Anaerobic Digestion of Lignocellulosic Wastes

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A Thesis Submitted to

Indian Institute of Technology Hyderabad

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The Degree of Master of Technology

Department of Civil Engineering

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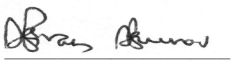

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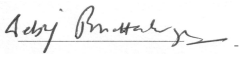

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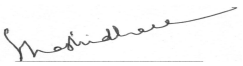

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Dedication

to my parents....

Abstract

One of the major problems faced by modern society is the shortage of energy supply. On the other hand, a large amount of energy-rich organic material is discharged into the environment as waste. Anaerobic digesters are considered as sustainable sources for alternate energy. Yard waste and other green waste constitute a major portion of municipal solid waste (MSW). Lignocellulosic biomass feed stocks, in particular yard waste, are highly desired for anaerobic digestion as they are widely available. High concentration of lignin presents a major challenge for utilizing yard wastes as a feedstock for anaerobic digestion as lignin is resistent towards anaerobic degradation . Therefore, additional steps are necessary to increase the biodegradability of yard waste.

This research assessed the potential of application of anaerobic digester in managing lignocellulosic wastes. The main focus was to increase the methane production of yard waste by co-digesting with widely available food waste and to study on degradation of lignocellulosic components. The food waste and yard waste obtained from IIT Hyderabad mess and lawn respectively, were co-digested in the ratios of 1:0, 1:0.5, 1:1, 1:1.5, 1:2, 1:3, 1:4 and 0:1. The reactor responses at four solid retention times (SRTs) - 30day, 20 days, 12 days, and 6 days, were studied. The research was performed in semi-batch mode using a respirometer. The reactors were initially inoculated with sludge obtained from a running anaerobic digester.

A biochemical methane potential test was done in order to find the methane potential of substrates. The methane potential obtained for food waste was 472.19 L/kg VS and that for yard waste was 265.71 L/kg VS. The destruction of volatile solids, lignin, cellulose, hemicellulose and volatile fatty acids were studied. Methane production showed a decreasing trend with increase in yard waste content.However, the performance of the reactor, in terms of biogas production and volatile solids destruction, was observed to be the best at a food to yard waste ratio of 1:0.5. Also a SRT of 20 days showed a positive increment to gas production and fiber degradation compare to other SRTs.Volatile solids degradation decreased with increase in VS loading. Similarly, the degradation of lignin, cellulose and hemicellulose also decreased with increase in VS loading. A maximum degradation of 21% in lignin, 32% in cellulose and 27% in hemicellulose was observed.

The volatile fatty acids were high in food waste, as the food waste facilitates faster fermentation. Acetic acid concentration showed an inverse relationship with methane production. Concentrations of propionic and butyric acids, which indicate the health of reactors, increased with increase in VS loading . Consequently, pH decreased. Carbon mass balance was performed at each SRT. Unaccounted was obtained to be within 10%.

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Chapter 1

Introduction

1.1 Introduction

One of the major problem that faced by the modern society is the shortage of energy supply. But on the other side, large amount of energy rich organic materials are disposed to the environment as wastes and we worry about the environmental consequences of such disposals. The present condition of our society is like short in energy but rich in waste.

A popular method of managing solid wastes is dumping into landfill sites. However, given the fact that land is precious and disposal costs would go up in the future due to stricter environmental regulations, ways to minimize land filling or alterations to landfill disposal are in a sought. Common alterations are aerobic composting, incineration, and anaerobic digestion. Aerobic composting preserves the nutrient content of the waste, but the process is slow and may require energy. Incineration causes air pollution. Anaerobic digestion process is sustainable, recover energy with very less emission (eg. CO_2 in biogas), retain fertilizer,kills pathogens and facilitates recovery of heavy metals.

This research assess the potential of application of anaerobic digester in managing lignocellulosic wastes. Lignocellulosic wastes is co-digested with food wastes which can be easily degraded. The campus of IIT Hyderabad has been used in this study as test bed. Lignocellulosic wastes in the form of yard wastes was collected from lawns and gardens, whereas food waste was obtained from institute mess. Currently, the yard trimmings are burned and wood wastes are dumped in outer areas. Anaerobic digestion is historically one of the oldest processing technologies used by mankind. It is a naturally occurring process which involves the decomposition of organic matter by microorganisms in an oxygen free environment. The most desirable product of anaerobic digestion is biogas, which consists of methane and carbon dioxide. Anaerobic digestion occurs in four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The rate limiting step in anaerobic digestion of lignocellulosic biomass is hydrolysis. Hydrolysis is slowed by the presence of lignin, which inhibits the hydrolytic enzymes from penetrating into the cellulose and hemicellulose. Out of many factors that affect the digestion, lignin content is regarded as the most problematic factors that affect the digestion of lignocellulosic wastes.

1.2 Research Objectives and scope of work

The primary objective of this study is to improve the methane production of yard wastes using food wastes as a co-substrate in anaerobic digestion. The specific objectives are:

- To study the methane production of yard waste with food wastes as a co-substrate in various proportions in a mesophilic atmosphere. Here food and yard wastes are mixed in the ratio 1:0; 1:0.5; 1:1; 1:1.5; 1:2; 1:3; 1:4; and 0:1.
- To study the biochemical methane potential of these wastes
- To study the reactor response to various solid retention time (SRT). Here 4 SRT are studied: 30 days,20 days,12 days and 6 days
- Responses were studied in terms of volatile solids destruction, methane production, volatile fatty acids synthesis, pH and degradation of lignin, cellulose,hemicellulose.

Chapter 2

Literature Survey

2.1 Introduction

Each year, millions of tons of lignocellulosic wastes, consisting mainly of agricultural and industrial residues and municipal solid wastes, are produced worldwide. Their conversion to useful feedstuff or fuels using chemical or biological methods has been proposed and explored [4, 3, 5, 6, 7]. Lignocellulose, the major component of biomass [4], makes up about half of the matter produced by photosynthesis. In nature, lignocellulose accounts for the major part of biomass and, consequently, its degradation is essential for the operation of the global carbon cycle Fig. 2.1 [1]. It consists of three types of polymers cellulose, hemi-cellulose, and lignin [4].

Lignin is a natural composite material in all vascular plants, providing the plant with strength and rigidity. Plant's resistance to biodegradation as well as to environmental stresses are increased by lignin by decreasing water permeation across the cell wall [8] . Lignin is an amorphous, aromatic, water-insoluble, heterogeneous, three-dimensional, and cross-linked polymer with low viscosity [4, 8, 5]. It consists of 20-30% of dry weight of vascular plant tissues [5]. Lignin is distributed throughout the cell wall, although, again, not uniformly [4]; the highest lignin content is in middle lamella, [8] however, the greatest amount of lignin is in the secondary wall.

Lignin is recalcitrant to anaerobic biodegradation [4, 5]. Also lignin can interfere with the biodegradability of associated cellulose and hemi-cellulose by limiting microbial access [8]. As a result, it seems that most natural materials with higher lignin contents would be less biodegradable. But lignocellulosic biomass feed stocks are highly desired for anaerobic digestion because of their abundance. Yard waste is extremely desirable because it makes up the third most abundant source of municipal solid waste. But lignin is recalcitrant to the anaerobic digestion (0-25% degradation), and also it act as a rate limiting component of methane production, So more study is required to increase the efficiency. But still literature says about the anaerobic degradation of lignocellulosic wastes by various methods like various pretreatments [6, 9], co-digesting with food wastes [3, 2, 10], co-digesting with ruminal fungus [11, 12, 13, 14] and incubating radio labelled lignocellulose from grasses and hardwoods. In aerobic environments, lignin biodegradation is accomplished by white-rot and brown-rot fungi.

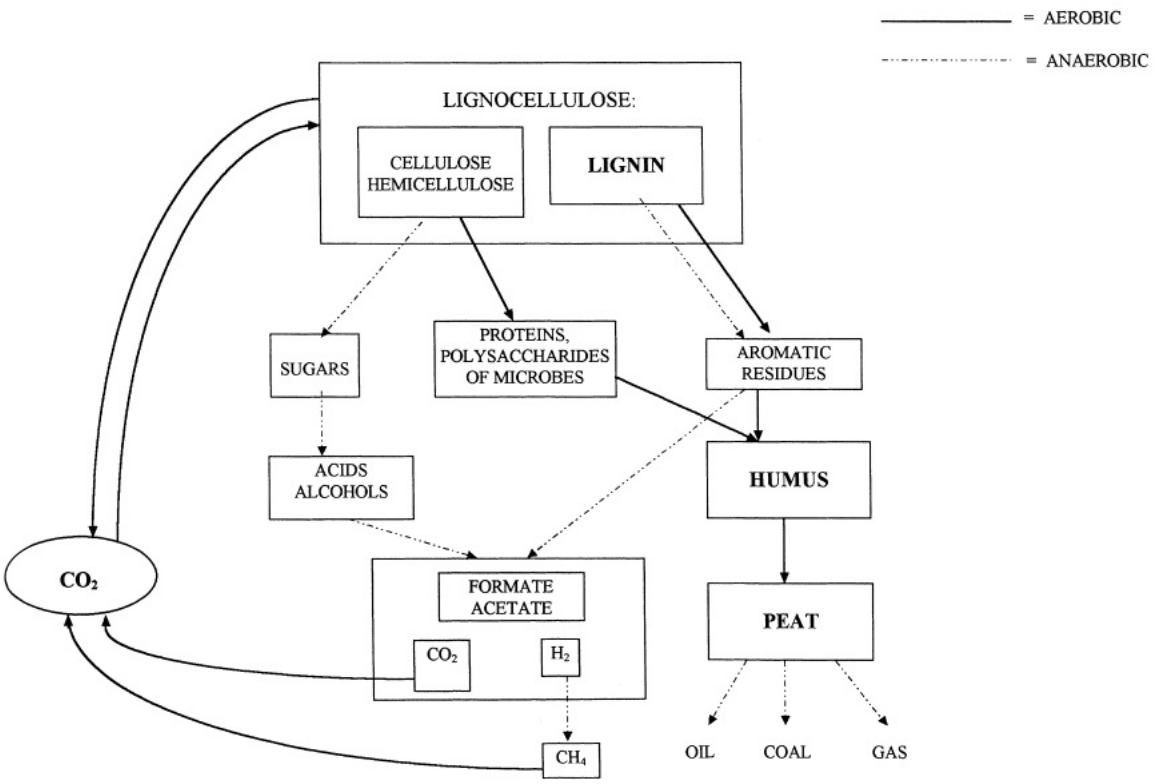

Figure 2.1: Global carbon cycle [1]

2.2 Anaerobic Digestion

Anaerobic digestion (AD) is a process by which organic material is decomposed by microorganisms under oxygen-free conditions [15]. Decomposing organic material is transformed into biogas, soluble nutrients, and other cell matter. AD is a viable method for waste treatment that can provide energy recovery from waste biomass as well as beneficial uses of all waste streams.

The most desirable product of anaerobic digestion is biogas which can be used for heat and electricity generation. Biogas generally contains 58-70% methane and 30-40% carbon dioxide [15]. Effluent, the liquid outflow containing digested solids, is another beneficial by-product of anaerobic digestion. The effluent can be used as soil amendments, fertilizers and sometimes composted and used as a bedding material. Biogas from AD can be used without treatment for heating, cooking, an running generators or internal combustion engines. After purification, biogas can be added to natural gas pipelines, used as vehicle fuel, or used in any application appropriate for natural gas. During full scale operation of a high-solids reactor treating source sorted organic wastes the methane yield from AD was 0.4 m^3CH_4/kg volatile solids (VS) fed [16]

2.2.1 Stages of Anaerobic Digestion

The anaerobic digestion process is a series of steps performed by numerous microorganisms working together. It involves four important stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The process begins with bacterial hydrolysis of the feedstock. This process breaks down insoluble organic polymers such as carbohydrates, fats and proteins into simple sugars, fatty acids, and amino acids respectively. These constituent parts or monomers are then readily available for use by other bacteria. Hydrolysis is typically the most rate-limiting stage, but may vary depending on the substrate. The composition of a substance affects the rate at which hydrolysis occurs. Hydrolysis constants differ for carbohydrates, proteins and lipids.

The second stage of the AD process is acidogenesis. Acidogenesis is the process by which acidogenic bacteria convert the sugars, fatty acids and amino acids produced by hydrolytic bacteria into carbon dioxide, hydrogen, ammonia, and organic acids. Typical reactions occurring at this stage are the following: - Conversion of the glucose to ethanol - Conversion of the glucose to propionate

Following acidogenesis is the third stage of AD, acetogenesis. In this stage acetogenic bacteria convert the organic acids into acetic acid, hydrogen, carbon dioxide and some additional ammonia. The presence of hydrogen is of critical importance in acetogenesis of compounds such as propionic and butyric acid. These reactions can only proceed if the concentration of H_2 is very low. Important reactions during the acetogenesis stage are as follow : - Conversion of glucose to acetate - Conversion of ethanol to acetate - Conversion of propionate to acetate - Conversion of bicarbonate to acetate

The final stage of AD, methanogenesis, is facilitated by methanogens, classified as a domain of microorganisms, which use the acetic acid, hydrogen and carbon dioxide to produce methane. Two thirds of the total methane produced is derived converting the acetic acid or by fermentation of alcohol formed in the second stage, such as methanol. The other one third of the produced methane is a result of the reduction of the carbon dioxide by hydrogen. There are two primary pathways for the production of methane: the first is the degradation of acetic acid to form methane and carbon dioxide, and the second is the reduction of carbon dioxide with hydrogen, which produces methane and water. Overall process of anaerobic decomposition is shown in Fig. 2.2.

2.2.2 Requirements for successful operation

Temperature: Anaerobic digestion has been successful under a wide array of temperature conditions. Although it progresses at temperatures between 4 and (100^0C), digestion proceeds most reliably at mesophilic(35^0C) and thermophilic (55^0C) temperatures [8]. Anaerobic degradation of wastewater at psychrophilic temperatures (around 15^0C) has also been demonstrated .

Research in AD of food waste and municipal solid waste (MSW) under a range of different moisture levels and reactor configurations has indicated that the best VS destructions and methane production results are achieved at thermophilic temperatures. [4, 17, 18]. But results of one study indicated that thermophilic digestion was slightly less stable at short mass retention times than mesophilic digestion. Also It needs additional energy, which is not feasible economically. Figure. 2.3 shows the effect of temperature in anaerobic digestion.

