ANAEROBIC DIGESTION OF FOOD WASTE IN A HORIZONTAL PLUG FLOW REACTOR

THESIS

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Abstract

Food waste is the single largest component of the waste stream by weight. These organic wastes needs to be managed in a sustainable way to avoid depletion of natural resources, minimise risk to human health, reduce environmental burdens and maintain an overall balance in the ecosystem. Anaerobic digestion is a promising technology which could effectively address the problem of food waste disposal yielding valuable outputs like biogas and fertilizers. The main focus of this research work was to evaluate and optimize the methane potential of food wastes and to have a process for sustainable energy production for small communities. Our institute is a residential campus with two cafeteria's catering to 2500 students per day and generating one ton of food waste per day. Four different categories of food waste collected from the institute's mess were studied: cooked vegetables (CV), cooked dals (CD), cooked rice (CR), and a mixture of these food wastes (MFW). Biochemical characterization of the waste fractions was carried out and linked to their anaerobic biodegradation. The biogas and methane yield of food waste was evaluated using biochemical methane potential assays and batch anaerobic digestion tests performed at 37^{o}C. The experimental study in batch reactors reveals that all the four wastes degrade to the same extent. Mixing different fractions of food wastes was neither detrimental nor synergistic to biodegradation in anaerobic digestion process.

This study was followed by construction of a horizontal plug flow reactor for anaerobic digestion of food wastes generated in the institute's cafeteria. The performance of this reactor was monitored for approximately two years and microbial community of the anaerobic digester was studied by terminal restriction fragment length polymorphism. The parameters monitored during the study were pH, solid content, chemical oxygen demand and biogas production. *Methanoculleus sp.* was found to be the dominant in all samplings done indicating hydrogenotrophic pathway of methanogenesis. Diversity indices like Shannon

Weaver diversity index (H), Simpson index (D) and Equitability index (E) describing various aspects of ecosystem was determined. The values indicated high diversity but less evenness.

Although food waste has been regarded as readily biodegradable because of its high volatile fraction (90 % of total solids), its hydrolysis reaction is still a rate limiting step. Enhancement of the hydrolytic reaction during anaerobic digestion could shorten the hydraulic retention time and thus improve the economics of the process. The commonly seen undigested solid fractions in the outlet of the pilot scale anaerobic digester set up in the institute's premises are cottage cheese, whole potatoes and whole eggs. The pre-treatments studied (thermal, chemical, thermo-chemical and enzymatic) effectively hydrolyzed cottage cheese into soluble organic compounds. The enzymes were especially suitable for protein and lipid rich cottage cheese with low dose requirement.Utilizing undigested, fractions by anaerobic digestion after pretreatment would capture residual methane and consequently, could reduce greenhouse gas emissions.

Further, the effect of microbial stimulants on anaerobic digestion of food waste was studied. Plant secondary metabolites namely caffeine and saponin which acts like metabolic stimulants are used in this study to see their effects on the anaerobic digestion of food waste. As the processes that take place in rumens are similar to anaerobic digestion, we studied the effect of saponin on anaerobic digestion. We also selected caffeine for our study as caffeine is known to induce different enzymes but phosphodiesterase enzyme is inhibited by caffeine, the stimulatory effect being the result of raised cAMP levels. cAMP, the cell signalling molecule is a general activator of cell activity. Addition of caffeine resulted in increase in biogas production whereas saponin had no beneficial effect.

Apart from food waste, agro wastes and energy crops represent an important source of biomass that can serve as a substrate in anaerobic digestion. The wastes selected for our study are coconut oil cake (residue obtained after oil extraction), cashew apple waste (crushed

apple waste obtained after extraction of “Feni” – “Feni” is a form of liquor i.e., distilled from the pure fermented juice of cashew apple without addition of spirit), and grass from lawn cuttings. The most productive agrowaste was, in terms of methane yield, was coconut oil cake and grass. For cashew apple waste single stage fermentation inhibited biogas production. However, phase separation showed better methane yield.

Anaerobic digestion of food waste gives an opportunity to take responsibility for the food waste generated while ensuring environmental and economic benefits. Anaerobic digestion of food waste in a horizontal plug flow reactor will help in solid waste management for a small community like that of the institute. The digested effluent will be more uniform and predictable product than untreated waste.

