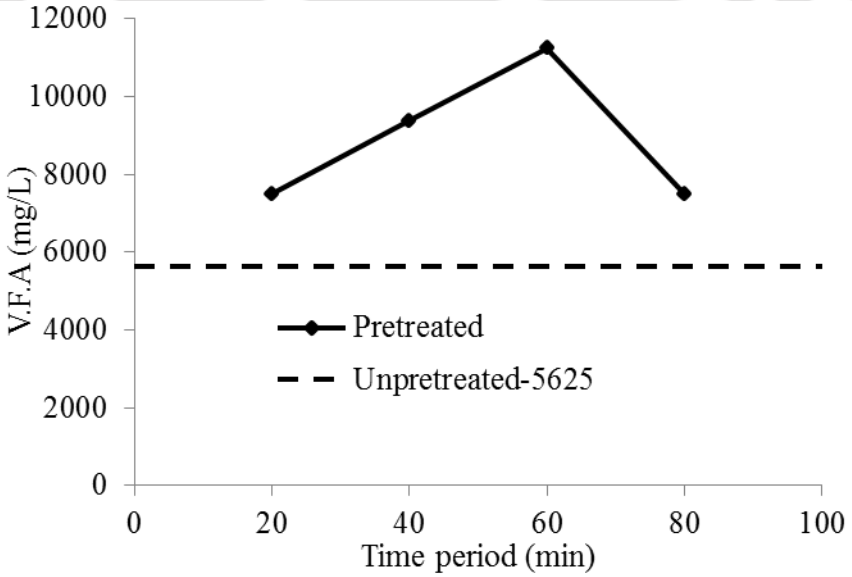


Fig. 5.26 Variation of VFA with autoclave time of exposure

Dosage Study

In the dosage study, each sample was given a different dosage of NaOH and kept in a horizontal shaker at 150 rpm for 24 h. The dosages given were 0.05, 0.1, 0.2, 0.5, 1, 2.5,

5 and 7.5% (w/w) of TS. It was found that at dosages of 1% (w/w) of TS and above the samples was getting solidified. Hence the samples with dosages of 1% (w/w) of TS and above were discarded.

- **pH**

The pH is gradually increasing with increase in dosage. But the increase is so minimal due to the lower dosages of NaOH. With the control having a pH of 5.36, the pH of the sample with 0.5% dosage increased to 6 (Fig. 5.27). This pH is within the range of optimum pH for acidogens to thrive. Since acidogens are responsible for hydrolysis, a favourable pH range in the substrate due to pretreatment will enhance the efficiency of hydrolysis. Zhu et al. (2010) reported an increase in pH to 8.5, 8.6, 8.9 and 9.5 for 1%, 2.5%, 5% and 7.5% (w/w of TS) respectively during the pretreatment of corn stover using NaOH. Erden (2012) studied on the effect of alkaline pretreatment on anaerobic digestion of meat processing wastewater. Samples were pretreated at various pH such as 8, 9, 10, 11, 12 and 13. But such high pH after pretreatment can be inhibitory for the acidogens and methanogens whose optimum pH range is 5.5-6.5 and 7.8-8.2 respectively.

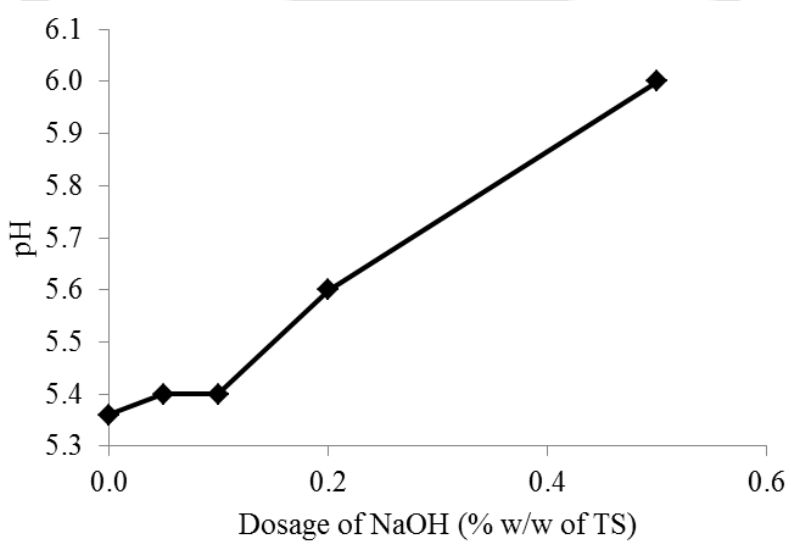


Fig. 5.27 Variation of pH with dosage of NaOH

- **Volatile solids (VS)**

The variation observed in the percentage of volatile solids between samples and the control was minimal. Volatile solids content of the control was 93.2% whereas the sample retreated with 0.5% (w/w of TS) of NaOH had the highest volatile solids content of 93.6% (Fig. 5.28). Alkali pretreatment didn't have any significant effect on the

volatile solids content. It can be inferred that food waste being already cooked and with a high volatile solids content, cannot undergo further increase in the volatile solids content.

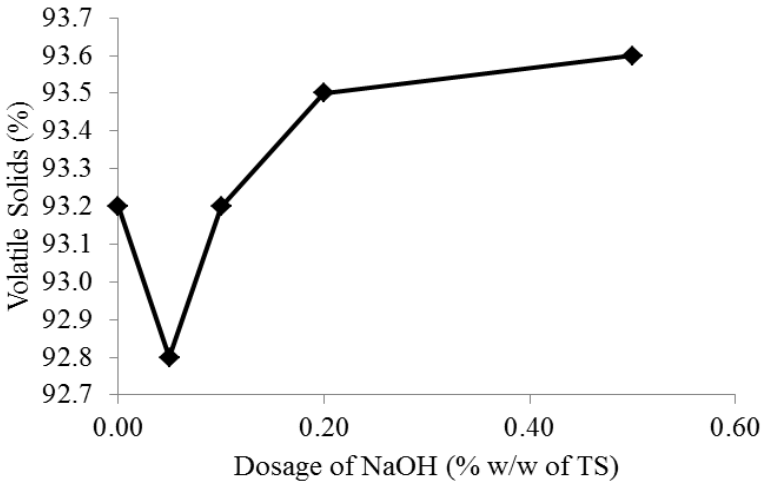


Fig. 5.28 Variation of VS with dosage of NaOH

- **Soluble COD**

Soluble COD of the pretreated samples increased with the increase in dosage of NaOH. The soluble COD improved from 650 g/kg of dry food waste for the control to 873.6 g/kg of dry food waste for the dosage of 0.5% (w/w of TS) of NaOH (Fig. 5.29). At this dosage the soluble COD became 1.34 times the control. There was no further increase in soluble COD after a dosage of 0.2% (w/w of TS) of NaOH. This may be due to the complete hydrolysis of available organic matter. Since samples are from cooked food waste with high soluble organic content, the amount of pretreatment required will be minimum. Hence 0.2% (w/w of TS) of NaOH was selected as the optimum dosage of alkaline pretreatment using NaOH. Florez-Juarez et al. (2010) reported a soluble COD increase of 2.94 times for the slaughter house solid waste pretreated at 0.6 g/g of VSS. This high increase in soluble COD is due to two reasons. One is due to the high dosage given and the other is due to the cellulose and lignin materials in ruminal content of slaughterhouse solid waste. Lin et al. (2009) found that a NaOH dose of 16 g/100 g TS will be the optimum for pretreatment of pulp and paper sludge. At this dose the floc structure of the sludge was well disrupted and the soluble COD increased by 8.8 times with respect to the control. This high increase in soluble COD is because since sludge primarily comprises of cells of microorganisms, the alkaline pretreatment causes swelling of these cells thus making it more susceptible to enzymatic attack by acidogens.

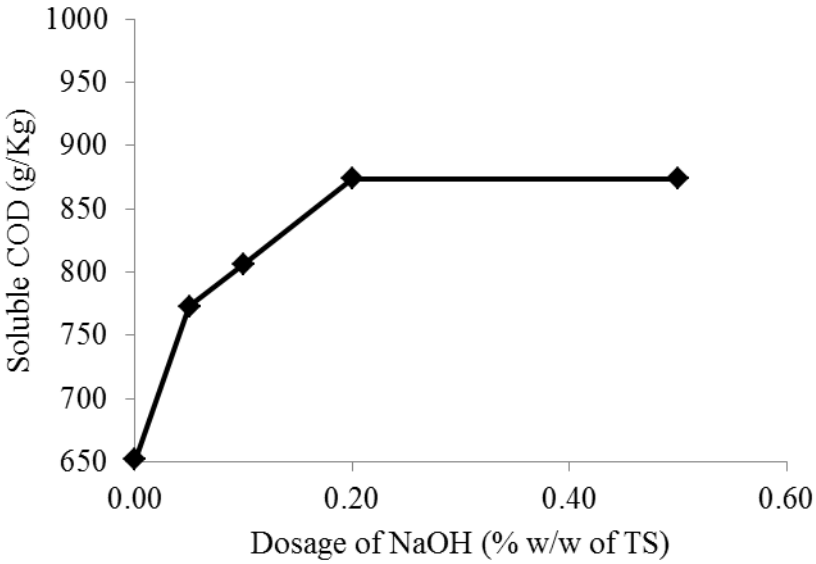


Fig. 5.29 Variation of soluble COD with dosage of NaOH

- **Volatile fatty acids (VFA)**

Volatile fatty acids content showed an increasing trend with increase in dosage of NaOH. It increased from 33.3 g/kg of dry food waste for the control to 86.7 g/kg of dry food waste for the sample pretreated with a dosage of 0.5% (w/w of TS) (Fig. 5.30). Even though the sample pretreated at 0.5% (w/w of TS) of NaOH had the highest volatile fatty acids content there was no significant increase after a dosage of 0.2% (w/w of TS) of NaOH. So it can be inferred that 0.2% (w/w of TS) of NaOH will be the optimum dosage for alkaline pretreatment.

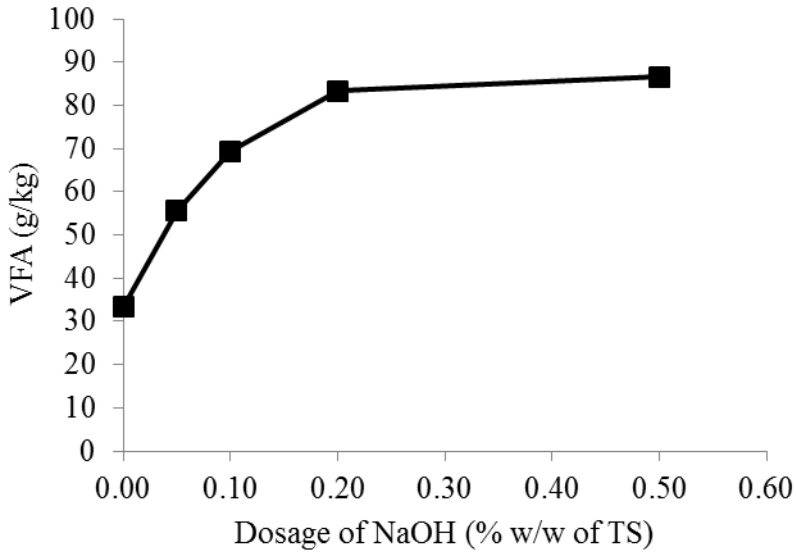


Fig. 5.30 Variation of VFA with dosage of NaOH

Temporal study

In the temporal study each sample was given the same NaOH dosage of 0.2% (w/w of TS) but was exposed to different time of exposures such as 1.5, 3, 6, 12 and 24 h. A sample with no pretreatment was kept as control. All samples were kept in a horizontal shaker at 150 rpm for their respective time of exposure.

- **pH**

The control had a pH of 5.9. For the sample exposed to alkali pretreatment for 1.5 h, the pH increased to 7 (Fig. 5.31). All the other samples with higher time of exposures showed a declining trend in pH. The increase in pH of the sample pretreated for a time period of 1.5 h is due to the addition of alkali. But the other samples which were given the same dosage of NaOH but with higher time of exposures showed a decrease in pH because of the acidification caused by aerobic degradation of organic matter. Zhu et al. (2010) exposed all the corn stover samples to NaOH pretreatment for a time period of 24 h and the samples had a pH ranging from 8.5 to 9.5. But these high pH are inhibitory to acidogens and methanogens whose optimum pH range is 5.5-6.5 and 7.8-8.2 respectively.