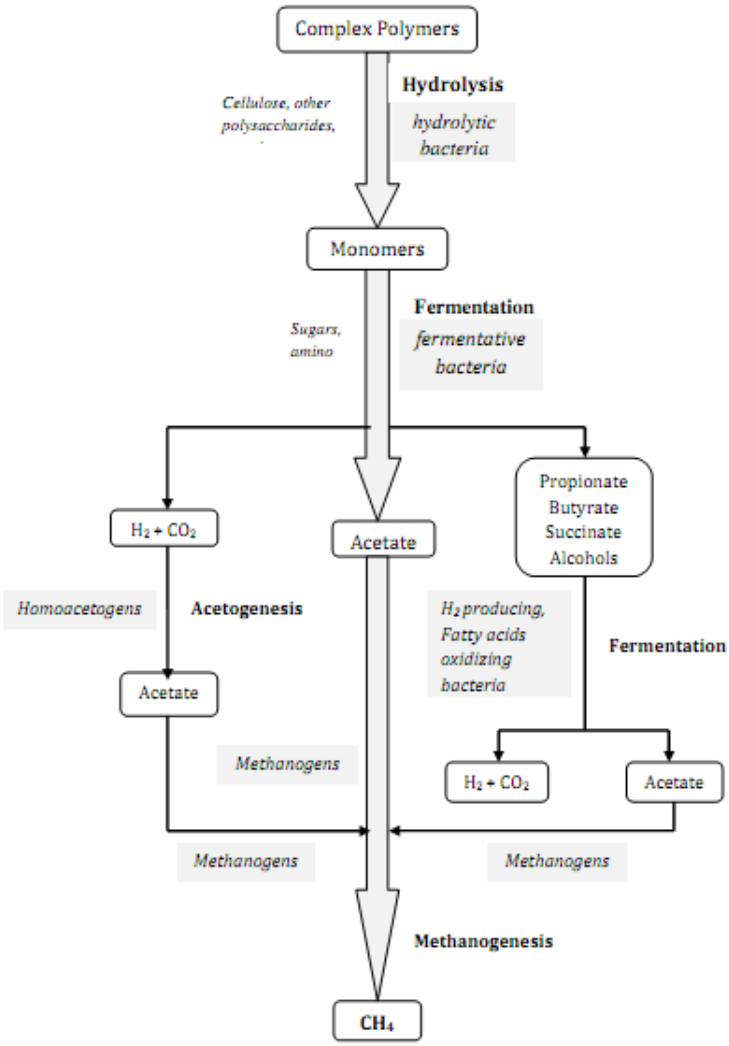

Figure 2.2: Overall Process of Anaerobic Decomposition

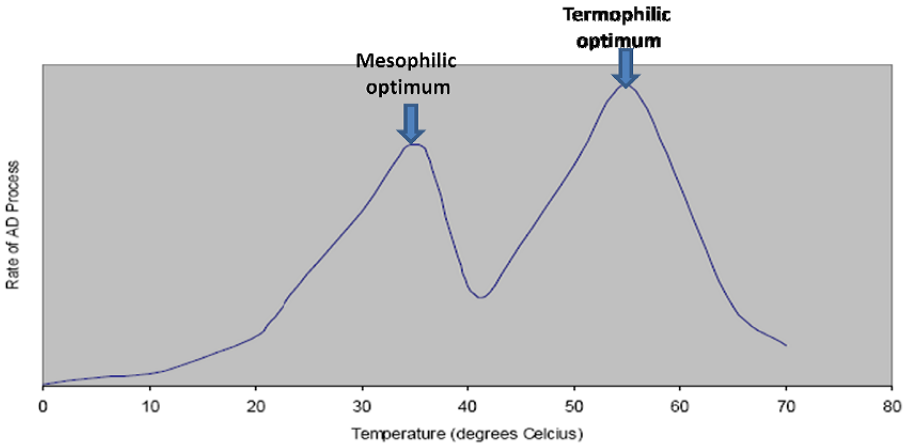

Figure 2.3: Rate of AD process vs temperature

Thermophilic digesters allow higher loading rate and yield higher methane production, substrate degradation and pathogen destruction [19]. Also, the higher temperature shortens the required retention time because it speeds up the reactions of degradation of the organic material. However, thermophilic bacteria are sensitive to toxins and small environmental changes. These systems are harder to maintain and are less attractive for commercial application because they require additional energy input for self-heating.

Mesophilic AD reactors operate with microbial environment that tolerate greater changes in the environment and are more stable and easier to maintain. Another advantage is that usually these systems do not need any additional energy input for heating the system. On the other hand, disadvantages of the mesophilic AD systems are longer retention time and lower biogas production [15]. However due to the fact that they are easier to operate and maintain, as well as the lower investment cost, they are more attractive for commercial scale plants.

pH: The optimal pH for methanogenic bacteria is between 6.3 and 7.8 , outside this range the process can slow or even stop [20]. Bicarbonate buffering capacity is extremely important in order to maintain stable pH in the desired range [20]. Alkalinity in excess of 2,500 to 5,000 mg/L as $CaCO_3$ is required to protect against a pH drop in the event of VFA accumulation . It may be necessary to add alkalinity to the digestion process to ensure adequate buffering if the substrate itself does not provide sufficient alkalinity.

The pH value of the reacting material is a process control factor in the AD of food waste. The importance of the pH is due to the fact that methanogenic bacteria are very sensitive to acidic conditions and their growth and methane production are inhibited in acidic environment. In batch reactors pH value is closer dependent of the retention time and loading rate.

Volatile Fatty Acids (VFA): The concentrations of the different volatile fatty acids serve as one of the best process indicators. They are either products of degradation steps from the hydrolysis and acidogenesis or serve as substrate for the acetogenesis and methanogenesis. As the different stages are linked and in equilibrium, the concentration of the volatile fatty acids is naturally low. With a change of the environmental conditions (pH drop, inhibition of the degradation, substrate overload, temperature instabilities), the bacteria can be inhibited and the concentrations can increase. In the literature, different rules and inhibition limits can be found for the concentrations. As a rule of thumb, especially the increase of propionic acid and other short chain fatty acids can be problematic, because they themselves inhibit the degradation even more.

Total Solids and Volatile solids: Historically, low-solids AD has been used for biomass reduction after the aerobic step in wastewater treatment, but in recent years an increasing number of projects have demonstrated successful digestion of solid waste at higher total solids (TS) concentrations . Although there is some variation in the solids cutoffs and terminology, High Solid Anaerobic Digester (HSAD) is the most commonly used term (others are dry fermentation and dry anaerobic digestion), and it is typically classified as digestion at solids content higher than 20% . An upper limit for solids content in AD is 40% . High solids digestion can include TS as low as 15% , or exclude any TS content lower than 25%ȦD at high solids eliminates the need for process water addition, effluent de-watering, and effluent water treatment . Under high solids operating conditions reactor heating requirements and size are likely to be lower, reducing both capital and operating costs . Phase separation in low solids digestion can result in equipment fouling. When floating or deposited materials are removed to protect equipment, VS are lost that could have been

digested . However, the low moisture content in HSAD may result in incomplete mixing, resulting in less contact between microbes and substrates, and decreased biogas and methane production rates . In general, material handling may be more challenging in HSAD, because of high reactor solids contents . Additionally, although HSAD potentially allows for higher loading rates than low-solids digestion (possibly up to 10 kgVS/m^3-day), effluent may require additional treatment for complete stabilization.

Organic loading rate (OLR): The organic loading rate refers to the daily feeding amount of fermentable biomass (VS), based on the digester volume. In a mesophilic operation, values between 3.5 and 5 kg VS $m^{-3}\, d^{-1}$ have proven to be successful. Most of the biogas plants still run under load to minimize the possibility of process errors and therefore exhibit a lot of unused potential. It could be shown, that doubling the OLR from 2.11 to 4.25 kg VS $m^{-3}\, d^{-1}$ can also double the plant capacity of 500 kW to 1000 kW without building any additional fermenters.

Solid Retention Time (SRT): Closely linked to the volumetric loading rate is the solid retention time (SRT). It species the statistically average residence time of the substrate in the digester. In conjunction with the digester temperature, the residence time is the decisive factor or the degree of conversion of biomass into biogas. With short residence times, only the easily degradable substances are methanized. At longer residence times of 20 or more days, also difficult degradable substrates can be converted to biogas. Solid retention times of 30 to 40 days have been proven to be satisfactory .

2.3 Methane Fermentation of Lignocellulosic Materials

The anaerobic biological conversion of lignocellulosic materials into methane is a complex process, not only because of the nature of the complex polymeric structure of the materials themselves, but also because of the many bacterial species involved . Since insoluble lignocellulosic materials cannot be assimilated into bacterial cells directly, they are first enzymatically hydrolyzed or depolymerized extracellularly into soluble components, which are then taken into bacterial cells and fermented into various volatile fatty acids (VFA), carbon dioxide, hydrogen,and possibly alcohols by a group of hydrolyzing and fermenting bacteria. A separate group of hydrogen- or formic-acid-producing acetogenic bacteria converts the products of the first group into hydrogen or formic acid, carbon dioxide, and acetic acid, which are then finally converted into methane and carbon dioxide by another distinct group of methanogenic bacteria.The fermentation of lignocellulosic materials into methane is, therefore, a strictly sequenced process and requires the coordinated action of many bacterial species .

In such a strictly sequenced system, the overall methane fermentation process will be controlled by the slowest step. It has been shown through numerous studies with lignocellulosic materials that the first step, the enzymatic hydrolysis of long-chain polymers into monomers, is the limiting step . Hydrolysis of lignocellulose is often a slow (on the order of weeks or months) and incomplete process under anaerobic conditions. However, once soluble components are released from the polymer, they can quickly be fermented into VFA and other intermediates, and do not accumulate to an appreciable extent in well-operating anaerobic digesters. The products of fermentation and acetogenesis are also usually present at low concentration, and converted into methane and carbon dioxide at very high turnover rates . Various pretreatment processes for lignocellulosic materials that result in pre-

hydrolysis also result in increased overall methane production rate and extent . The hydrolysis step, therefore, has been shown in various ways to control the rate and extent of methane fermentation of lignocellulosic materials.

2.4 Hydrolysis of Lignocellulosic Materials

The study on lignocellulosic hydrolysis involves a wide variety of works on purified and chemically modified individual lignocellulosic components to various types of lignocellulosic materials, using enzymes extracted from fungi and bacterial species, pure microbial cultures, mixed cultures from the rumen of animals, anaerobic digesters, and natural ecosystems, and also pure chemical methods, such as acid hydrolysis [13, 14, 2, 3] . While these studies differ from each other in many respects, they are common in that the results are the same long-chain polymers are depolymerized into soluble components.The depolymerization of cellulose and hemicellulose is a hydrolysis process in which the original monomers are the products. The depolymerization of lignin is, however, not a hydrolysis process. Whether under aerobic or anaerobic conditions, or by chemical or biological means, degradation generally does not yield the original monomers.

2.5 Factors Affecting Lignocellulosic Material Degradation

The process of hydrolysis and methane fermentation of lignocellulosic materials is subject to the influence of numerous factors, such as the property of bacterial cultures, pH, temperature, water content, substrate particle sizes, availability of various nutrients, and the presence of toxic materials.

2.5.1 The Influence of Culture

There are many bacterial species that are capable of hydrolyzing holocellulose. Obviously, not all will be present in a given mixed culture. Anaerobic digesters usually start with bacterial cultures from other active digesters, rumen contents, manures, or natural anaerobic sediments. Feed materials are seldom kept sterile and can therefore continuously introduce different microorganisms into the digester. Bacteria with the best competitive advantages for a given lignocellulosic material under given operational conditions are generally expected to dominate in a digester.

After a digester has been fed with a specific substrate for a sufficient time, it is likely to develop a culture that is highly selected toward the substrate. Such enrichment process is a complex one and involves selection and adaptation of bacterial species toward a given condition, production of special enzymes necessary for the degradation of the substrate,and even possibly genetic mutations. This is especially interesting for lignocellulosic material degradation because of the complexity and diversity of the materials themselves and the enzymes and microbial species involved in the degradation process.

2.5.2 The Influence of pH

Most holocelluloses isolated from fungi and bacteria have an optimum pH on the acidic side, usually from 4 to 6 , although a few have been reported to have optimum pH above 9 . Fungi generally grow better at acidic pH . For holocellulolytic bacteria, active growth can be found at pH anywhere

from as low as 3 to as high as above 11, depending on the species . Each species generally exhibits a maximum activity at a certain pH. However, anaerobic digesters are almost exclusively operated at neutral pH. This pH restriction is not generated for the hydrolytic and fermentative bacteria, but for the maximum overall activity of methanogenic bacteria. Therefore, holocellulolytic bacteria which have advantage at neutral pH are likely to be enriched for in anaerobic digesters.

2.5.3 The Influence of Temperature

Temperature can have a significant influence on the fermentation of lignocellulosic materials. Holocelluloses have optimum temperatures at which they exhibit maximum activity. The optimum temperature for most holocelluloses is between 40 to 65^0C although the holocelluloses extracted from some extreme thermophilic anaerobic bacteria exhibit maximum activity above 80^0C . As with pH, the optimum temperature for holocellulose in the cell free state may not necessarily be the same as when enzymes are associated with living cells. For the growth of holocellulolytic bacteria, each species has a clear upper temperature limit beyond which growth and the production of holocellulose will stop [18]. The lower temperature limit is usually not well defined. Within the operating temperature range for each species, growth rate will roughly double for each 10^0C increase in temperature . Based on the upper temperature limit, anaerobic holocellulolytic bacteria are often classified as either mesophilic, with optimum growth temperature between 30 to 40^0C, or thermophilic, with optimum growth between 50 to 60^0C . Mesophilic bacteria will normally be enriched in digesters operating between 30 and 40^0C, and thermophilic bacteria in digesters operated between 50 and 60^0C. Numerous studies have shown that hydrolysis proceeds faster in thermophilic digesters than in mesophilic digesters, when other conditions are similar [17] .

2.6 Pretreatment Methods of Lignocellulosic Wastes

2.6.1 Mechanical pretreatment

Mechanically based pretreatment technologies are aimed at reducing the size of LCW to facilitate subsequent treatments. Reduction of biomass size below 20 sieves shows the best mechanical performance . Mechanical pretreatment technologies increase the digestibility of cellulose and hemicellulose in the lignocellulosic biomass. The use of mechanical chopping ; hammer milling ; grind milling ; roll milling ; vibratory milling and ball milling have proved success as a low cost pretreatment strategy [9]. The pulverized materials with increased surface area have been found to facilitate the subsequent physicochemical and biochemical pre treatments of corn stover, barley straw sugar cane baggase,wheat straw, wood waste and municipal solid waste. Mechanical pretreatment also result to substantial lignin depolymerization. Solubility and fermentation efficiency of the natural lignocellulosic residues is also substantially increased by mechanophysicochemical pretreatment, leading to value-added utilization of these residues.

2.6.2 Physical pretreatment

Elevated temperatures and irradiation are the most successful physical treatments in the processing of lignocellulosic wastes (LCW). Thermogravimetric treatment of wood waste under both inert and

oxidant atmospheres from room temperature up to 1100 K leads to moisture loss; hemicellulose, cellulose and lignin decomposition . On the other hand, pyrolysis of nutshells, straws, sawdust and municipal solid wastes at temperatures of 600 - 1200 K result to yields of char, liquid and gaseous products of up to 55% of the original LCW . Irradiation can cause significant breakdown of the structure of LCW. Microwave irradiation at a power of up to 700 W at various exposure times resulted to weight loss due to degradation of cellulose, hemicellulose and lignin, and the degradation rates are significantly enhanced by the presence of alkali.

2.6.3 Chemical pretreatment

Chemicals ranging from oxidizing agents, alkali, acids and salts can be used to degrade lignin, hemicellulose and cellulose from LCW. Powerful oxidizing agents such as ozone and H_2O_2 effectively remove lignin; does not produce toxic residues for the downstream processes; and the reactions are carried out at room temperature and pressure . Alkali (NaOH, $Ca(OH)_2$, NaOH-urea,Na_2CO_3) hydrolyses of rice straw; spruce wood waste ; sugarcane, cassava and peanuts wastes ; corn cob ; organic fraction of municipal solid waste have been investigated. When these pretreatments are performed by using 0.5 - 2 M alkali at 120 - 200^0C, they substantially facilitate breaking of carbohydrate to sugar molecules and improve enzymic hydrolysis of LCW [6].

Dilute and concentrated acids at high temperature are suited for hydrolysis of LCW. Usage of more concentrated H_2SO_4 has been shown to be able not only to hydrolyse cellulose and hemicellulose, but also in separating lignin and other organic components from LCW . SO_2 and fly ash in flare gas; HNO_3, HCl and polyhydric alcohol in the presence of sulfuric acid are also useful in LCW pretreatment. Recent studies have shown that when acids are combined with alkali, they play a more effective role in LCW pretreatment than acids and alkalis alone [6] .

Organic acids such as oxalic, acetylsalicylic and salicylic acid can be used as catalysts in the organo-solv process whereby an organic or aqueous organic solvent mixture with inorganic acids (HCl or H_2SO_4) are used to break the internal lignin and hemicellulose bonds. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol . The use of a di-carboxylic acid catalyst, maleic acid, for hemicellulose hydrolysis in corn stover overcomes the technical and economic hurdle of hemicellulose hydrolysis .