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ABBREVIATIONS

AD	: Anaerobic Digestion
ADF	: Acid Detergent Fiber
ADL	: Acid detergent Lignin
APHA	: American Public Health Association
ATCC	: American Type Culture Collection
BLAST	: Basic Local Alignment Search Tool
BMP	: Biochemical Methane Potential
BMP_NDF	: Biochemical Methane potential of Neutral Detergent Fiber fraction
BMP_SOL	: Biochemical Methane potential of soluble fraction
BPR	: Biogas Production Rate
CD	: Cooked Dals
CEL	: Cellulose-like fraction
CFS	: Cell Free Supernatant
CMC	: Critical Micelle Concentration
COD	: Chemical Oxygen Demand
CPCB	: Central Pollution Control Board
CR	: Cooked Rice
CV	: Cooked Vegetables
DGGE	: Denaturing Gradient Gel Electrophoresis
DNA	: Deoxyribonucleic acid
EPA	: Environment Protection Agency
FAO	: Food and Agricultural Organization
FVW	: Fruit and Vegetable waste
GHG	: Greenhouse Gas

HEM : Hemicellulose -like fraction

HRT : Hydraulic Retention Time

ISWA : International Solid waste Association

KVIV : Khadi and Village Industries Commission

LIGN : Lignin-like fraction

MFW : Mixed Food Wastes

MSW : Municipal Solid Waste

NEERI : National Environmental Engineering Research Institute

NDF : Neutral Detergent Fiber

NDRI : Non Dispersive Infrared Analyzer

NMWCO : Nominal Molecular weight cut off

NTYE : NaCl, Tryptone and Yeast extract

OFMSW : Organic Fraction of Municipal Solid Waste

OLR : Organic loading rate

OTUs : Operational Taxonomic Units

PAST : Paleontological Statistics Software

PCR : Polymerase Chain Reaction

PPM : Parts Per Million

SCOD : Soluble Chemical Oxygen Demand

SEM : Scanning Electron Microscopy

SOL : Soluble – like fraction

STP : Standard Temperature and Pressure

TCD : Thermal Conductivity Detector

TCOD : Total Chemical Oxygen Demand

TOC : Total Organic Carbon

T-RFLP : Terminal Restriction Fragment Length Polymorphism

TRFs : Terminal Restriction Fragments

TS : Total Solids

UASB : Upflow Anaerobic Sludge Blanket Reactor

UPGMA : Unweighted Pair Group Method with Arthematic Mean

VFA : Volatile Fatty Acids

VS : Volatile Solids

VSS : Volatile Suspended Solids

WTE : Waste to energy

1 INTRODUCTION

Origin of the research problem

Food waste is the single largest component of the waste stream by weight. The food waste includes uneaten food and food preparation leftovers. In light of rapidly rising costs associated with energy supply, waste disposal and increasing concern with environmental quality degradation, conversion of food wastes to energy is a more economically viable solution (Zhang *et al.*, 2007; Nikolausz *et al.*, 2013). Due to the relatively high moisture content of food waste, bioconversion technologies such as anaerobic digestion are more suitable compared to thermochemical conversion technologies such as combustion and gasification.

Anaerobic digestion (AD) is a biological process in which microorganisms break down organic matter with biogas as the end product. It is a series of chemical reactions during which organic material is decomposed through the metabolic pathways of naturally occurring microorganisms in an oxygen depleted environment. The full process occurs, in four stages namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Amani *et al.*, 2010). AD is a promising technology which could effectively address the problem of food waste disposal thereby yielding valuable outputs like biogas and fertilizers. Thus AD could be an alternative for processing huge amount of food waste. AD without any pretreatment, but with energy recovery is the most attractive method for treating solid wastes (Lastella *et al.*, 2002). Food wastes have high ratios of volatile solids/total solids (80-90%). It is estimated that a methane yield of 0.80-0.96 m^3/kg volatile solids could be achieved by anaerobic digestion of food wastes (Scott and Ma, 2004).

The principal reasons of AD for food waste are:

On-site energy production: By recovering biogas and producing energy, operators can reduce or eliminate monthly energy expenses. Thermal energy for cooking could be acquired by

directly burning biogas or electricity can be produced by utilizing biogas in an engine generator.