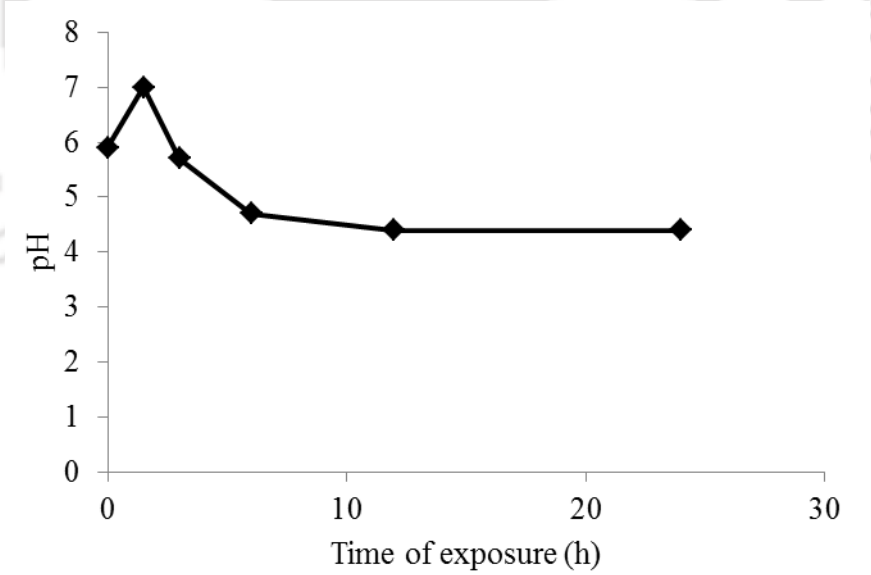


Fig. 5.31 Variation of pH with NaOH time of exposure

- **Volatile solids (VS)**

The variation observed in the percentage of volatile solids between samples exposed for different time periods and the control were minimal. Volatile solids content of the

control was 93.2% whereas it had increased to 93.7% for the sample exposed to alkali pretreatment for 24 h (Fig. 5.32). Alkali pretreatment did not have any significant effect on the volatile solids content. It can be inferred that food waste being already cooked and with a high volatile solids content, cannot undergo further increase in the volatile solids content.

- **Soluble COD**

The soluble COD showed an increasing trend with the exposure time. The soluble COD of the control was 888.9 g/kg of dry food waste and it increased to 1170 g/kg of dry food waste for samples exposed to NaOH pretreatment for time durations of 1.5, 3 and 6 h (Fig. 5.33). The soluble COD further increased to 1191 g/kg of dry food waste for samples exposed to a time period of 12 and 24 h. This proves that as the time of exposure increased the alkali got more time in hydrolysing the organic matter thus breaking the bonds between polymers and converting them into soluble monomers shown by the increase in soluble COD. Fig. 3.3 shows that the sample pretreated for 1.5 h has a soluble COD content which is about 98% of the sample pretreated for 24 h. So the difference in soluble COD between these samples is very less. This small difference in soluble COD may be because food waste being already cooked and with high soluble organic content will hydrolyse easily even in small exposure times reaching a saturation early. Hence it can be inferred that an exposure time of 1.5 h will be optimum for alkali pretreatment using NaOH.

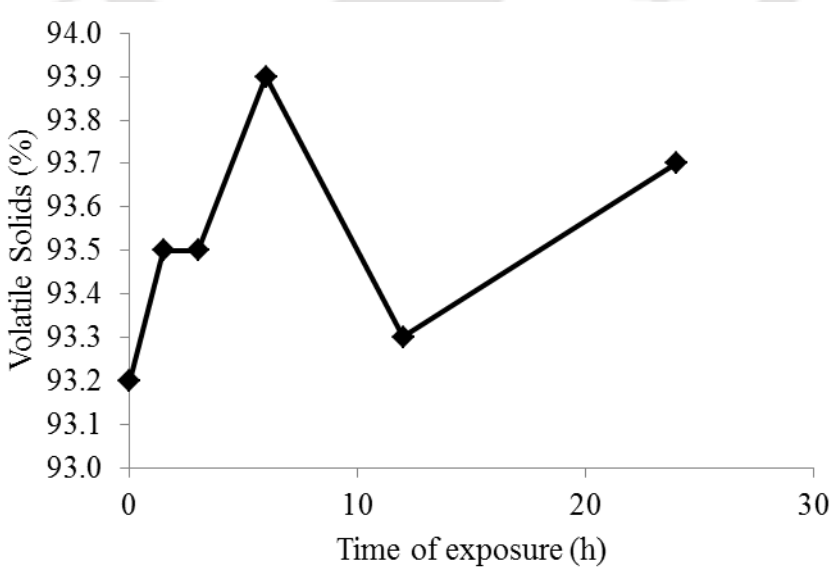


Fig. 5.32 Variation of VS with NaOH time of exposure

Florez-Juarez et al. (2010) reported a soluble COD increase of 2.94 times for the slaughter house solid waste pretreated for 24 h. Slaughterhouse wastes require high time of exposure due to the cellulose and lignin materials in ruminal content of slaughterhouse solid waste. Lin et al. (2009) exposed the samples to 6 h for pretreatment of pulp and paper sludge using NaOH. It was found that the soluble COD increased by 8.8 times with respect to the control. Sludge pretreatment requires more time of exposure because it primarily comprises of cells of microorganisms, which are difficult to digest due to their cell membranes which provide intrinsic strength to the cell against degradation.

- **Volatile fatty acids**

Volatile fatty acids content showed an increasing trend with increase in time of exposure. The volatile fatty acids content increased from 33.3 g/kg of dry food waste for the control to 83.3 g/kg for the sample exposed to a time period of 24 h (Fig. 5.34). This increase in volatile fatty acids due to the acidification caused by aerobic degradation made possible by the swelling of organic molecules caused by alkaline pretreatment.

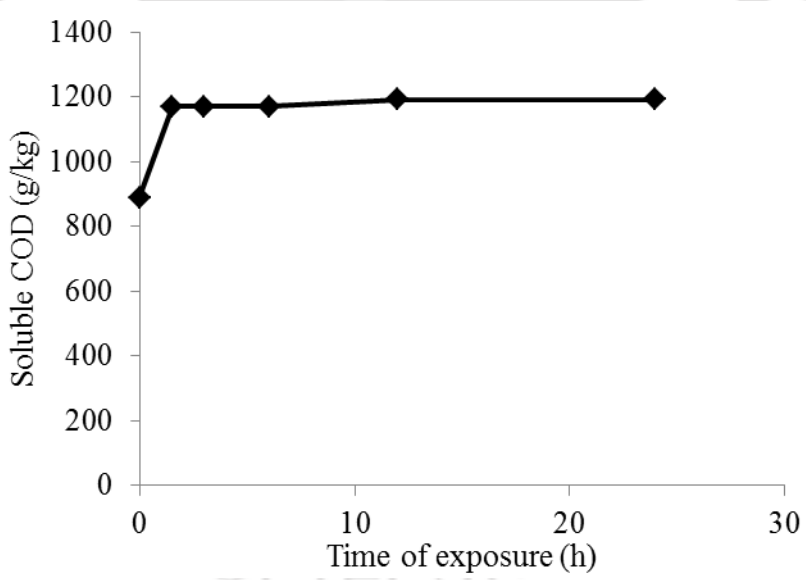


Fig. 5.33 Variation of soluble COD with NaOH time of exposure

5.1.3 Electrohydrolysis

Electrohydrolysis pretreatment was done using graphite electrodes. This pretreatment study was divided into two parts. They are

- Voltage study
- Total fat, protein and carbohydrate analysis

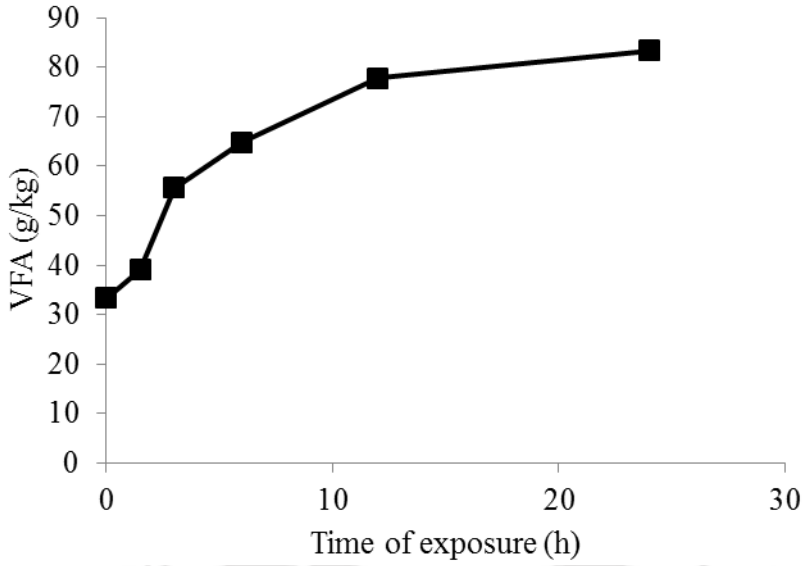


Fig. 5.34 Variation of VFA with NaOH time of exposure

Voltage study

In the voltage study, each sample was exposed to a different voltage. The voltages considered for this study are 10, 15, 20, 25, 30, 35 and 40 V. A sample was kept as control without giving any pretreatment. All samples were exposed to a time period of 60 min. The sample pretreated at 40 V was discarded due to excessive foaming.

- **Variation of current and resistance with time**

It was found that at the same applied voltage, the current is gradually increasing with the time of exposure (Fig. 5.35). This is due to the increase in conductivity caused by solubilisation of particulate organic matter by the breaking of bonds between polymers induced by application of current. It can also be inferred that as the time of exposure increased the solubilisation also increased. The next observation made was that as the applied voltage increased the slope of the current versus time curve also increased. When the sample pretreated at 10 V showed an increase of just 0.03 amp, the sample pretreated at 35 V showed an increase of 1.65 amp. This is because at higher voltages, there is more energy available to cause rapid solubilisation of organic matter. Solubilisation of organic matter will reflect as an increase in soluble COD. Importance of soluble COD lies in the fact that soluble COD can be readily used to produce methane during anaerobic digestion (Wang et al., 2005).

From analysing the resistance versus time curve it can be observed that at the same applied voltage, the resistance is gradually decreasing as the time of exposure increases

(Fig. 5.36). This is due to the decrease in resistance due to the hydrolysis of polymers into simple soluble monomers. So as the time of exposure increased more and more particulate matter is getting solubilised. The other observation made was that as the applied voltage increased the slope of the resistance versus time curve also increased. When the sample pretreated at 10 V showed a decrease of just 2.2 Ω, the sample pretreated at 35 V showed a decrease of 9.2 Ω. This is huge difference in decrease of resistance is because at higher voltages, there is more energy available to break the bonds between polymers.

- **pH**

The pH showed an increasing trend with increase in voltage applied. But the increase in pH was limited with control having a pH of 3.8 while the sample pretreated at 35 V showing a pH of 4.4 (Fig. 5.37). Even the pH of sample pretreated at the highest applied voltage is less than the range of pH required for the co-existence of acidogens and methanogens which is 6.8 to 7.4. So it can be inferred that electrohydrolysis does not have much influence on pH.

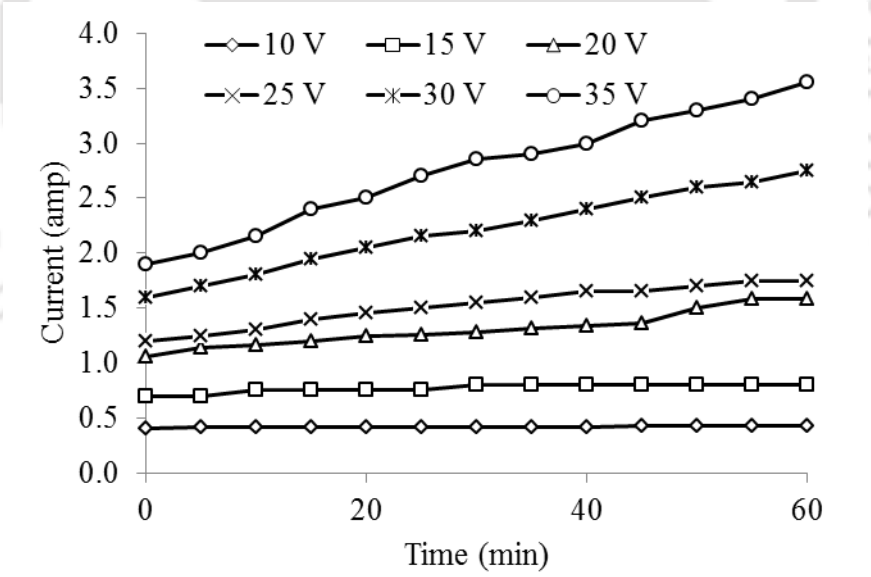


Fig. 5.35 Variation of current with time

- **Soluble COD**

Soluble COD is showed an increasing trend with increase in applied voltage. While the control had a soluble COD of 889 g/kg of dry food waste, the sample pretreated at 35 V improved 1.33 times to become 1185 g/kg of dry food waste (Fig. 5.38). The sample pretreated at 40 V was discarded due to excessive foaming. Thus 35 V which showed the

highest increase in soluble COD was selected as the optimum voltage for electrohydrolysis pretreatment of food waste using graphite electrodes.