2.6.4 Biological pretreatment

Biological treatment involves the use of whole organisms or enzymes in pretreatment of LCW. Both fungi and bacteria are used for biotreatment of LCW. Commercial preparations of fungal and bacterial hydrolytic and oxidative enzymes are also widely used instead of these microorganisms. Fungal pretreatment of agricultural residues is a new method for improvement of digestibility. White- , brown- and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials whereby brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials . The post treatment by anaerobic bio-processes of LCW effluents that have been pre-treated with fungi can lead to higher biogas than the original effluents. In bio-organo-solv process, fungal pretreatment of wood waste for 2 - 8 weeks followed by organic solvent treatment at 140 - 200^0C for 2 hrs has achieved considerable energy efficient delignification and hemicellulose hydrolysis .

Bacterial pretreatment of LCW involves both anaerobic and aerobic systems. Anaerobic degradation utilizes mainly mesophillic, rumen derived bacteria [13, 14]. Aerobic-anaerobic systems have an upper hand when it comes to degradation of LCW richer in lignin content while in aerobic system alone,fungi is able to produce high levels of extracellular hydrolytic enzyme that degrade lignocellulose .

2.7 Case studies on Anaerobic degradation of lignin using co-substrates

2.7.1 Co-digestion with food wastes

Lignocellulosic biomass feed stocks are highly desired for anaerobic digestion because of their abundance. Yard waste is extremely desirable because it makes up the third most abundant source of municipal solid waste. But the usage of yard waste as a feedstock for anaerobic digestion can be effective only if the hydrolysis is increased, which in turn increases methane production.Because bacterial hydrolysis is typically the rate limiting step, anaerobic digestion of solid waste is traditionally very slow and inefficient due to the rigid cell structure of the cellulosic biomass. The main studies to improve the biogas production concentrates on pretreatments and co-digestion. As pretreatments of lignocellulosic wastes require more energy and cost, its usage in turn increases the cost of anaerobic digestion.

Anaerobic co-digestion is the process of combining two or more substrates for simultaneous digestion. Co-digestion of solid waste can utilize diverse nutrient and bacterial colonies in various materials to establish a positive synergism,supporting microbial growth. Co-digestion also provides the ability to balance nutrients. The addition of substrates with higher C/N ratios to those with lower C/N ratios leads to more balanced nutrients within the digester. Food waste is major part in municipal solid waste. Also anaerobic digestion is especially suitable for treating such wastes [21]. Studies were done in co- digestion of yard wastes with food waste to produce methane [3, 10, 2]. Even though the degradation of lignocellulose components are not so satisfactory, the results gives a hopeful opening for research in this area. Methane potential of food waste varies depending on the specific content but mixed food waste has an average potential methane yield of 472L methane/ kg VS added. [22, 2]. And methane potential of yard waste is reported as 209 L methane/ kg VS added. [23].

The study also involves the characterization of yard wastes, food wastes and inoculum which is used as the seeding for the reactor. The co-digestion was done for different ratios of food to yard waste such as 1:1, 3:1, 1:0,0:1, 1:9 and 1:4 [2, 3]. f/m ratios considered were 1,2,3,4 and particle sizes of 0.25 inch and 5 inches.

Table 2.1: Characterization of feed stock and inoculum from Literature

Parameter	Yard Waste	Food waste	Inoculum	Reference
Total solids (%)	94.11 ± 0.03	20.83 ± 0.62	8.58 ± 0.08	[2, 3]
Volatile solids (%)	93.37 ± 0.03	19.95 ± 0.60	3.52 ± 0.08	[2, 3]
Total carbon (%)	47.90 ± 1.00	9.72 ± 0.28	2.07 ± 0.07	[2, 3]
Total Nitrogen (%)	0.35 ± 0.08	2.74 ± 0.12	0.300 ± 0.12	[2, 3]
C/N	143.27 ± 9.50	3.58 ± 0.13	7.0 ± 0.02	[2, 3]
Lignin (%)	27.1 ± 0.5	6.1 ± 0.8	N/D	[2, 3]
Cellulose (%)	23.3 ± 1.3	8.7 ± 0.8	N/D	[2, 3]
Hemicellulose (%)	11.5 ± 0.9	2.3 ±0.8	N/D	[[2, 3]
pH	N/D	N/D	8.0 ± 0.01	[2, 3]

The study shows that as the f/m increases there is a substantial decrease in methane production keeping other parameters same [2, 3]. f/m = 1 shows a favourable increase in methane production [3]. The ratio of food to yard also affects the methane production widely. Digestion of substrates in the ratio F:Y :: 3:1 produces 70% and 50% more methane than that of 1:1 and 1:0 respectively [2]. For f/m =1, co-digestion in the ratio f:y::1:4 shows a increment in methane production of 2.8 and 1.3 times that of 0:1 and 1:9 respectively. But as f/m increases, the methane production decreases as the presence of food waste increases [3]. Also an increase of 1.6 time of VS reduction was achieved by increasing the food waste ratio from 0 to 20% [3]. The analysis of volatile fatty acids, mainly acetic acid and propionic acid, gives an idea about reactor health. The peaks of methane production approximately correlate inverse to the peak of acetic acid level [3]. Since propionic acid is much more persistent that acetic acid, high levels indicate a more serious instability within the digester. Figure 2.4 shows the methane production for different Co-digestion ratio.

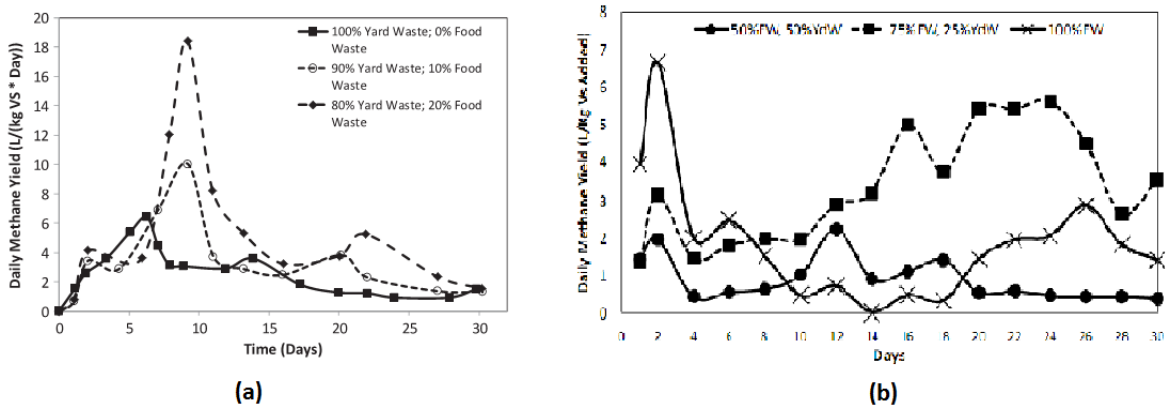

Figure 2.4: Methane Production for different Co-digestion ratio (a) f/m = 1 (b) f/m = 2 [2, 3]

2.7.2 Degradation of Lignocellulose using rumen microorganisms as inoculum

The rumen microorganisms are found to be more active for the degradation of lignocellulosic wastes over other microbes [13]. It composes of bacteria, fungi and protozoa which are the needed enzyme components for lignocellulosic degradation to microbial end products [12]. The studies shows that wastes with a lignin content of 10-15% were degraded with high efficiency using rumen microorganisms [14]. It was found that there is a correlation between lignin content as %TS and its degradation by rumen microorganisms in batch cultures with a correlation coefficient of 0.9834 [14]. The fermentation of wheat straw in rumen causes a lignin degradation of 25.5%, cellulose degradation of 44% and hemicellulose degradation of 43% in 13 days [13]

2.8 Summary

By broad definition, lignocellulosic materials include almost all forestry products, agricultural crop residues, and other grass materials. The organic fraction of municipal solid wastes is primarily a mixture of forest products, including waste paper and cardboard. Chemically, all lignocellulosic materials consist, in different proportions, of the following components: cellulose, hemicellulose, lignin, extractives, and ash. Cellulose and hemicellulose are collectively called holocellulose. Lignin is a highly branched and high molecular weight polymer consisting of phenylpropane-based monomeric units. Cellulose, hemicellulose, and lignin are the structural components of lignocellulosic materials, which, together with extractives and ash, form intimate physical and chemical relationships in lignocellulosic materials.

Methane fermentation of lignocellulosic materials is a sequential process. The insoluble polymers are first enzymatically hydrolyzed into soluble component units, which are then fermented into other intermediates, and then into acetic acid, carbon dioxide, and hydrogen (or formic acid), which are, in turn, converted into methane and carbon dioxide.Each step is performed by a distinct group of bacteria. The first step, the hydrolysis of insoluble lignocellulosic polymers into soluble component units, is known to be the limiting step for these materials. However, quantitative determination is still lacking as regard to the actual rate potentials of each step in the sequential fermentation process of a lignocellulosic material in well developed methanogenic cultures.

Under anaerobic conditions, lignin is not readily broken into soluble monomeric components, and is therefore considered refractory. Pure holocellulose is fully hydrolyzable. However, when it is combined with lignin to form lignocellulose, it may become only partially available to enzymatic action or even completely refractory. Extractives are more easily hydrolyzed than holocellulose, and their biodegradability is much less affected by lignin. By forming certain chemical bonds with hemicellulose, lignin blocks the hydrolysis of a small portion of hemicellulose. Lignin also physically covers part of the particle surface and thus limits the exposure of holocellulose molecules to hydrolytic enzymes. Lignin also controls the hydrolysis of holocellulose predominantly by maintaining a rigid cell wall structure, which limits the hydration and conformational changes in holocellulose molecules required by hydrolytic enzymes, resulting in either a reduction in the hydrolysis rate or its complete prevention. Accordingly, either delignification or lignin depolymerization by pretreatment can reduce lignin's interference. For an individual lignocellulosic material without pretreatment, the

following factors will govern the lignin's ability to control the hydrolysis rate and extent: (1) the amount of lignin present (lignin content); (2) the degree of lignin polymerization; (3) the structure of the lignin polymer;(4) the distribution of lignin within the material; and (5) the degree of intimacy between lignin and the holocellulose molecules. Thus, the biodegradability of a lignocellulosic material may not be predicted accurately based on its lignin content alone. Further experiments are necessary to evaluate the relationship between the nature of lignocellulosic materials and their biodegradability. Although hydrolysis is an enzymatic process, the presence of living cells also has many other beneficiary effects on hydrolysis efficiency, such as producing fresh enzymes and removing adsorbed deactivated enzymes from the substrate surface. Therefore, hydrolysis can generally be achieved to a greater extent in the presence of living microorganisms than with cell-free enzyme systems. The presence of living cells also provides an opportunity for enzymatic adaptation.

Although many anaerobic bacterial species are known to possess holocellulolytic activity, only those species that have the best competitive advantages will be enriched for in an anaerobic digester fed with a given lignocellulosic material and operated at a given pH and temperature. Differences exist between cultures enriched under different feed and environmental conditions. Hydrolysis generally proceeds faster with thermophilic cultures than with mesophilic cultures. Although most isolated holocellulolytic enzymes exhibit maximum activity at acidic pH, this may not necessarily be true for anaerobic enrichments. Laboratory evidence indicates that hydrolytic bacteria enriched at neutral pH exhibited maximum activity at neutral pH. Cultures enriched at acidic pH also performed more poorly than cultures enriched at neutral pH. It is possible that holocellulolytic enzymes,once associated with living cells, may require different environmental conditions for maximum activity than when the enzymes are in the free state. For this reason, the results gained from the study of cell-free extracts may not be useful for interpreting the actual situation occurring in operating anaerobic digesters. However, previous studies were less and non-systematic, and bacterial cultures used were not clearly characterized, which make it difficult to compare the results between different studies and to draw any firm conclusions from them.

Chapter 3

Methods and Methodology

3.1 Introduction

In this chapter, the methods followed in experiments are described. It includes the experimental design, material selection and all the analysis tests for substrates, inoculum and mixed liquors namely, total solids, volatile solids, total carbon,inorganic carbon, total nitrogen, Lignocellulosic components, volatile fatty acids , pH, SMA, BMP, biogas and methane content.

3.2 Design of experiment

The study was conducted in a respirometer (Model No: PF-8000) as shown in the Fig. 3.1. A set of 24 no.s 500mL Wheaton bottles were used as test reactors. The reactors were inoculated with 250 mL sludge obtained from a running anaerobic digester at Amberpet waste water treatment plant. Prior to the actual test, the rectors were dosed with dilute ethanol solution for 20 days at 35^0C for facilitating acclimation under new condition.

Figure 3.1: Respirometer setup

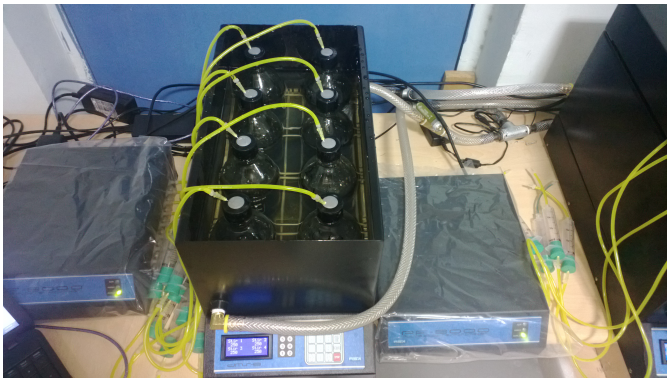

Figure 3.2: Reactor arrangement

3.3 Semi batch study

The substrate for the test was prepared by mixing dry food waste and dry yard waste in the proportion of 1:0; 1:0.5; 1:1;1:1.5; 1:2; 1:3; 1:4 and 0:1 on dry weight basis.The yard waste (grass and leaves) and food wastes were obtained from lawn and mess of IIT Hyderabad respectively. The wastes were separately dried , grinded, sieved to 2mm size and then mixed in the above proportion on dry weight basis. The teat reactor was fed with the substrate once in 24 hours. The volume of feed added or mixed liquor withdrawn was determined based on solid retention time and volume of the reactor (volume of mixed liquor). Altogether 4 SRTs were tried - 30days, 20 days, 12 days and 6 days. The reactor was kept in a water bath whose temperature was maintained at 35^0C. The tests were run in duplicate. Figure. 3.3 explains the experimental design.

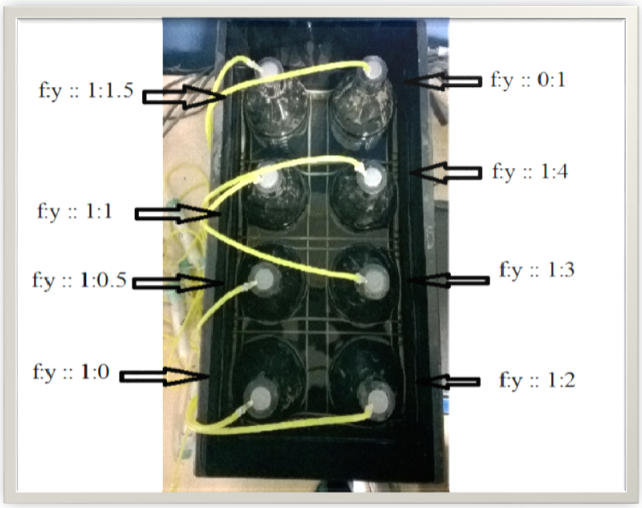

Figure 3.3: Reactor Design

3.4 Biochemical methane production (BMP) test

Biochemical methane production is a simple and rapid test which demonstrates both the suitability of a feedstock for anaerobic digestion and its potential methane yield. A set of 8 Wheaton bottles were used as test reactors. The reactor were inoculated with 250mL anaerobic sludge. The inoculum was dosed with ethanol for 20 days for acclimation. Before beginning of the test, the inoculum was diluted with tap water to bring the final mixed liquor volume to 400 mL in the test bottles. The bottles were fed with 10.28 gm of substrate consisting of food and yard waste which are mixed in different proportions as mentioned in the previous section. Cumulative methane production and daily gas production were monitored using respirometer. Once the gas production is subsided, the reactors were again fed with the substrate and the procedure was repeated. In all, the test reactors were fed three times. Figure. 3.3 also explains the experimental design for the BMP setup.