Generation of fertilizers: AD process converts the chief nutrients in food waste namely nitrogen, phosphorus and potassium into a soluble form that is more readily available to plants (Lusk, 1999; Cantrell *et al*., 2008).

Reduction in pollution: Digested effluent is a more uniform and predictable product than untreated waste. Its higher ammonium content allows better crop utilization and its physical properties will allow easier land application.

Apart from the above benefits adopting AD also gives an opportunity to take responsibility for the food waste generated while ensuring environmental and economic benefits. The present research work focuses on interrelated scientific topics, like anaerobic digestion, microbial ecology and chemical engineering. This work consists of fundamental research (implementation of indicators for the prediction of methane production from solid wastes) and applied research (optimization of a pilot scale horizontal plug flow reactor for solid food wastes).

The main focus of this research work was anaerobic digestion of food waste with the following objectives:

1. Characterization of food waste and the role of individual organic fractions of food wastes on anaerobic digestion.
2. Pilot scale and microbial diversity studies of anaerobic digestion of food waste in a horizontal plug flow reactor.
3. To study the effect of pre-treatments on undigested food waste fractions.
4. To study the effect of microbial stimulants on anaerobic digestion of food waste.
5. To study the biochemical methane potential of other wastes like agro wastes.

2 REVIEW OF LITERATURE

2.1 History of Anaerobic Digestion

Anaerobic digestion is historically one of the oldest processing technologies used by mankind. In modern age, after the discovery of methane emissions from natural anaerobic habitats by Volta in 1776, people started to collect the natural biogas and used it as a fuel, basically for lighting. In 1808, Sir Humphry Davy demonstrated the production of methane by the anaerobic digestion of cattle manure (Lusk, 1997). The first anaerobic digestion plant was reported to have been built at a leper colony in Bombay, India in 1859. Anaerobic digestion reached England in 1895, when biogas was recovered from sewage treatment facility to fuel street lamps in Exeter (Residua, 2009). The development of microbiology as a science led to research by Buswell and others in 1930's to identify anaerobic bacteria and conditions that promote methane production. The primary aim of waste stabilization in due course of time led to the basic municipal sludge digester. However, it took until the end of the 19^{th} century when anaerobic digestion was applied for the treatment of wastewater and solid waste (Gijzen, 2002). In developing countries such as India and China there was a gradual increase in small-scale AD systems used mostly for energy generation and sanitation purpose.

2.2 International status

An important fraction of the municipal solid waste stream can be defined as organic fraction of municipal solid waste (OFMSW). The food waste which includes both cooked and uncooked leftovers constitutes the largest component of waste coming from the restaurants and fruit/vegetables markets, residences, cafeterias etc. At present, the world wide municipal solid waste generation is about two billion tons per year, which is predicted to increase to three billions tons by 2025 (Charles *et al.,* 2009). A study of global food waste published in 2011 by the Food and agriculture organization (FAO) of the UN found that one third of all food produced for human consumption each year goes to waste which amounts to 1.3 billion tons. This waste is distributed fairly evenly between developing and industrialized nations with 40% of the food waste in the developing nations occurring in the production and processing phases of consumption while in the industrialized nations, 40% occurs at the retail and consumer levels of consumption.

In 2009, USA generated 243 million tonnes of municipal solid waste (MSW) comprising mainly of food scraps, yard waste, plastic packaging, furniture, tyres, appliances, paper, and cardboard. The discarded MSW came from two main sources: residential (55-65%) and commercial/institutional (35-45%). Nearly half of this waste was recycled or reclaimed with 132 million tonnes (54%) going to landfill (EPA, 2009).

The per capita food waste in Europe and North America is 95-115 kg/year (Global, 2011). The average solid waste generation rate in 23 developing countries is 0.77 kg/person/day (Troschinetz and Michelcic, 2009). The municipal solid waste from urban areas of Asia would rise from 760,000 tonnes/day in 1999 to 1.8 million tonnes/day in 2025 (Chandrappa and Das, 2012). These organic wastes requires to be managed in a sustainable way to avoid depletion of natural resources, minimise risk to human health, reduce environmental burdens and maintain an overall balance in the ecosystem (Khalid *et al.,* 2011). Anaerobic digestion

could be an appealing option for converting solid organic waste into useful product like biogas, which will play an important role in meeting the world's ever increasing energy demand in the future. In addition, where anaerobic digestion technology is applied, food waste would not be sent to landfills reducing transportation costs and greenhouse gas emissions (Curry and Pillay, 2012). As a global measure of the expected impacts, if present waste management trends are maintained, landfilled food waste is predicted to increase the landfill share of global anthropogenic emissions from 8 to 10.5% (ISWA, 2012).