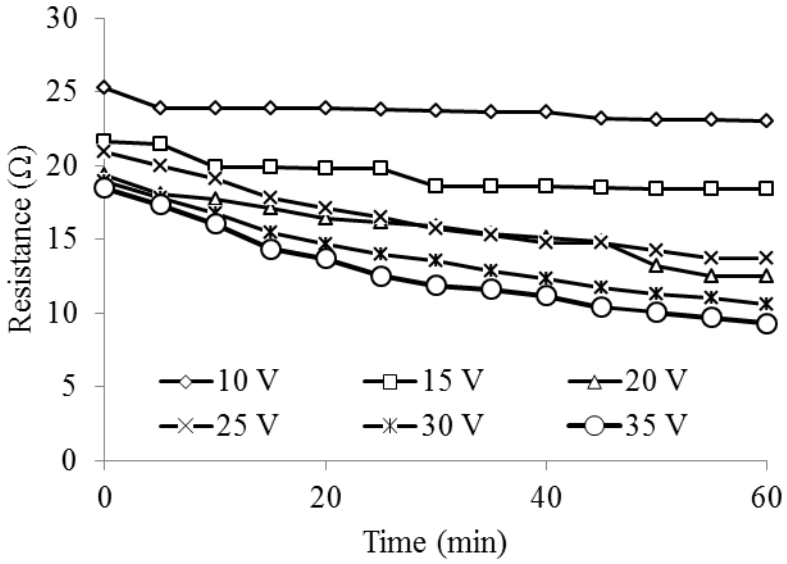


Fig. 5.36 Variation of resistance with time

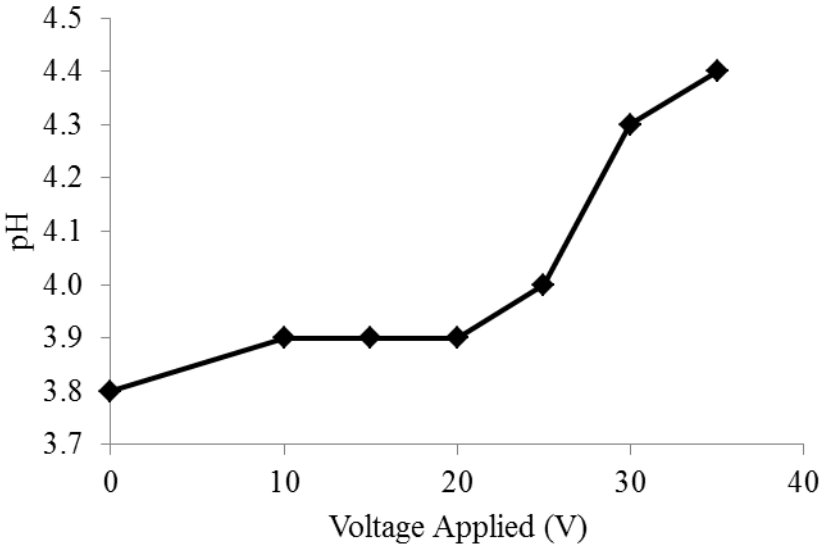


Fig. 5.37 Variation of pH with applied voltage

Zhen et al. (2014) employed a pair of Ti/RuO_2 as anode and cathode for the electrohydrolysis pretreatment of waste activated sludge. The voltages studied were 5 to 20 V. In their work it was found that the soluble COD of the sample pretreated at 20 V improved by 1.5 times when compared to the sample pretreated at 5 V. This increase is higher than the value obtained in the present study for 35 V. This may be due to the difference in substrate of the two works. Since food waste is already cooked and having

very high soluble organic content, the increase in soluble COD that can be achieved is very limited. Whereas waste activated sludge being rich in microbial cells, during pretreatment get their partial extracellular biopolymer surrounding the microbial cells solubilised thus increasing the soluble COD considerably (Zhen et al., 2014).

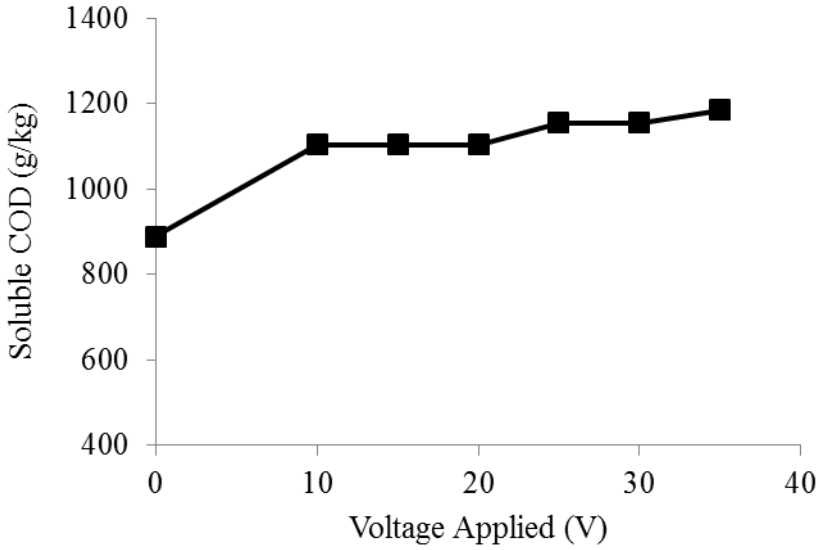


Fig. 5.38 Variation of soluble COD with applied voltage

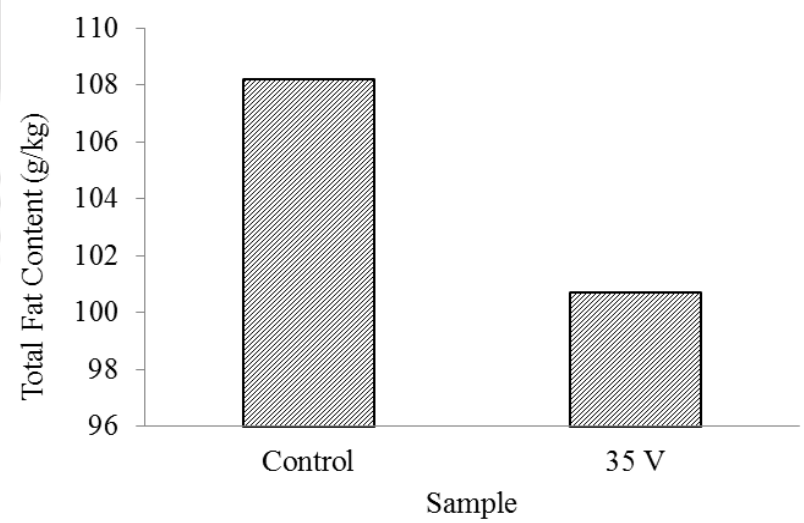


Fig. 5.39 Variation of total fat content with sample

- **Total fat analysis**

The results of the total fat analysis showed a decline in fat content by 7.4% for the sample pretreated at 35 V when compared to the control (Fig. 5.39). The reason for the decline in fat content lies in the properties of fats. Triglycerides like fat and oil are

hydrophobic like any other lipids. During pretreatment the oil and fats gets hydrolysed into fatty acids which are water soluble unlike triglycerides. Thus after pretreatment the total fat content decreases as a consequence of hydrolysis.

- **Protein analysis**

The results showed an increase in soluble protein content for the pretreated sample with respect to the control (Fig. 5.40). The soluble protein content of the sample pretreated at 35 V was 1.2 times that of the control. Zhen et al. (2014) reported an increase of soluble protein content by 1.37 times for the sample pretreated at 20 V with respect to that of 5 V. Even at lower voltage a higher solubilisation of proteins was achieved when compared to the present study because the substrates used in the present study is food waste whereas Zhen et al. (2014) studied on waste activated sludge. Sludge being rich in microbial cells, during pretreatment get their partial extracellular biopolymer surrounding the microbial cells solubilised (Zhen et al., 2014). While food waste is already cooked and having very high soluble organic content, thus the increase in soluble proteins that can be achieved is very limited

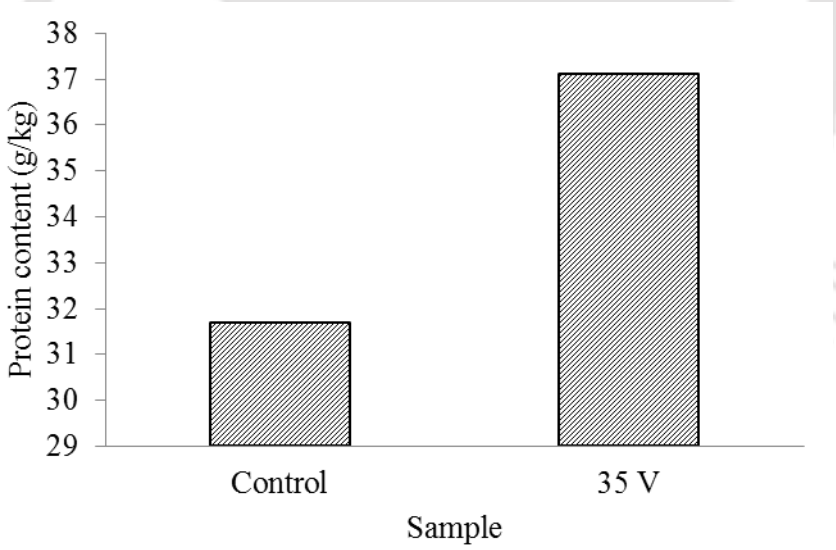


Fig. 5.40 Variation of soluble protein with sample

- **Carbohydrate analysis**

Carbohydrate analysis showed an increase in soluble carbohydrate content by 1.08 times for the sample pretreated at 35 V with respect to the control (Fig. 5.41). Zhen et al. 2014 has reported an increase in polysaccharide content by 1.45 times for a sample

pretreated at 20 V with respect to the sample pretreated at 5 V. This huge variation in increase of carbohydrate content between the present study and Zhen at al. (2014) is due to the different substrate used in both studies. Since the food waste is already cooked and with a very high soluble organic content, the increase in soluble carbohydrate is very limited. Whereas sludge comprises mainly of microbial cells which during pretratment get their partial extracellular biopolymer surrounding them solubilised (Zhen et al., 2014).

5.2 BATCH STUDY FOR BEST PRETREATMENT METHOD

Batch study was done with four batch reactors running with untreated, best thermally treated, alkali treated and electrohydrolysis treated FW. The pH of all the reactors was kept between 6.8 and 7 by the addition of sodium bicarbonate. Parameters used for comparison are VFA, methane production and VS reduction.

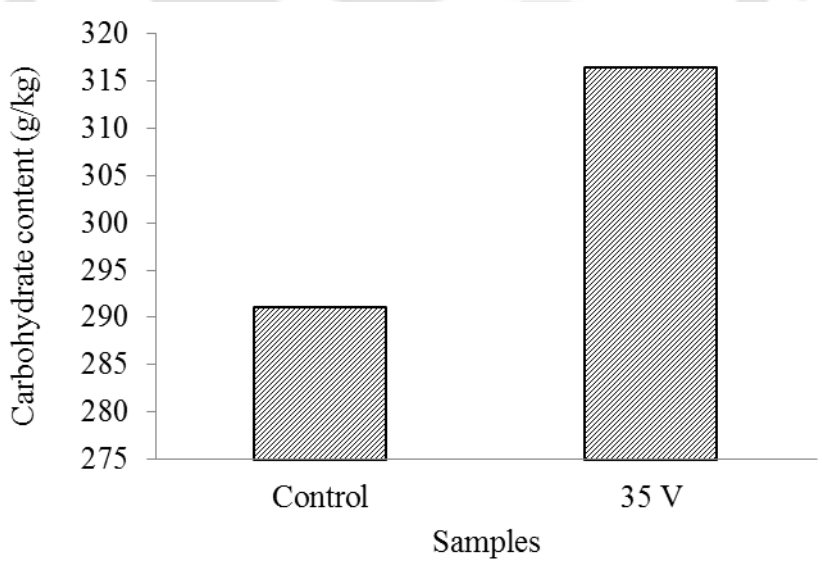


Fig. 5.41 Variation of soluble carbohydrate with sample

5.2.1 Volatile fatty acids

The results of the VFA analysis showed an exponential increase in VFA concentration in batch reactors running on pretreated FW when compared to untreated FW (Fig. 5.42). Within the first four days itself VFA became 20,750 mg/L from 3475 mg/L in the batch reactor running on thermally pretreated FW while the VFA increased to just 3965 mg/L for the batch reactor running on untreated FW. This is in line with the

VS reduction in batch reactors of pretreated and untreated FW. The first four days had high reduction in VS for the batch reactors running on pretreated FW (Fig. 5.46). When the VFA concentration exceeds 13000 mg/L, AD stops (Vieitez et al., 1998). This is backed by the sudden decrease in methane production of the reactors running on pretreated FW. The cumulative methane production of pretreated FW increased exponentially in the initial days and then flattened out by the 6th day. Whereas VFA in the batch reactor with untreated FW never exceeded 13000 mg/L and it showed a steady increase in cumulative methane production (Fig. 5.43). This clearly indicates that the AD in the batch reactors running on pretreated FW was adversely affected by the increase in VFA above 13000 mg/L. Due to pretreatment, huge amounts of readily degradable organic matter were produced in the batch reactors with pretreated FW which were consumed by the acidogens to produce VFA.