3.5 Specific Methanogenic Activity

Specific Methanogenic Activity evaluates the capacity of anaerobic sludge to convert an organic substrate into methane. Prior to the begin of semi-batch and BMP studies an activity test of the inoculum was performed. Respirometers were used for assessing the activity of anaerobic cultures in reactors. The test protocols involves addition of a highly biodegradable substrate to a sample of biomass in a sealed container. Methane production was measured over two to three feed cycles. The test usually is repeated with second and third doses of substrate because the first dose often is required to activate the culture and overcome any transfer shock or adjustments to test condition. Specific methanogenic production rate is calculated as follows [16]:

$$SMP, gmethaneCOD/gVS/d = \frac{\frac{L}{d} \times methane \times 2.53}{gVS} \tag{3.1}$$

L/d is the maximum slope of the methane production curve. The maximum SMP represent the specific methanogenic activity (SMA) or the maximum rate at which the culture produces methane from the substrates. SMA values indicates the concentration of percentage acetoclastic methanogens in a biomass sample. Since the maximum acetate conversion rate is 6g COD/g VS -d, a SMA of 6 represents 100% acetoclastic methanogens.

$$\%Methanogens = \frac{SMA}{6} \times 100 \tag{3.2}$$

All 16 bottles of respirometer where fed with 400mL inoculum. As per the reactor design, 8 bottles were fed with different ratios of substrate and other 8 bottles were the duplicate. Here ethanol feed stock were added to inoculum at a f/m ratio of 0.4 g COD/g VS. The ethanol feedstock was prepared by adding 13 mL of 95% ethanol solution to 800 mL of distilled water and diluted to 1 L using distilled water. The temperature was maintained at 35^0C. The test was repeated three times. The biogas produced was measured overtime by respirometer.

3.6 Analytical methods

The substrates, inoculum and mixed liquor are analyzed for TS, VS, TC, IC, COD, TN, lignin, cellulose, hemicellulose, pH and VFA.

3.6.1 Total Solids and Volatile Solids

The TS and VS tests were done according to Standard Methods for the Examination of Water & Wastewater (2005) [24]. This method is applicable to the determination of total solids ,fixed and volatile fractions in such solid and semisolid samples as soils, sediments, bio solids. Here the samples were yard wastes (grass, leaves), food wastes and sludge. Total solids are the residue left in the vessel after evaporation of liquid from a sample and subsequent drying in an oven at 103^0C to 105^0C. It consists of volatile and fixed solids.

For volatile solids measurements, the crucibles were ignited at 550^0C for 1 hour in a muffle furnace. The dried crucibles were cooled and stored in a desiccator. Each dish was weighed prior to use (recorded weight as W dish). The sludge sample was stirred to homogenize, and 100 mL was taken in a prepared crucible. The samples were added to different crucibles and weighed. The weight was recorded as "W sample". After this the samples were placed in a preheated oven and evaporated at 103^0C to 105^0C for over night. After drying, the samples were allowed to cool to room temperature in a desiccator.The residue was again heated for 1 hour, and cooled to balance temperature, and weighed. This heating, cooling, desiccating, and weighing procedure was repeated until the weight change is less than 4% or 50 mg, whichever is less. The final weight was recorded as W total.

$$TS = \frac{Wtotal - Wdish}{Vol.of sample} \tag{3.3}$$

To measure the volatile solids, the crucibles containing the dried residues after TS measurement were transferred to a cool muffle furnace. The furnace was ignited to 550^0C for 1 hour. The residue was cooled in a desiccator to balance the temperature, and weighed. Igniting is repeated (30 min), with cooling, desiccating, and weighing steps until the weight change is less than 4% or 50 mg, whichever is less. The final weight was recorded as W volatile.

$$VS = \frac{Wtotal - Wvolatile}{Vol.of sample} \tag{3.4}$$

- Wdish=Weight of crucible (mg)
- Wsample=Weight of wet sample and crucible (mg)
- Wtotal=Weight of dried residue and crucible (mg)
- Wvolatile=Weight of residue and crucible after ignition at 550^0C (mg)

3.6.2 Lignin, Cellulose and, Hemicellulose

The analysis of fibre composition, lignin, cellulose and hemicellulose were done according to Van Soests neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL)

analysis [25]. The acid-detergent fibre procedure provided a rapid method for lignocellulose determination. The fibre residues are primarily cellulose and lignin, but can also include silica. The difference between the neutral-detergent and acid-detergent fibre is an estimate of hemicellulose; however, this difference includes some protein attached to the cell walls. The acid detergent fibre was used as a preparatory step for lignin determination. The ADF content is expressed as a dry mass basis fraction in percentage.

Approximately 0.50-1.00±0.01 g air-dry sample extract was placed in a beaker, to which 100±1 mL of room temperature acid-detergent solution was added. Acid-detergent solution was prepared by adding 20.00± 0.01 g cetyl trimethyl ammonium bromide (CTAB), technical grade, to 1 L ± 0.3 mL of 1±0.001 N H_2SO_4 previously standardized and mixed. After adding ADS to sample, 2mL of Decalin was added in the mixture and the mixture was heated to constant boiling for 1hour. After boiling, the mixture was transfer into a previously tarred glass fibre filter paper and vacuum filtered, using a low vacuum at first, increasing it only as needed. Next, the residue in the filter paper was washed with 50± 1 mL of hot water ($80-90^0$C) and the liquid was filtered. The washing procedure was repeated 3 times, and then the sample was washed and filtered dry with acetone in same manner . After filtering, the residue with filter paper was transferred into a previously tarred crucible and was dried at 100^0C for 8 hours or overnight in the drying oven and cooled in a desiccator and then weighed.The acid-detergent fibre was computed as per Eqn. [3.5]

$$ADF = \frac{W_0 - W_t - W_f}{S} \times 100 \tag{3.5}$$

- W_0 Weight of dry crucible including filter paper and fibre (g)
- W_t - Tared weight of dry crucible (g)
- W_f - Tared weight of filter paper (g)
- S - Sample weight (g)

In the acid-detergent lignin procedure, the acid-detergent fibre (ADF) procedure is used as a preparatory step. The detergent removes the protein and other acid-soluble material that would interfere with the lignin determination. The ADF residue consists of cellulose, lignin, cutin and acid-insoluble ash (mainly silica). Treatment with 72% H_2SO_4 dissolves cellulose. Ashing of the residue will determine the crude lignin fraction including cutin. The ADL content is expressed as a dry mass basis fraction in percentage.

The ADF was transferred to a 100mL beaker with 50 mL of 72% sulphuric acid. It was kept for 3h with intermittent stirring with a glass rod. The acid was diluted with distilled water and filter with pre-weighed filter paper. The glass rod and the residue is washed several times to get rid of the acid. The filter paper is transferred to a pre-weighed silica crucible and dried at 100^0C and weighed after cooling in a desiccator. The filter paper with the content was ashed in a muffle furnace at 550^0C for about 3h. The crucible was cooled in a desiccator and weighed. The ash content was calculated. The acid-detergent lignin was computed as per Eqn. [3.6]

$$ADL = \frac{W_d - W_a}{S} \times 100 \tag{3.6}$$

- W_d Weight of sample including crucible and filter paper after 100^0C ignition (g)

- W_a - Weight of ash including crucible and filter paper after 550^0C ignition (g)
- S - Sample weight (g)

NDF fibre is the residue remaining after digestion in a detergent solution. The fibre residues were predominantly hemicellulose, cellulose and lignin. The NDF content is expressed as a dry mass basis fraction in percentage.To 1g of the powdered sample in a beaker,100 mL of cold neutral detergent solution was added. To prepare neutral detergent solution, 18.61g of disodium ethylene diamine tetra acetate and 6.81g of sodium borate Decca hydrate was dissolved in about 200mL of water by heating. To this, about 100-200ml of a solution containing 30g of sodium lauryl sulphate and 10 mL of 2-ethoxy ethanol was added. Then about 100 mL of a solution containing 4.5g of disodium hydrogen phosphate was mixed. The solution was made up to one liter. After adding NDS to sample, 2ml of decahydronapthalene and 0.5g of sodium sulphite was added and heated to boiling to 60 min. After heating it was filtered by suction and washed with hot water.Also it was washed with acetone. The residue is transferred to crucible and dried at 100^0C for 8h. The crucible is cooled in a desiccator and weighed. The ash content is calculated. The acid-detergent lignin was computed as per Equation [3.7]

$$NDF = \frac{W_0 - W_t - W_f}{S} \times 100 \tag{3.7}$$

- W_0 Weight of dry crucible including filter paper and fibre (g)
- W_t - Tared weight of dry crucible (g)
- W_f - Tared weight of filter paper (g)
- S - Sample weight (g)

Cellulose= ADF - ADL
Hemicellulose = NDF - ADF
Lignin = ADL

3.6.3 Total carbon and Inorganic carbon

Total carbon and inorganic carbon is measured with Solid Sample Combustion (SSM) unit combined with a TOC-L analyzer (Model : SSM - 5000A). Total carbon include both organic and inorganic carbon. Inorganic carbon includes corbonate, bicarbonate and dissolved carbon dioxide. The calibration of the TC range was done with glucose, since it is available to very high degrees of purity and is oxidized fast and completely.The TC was measured at 900^0C. Five glucose sample measurements were taken for TC calibration namely, 10mg, 15mg, 20mg, 25mg and 30mg. The glucose employed for the calibration has a carbon content of 40%. Sodium carbonate was used for the calibration of the inorganic carbon and IC is measured at 250^0C. Five sodium carbonate sample measurements were taken for TC calibration namely, 10mg, 15mg, 20mg, 25mg and 30mg. The sodium carbonate employed for the calibration has a carbon content of 47%. The samples were dried, powdered and sieved to 2mm size. Zero air is used as carrier gas. Once the calibration curves are set, the samples to be tested are taken in the sample boat and software procedure is done.

3.6.4 Total Nitrogen

Total nitrogen was measured with TNM-L unit combined with TOC-L analyzer. In this 720^0C catalytic thermal decomposition methods are adopted for TN measurement. Total nitrogen is the sum of total kjeldahl nitrogen (ammonia, organic and reduced nitrogen) and nitrate-nitrite. Total Nitrogen is an essential nutrient for plants and animals. However, an excess amount of nitrogen in a waterway may lead to low levels of dissolved oxygen and negatively alter various plant life and organisms.

The calibration of TN was done with potassium nitrate. Five sample points were used for calibration with concentration of nitrogen namely 500ppm, 1000ppm, 1500ppm, 2000ppm, 3000ppm, and 4000ppm. The solid samples where digested using Digesdahl digestion apparatus. The samples were dried, powdered and sieved to 2mm size before digestion. Zero air is converted to ozone and is used as carrier gas.Once the calibration curves are set, the samples to be tested are taken in the auto sampler and software procedure is done.

3.6.5 Volatile Fatty Acids

The gas chromatography (Model : Bruker Scion TQ) was used to determine the individual concentrations of following fatty acids: acetic acid, propionic acid and butyric acid. The samples are filtrated and is analyzed by direct injection into a gas chromatograph equipped with a flame ionization detector. Initially a particular operation method was created for gas chromatograph. The column used wa BR-QPlot of 30m length and 0.53mm diameter. The temperature and flow rates were optimized such that the optimal injector temperature would allow vaporization of prepared sample. The gases used were, hydrogen to fuel the FID, GC grade air,and Helium as carrier gas . The injector temperature given was 200^0C. For the oven, the temperature was holden at 200^0C for 5 min. The detector temperature was adjusted to 240^0C. The gas flows , namely hydrogen flow rate was 30ml/ min and air flow rate was 300 ml/min. Carrier gas flow rate was 15ml/min. The samples were injected by auto sampler.

Volatile fatty acids standard mixture of 20ppm, 40ppm, 60ppm, 80ppm and 100ppm were prepared by diluting a stock solution containing a mixture of acetic, propionic and butyric acids . It was stored in 4^0C. To prepare the calibration, 1 μL each of VFA calibration standard mixture was injected into GC. A calibration curve from the five calibration standards was constructed. The correlation coefficient was greater than 0.995. Each calibration curve could be used upto 1 month. The samples are injected into GC along with blank. All the data was collected using chromatographic data system software.

3.6.6 Methane Analysis

Sample were taken from each reactors were taken by a syringe and collected in tedlar bags and later was injected into gas chromatography system. The column used was BR-QPlot of 30m length and 0.53mm diameter. The injector temperature given was 100^0C. For the oven, initially the temperature was holded at 40^0C for 3 min and then increased to 100^0C at a rate of 10^0C/ min. The detector temperature was adjusted to 240^0C.The samples were injected directly by syringe.

Chapter 4

Results and Discussion

4.1 Characterization of feed stock and inoculum

Feedstock and inoculum were tested for TS,VS, carbon, nitrogen,lignin, cellulose, hemicellulose and COD. The summary of characterization is given in Table 4.1

Table 4.1: Characterization of feed stock and inoculum

Parameter	Yard Waste	Food waste	Inoculum
Total solids (%)	63.5	35	16.194
Volatile solids (%)	55.48	27.19	5.14
Total carbon (mg/g)	488	98.1	21.1
Total carbon (%)	48.8	9.81	2.11
Total Nitrogen (ppm)	11.1	31.2	38.1
Total Nitrogen (%)	0.37	1.04	0.38
C/N	131.89	9.43	5.55
Lignin (%)	18.14	3.44	1.7364
Cellulose (%)	24.43	6.02	2.114
Hemicellulose (%)	14.12	1.96	1.372
COD (mg/g)	162.1	391.95	265.5
VS/TS	0.87	0.77	0.32
Lignin/ TS	0.29	0.1	0.11
Cellulose/TS	0.38	0.17	0.13
Hemicellulose/TS	0.22	0.056	0.08

4.2 Specific Methanogenic Activity

The test bottles were dosed three times with 50mL of dilute ethanol (COD = 20000 mg/L). In other words the bottles were fed with 1 gm of COD. Theoretically, this should produce 400mL of methane under normal atmospheric pressure and at 35^0C. Figure. 4.1 shows the cumulative methane production which is obtained by multiplying cumulative biogas production (obtained from respirometer) with the percentage of methane in the biogas. Methane percentage in biogas was

found out to be 67% by GC analysis.

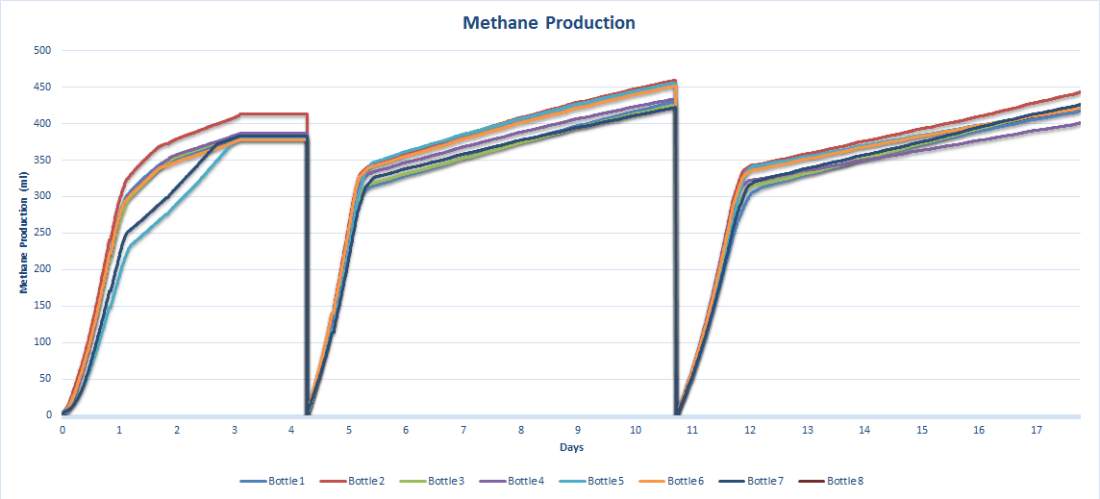

Figure 4.1: Total gas Production in mL

Figure. 4.2 is derived from Fig. 4.1. In Fig. 4.2 specific methane production (SMP) is plotted against time for three feedings. The highest ordinate in the graph gives the specific methanogenic activity. As per the Fig. 4.2, SMA was found to be 1.54 g COD/ g VS-d. Using Eqn.4.2, the fraction of acetoclastic methanogens in the inoculum was estimated to be around 25.6%.