A comparison between aerobic and anaerobic treatment methods show that with anaerobic treatment, 80% of the organic load can be reduced to CH_4 and CO_2 with a very small amount of surplus sludge production. In the aerobic treatment, 45% of the organic load is mineralized to CO_2 while 50% is transformed into biomass (Zupančič and Grilc, 2012). Studies carried out by Zhang *et al.,* (2007) indicate that food waste is a highly desirable substrate for anaerobic digesters with regards to its high biodegradability and methane yield. Anaerobic digestion of different types of food waste has been studied extensively. Schober *et al.,* (1999) investigated digestion of kitchen refuse with a total solid concentration of 7-8% VS. With OLR 6.0 $gVSl^{-1}d^{-1}$ and HRT 11d, the maximum VS reduction under steady state conditions reached 72% at mesophilic and 80% at thermophilic conditions. At 55°C the yield of gas, were about 830L gas/kg/VS, whereas at mesophilic conditions only about 800L gas kg/VS were generated. They conducted the digestion in a two stage plant with a concentration unit between the two stages. Under these optimized process conditions a turnover of the organic matter of 90% with low retention time could be realized.

Food waste has high ratios of volatile solids/total solids (80-90%) and has a very interesting methane potential (Scot and Ma, 2004). Cho *et al.,* (1995) conducted batch digestion tests of food wastes at 37^oC and 28 days retention time. The methane yields were 0.48, 0.29, 0.28 and 0.47 L/g VS for cooked meat, boiled rice, fresh cabbage and mixed food wastes, respectively.

Heo *et al.*, 2004 evaluated the biodegradability of a traditional Korean food waste 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 food waste from a restaurant, showing that the food waste contained appropriate nutrients for anaerobic microorganisms, as well as reported a methane yield of 0.44L/g VS of food waste in batch digestion test under thermophilic conditions (50℃) after 28 days. Anaerobic digestion of food waste is achievable, however different types of food waste result in varying degrees of methane yields, and thus the effects of mixing various types of food waste and their proportions should be determined. Neves *et al.*, (2008) reported that the low hydrolysis rate constants were obtained in the assays fed with kitchen waste that contained an excess of lipids.

Bouallagui *et al.*, (2003) tested the conversion of fruit and vegetable waste (FVW) into biogas in a semi continuously mixed mesophilic tubular anaerobic digester. By applying a feed concentration of 6% and HRT of 20 days in the tubular digester, 75% conversion efficiency of FVW into biogas with a methane content of 64% was achieved. In a tubular digester FVW could be treated anaerobically with a high stability, a high depuration rate and energy recovery with a good process economy.

Lastella *et al.*, (2002) evaluated the effects of using different bacterial inocula at identical technical settings on the AD process for the treatment of semisolid organic waste available from the fruit market. They purified the biogas produced by means of CO_2 adsorption, resulting in a higher CH_4/CO_2 ratio and thus improving its use as fuel for power generation. Designs such as batch and plug flow types have significant potential to produce biogas with lower capital investment and higher efficiency.

2.3 National Status

In India, solid waste management falls under the jurisdiction of the municipal authorities (World Bank, 2007). Municipal solid waste (MSW) in India is defined as the non-industrial, non-hazardous solid waste. The municipal solid waste amount is expected to increase significantly in the near future as India strives to attain an industrialized nation status by the year 2020 (Kaushal *et al.,* 2012). According to central pollution control board (CPCB, 2000) report more than 90% of MSW in India is directly disposed of on land in an unscientific manner. As per the Municipal Solid Wastes (Management and Handling) Rules, 2000, "Land- filling shall be restricted to non-biodegradable, inert waste and other waste that are not suitable either for recycling or for biological processing". This is factual in an emerging economy like India because of two important reasons: (a) unavailability of land for disposal of MSW due to rapidly growing population; and (b) disorganized way of MSW disposal which results into emission of greenhouse gases mainly methane (Saini *et al.,* 2012). Since energy is the key for any sustainable economic development, India is losing prospective organic resource by way of improper MSW disposal. It is therefore necessary to harness the locked energy resource from the organic fraction of MSW. Adoption of environment-friendly waste-to-energy (WTE) technologies is one such effective alternative which will help in reducing the space required and will allow treatment and processing of wastes before their disposal.