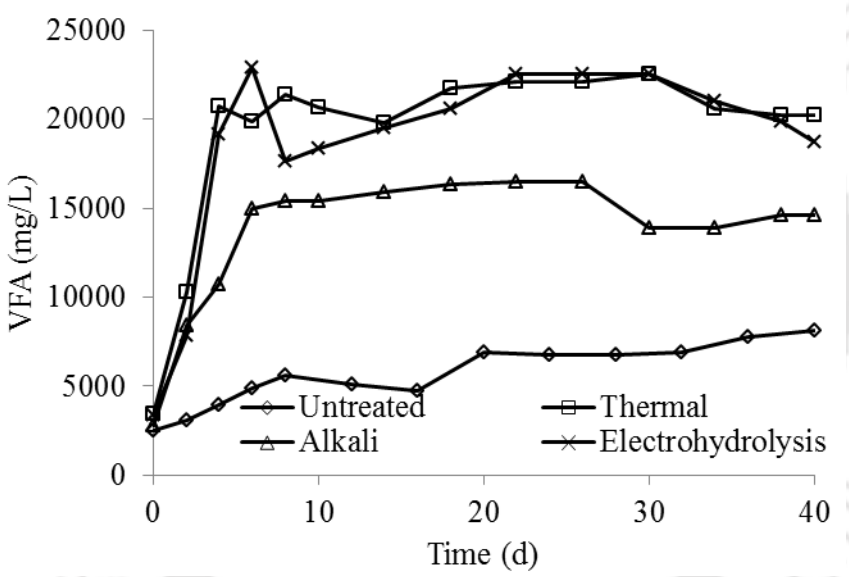


Fig. 5.42 Variation of VFA with time during batch study

This huge production of VFA resulting from the rapid hydrolysis and acidification caused the inhibition of methanogenesis leading to huge decrease in methane production (Wang et al., 2009). Acetoclastic methanogens are severely affected by the accumulation of VFA (Zhu et al., 2010). Batch study on AD of corn stover using alkali pretreatment showed that the batch running on 7.5% NaOH pretreated substrate stopped digestion on the 13th day due to the increase of VFA concentration to 22 g/kg (Zhu et al., 2010). The VFA concentration varied between 365 to 1040 mg/L during AD of alkaline pretreated pulp and paper sludge. This study observed that when the VFA concentration decreased, the methane generation rate increased (Lin et al., 2009). The current study also observed similar results. For instance, the decrease in VFA concentration in the batch reactor

running on electrohydrolysed FW from the 34th day was reflected as increased methane production.

5.2.2 Methane production

The results of cumulative methane production showed that the batch reactor with untreated FW had the highest cumulative methane production (Fig. 5.43). The cumulative methane production of the batch with untreated FW was 50.1 L whereas the batch reactors running on thermal, alkali and electrohydrolysis pretreated FW were 22.8, 27.2 and 25.2 L respectively. This huge decrease in methane production of batch reactors running on pretreated FW is due to the inhibition of AD caused by VFA accumulation. But it was observed while comparing the methane production of initial few days, the batch reactors with pretreated FW showed much higher methane production. But the cumulative methane yield of the initial few days showed that even though the methane production was higher for batch reactors running on pretreated FW, the cumulative methane was lesser when compared to the batch with untreated FW (Fig. 5.44). This implies that the reduction in VS did not amount to a proportionate increase in methane production but rather caused increased accumulation of VFA. This clearly shows that the rapid hydrolysis and acidogenesis seen in the initial few days were not followed by an increased rate of methanogenesis causing accumulation of VFA. This is because the inoculum used in the current study was not acclimatised to handle the high amount of available organic matter due to pretreatment. At the end of 40 days, the cumulative methane yield of the batch reactors running on untreated, thermal pretreated, alkali pretreated and electrohydrolysis pretreated FW were 336, 110, 151 and 134 mL/g VS respectively. This indicates that the reactors running on pretreated FW never recovered from the inhibition caused by VFA accumulation. But after 30 days, the reactors running pretreated FW showed an increase in methane production suggesting that the methanogens had started getting acclimatised to the adverse condition. A study on AD of FW using hot air oven pretreatment showed an increase of 22.2% increase in methane production (Ariunbaatar et al., 2014). The sample treated at 80°C showed a specific methane potential of 540 mL/g VS while the untreated had a specific methane potential of 426 mL/g VS. This study used inoculum from a full scale AD plant and an F/M ratio of 0.5 $gVS_{substrate}/gVS_{inoculum}$. This might be the reason that the VFA accumulation did not occur in their study, as the present study had used cow dung as inoculum and a F/M

ratio of 2 $gVS_{substrate}/gVS_{inoculum}$. An inoculum from an AD plant will be acclimatised to handle the high amount of available organic matter produced during pretreatment thus effectively converting the VFA produced to subsequent products, thus preventing VFA accumulation. This implies that the F/M ratio and inoculum used in the present study is suitable for untreated FW but not for batch reactors running on pretreated FW. The results of a study on the effect of autoclave pretreatment on FW showed that at the end of 35 days the reactor running on untreated FW had a cumulative methane yield of 500 mL/gVS whereas for the pretreated FW it reduced to 445 mL/gVS (Tampio et al., 2014). This reduction happened due to the formation of refractory compounds at 160°C due to Maillard reactions. These refractory compounds are difficult to degrade thus resulting in lesser methane production. Similar results were observed where autoclave pretreatment (170°C) was used to hydrolyse FW before BMP studies (Qiao et al., 2011). The results showed a decrease in methane production by 6.9% for the reactor running on pretreated FW. The methane yield reduced from 531 mL/gVS for the untreated to 491 mL/gVS for the autoclaved FW. For wastes having high lignocellulose content the negative effects due to Maillard reactions can be overcome due to solublisation of lignocellulosic material at high temperatures. But since FW has much lower lignocellulose content the negative effects due to Maillard reactions caused the reduction in methane yield (tampio et al., 2013). The study on the effect of microwave pretreatment on AD of organic fraction of municipal solid wastes showed an increase of 4-7% in biogas production for samples pretreated at 115°C and 145°C (Shahriari et al., 2011). But the sample pretreated at 175°C showed a decrease in biogas production due to the formation of refractory compounds due to Maillard reactions. A study on the alkali pretreatment of slaughter house waste showed a total biogas production of 36.5 L and a biogas yield of 300 mL/gVS for the substrate pretreated with 0.6 g/g VSS of NaOH (Flores-Juarez et al., 2014). The current study had obtained a higher methane yield of 336 mL/g VS for the untreated FW indicating the superior biodegradability of FW over slaughter house solid wastes which have lignocellulose materials in the ruminal content. A study on the effect of alkaline pretreatment on AD of corn stover showed that the sample pretreated with 5% NaOH had a biogas yield of 372 mL/gVS at the end of 40 days period (Zhu et al., 2010). Whereas for the substrate pretreated at 7.5% NaOH, methane production was inhibited due to VFA accumulation. A work on the effect of alkaline pretreatment on AD of pulp and paper sludge found a peak methane yield of 320 mL/gVS at the optimum dosage of 8

gNaOH/100 gTS (Lin et al., 2009). This result also indicates the superior biodegradability of FW over pulp and paper sludge.

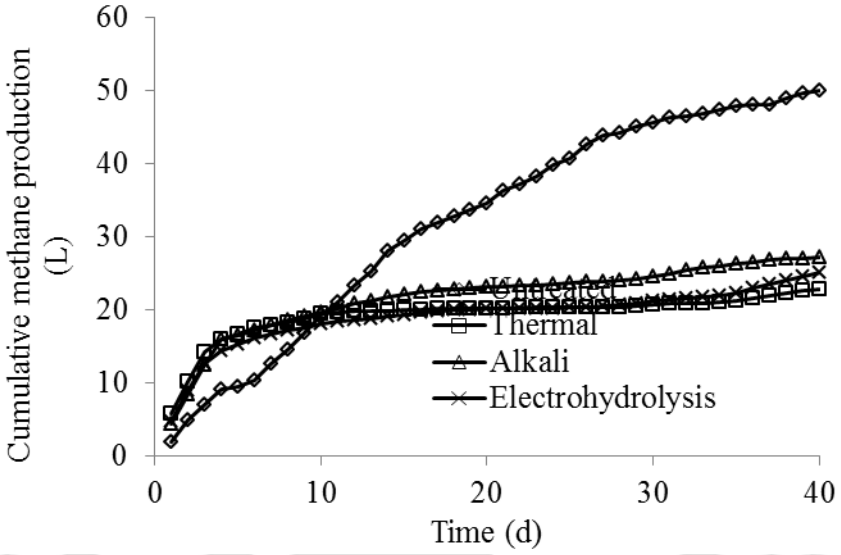


Fig. 5.43 Variation of cumulative methane production with time during batch study

When analysing the first 6 days of running the batch reactor it can be seen that while the batch reactor with untreated FW had just 17.5% reduction in VS, the thermal, alkali and electrohydrolysis pretreated FW had 31.8, 22.6 and 29.1% VS reduction respectively. The batch reactor running thermal and electrohydrolysis pretreated FW has double the reduction in VS obtained by the batch reactor running untreated FW. The final VS reduction achieved by thermal, alkali and electrohydrolysis pretreated FW at the end of 40 day time period was 36.6, 31.8 and 33.1% respectively. The VS reductions obtained by these reactors within 6 days were higher with 86.9, 71.0 and 87.9% of their 40 day respective VS reduction. While the cumulative gas production at the end of 6 days was just 17.6 L for the batch reactor running untreated FW, the reactors running thermal, alkali and electrohydrolysis pretreated FW had 17.6, 17.1 and 16.1 L respectively (Fig. 5.45). These values are 35.1, 34.2 and 32.1% of the total cumulative gas production achieved by the batch reactor running for 40 days. From this it can be inferred that a continuous reactor running with 6 days HRT will be able to achieve around 32% VS reduction and 35% of the 40^{th} day cumulative gas production attainable with untreated FW. But post treatment of the effluent should be ensured for the utilisation of high VFA content.