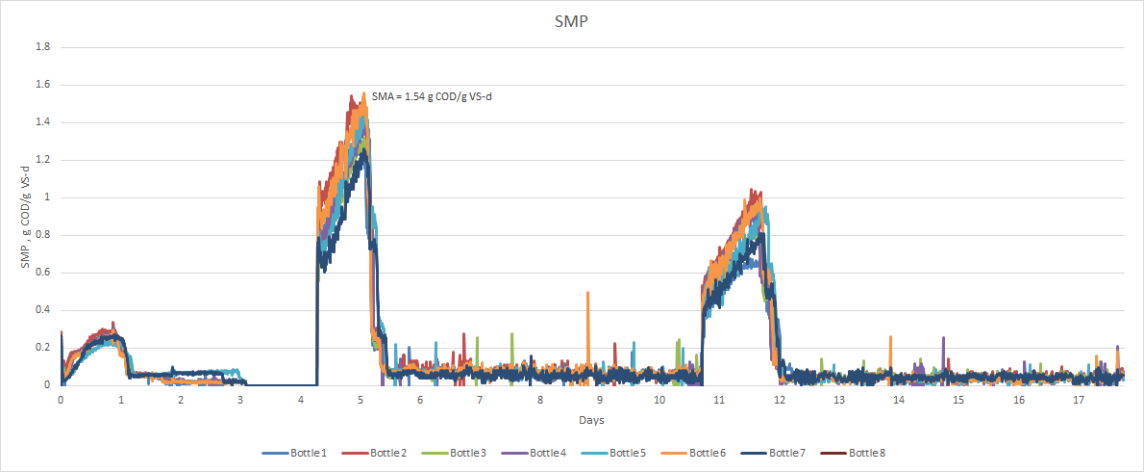

Figure 4.2: Specific Methanogenic Production

4.3 Biochemical Methane Potential

The methane potential of food waste and yard waste were correlated with the values obtained from literature. The change in methane potential may be due to the difference in VS used in this study and other literature studies. BMP test data is shown in Fig. 4.3. Methane production from 100% food waste was almost same as those from literature. But the methane production from 100% yard

waste was slightly higher than those of literature. The difference may be due to the difference in material composition.

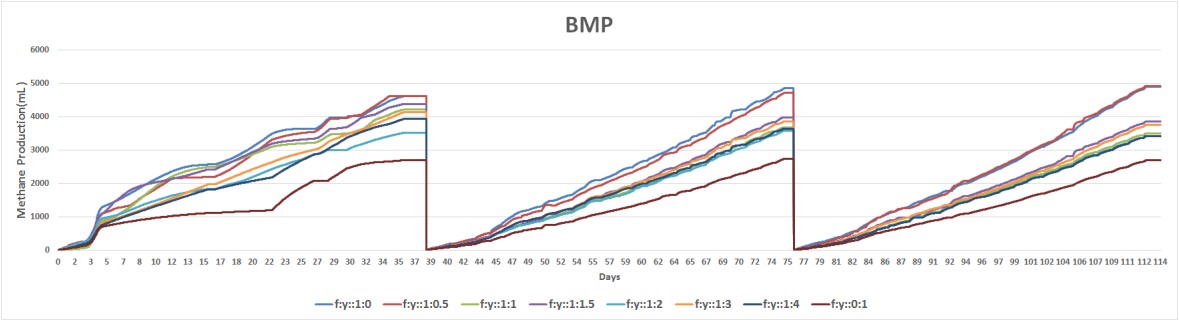

Figure 4.3: Biochemical Methane Potential of substrates

Figure. 4.3 shows the cumulative methane production for the successive feeding of of substrate. The graph is obtained by multiplying the cumulative biogas production (obtained directly from respirometer) by the percentage of methane in the biogas of the test reactors. Table 4.3 shows the COD added, methane % and the actual methane production. Figure 4.4 is derived from Fig. 4.3. It shows the daily methane yield over time.

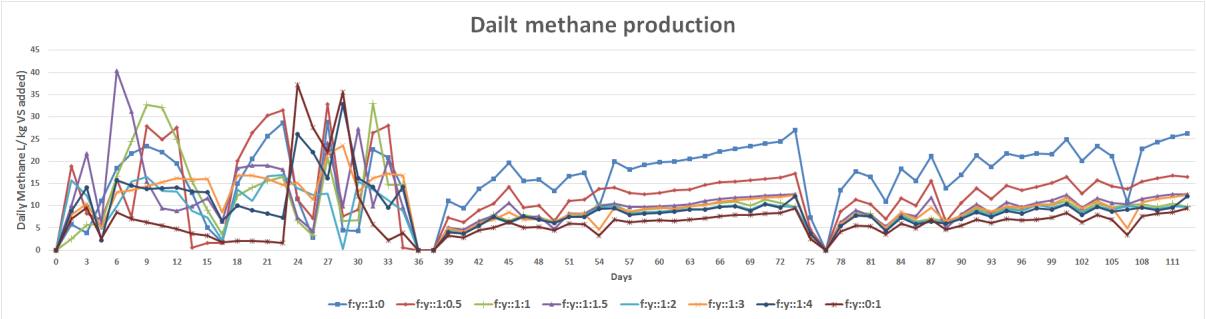

Figure 4.4: Daily Methane Potential of substrates

Ratio	COD added (g)	Methane Content (%)	Theoretical Methane (mL)	Actual Methane (mL)	Convrsion (%)
f:y::1:0	13.20	68	5281.11	4854.113	91.9
f:y::1:0.5	11.84	66	4734.58	4714.12	99.6
f:y::1:1	11.15	60	4461.31	3686.614	82.6
f:y::1:1.5	10.74	64	4297.35	3990.388	92.9
f:y::1:2	10.47	61	4188.04	3580.113	85.5
f:y::1:3	10.13	63	4051.41	3857.878	95.2
f:y::1:4	9.92	65	3969.43	3646.83	91.9
f:y::0:1	9.10	62	3641.51	2731.499	75

4.4 Bio methane Production

The respirometer gave an actual response of reactors by producing a daily biogas production data. The initial days shows a variation in biogas production and later it started to follow a trend. For all the SRTs, the reactor with f:y::1:0.5 showed a higher production of bio methane than others. The biogas production of reactors for 30,20,12,6 days SRTs are shown in Fig. 4.5 to Fig. 4.8 respectively. During 20 days SRT, the biogas production was more than 30d SRT. But as the SRT decreases, the gas production also showed a decrement. For 30 days SRT, the reactor were operated for 44 days, by which the biogas production attained a steady state. For 20 days SRT, 12days and 6 days, the reactors came to a steady state by 40, 36and 25 days respectively

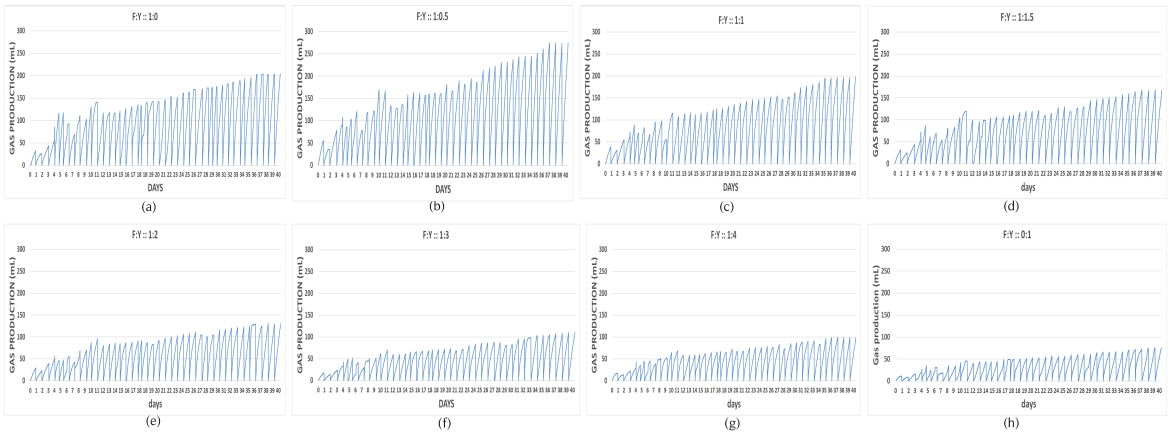

Figure 4.5: Bio methane production in mL for 30 days SRT

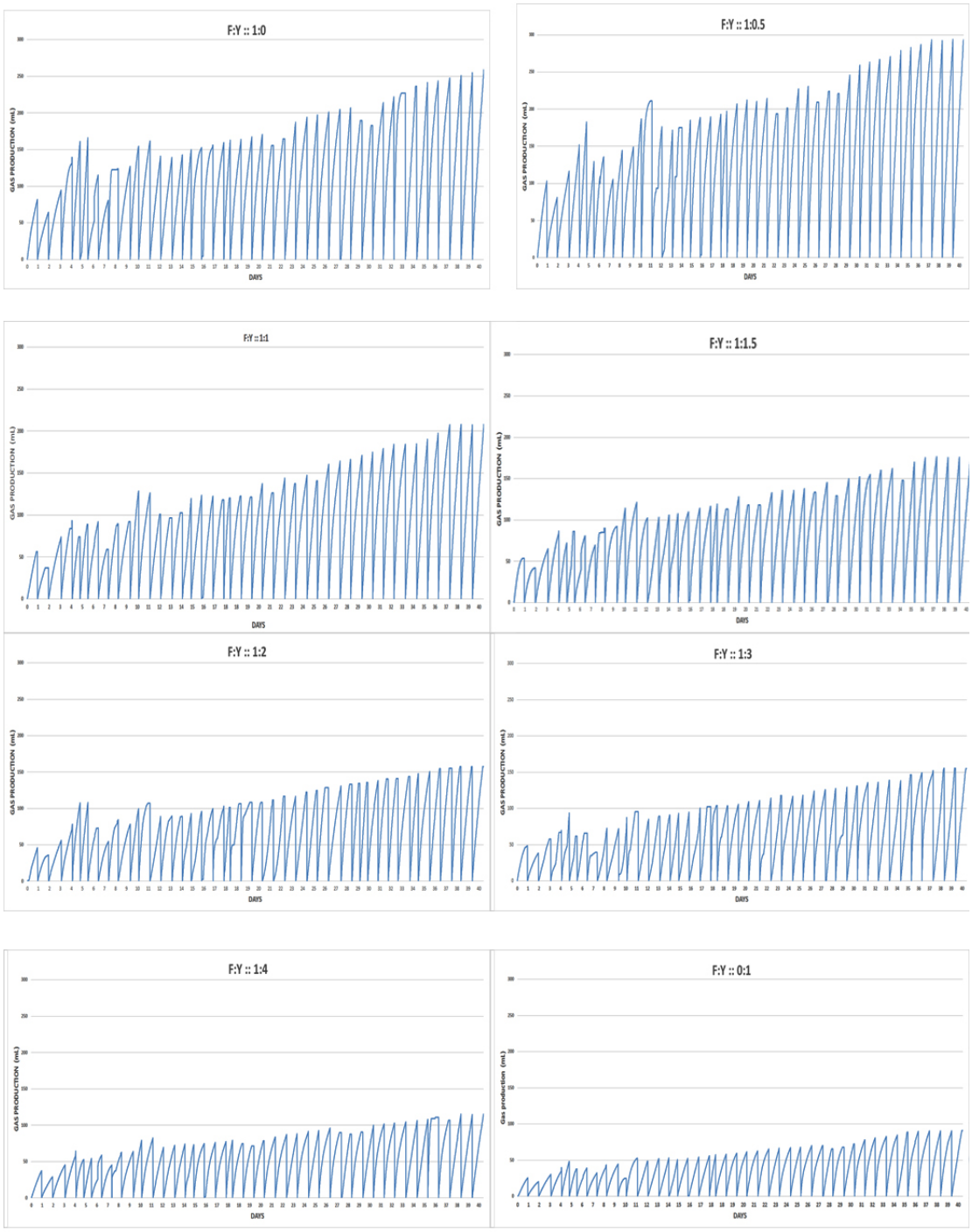

Figure 4.6: Bio methane production in mL for 20 days SRT

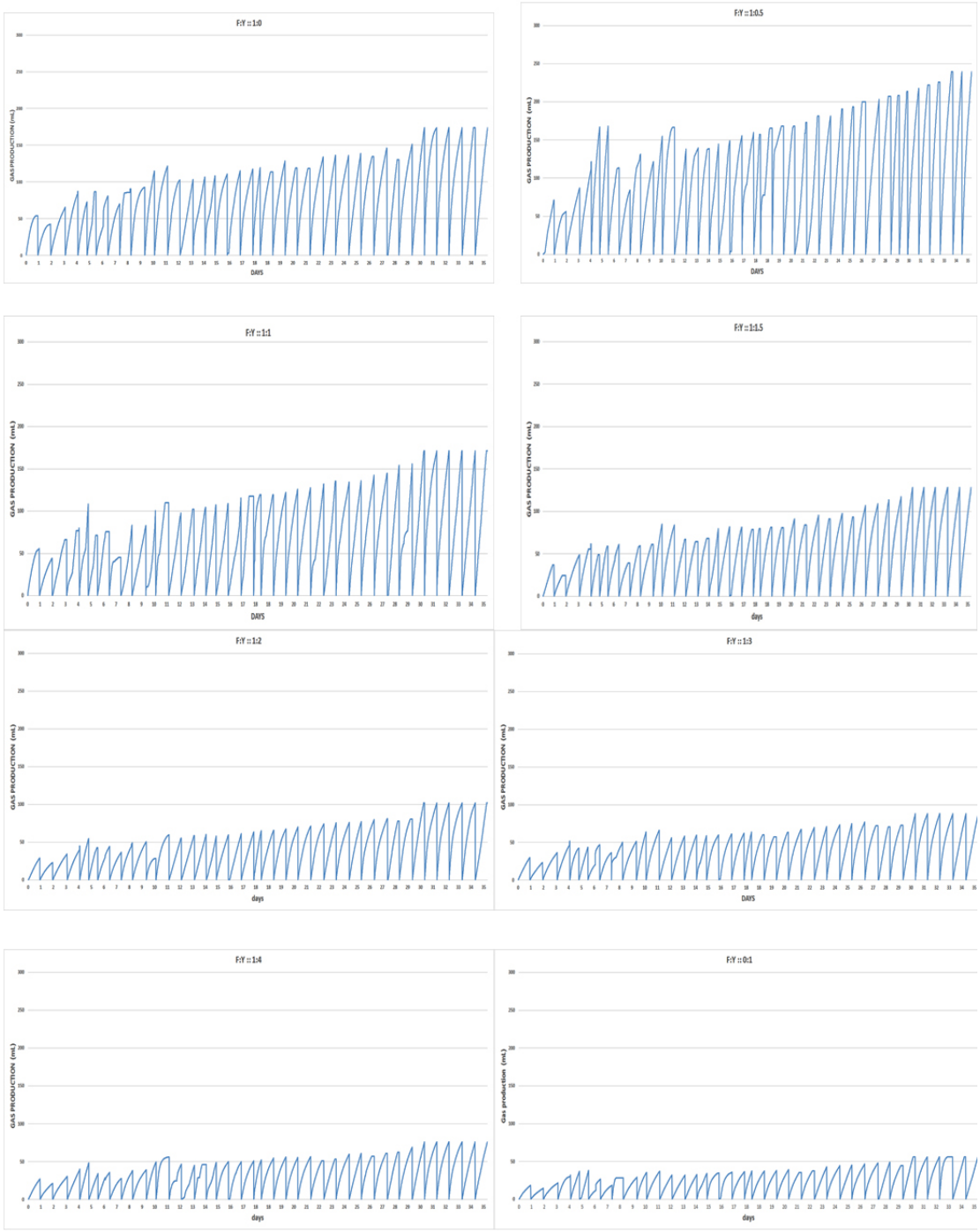

Figure 4.7: Bio methane production in mL for 12 days SRT

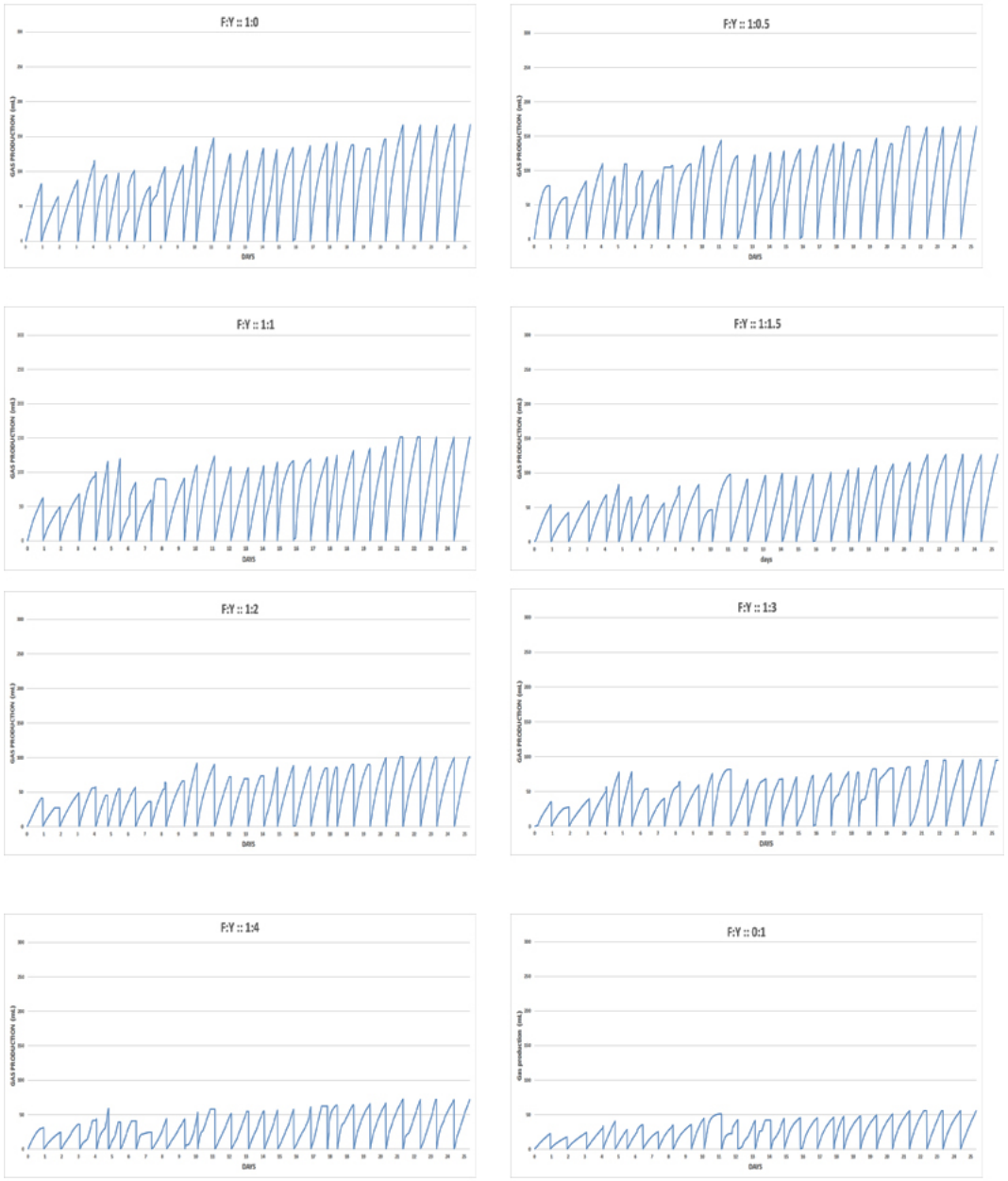

Figure 4.8: Bio methane production in mL for 6 days SRT

4.5 Methane content in biogas

The methane content obtained were n a range of 60-70% at steady state,which matches with the vales of literature. Figure 4.9 shows the methane content n biogas in a reactors at a SRTs.

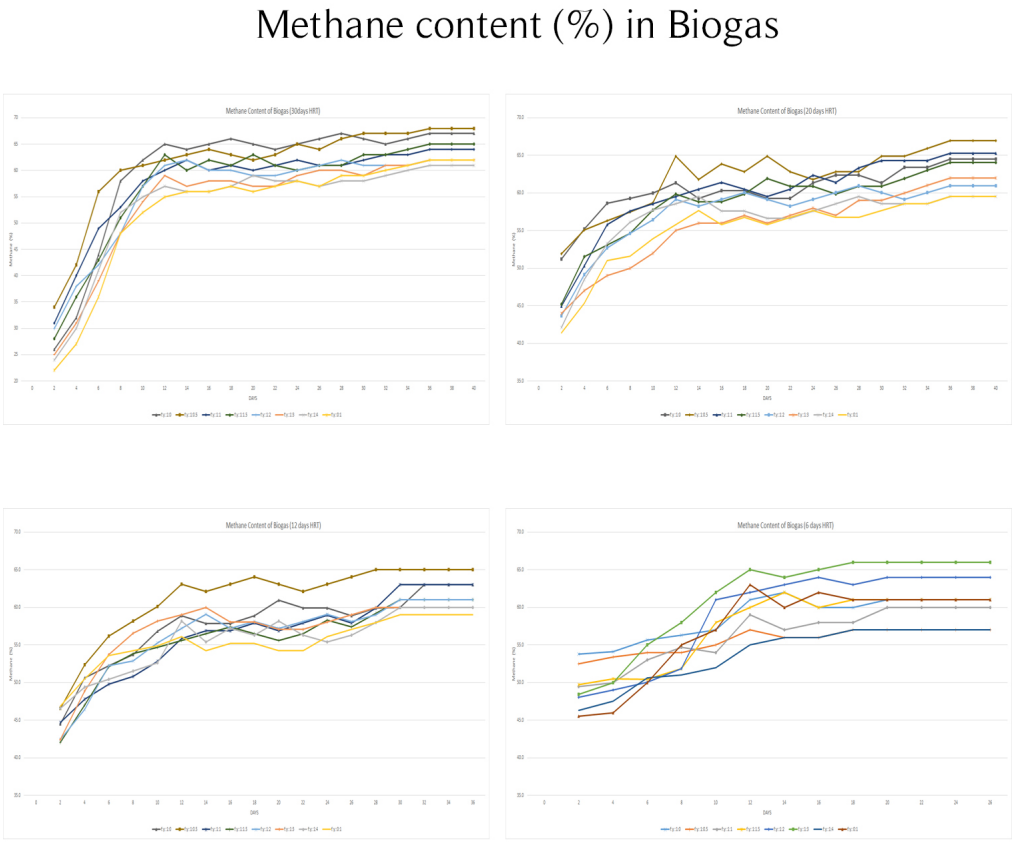

Figure 4.9: Methane content in % for all SRTs

4.6 Volatile solids destruction

Volatile solid reduction of the feed stocks in all reactors against the VS loading are shown in Fig. 4.10. Maximum VS destruction was observed as 75% at 20 days SRT. And also, as the VS loading increases, the degradation of VS started to decrease. The VS destruction is co-related with methane production. Mainly, higher VS destruction was seen in reactors with higher methane production.

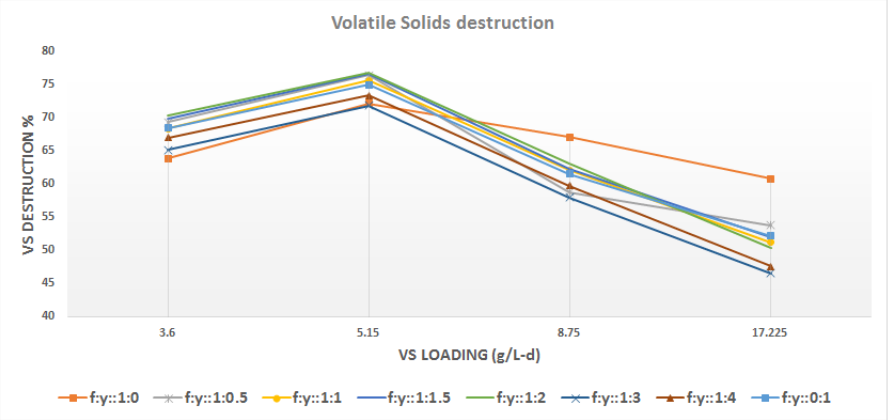

Figure 4.10: Volatile solids destruction against VS loading

4.7 Fibre Analysis

4.7.1 Lignin, cellulose and hemicellulose destruction

Nature of lignin degradation was also studied. The results were matching to previous studies. Maximum lignin degradation of 21% was observed for reactor with f:y::1:0.5. The percentage of degradation reduces with a decrease in food content and increase in VS loading. Figure 4.11 to Fig 4.13 shows the trend of lignocellulosic components degradation with VS loading.

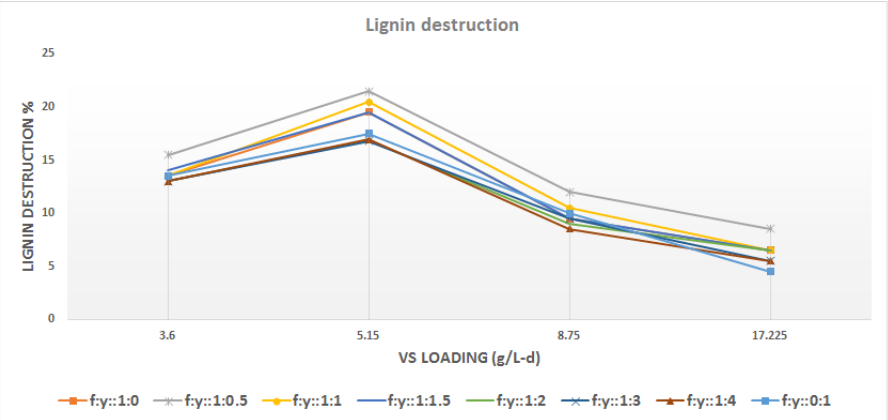

Figure 4.11: Lignin destruction against VS loading

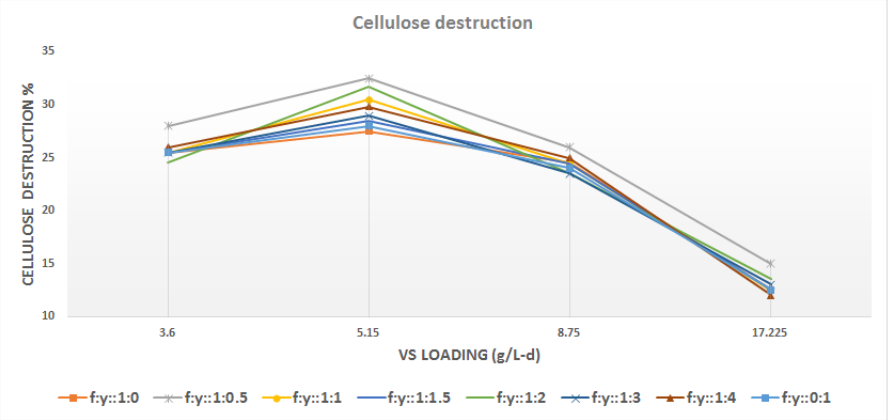

Figure 4.12: Cellulose destruction against VS loading

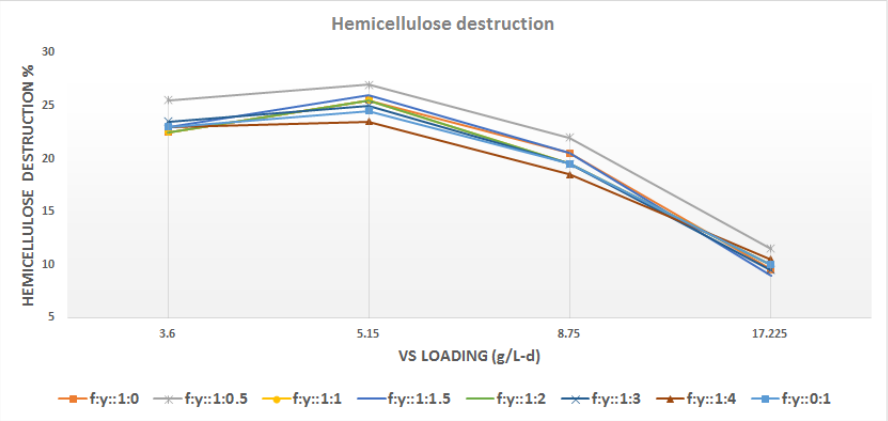

Figure 4.13: Hemicellulose destruction against VS loading

4.8 Volatile Fatty Acids

The VFA, which is byproduct of hydrolysis and acetogenesis was measured through out the experiment.Acetic acid, propionic acid and butyric acid were the major VFA observed in the reactors. The peaks of methane production and acetic acid concentrations are almost inversely correlated. Initially the reactors were having a fluctuation in gas production and subsequently had fluctuations in acetic acid concentrations too. As the SRT decreased, the gas production decreases and an increment in corresponding acetic acid concentrations were seen. High level of propionic acid and butyric acid indicates the condition of reactor health. These acids' concentrations were less for the reactors running in 20 days SRT, compared to other SRTs. The acid concentrations are shown in Fig 4.14 to Fig 4.16 for different VS loading.

VFA for reactor with only food waste was found to be more than other bottles. This indicates that food wastes are more amenable to hydrolysis and acidification than yard wastes. the accumulation of acids in the reactors didn't increase significantly with increase in VS loading. So this indicates that hydrolysis was the rate limiting process in this experiment. Table 4.2 and Table 4.3 shows the ratios between acid obtained, for different VS loadings

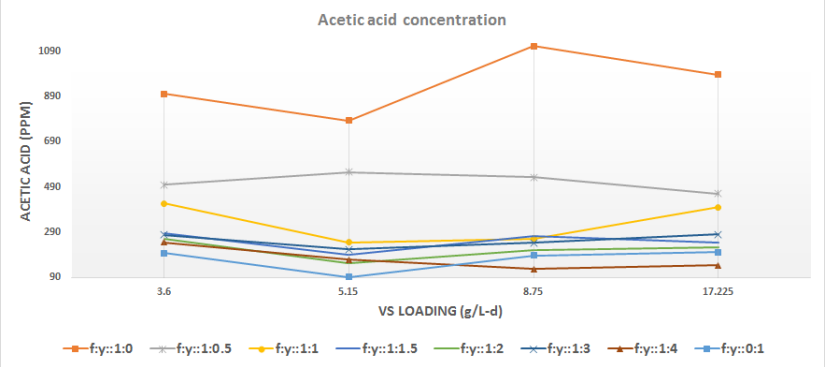

Figure 4.14: Acetic acid concentration against VS loading

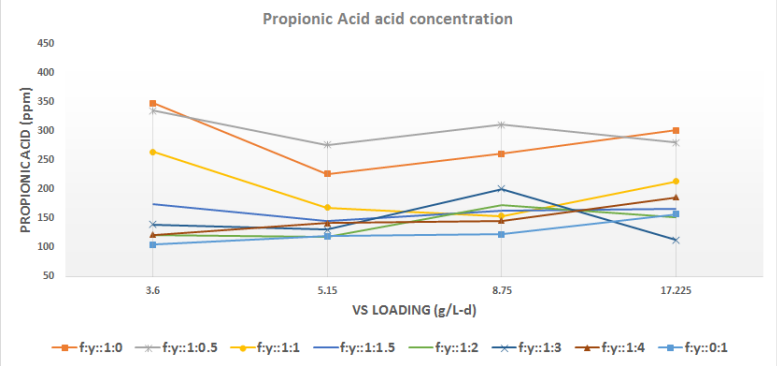

Figure 4.15: Propionic acid concentration against VS loading

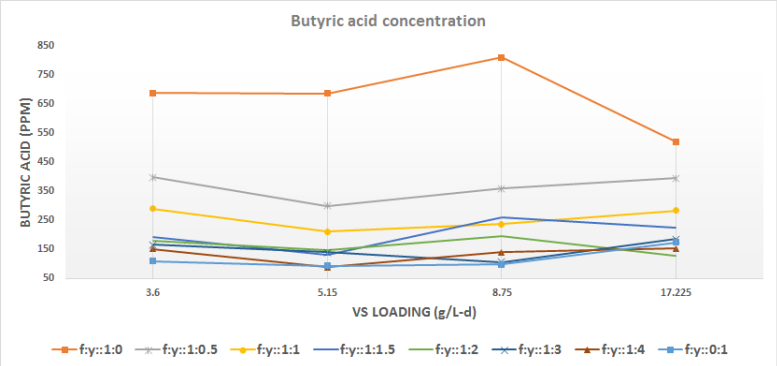

Figure 4.16: Butyric acid concentration against VS loading

Table 4.2: Ratios between VFAs

vs loading (g/L-d)	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5
Ratio	A : P : B	A : P : B	A : P : B	A : P : B
3.6	1: 0.4: 0.75	1 : 0.67 : 0.8	1 : 0.6 :0.7	1 : 0.61: 0.67
5.15	1: 0.3 : 0.88	1 : 0.5: 0.54	1:0.68 :0.86	1:0.76 : 0.68
8.75	1: 0.23: 0.73	1:0.6 :0.7	1 : 0.58: 0.9	1: 0.6 :0.95
17.225	1: 0.31: 0.53	1 : 0.6 : 0.85	1: 0.54 : 0.7	1 : 0.68 : 0.92