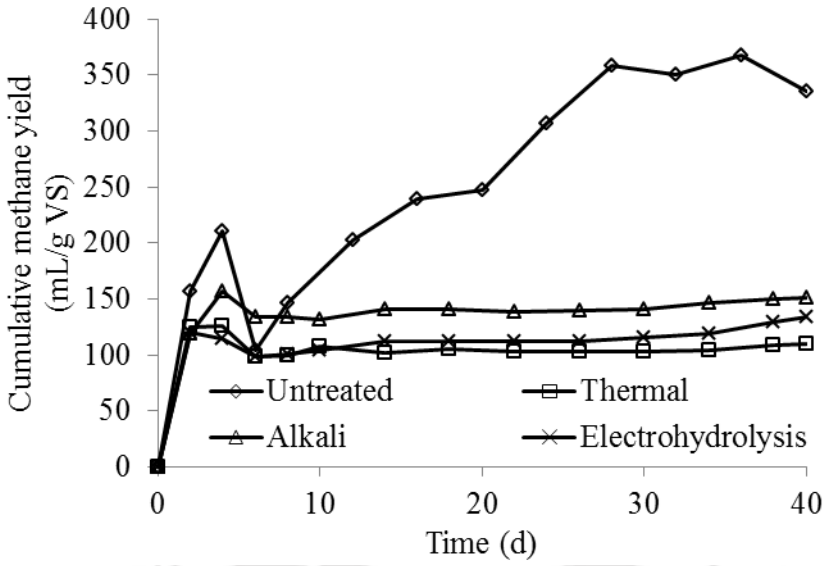


Fig. 5.44 Variation of cumulative methane yield with time during batch study

5.2.3 Volatile solids reduction

The analysis of the results of batch studies showed that thermally pretreated FW had the highest VS reduction of 36.6% while untreated had only 26.4% (Fig. 5.46). Electrohydrolysis closely followed thermal by 33.1% while the alkali pretreated had 31.8% VS reduction. Similar results were obtained during a study on the effects of alkali pretreatment on slaughterhouse solid waste where it was observed that a VS reduction of 48% was achieved at the end of 30 days duration (Florez-Juarez et al., 2014). A VS reduction of 44.4% was obtained at the end of 40 days for the batch study of a corn stover sample pretreated with 5% NaOH (Zhu et al., 2010). In the current study, it is interesting to note that thermal pretreatment had the highest solubilisation of the three pretreatments with 1.38 times improvement with respect to the untreated FW. Even then it cannot be concluded that the thermal pretreatment solubilised particulate organic matter which the other two pretreatments are not capable of. It is because from the trend of variation of volatile solids it can be seen that even on the 40^{th} day reduction in VS is still going on. So even though at the 40^{th} day, batch reactor with thermally pretreated substrate had higher VS reduction, the highest possible VS reduction of all the batch reactors might be similar. This is applicable even to the batch reactor running on untreated FW because as FW being high in VS content and very low in non-degradable VS, every reactor is expected to have a similar highest possible VS reduction even though the HRT required to reach that particular VS reduction may vary.

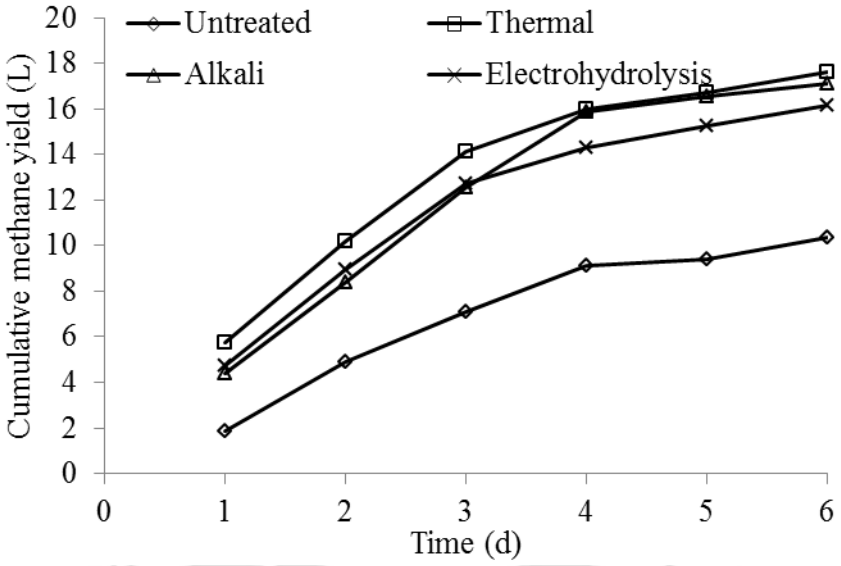


Fig. 5.45 Variation of VS reduction with time during batch study

The next observation that can be made from variation of VS with time is that for the batch reactors running on pretreated substrates showed exponential decrease in VS content in the first four days when compared to the untreated FW. While the batch reactor with untreated FW achieved just 8% VS reduction, the batch reactor with thermally treated FW had 22.5% reduction in VS. The other two batch reactors with alkali and electrohydrolysis pretreated FW also showed similar results. This implies that due to the availability of high amounts of solubilised organic matter in the batch reactors with pretreated FW, acidogens were able to convert them rapidly into VFA and subsequent conversion to methane by methanogens. This is backed by the results obtained for VFA variation and methane production. In the initial days, the VFA increased exponentially for the batch reactors with pretreated FW when compared to the batch reactor with untreated FW. For instance, the 4^{th} day VFA of the untreated batch reactor was just 3965 mg/L whereas the batch with thermally pretreated FW had 20750 mg/L. In the case of methane production, the initial days showed high methane evolution for batch reactors running on pretreated FW than untreated FW. For instance, the batch running on thermally pretreated FW had a 3^{rd} day cumulative methane production of 14.1 L, whereas the batch running on untreated FW had just 7.1 L. This huge difference in methane production goes in line with the difference between VS reduction for batch reactors running on pretreated and untreated FW.

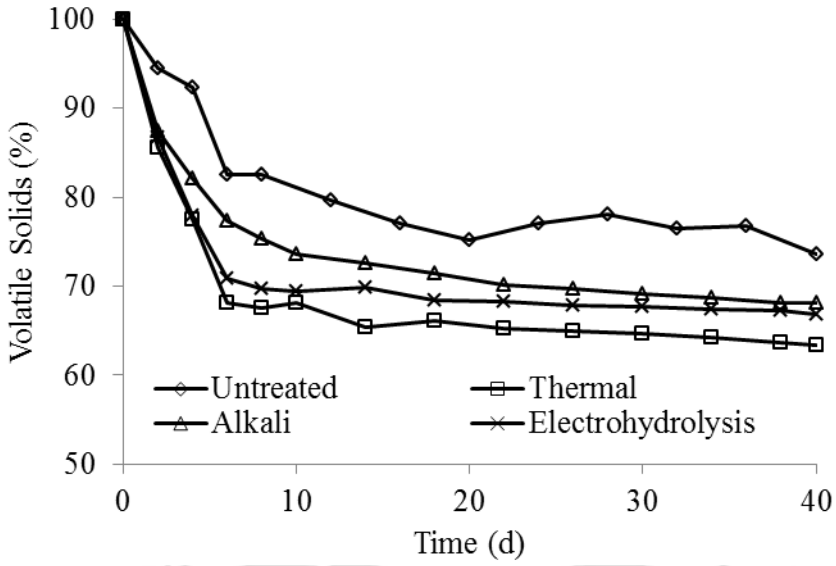


Fig. 5.46 Variation of VS reduction with time during batch study

5.3 CONCLUSION

From pretreatment study for solubilization, it was inferred that hot air oven pretreatment of food waste at temperature of 75°C with a time of exposure of 90 min is the optimum condition and it concludes that thermal pretreatment using hot air oven is the best among the five pretreatments studied for hydrolysis of FW. The results of the batch study revealed that the thermally pretreated FW had the highest VS reduction of 36.6% followed by electrohydrolysis, alkali and untreated with 33.1, 31.8 and 26.4% respectively. High solubilization of organic matter facilitated by pretreatment lead to the accumulation of VFA and caused the inhibition of AD process. Since the microbial consortia in the inoculum used were not acclimatized to handling high organic loads, the accumulation of VFA caused the inhibition of acetoclastic methanogens. It can be concluded that a lower F/M ratio or an acclimatized inoculum such as those from a full scale AD plant will be able to handle the high availability of degradable organic matter arising due to pretreatment.

CHAPTER 6

LAB AND PILOT SCALE ABBR AND METAGENOMICS STUDY

This chapter deals the results and discussion during lab scale and pilot continuous reactor operation with best inoculum concluded from batch study and the microbial succession during lab scale continuous anaerobic digestion through 16s rRNA gene sequence method.

6.1 PHASE VI AND VII: LAB SCALE ABBR STUDIES WITH DIGESTED SLUDGE AND COW DUNG AS INOCULUM (HRT – 8 TO 14 DAYS)

Lab scale ABBR studies have been performed for anaerobic digestion of food waste with two different inoculums i.e. digested sludge (DS) and cow dung (CD). The study has been conducted to find the optimum degradation in ABBR and its hydraulic retention time to degrade.

6.1.1 Acclimatization of ABBR with two different inoculum

ABBR performed well at the operational temperature of 26 to 30 ^{o}C. The reactors were fed with FW at an OLR of 10 g_{SCOD}/L/d initially; OLR was increased upto 40 g_{SCOD}/L.d by increasing the flow. COD load applied during the startup period in DS reactor and CD reactor was 40 g_{SCOD}/L/d. During the initial period, COD removal efficiency was only about 20 to 30%. The COD removal efficiency increased to 50 and 62% in DS and CD reactor respectively within 40 days as the bacteria got acclimatized with the substrate. It was observed that pH of influent was in the range of 5.2-5.8 and it was increased to 7-8.2 during startup process of the continuous reactor.

6.1.2 Soluble COD reduction profile on different HRT days

ABBR performed well at the operational temperature of 26^{o}C. The final effluent soluble COD of ABBR with digested sludge as inoculum ranged from 9.87 g_{SCOD}/L at a HRT of 14 days to 13.4 g_{SCOD}/L at a HRT of 8 days. Parawira et al. (2006) reported that the UASB can provide stable process conversion rate up to an OLR of 6.1 kg COD/m^3/d. Single stage reactor operation at an OLR of 9.2 kg VS (15.0 kg COD) /m^3/d was achieved with higher VS reduction (91.8%) and maximum methane yield (455 mL/g VS)

(Nagao et al., 2012). ABBR studies with CD as inoculum had the final effluent soluble COD of 7.3 g_{SCOD}/L at HRT of 14 days to 11.2 g_{SCOD}/L at HRT of 8 days. The COD reduction was low at the startup period, later it was increased to more than 50% at HRT 14 days during first 30 days of reactor in both studies. Next 30 to 60 days, the HRT was maintained at 8 days, further COD reduction was dropped to an average of 45.2 and 46.4 % in DS and CD study respectively. In 60 to 90 days, the HRT was maintained at 9 days to increase the COD reduction percentage in both inoculum studies. At HRT 9 days, the reactor achieved maximum COD reduction of 57.73% which was similar to HRT 14 days. Methane yield (mL/gVS) can only represent a constant reaction rate when there is no accumulation of intermediary products (Veeken and Hamelers, 1999). Later the HRT was increased to 10 days on 90 to 120 days; then there was more stabilized reduction than 9 days HRT at its maximum percentage. Fig. 6.1 shows the SCOD profile of ABBR at various HRT days. Ince (1995) and Sosnowski et al. (2002) reported that the single stage system requires 37 days HRT whereas the two-stage requires 30 days HRT for maximum COD removal (80%). In ABBR with CD as inoculum achieved the highest percentage of SCOD reduction up to 72% on HRT 10 days.

6.1.3 Biogas production from ABBR on different HRT

Methane gas production is the most important parameter that needs to be optimized in anaerobic digestion process. The methane production varies with many factors such as inoculum, VS, VFA, and temperature. The biogas production followed the same trend as COD reduction percentage. At HRT 10 days the average biogas production was higher as 13.63 L/d and 16.16 L/d in DS and CD studies respectively. To estimate the inclination of sludge acclimation and the change in methane-generation activity, a reaction-rate constant for methane conversion was calculated. This was used to define the methane yield from the substrates; then this yield was compared with the OLRs (Veeken and Hamelers, 1999). At HRT 8 days, biogas production was very low in both inoculum studies. At 9 days HRT, the average biogas production was almost close to 10 days HRT as 0.438 L/g_{SCOD}/d in DS, but in CD studies the gas production had been increased in 10 days HRT i.e. 0.528 L/g_{SCOD}/d than 9 days HRT. The biogas production profile of ABBR at different HRT is shown in Fig. 6.2. The Anaerobic phased solids-digester was started at an organic loading rate (OLR) of 3.1 g /L/d and operated at three higher OLRs of 4.6, 7.7 and 9.2 g VS/L/d. At the OLR of 9.2 g VS/L/d the system

biogas production rate was 3.5 L/L/d; the biogas and methane yields were 0.38 and 0.19 L/g VS, respectively (Zhu et al., 2010).

6.1.4 Volatile fatty acids profile at different ports on different days

VFA formation is the second stage of anaerobic reaction resultant due to acidogenesis process. Readily available VFA facilitate the methanogens process as a substrate and so VFA formation is supposed to be one of limiting step for methane production. But high VFA concentration also affects methanogenesis stage of anaerobic process which reduces methane production. Increasing VFA concentration was absorbed during initial days due to production of acid at a faster rate than methanogenesis step (consuming fatty acids); which led to the accumulation of fatty acid inside system that consequently increased the VFA concentration. During degradation it got mixed with the reactor solution to form ammonium bicarbonate and helped in buffering the pH of the digester (Murto et al. 2004).