Table 4.3: Ratios between VFAs

vs loading (g/L-d)	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
Ratio	A : P : B	A : P : B	A : P : B	A : P : B
3.6	1 : 0.46 : 0.68	1: 0.5: 0.6	1: 0.5 : 0.62	1 : 0.5: 0.55
5.15	1 : 0.77: 0.97	1: 0.61 :0.65	1: 0.83 :0.53	1: 1.3 : 1.02
8.75	1: 0.82 : 0.93	1: 0.82 : 0.44	1: 1.15 : 1.1	1: 0.65 :0.53
17.225	1: 0.67:0.57	1: 0.4 : 0.66	1 : 1.3 : 1.05	1: 0.78 :0.86

4.9 pH

Figure 4.17 shows the variation of pH against VS loading for various co-substrates combination. It clearly shows that, the pH decreases with increase in VS loading. For a successful AD, pH range should fall in 6.3 to 7.8. pH in all reactors other than f:y :: 1:0 falls in this range. The reduction in pH was more in reactor with f:y::1:0, which indicates a faster hydrolysis and acidification in food waste.

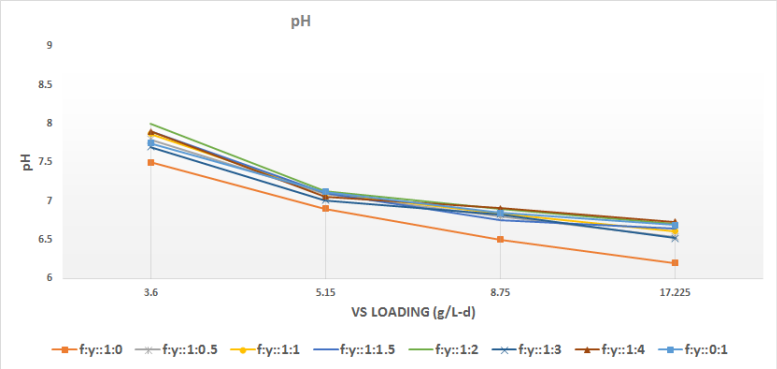

Figure 4.17: pH against VS loading

4.10 Summary of reactors

The overall summary of the reactors are given below. The SRTs in which reactors operated were 30 days, 20 days, 12 days and 6 days and corresponding VS loading were 3.6, 5.15, 8.75, 17.225 g/ L-d. The various parameters achieved by the reactor is given in Table 4.4. For 20 days SRT, the reactors came to a steady state by 40 days and the details are given in Table 4.5. For 12 days and 6 days SRT, the reactors run for a total of 36 and 25 days respectively. [Table 4.6 and Table 4.7].

Table 4.4: Summary for operational result for 30 days SRT

Parameters	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
Methane Content(%)	67	68	64	65	62	62	61	62
Methane (L/kgVS)	308.8	385.50	284.20	250.20	200.20	157.80	148.70	109.10
Vs Degradation(%)	31	33	29	27	27	25	24	23
Lignin Degradation(%)	13.5	15.5	13.5	14.0	13.0	13.0	13.0	13.5
Cellulose Degradation(%)	25.50	28.0	25.5	25.5	24.5	25.5	26.0	25.5
Hemi Cellulose Degradation(%)	22.49	25.5	22.5	23.0	22.5	23.5	23.0	23.0
Acetic Acid(ppm)	900	500	415.89	287.12	261.28	278.19	245.17	197.15
Propionic Acid(ppm)	348.48	335.60	264.25	175.18	121.75	139.71	121.79	105.02
Butyric Acid(ppm)	689.6	398.2	289.8	193.1	179.5	165.3	151.2	109.1
pH	7.5	7.8	7.87	7.9	8	7.7	7.9	7.75

Table 4.5: Summary for operational result for 20 days SRT

Parameters	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
Methane Content(%)	64.48	66	65.28	64.05	62	62	61	61
Methane (L/kgVS)	352.0	417.98	333.63	303.59	274.81	263.91	200.98	150.10
Vs Degradation(%)	38	41	36	35	34	31	30	29
Lignin Degradation(%)	19.50	21.5	20.5	19.5	16.9	16.8	17.0	17.5
Cellulose Degradation(%)	27.50	32.5	30.5	28.5	31.7	29.0	29.8	28.0
Hemi Cellulose Degradation(%)	25.52	27.0	25.5	26.0	25.5	25.0	23.5	24.5
Acetic Acid(ppm)	783.37	555.21	244.99	189.85	153.17	214.38	169.27	91.43
Propionic Acid(ppm)	226.50	276.60	168.60	145.50	118.62	130.62	141.99	119.12
Butyric Acid(ppm)	687.7	300	210.8	130.2	148.7	140.4	88.8	93.2
pH	6.9	7.09	7.1	7.11	7.13	7.01	7.06	7.12

Table 4.6: Summary for operational result for 12 days SRT

Parameters	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
Methane Content(%)	63	65	63	61	61	60	60	59
Methane (L/kgVS)	282.2	292.03	259.89	221.69	166.24	155.40	135.29	97.67
Vs Degradation(%)	24	25	23	20	20	19	18	17
Lignin Degradation(%)	9.50	12.0	10.5	9.5	9.0	9.5	8.5	10.0
Cellulose Degradation(%)	24.50	26.0	24.5	24.5	23.5	23.5	25.0	24.0
Hemi Cellulose Degradation(%)	25.52	27.0	25.5	26.0	25.5	25.0	23.5	24.5
Acetic Acid(ppm)	1112.39	532.22	261.34	273.37	209.91	243.81	126.85	186.32
Propionic Acid(ppm)	261.25	311.80	153.80	163.26	172.47	200.74	146.36	122.32
Butyric Acid(ppm)	811.8	359.1	236.4	260.3	195.8	106.2	140.75	98.65
pH	6.50	6.8	6.84	6.75	6.9	6.83	6.91	6.85

Table 4.7: Summary for operational result for 6 days SRT

Parameters	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
Methane Content(%)	61	57	60	61	64	66	57	61
Methane (L/kgVS)	192.32	197.63	162.17	145.56	124.01	106.70	99.33	68.63
Vs Degradation(%)	20	20	18	18	16	15	14	14
Lignin Degradation(%)	6.50	8.5	6.5	6.5	6.5	5.5	5.5	4.5
Cellulose Degradation(%)	12.50	15.0	12.5	12.5	13.5	13.0	12.0	12.5
Hemi Cellulose Degradation(%)	9.51	11.5	10.0	9.0	10.0	9.5	10.5	10.0
Acetic Acid(ppm)	985.01	459.73	399.10	243.61	225.18	279.51	143.90	201.95
Propionic Acid(ppm)	302.18	280.57	213.99	166.53	152.55	113.06	186.29	157.67
Butyric Acid(ppm)	521.37	395.37	284.29	224.37	128.15	185.41	153.25	173.46
pH	6.20	6.54	6.61	6.64	6.7	6.52	6.73	6.69

4.11 Carbon mass balance

The carbon mass balance of the system is shown in Table 4.8 to Table 4.11 for various SRTs. TCin is the total carbon entering the system through influent that is the daily feeding. TC out is the total carbon leaving out of system through effluent. Some carbon is escaped from the system as biogas. It is considered as TC in biogas. Since this is a closed system, the loss of carbon is very less. So the unaccounted carbon amount is very less.

Table 4.8: Carbon mass balance for 30 days SRT

Carbon data	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
TC in (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
TC out (%)	23.9	37.9	57.8	65.6	70.8	75.6	78.9	84.1
TC in methane (%)	46.9	38.2	25.0	19.0	14.3	11.5	9.9	6.5
TC in CO2 (%)	25.8	19.7	13.3	10.6	8.8	7.1	6.3	4.1
Unaccounted (%)	3.4	4.3	3.9	4.8	6.1	5.8	4.8	5.3

Table 4.9: Carbon mass balance for 20 days SRT

Carbon data	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
TC in (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
TC out (%)	16.9	34.0	58.2	66.5	69.1	70.4	77.5	81.5
TC in methane (%)	54.2	42.4	24.2	19.4	16.2	14.9	10.7	7.6
TC in CO2 (%)	26.7	19.9	13.6	10.5	9.9	9.2	6.9	4.7
Unaccounted (%)	2.2	3.7	4.1	3.6	4.8	5.5	4.9	6.2

Table 4.10: Carbon mass balance for 12 days SRT

Carbon data	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
TC in (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
TC out (%)	67.1	69.4	82.6	85.6	85.8	85.2	88.4	89.7
TC in methane (%)	19.1	16.9	10.2	7.0	5.3	4.3	3.6	2.1
TC in CO2 (%)	11.2	9.1	6.0	4.5	3.4	2.9	2.4	1.4
Unaccounted (%)	2.5	4.7	1.2	2.9	5.6	7.6	5.7	6.8

Table 4.11: Carbon mass balance for 6 days SRT

Carbon data	f:y::1:0	f:y::1:0.5	f:y::1:1	f:y::1:1.5	f:y::1:2	f:y::1:3	f:y::1:4	f:y::0:1
TC in (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
TC out (%)	80.4	83.6	85.0	88.7	90.1	92.7	95.2	92.2
TC in methane (%)	8.7	5.6	4.4	3.4	2.6	2.3	1.7	1.1
TC in CO2 (%)	5.6	4.2	2.9	2.2	1.4	1.2	1.3	0.7
Unaccounted (%)	5.3	6.5	7.7	5.7	5.9	3.8	1.9	5.9

Chapter 5

Conclusion

Lignocellulosic biomass can be used as a renewable source of energy using anaerobic digestion technology. This will reduce our dependence on fossil fuel and petroleum based fuels and will contribute a cleaner environment. The efficiency of anaerobic degradation of lignocellulosic wastes can be enhanced by co digesting the wastes with other substrates like food wastes.Codigesting wastes with food wastes significantly increases the methane production. Co digestion of LCW with food wastes, when mixed in a ratio of 1:0.5 produced 1.1 and 3.2 times more methane than food and yard waste alone, respectively, under same VS loading. A SRT of 20 days was found to be suitable for maximising methane production and VS destruction. A 21% degradation in lignin, 32% degradation in cellulose, 27% degradation in hemicellulose and 75% destruction in VS was observed when reactor was operated at 20 days SRT using a f:y::1:0.5.