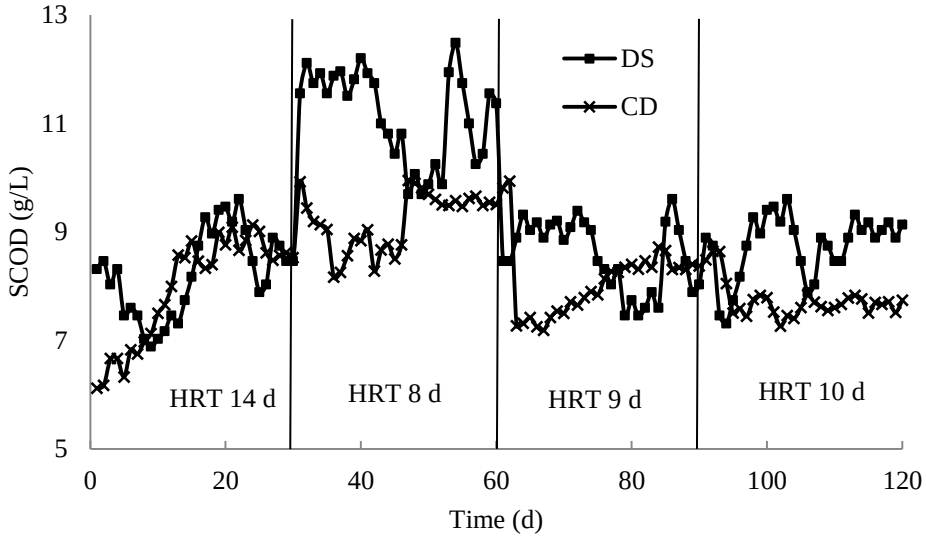


Fig. 6.1 SCOD profile of ABBR at various HRT

Climenhaga and Banks (2008) reported that anaerobic digestion of food waste was successful only at long retention time (HRT 100 day) and lower organic loading rate (1.0 g VS/L/d) with high level VFA concentration. These results further confirmed that anaerobic digestion of food waste was not successful in single-stage reactors. Unfortunately, with the prolonged operation, noticeable decrease of methane productivity and methane content were observed after day 60, even though pH was still

in the optimal range. Concomitantly, the total VFA concentration increased from 1500 to 10000 mg/L as shown in Table 6.1. Surprisingly, acetate concentration did not significantly increase as compared with propionate and iso-butyrate and iso-valerate, which were accumulated in large amount. The accumulation of propionate and iso-form fatty acids indicated the upset of anaerobic process. These results also suggested that the acetogenesis might be limited step other than aceticlastic methanogenesis during the bio-methanization of food waste. In the present study, accumulation of VFA was very low in the second phase where the methanogenesis was active. Table 6.1 shows the profile of acetic acid, butyric acid, propionic acid, valeric acid and isobutyric acid respectively in four different ports (inlet, sampling port 1 (sp1), sampling port 2 (sp2) and outlet). At the end of day 120, the increase in utilization of every acid by the acetogenic and methanogenic bacteria was confirmed by the VFA analysis. pH values indicated the acids accumulation and utilization in port 2 and 3.

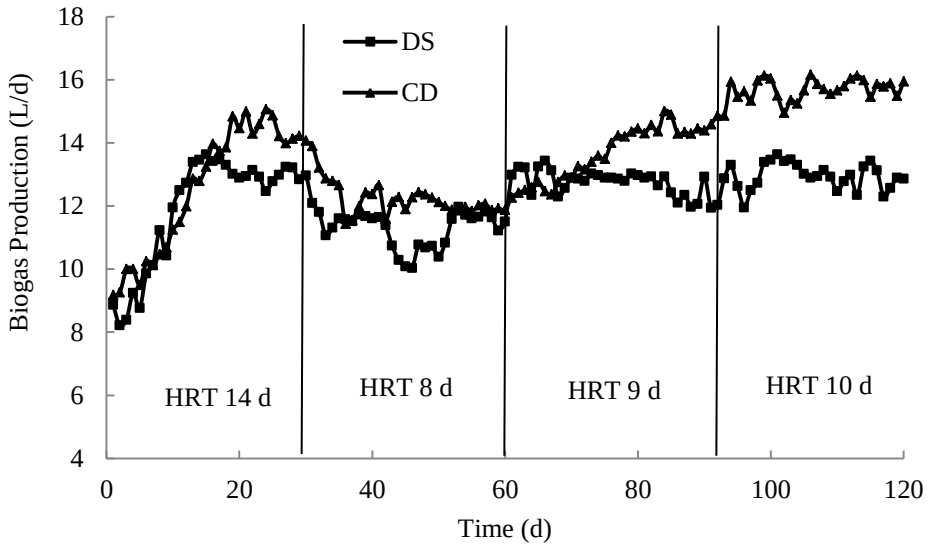


Fig. 6.2 Biogas production profile of ABBR at various HRT

6.2 16S METAGENOME SEQUENCING OF COW DUNG INOCULUM REACTOR SLUDGE

The aim of the study was focused on the enumeration and identification of different bacterial and archaeal communities involved in the degradation of food waste during anaerobic digestion process using cow dung as inoculum. The sludge was collected from

the lab scale continuous ABBR and analyzed using the 16S Metagenome sequence method.

Table 6.1(a) VFA at various ports at different days in ABBR with DS

VFA(mg/L)	Day 0				Day 40			
	Inlet	sp1	sp2	outlet	Inlet	sp1	sp2	outlet
Acetic	800	4480	6520	1800	760	5620	8980	2860
Butyric	1120	5280	3800	1280	830	6348	4340	1880
Propionic	0	1800	1400	860	50	2830	2190	1020
valeric	120	1690	1280	690	80	1240	1620	980
isobutyric	0	2430	1960	1230	20	1890	1430	860
	Day 80				Day 120			
	Inlet	sp1	sp2	outlet	Inlet	sp1	sp2	outlet
Acetic	822	5580	6780	1980	846	5400	6690	1890
Butyric	896	6248	3830	1650	782	6480	3670	1560
Propionic	54	3270	1890	1020	68	2890	1820	980
valeric	90	1340	1596	810	84	1260	1490	820
isobutyric	25	1260	1280	780	38	1080	890	760

Table 6.1(b) VFA at various ports at different days in ABBR with CD

VFA(mg/L)	Day 0				Day 40			
	Inlet	sp1	sp2	outlet	Inlet	sp1	sp2	outlet
Acetic	1100	6280	6520	1200	720	8480	7280	1430
Butyric	840	5260	3800	800	1020	6986	5396	1186
Propionic	200	2280	1800	660	120	2258	1660	814
valeric	180	1520	1360	840	260	1390	920	438
isobutyric	400	240	1820	980	220	1620	1040	440
	Day 80				Day 120			
Acetic	980	9328	8008	1573	1210	6908	7172	1320
Butyric	1128	7686	5938	1230	924	5786	4180	880
Propionic	420	2484	1826	898	220	2508	1980	726
valeric	240	1529	1012	490	198	1672	1496	924
isobutyric	120	1782	1144	484	440	264	2002	1078

6.2.1 QUALITATIVE AND QUANTITATIVE ANALYSIS OF gDNA AND PREPARATION OF LIBRARIES FOR 2 X 300 bp RUN CHEMISTRY

DNA was isolated using modified xcelgen soil gDNA kit. Quality of gDNA was checked on 1% agarose gel (loaded 5 µl) for the single intact band. The gel was run at 110 V for 30 mins. 1 µl of each sample was loaded in nanodrop 8000 for determining A260/280 ratio. The DNA was quantified using Qubit dsDNA BR Assay kit (Thermo

Fisher Scientific Inc.). 1 µl of each sample was used for determining concentration using Qubit® 2.0 Fluorometer. The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc.) as per the 16S Metagenomic Sequencing library preparation protocol (Part # 15044223 Rev. B). Primers for the amplification of the V3-V4 hyper-variable region (Table 6.1) of 16S rDNA gene of Eubacteria and Archaea were designed in Xcelris NGS Bioinformatics Lab. These primers were synthesized in Xcelris PrimeX facility. The amplicons with the Illumina adaptors were amplified by using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were purified by 1X AMpureXP 2 beads and checked on Agilent DNA 1000 chip on Bioanalyzer 2100 and quantified on fluorometer by Qubit dsDNA HS Assay kit (Life Technologies).

- **Cluster Generation and Sequencing**

After obtaining the Qubit concentration for the library and the mean peak size from Bioanalyser profile, the library was loaded onto MiSeq at appropriate concentration (10-20pM) for cluster generation and sequencing. Paired-end sequencing allows the template fragments to be sequenced in both the forward and reverse directions on MiSeq. The kit reagents were used in binding of samples to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

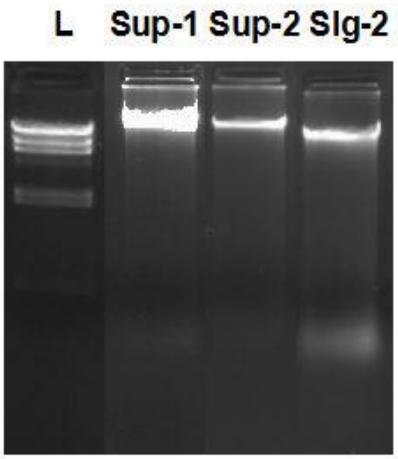
- **QC on Agarose Gel**

Primers designed to amplify the eubacterial 16S rRNA gene sequences including the variable V3 and V4 regions yielded complex single-strand-conformation polymorphism (SSCP) patterns on polyacrylamide gels. The intensity of bands in the gel indicated good reproducibility of the DNA extraction method and PCR amplifications. With primers targeting the V3 region of actinomycete 16S rRNA genes, the SSCP patterns consisted of strong bands and also a cluster of other bacterial species of the V4 region (Fig. 6.3).

6.2.2 TAXONOMIC HITS DISTRIBUTION

The pie charts (fig. 6.4) illustrates the distribution of taxonomic domains, phyla, and orders for the annotations. Each slice indicates the percentage of reads with predicted proteins and ribosomal RNA genes annotated to the indicated

taxonomic level. This information is based on all the annotation source databases used by MG-RAST. Domain level taxonomic hits distribution shows that sample DGS has 87.6% Bacteria, 11.1% Eukaryota and 1.3% Archea as represented in the figure 8. The higher bacterial populations are reported to actively involved in the hydrolysis and acidification processes of anaerobic fermentation for food waste (Jing yi et al., 2014).



Sup = Supernatant, Slg = Sludge pellet

Fig. 6.3 Q C of gDNA on 1% Agarose gel

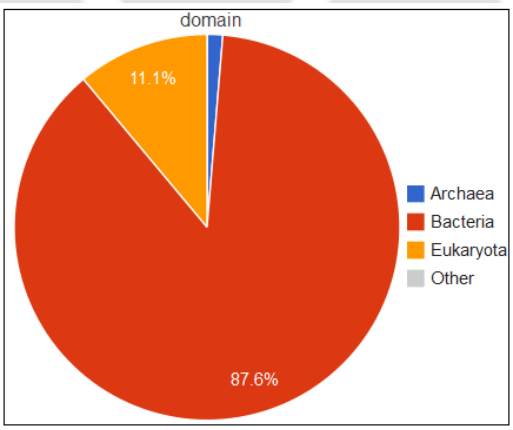


Fig. 6.4 Taxonomic hits distribution at domain level of sample DGS

- **Taxonomic Hits Distribution at Phylum Level**

Taxonomic hit distribution at phylum level shows that sample DGS has 28.5% Firmicutes, 25.6% Bacteroidetes and 7.1% Arthropoda etc. as represented in the figure 6.5. The higher abundance of bacteria can be considered due to the presence of readily available organic matter and lignocellulosic fraction because of vegetables. Actinobacteria were reported to the major populations at phylum level in the

mesophilic anaerobic digester treating food waste (Guo et al., 2014). The dominance of Bacteroidetes, Firmicutes and Chloroflexi was also found in other previous studies (Chouari et al., 2005; Ariesyady et al., 2007). Although most bacteria in reactors were affiliated to these dominant phyla, the relative abundances of these phyla in each reactor were different and each digester had its own characteristic bacterial community composition. The proportion of phylum Chloroflexi in the reactor was in abundant proportion. This was in good accordance with previous reports that Choroflexi populations were abundant in anaerobic digesters, as determined by membrane hybridization (Chouari et al., 2005), FISH (Ariesyady et al., 2007) and 16S rRNA gene clone analysis (Ariesyady et al., 2007; Yamada and Sekiguchi, 2009) The proliferation of Choroflexi (formerly known as Green Nonsulfur Bacteria), is a well-known scavenger biomass-derived organic carbon such as soluble microbial products (SMP), suports a greater influence of difficult-to-biodegrade organic materials from the input substrates and from endogenous decay of the anaerobic biomass (Kindaichi et al., 2004; Rivie`re et al., 2009). The phylum Bacteroidetes are proteolytic bacteria and were probably involved in the degradation of various proteins used for anaerobic digestion studies (Kindaichi et al., 2004; Riviere et al., 2009). The majority of proteolytic microorganisms are able to metabolize amino acids to produce VFA such as acetate, propionate and succinate and NH_3 (Riviere et al., 2009).