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APPENDIX

5.1 Total Solids and volatile solids

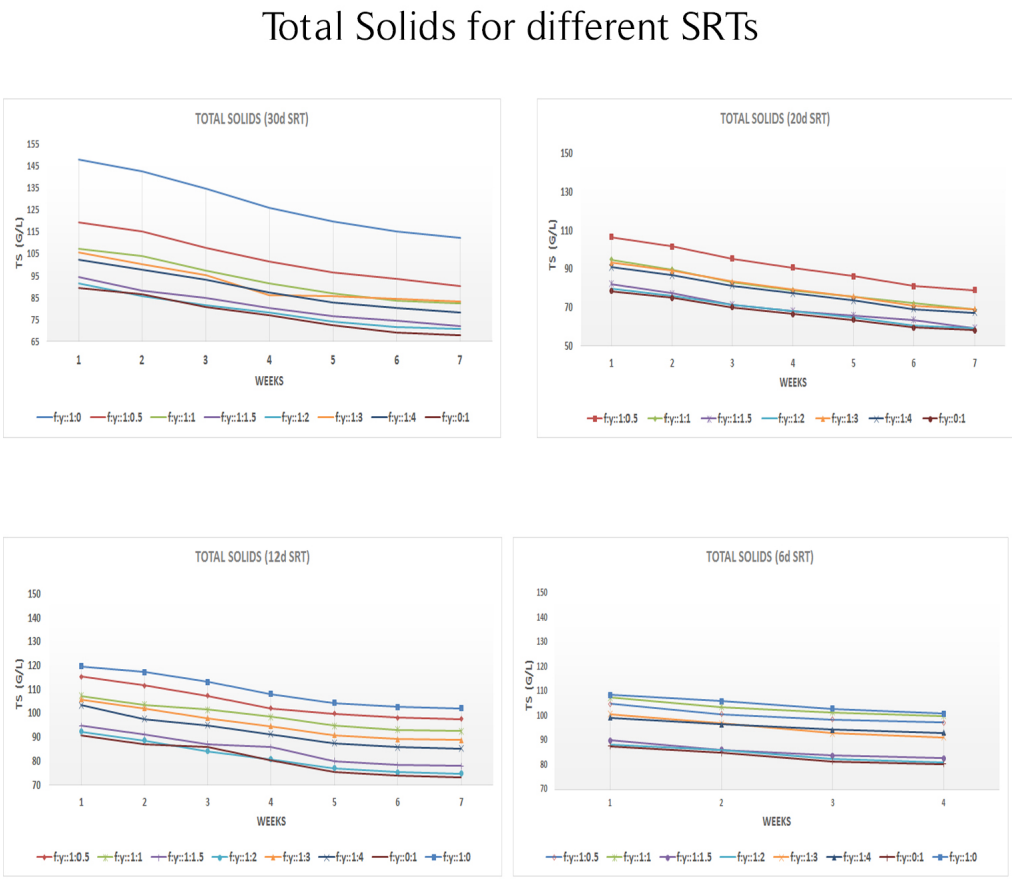

Figure 5.1: Total Solids of reactors for all SRTs

Volatile solids and its destruction for different HRTs

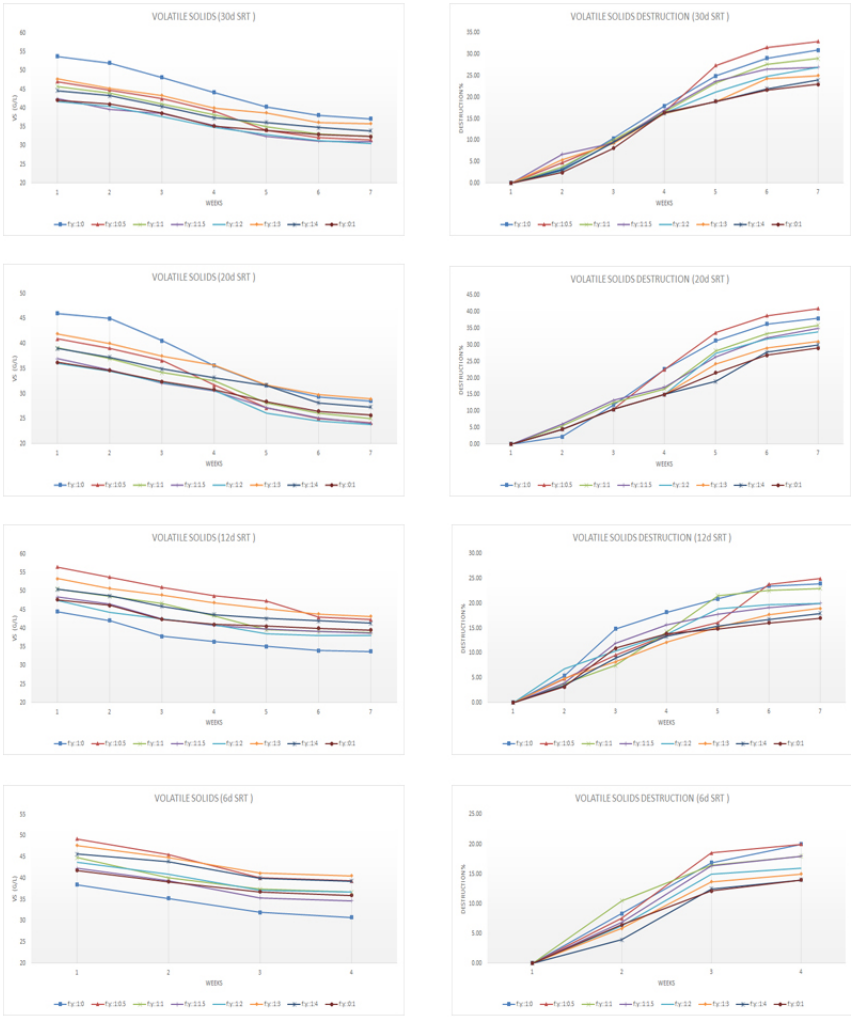

Figure 5.2: Volatile Solids and its destruction with time

5.2 Lignocellulosic Contents and their Degradation

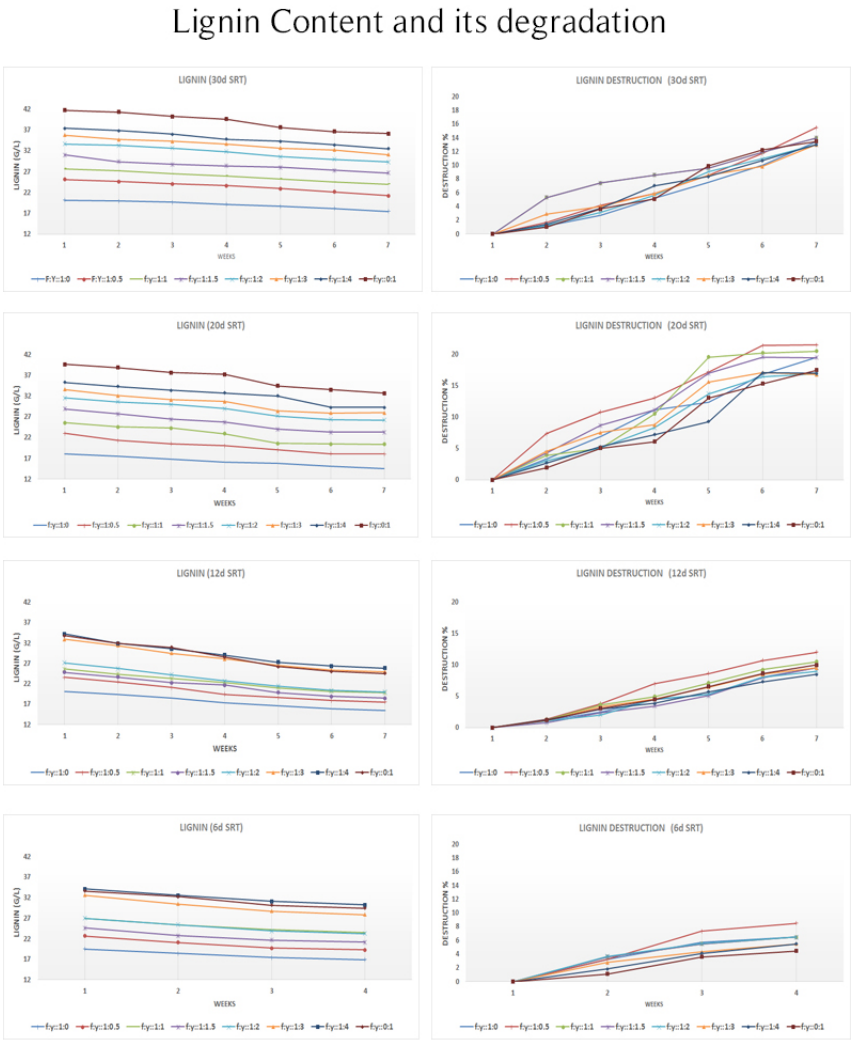

Figure 5.3: Lignin and its destruction with time

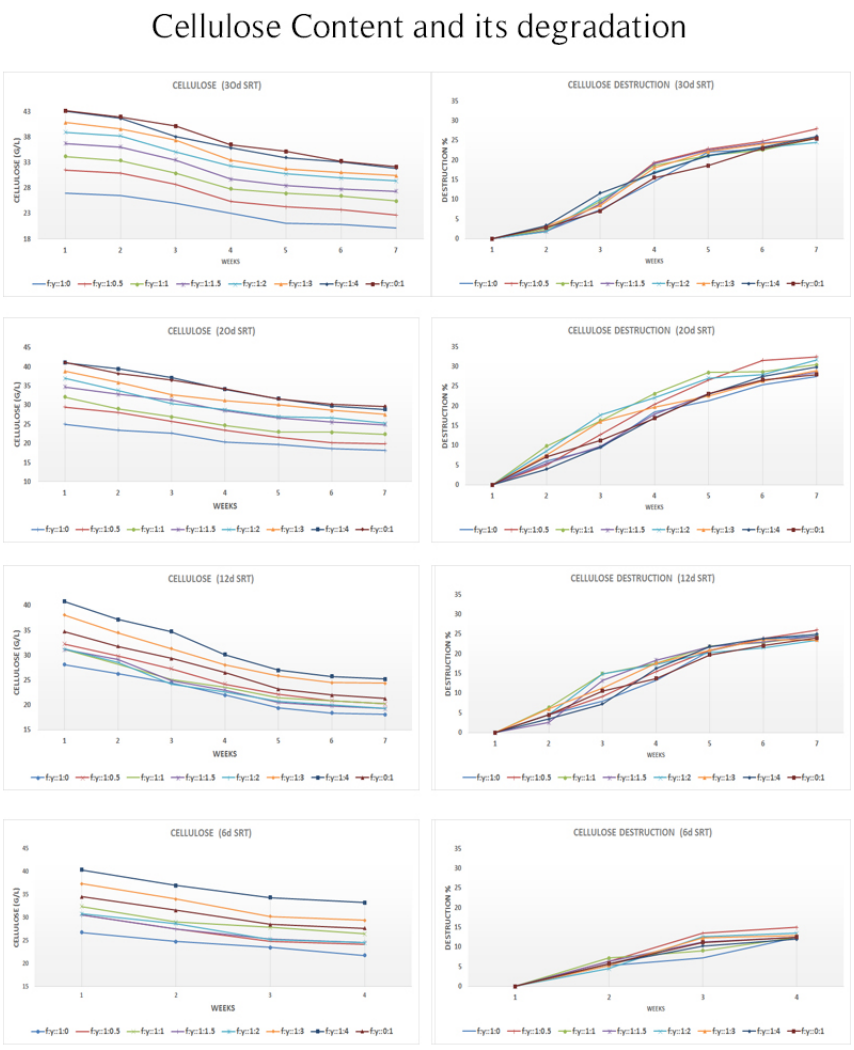

Figure 5.4: Cellulose and its destruction with time

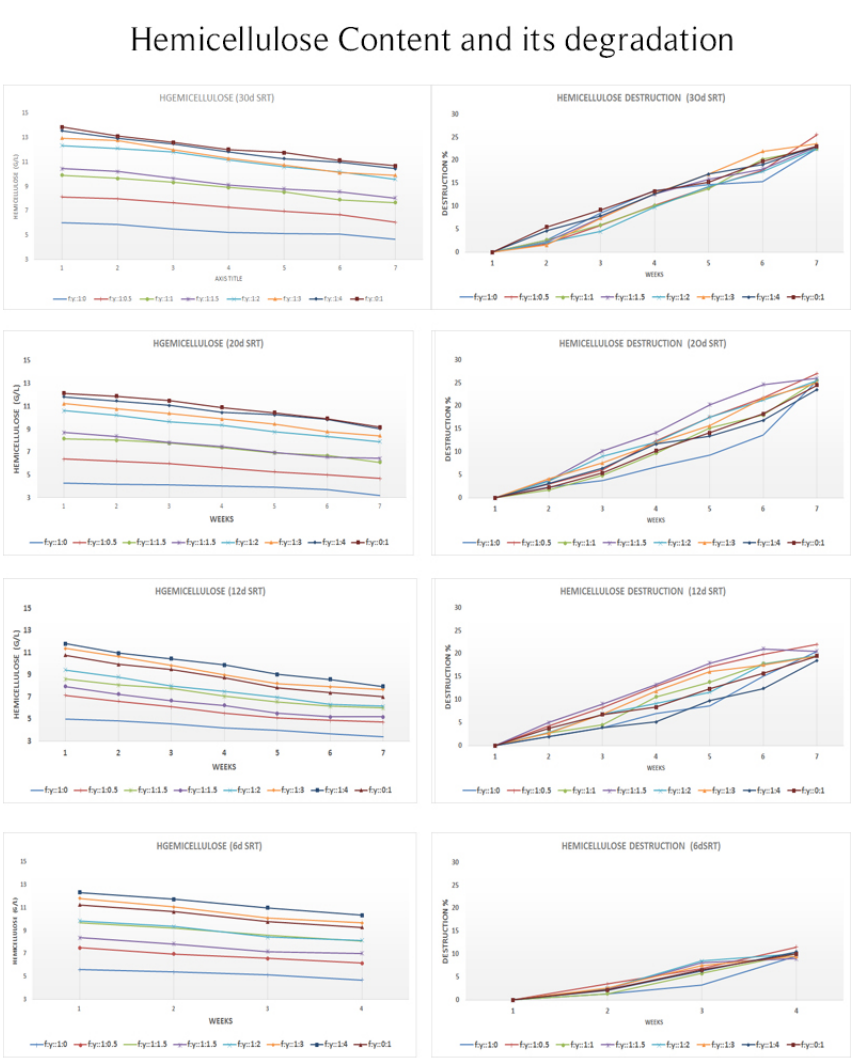

Figure 5.5: Hemicellulose and its destruction with time

5.3 Volatile fatty acids

Figure 5.6: Acetic acid concentration

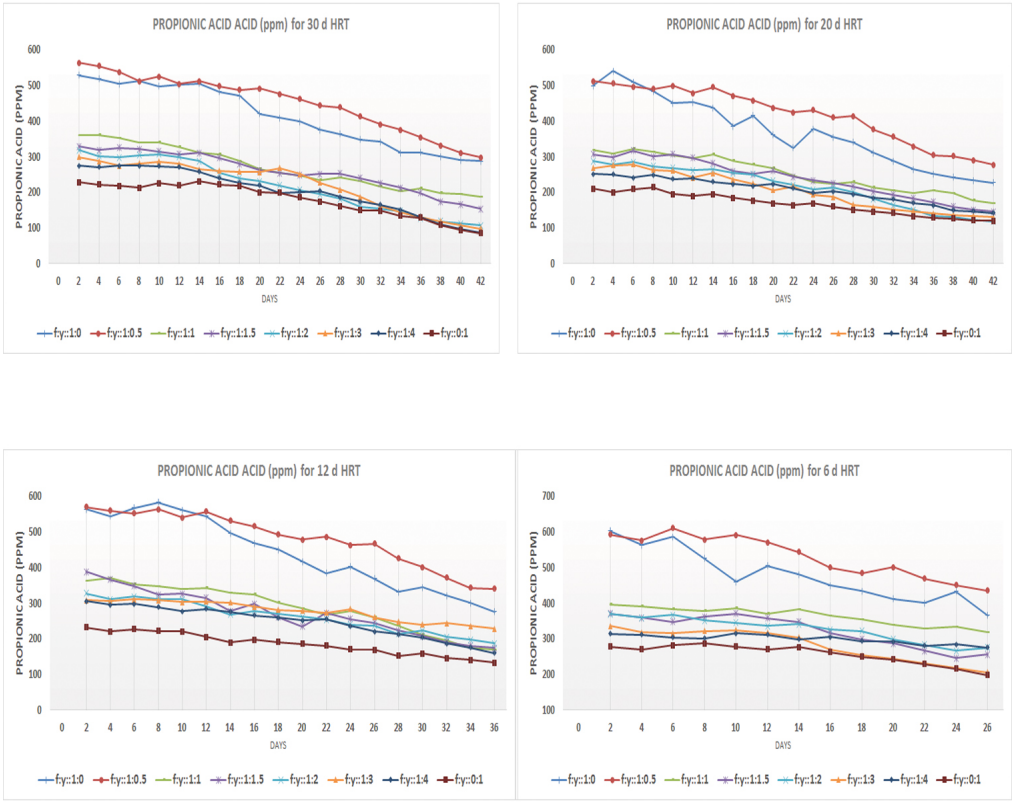

Figure 5.7: Propionic acid concentration

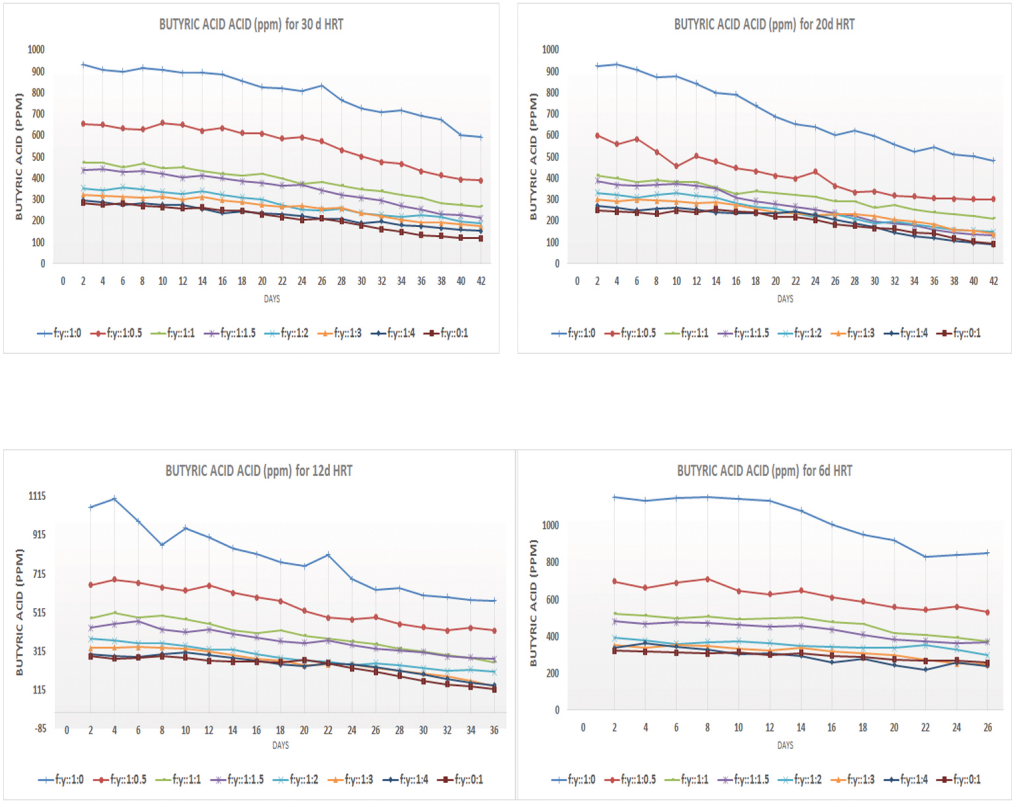

Figure 5.8: Butyric acid concentration