Interestingly, their selective enrichment at high TS contents seems to be in consistent with the observation of high protein-input rate and VFA production in the reactors with higher TS contents. The source of proteins was due to sprouts and pulses (daal). And the concentration was 30 to 60 g/kg. This result indicated the importance of the Bacteroidetes performing protein hydrolysis. The changing trend of relative abundance of the phylum Firmicutes was obvious with increasing TS contents. The higher value of Firmicutes proportion was 28.5% in the reactor. Firmicutes are well-known to be acetogenic and syntrophic bacteria that can degrade VFA, such as butyrate and its analogs. The prevalence of organisms belonging to Firmicutes suggested that these products are readily available due to the prior fermentation of these simple VFA and played a critical role in anaerobic digestion of FW, especially on the production of acetic acid, an essential step for methane production by acetoclastic methanogenic microorganisms. In addition, the relative abundances of other phyla including Proteobacteria, Spirochaetes and Tenericutes obviously increased with the feeding TS contents increasing. It has been suggested that they might play important roles in the

degradation of FW. Proteobacteria are also involved in the first step of the degradation of organic wastes and they are important consumers of propionate, butyrate, and acetate (Ariesyady et al., 2007). Spirochaetes are reported to ferment carbohydrates or amino acids into, mainly, acetate, H_2 and CO_2 (Jing et al., 2014) and Tenericutes was found to be related with lignin utilization (Boucias et al., 2013).

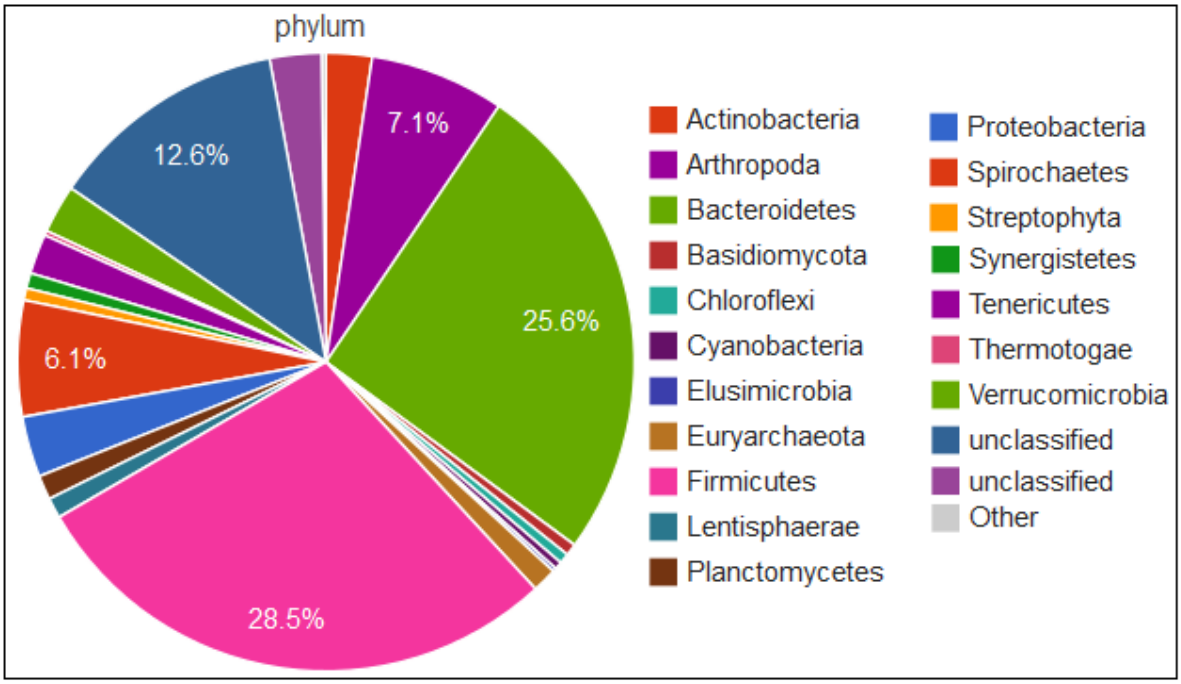


Fig. 6.5 Taxonomic hits distribution at phylum level of sample DGS that contains maximum hits from Firmicutes followed by Bacteroidetes and Arthropoda

- **Taxonomic Hits Distribution at Class Level**

Taxonomic hit distribution at class level shows that sample DGS has 20.7**%** Clostridia**,** 16.2% Bacteroidia**,** 6.1% Spirochaetia, etc. as represented in the figure 6.6. The proportion of the reigning genera Spirochaetes within the abundant phylum Spirochaetes increased obviously with TS contents increasing. From the analyses made above, it can be seen that the changing patterns of main microbial population abundances were closely related to the performance variations with TS contents increasing, especially for VS reduction. The increasing degradation of organic matter to precursors for methanogenesis was jointly accomplished by the compatible collaborations of these microorganisms which played their respective roles in one of several trophic levels including hydrolysis, fermentation and acetogenesis.

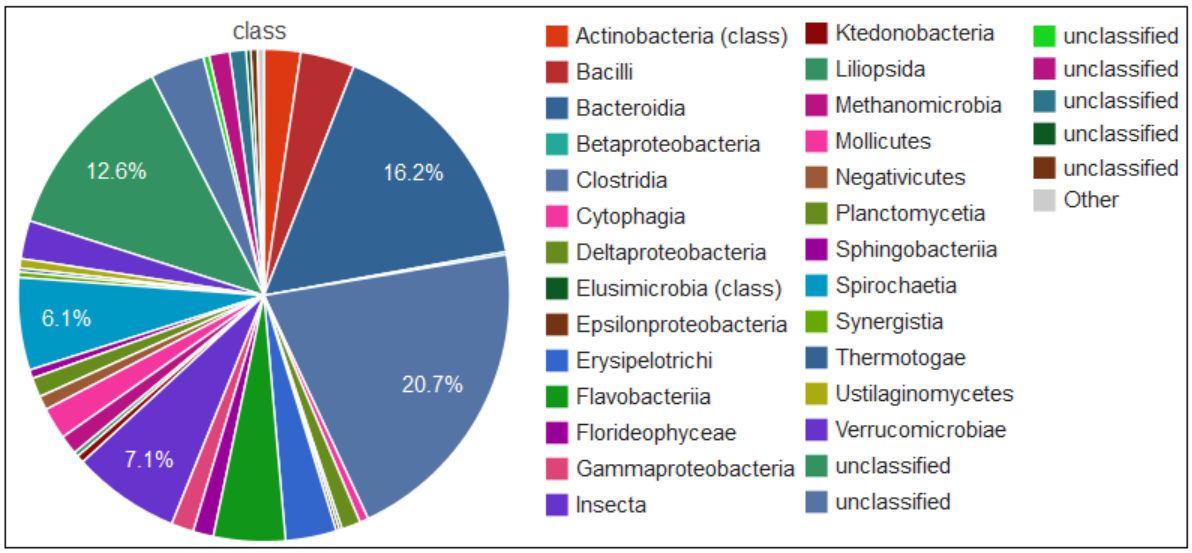


Fig. 6.6 Taxonomic hits distribution at class level of sample DGS that contains maximum hits from Clostridia followed by Spirochaetia and Bacteroidia.

- **Taxonomic Hits Distribution at Family Level**

Taxonomic hit distribution at family level shows that sample DGS has 10.7% Porphyromonadaceae, 7.1% Rhinotermitidae and 6% Spirochaetaceae etc. as represented in the figure 6.7. The genus Porphyromonas, which was formed by reclassification of various species of Bacteroides (Shah and Collins, 1988), is the type for this family. Originally, this family comprised the genera Porphyromonas, Dysgonomonas, and Tannerella (Garrity et al., 2005). The genus Porphyromonas comprises five subclusters: (1) the type species Porphyromonas asaccharolytica and Porphyromonas circumdentaria , endodontalis, gingivicanis, and uenonis; (2) Porphy-romonas cangingivalis, canoris, levii, and somerae; (3) Porphyromo-nas crevioricanis, gingivalis, and gulae; (4) Porphyromonas catoniae and macacae; and (5) Porphyromonas cansulci. Members of the family Porphyromonadaceae are obligately anaerobic, heterotrophic, non-spore-forming, non- motile, Gram-staining-negative rods that ferment sugars. Species from the family Porphyromonadaceae are known to be involved in the degradation of proteins and amino acids, eschewing saccharides.

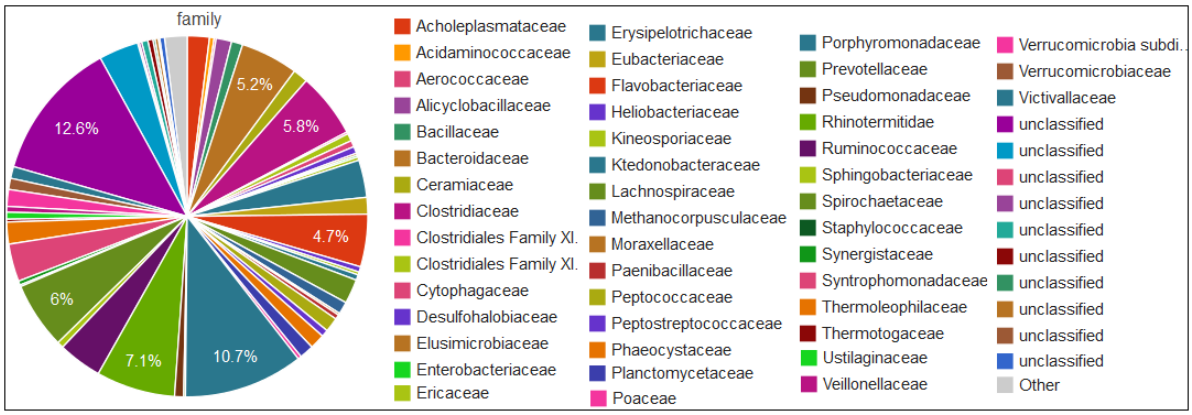


Fig. 6.7 Taxonomic hits distribution at family level of sample DGS that contains maximum hits fromPorphyromonadaceae followed by Rhinotermitidae and Spirochaetaceae.

- **Taxonomic Hits Distribution at Genus Level**

Taxonomic hit distribution at genus level shows that sample DGS has 7.1% Coptotermes, 5.4% Porphyromonas and 5.1% Bacteroides etc. as represented in the fig. 6.8. The lower termite, Coptotermes species is one of the most prominent plantation pests that feed upon, digest, and receive nourishment from exclusive lignocellulose diets. The microbial dynamics showed that Clostridium, Bacteriodes, Porphyromonas, Prevotella, Lactobacillus population were found very abundant during the study. These are hydrolytic bacteria involved in the depolymerization of carbohydrates, proteins and lipids into monomers by their extracellular enzymes. So their population was seen high at hydrolytic stage. During hydrolysis, complex insoluble substrate such as polysaccharides are hydolysed into smaller units by a large number of hydrolytic microorganisms such as Clostridia, Micrococci, Bacteroides, Butyrivibrio, Fusobacterium, Selenomonas, and Streptococcus by secreting different hydrolyzing enzymes like cellulase, cellobiase, xylanase, amylase, protease and lipase.

- **LCA computed phylum level bacterial diversity**

The higher abundance of verrucomicrobia is actively involved in the macromolecule degradation (Fig. 6.9). Tenericutes was found to be related with lignin utilization (Boucias et al., 2013). The prevalence of organisms belonging to Firmicutes suggested that these products are readily available due to the prior fermentation of these simple VFA and played a critical role in anaerobic digestion of FW, especially on the production of acetic acid, an essential step for methane production by acetoclastic methanogenic microorganisms. In addition, the relative abundances of other phyla

including Proteobacteria, Spirochaetes and Tenericutes obviously increased with the feeding TS contents increasing. It has been suggested that they might play important roles in the degradation of FW. Proteobacteria are also involved in the first step of the degradation of organic wastes and they are important consumers of propionate, butyrate, and acetate (Ariesyady et al., 2007).

Fig. 6.8 Taxonomic hits distribution at order level of sample DGS that contains maximum hits from Coptotermes followed by Porphyromonas and Bacteroides.

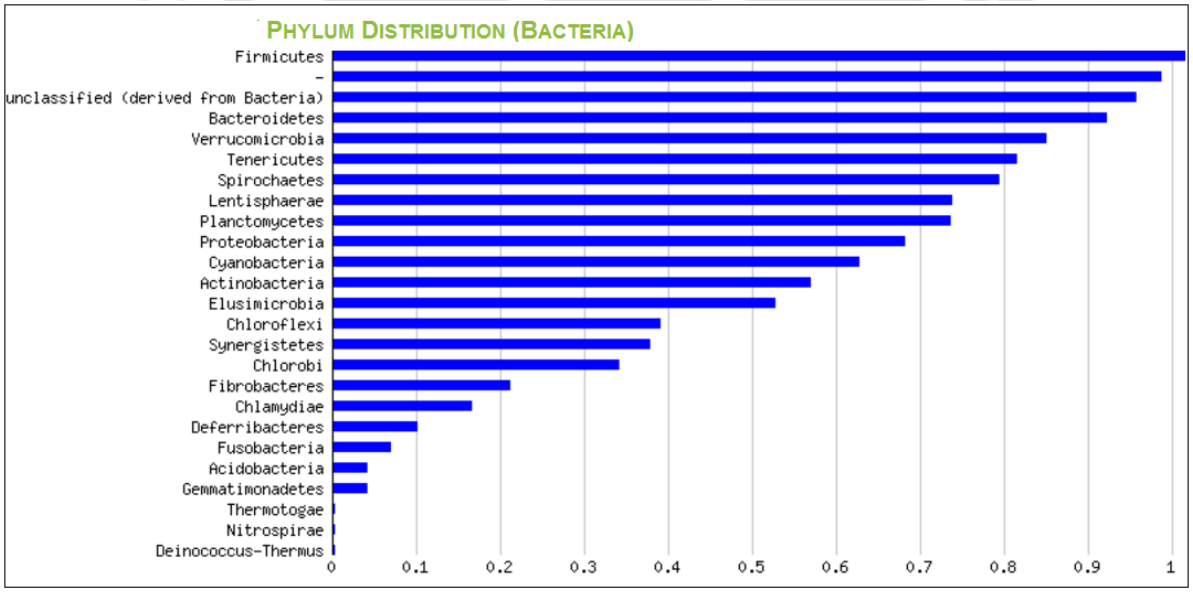


Fig. 6.9 LCA computed phylum level bacterial diversity distribution of sample DGS shows that the phylum Firmicutes is the most abundant followed by Bacteroidetes.

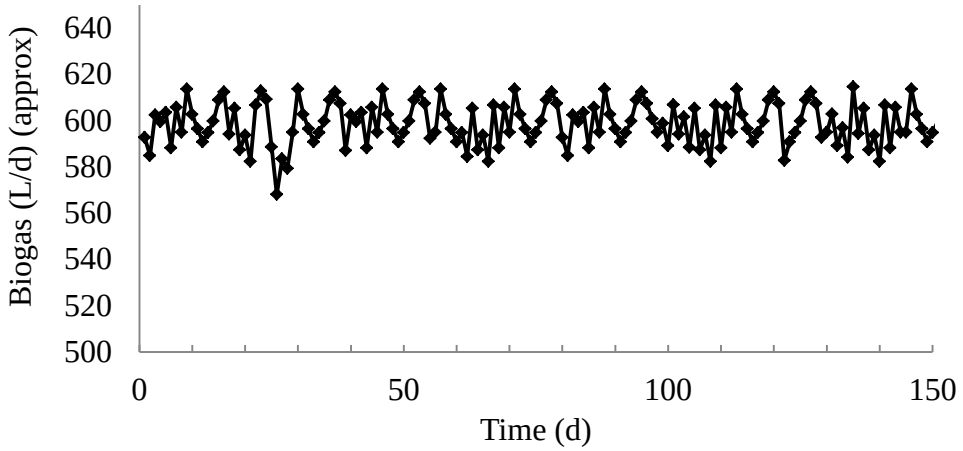


Fig. 6.10 Biogas production profile of pilot scale ABBR

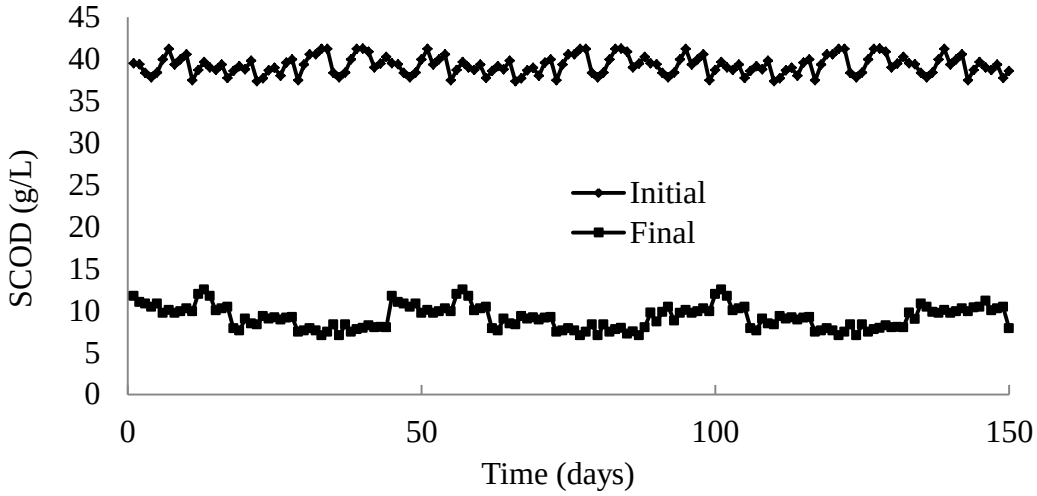


Fig. 6.11 SCOD profile for pilot scale ABBR

Fig. 6.12 Biogas filled from Pilot scale ABBR

6.2.3 Summary

Amplicon sequencing analysis has been carried out for DGS sample on Mi-Seq, with following details:

- In total 348 mb has been generated for DGS samples respectively.
- MG-RAST analysis shows that DGS amplicon shows α-Diversity = 99.239 species.
- Taxonomic hit distribution at phylum level shows that sample DGS has 28.5 % Firmicutes, 25.6 % Bacteroidetes and 7.1% Arthropoda
- Taxonomic hit distribution at genus level shows that that DGS sample has 7.1% Coptotermes, 5.4% Porphyromonas and 5.1% Bacteroides etc.

6.3 PILOT SCALE ABBR STUDIES

The pilot scale ABBR studies followed the same trend as lab scale ABBR with cow dung as inoculum. The pilot scale reactor was acclimatized for initial 60 days with gradual increase of Soluble COD concentration 10 g/L to 40 g/L. At HRT 10 days the average biogas production was higher (0.58 m^3/d). The biogas production profile of ABBR at different HRT is shown in Fig. 6.10. ABBR performed well at the environmental temperature varies from 22 to 34^oC. The COD reduction was less at the startup period, later it was increased to an average of 68-72 % at HRT 10 day during first 30 days of reactor (Fig. 6.11).

The gas samples collected in 2 m^3gas bags to found the production rate. The gas bags were filled in every two days. The gas bags were shown in Fig. 6.12. Few gas samples were collected at different days and analyzed using gas chromatography, showed the methane percentage of 55-62 % and carbon dioxide of 36-44 %.

6.4 CONCLUSIONS

Highest methane production of 16 L/d was achieved in lowest HRT of 10 days was achieved in Lab scale ABBR with CD as inoculum. ABBR provides the favorable conditions for both the acidogens and methanogens to achieve their best. Phase II in ABBR provided good conditions for methanogens as like Upflow anaerobic sludge blanket process. Phase I increase the travelling length and improve the attached growth process to improve hydrolysis and acidogenesis. Pilot scale study confirmed the trend on VS reduction percentage and biogas production. In pilot scale reactor maintained in open environmental conditions with varying temperature from 18 to 30^oC achieved the maximum gas production yield was 0.58 m^3/d at optimum HRT of 10 days.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

This chapter dealt with conclusion achieved from the all phases conducted with different inoculums in BMP and batch studies and optimization of anaerobic bi-phased baffled reactor for anaerobic digestion of food waste in both lab and pilot scale studies.

7.1 CONCLUSIONS

In Anaerobic BMP of FW with DS as inoculum, the highest of 59 % VS reduction was observed in F/M ratio 2 maintained reactors with higher methane yield. In the F/M ratio 1.5 reactors, the VS reduction was less due to deficiency of microbial community population compare to F/M ratio 2. In F/M 2.5 reactor the VS reduction is lesser than other reactors and control it may be due to high acid accumulation. The results concluded that only the requisite amount of food is mandatory for microorganisms to get better anaerobic digestion of FW neither more nor less to achieve higher methane yield.

In AD of FW, highest of 68% VS reduction was observed in 500 mg/L nitrogen added reactor with higher methane yield. In the 250 mg/L nitrogen added reactor, the VS reduction was less due to nitrogen deficiency. In 1000 mg/L nitrogen addition reactor the VS reduction is lesser than the entire nitrogen added reactor and control it may be due to ammonium toxicity. The kinetics studies revealed the perfect curve fit with high R^2 value in all the reactors. The results concluded that only the requisite amount of nitrogen is mandatory for better AD of FW neither more nor less to achieve higher methane yield, although F/M ratio was optimized.

In BMP studies with different livestock dung as inoculum, the results of study demonstrated that there were significant differences between different inoculums. The reactors inoculated with CD had shorter initial time and achieved higher biogas production than reactors inoculated with other inoculums. Livestock inoculum such as GD and RD failed to create a favourable condition for FW digestion. Methane production was unsteady and retained in a low level in reactors inoculated with GD and RD in 30 days period of AD. CD followed by PGD with the highest activity and most suitable nutrient content, achieved the highest methane production and showed the best degradation among all livestock inoculums.

In batch studies FW with DS as inoculum, percentage of VS reduction followed the same trend as the best F/M ratio 2 in BMP of food waste. Maximum of 996 mL/d methane production rate was achieved in this study. The VFA productio was high at initial days later it was stabilized by the methane producers. In batch studies with different livestock dung as inoculum, the results of study validated that there were substantial differences between different inoculums. The reactors inoculated with CD and PGD have achieved higher biogas production than reactors inoculated with PD, RD and GD. PGD and PD inoculated reactor had shorter lag time than others. Inoculum such as RD failed to create a suitable environmental for FW digestion. Biogas productions was unstable at initial and performed well at final days in reactors inoculated with RD and GD. CD, with the highest volatile solids reduction and most suitable nutrients content, achieved the highest biogas production and showed the best adaptability among all inoculums.

Highest methane production of 16 L/d was achieved in lowest HRT of 10 days was achieved in Lab scale ABBR with CD as inoculum. ABBR provides the favorable conditions for both the acidogens and methanogens to achieve their best. Phase II in ABBR provided good conditions for methanogens as like Upflow anaerobic sludge blanket process. Phase I increase the travelling length and improve the attached growth process to improve hydrolysis and acidogenesis. Pilot scale study confirmed the trend on VS reduction percentage and biogas production. In pilot scale reactor maintained in open environmental conditions with varying temperature from 18 to 30^{o}C achieved the maximum gas production yield was 0.58 m^{3}/d at optimum HRT of 10 days.

Amplicon sequencing analysis has been carried out for CD sample on MiSeq and total 348 mb has been generated. MG-RAST analysis shows that DGS amplicon shows α-Diversity = 99.239 species. Taxonomic hit distribution at phylum level shows that sample DGS has 28.5% Firmicutes, 25.6% Bacteroidetes and 7.1% Arthropoda. Taxonomic hit distribution at genus level shows that that DGS sample has 7.1% Coptotermes, 5.4% Porphyromonas and 5.1% Bacteroides etc.

Therefore, the study concludes that the anaerobic digestion of food waste produces the energy valuable gas. According to the activity of the inoculum the amount of gas production and percentage of VS reduction varies. Adaptability of inoculum to the substrate enhances the degradation. The new reactor ABBR design for the anaerobic degradation of organic waste enhanced the degradation in continuous phase.

7.2 RECOMMENDATIONS FOR FUTURE WORK

- Enhancing the activity of microbial community in the livestock dung to improve the anaerobic digestion of food waste for higher methane production.
- Isolation of methanogens from livestock dung and utilization in the industrial level methane production.
- Optimization of ABBR for other organic waste treatment with highly available organic matter.
- Improvisation of ABBR for the treatment of lignocellulose waste.
- More studies on pilot scale ABBR with food waste as substrate and optimization to improve more methane percentage.

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