Indian urban dwellers generate 0.2-0.6 kg per person per day of solid waste resulting into a total generation of nearly 105,000 metric tons of solid waste per day nationally (Kumar, 2010). The per capita waste generation is increasing by about 1.33% annually. The total waste quantity generated by the year 2047 is estimated to be about 260 million tons per year. It is estimated that if the waste is not disposed off in a more systematic manner, more than 1,400 km^2 of land, would be required in the country by the year 2047 for its disposal

(European Business and Technology Centre, 2011). The Municipal bodies spend approximately Rs.500 to Rs.1500 per ton for solid waste management (Jha *et al.,* 2011). Studies carried out in the 59 selected cities have revealed that there are many shortcomings in the existing practices followed for the management of MSW. The main causes for these are inadequate manpower, financial resources, and implements/machinery required to effectively carry out various activities of MSWM (Kumar *et al.,* 2009). Some good efforts regarding municipal solid waste management are observed in the state of Andhra Pradesh (Hyderabad, Guntur and Vijaywada); Goa; Gujarat (Ahmedabad, Surat, Rajkot, Vadodara); Madhya Pradesh (Bhopal and Gwalior); Maharashtra (Pune, Nashik, Nagpur and Mumbai); Uttar Pradesh (Lucknow and Kanpur) and Kolkata (39 Urban Local Bodies) (MoEF, 2011). The National Environmental Engineering Research Institute (NEERI) has carried out studies in more than 50 cities and towns in India. Characterization of MSW indicated that the waste consists of 30-45% organic matter, 6-10% recyclables, and the remaining as inert matter. The organic matter in solid waste in developing countries is much higher than that in the waste in developed countries (CPCB, 2000).The larger proportion of organic matter in MSW indicates the desirability of biological processing of waste. Although composting is a prevalent biological processing practice in India, it is been discouraged due to non-availability of adequate land in the urban centers and poor segregation of wastes.

About 135.5 million tonnes per year of municipal solid waste is generated in India and food waste alone constitutes of about 30-40 % (Kashyap *et al.,* 2003). Anaerobic digestion of food waste has the potential to generate 367m^3 of biogas per dry ton at about 65% methane with an energy content of 6.25 kWh/m^3 of biogas yielding 894 TWh annually (Curry and Pillai, 2012).

India has about 2 million biogas plants of various sizes and capacities ranging from 1m^3 to about 150m^3. These plants have either fixed dome type or floating drum type gas holders.

Most of the plants are cow dung based. The different types of biogas digesters used in India are KVIC design, IARA design, PRAI design, Kamadhenu, Astra model, Jwala model, Ganesh model, Khira model, FRP model, Ferro cement digester model, SERC model, SPRERI model. Detailed description of few of the above mentioned models are given below.

KVIC floating drum

KVIC (Khadi and Village Industries Commission) developed this model in the early sixties. It has an underground cylindrical digester with inlet and outlet connection at the bottom on either side of a masonary wall. An inverted metal drum, which serves as the gas holder rest on a wedge–type support on the top of the digester and as the gas being accumulated, the drum starts rising in height . The gas flows out, the drum gradually moves down. Due to this smooth two-way motion, the gas remains at constant pressure, which ensures the efficient use of the gas.

Fixed-Dome Bio-Gas Plant

This is a spherical type fixed-dome bio-gas plant which ensures that minimum energy is wasted when working with waste. The spherical shape of the plant merges the digestion and gas storage spaces to a single dimension, making their construction easier. It also minimizes the surface area for a given volume, thereby reducing the cost while increasing the gas production rate. The plants have been designed for high efficiency and low maintenance.

Deenbandhu

The Deenbandhu model, developed in 1984, was probably the most significant development in the entire bio-gas program of India, as it reduced the cost of the plant to almost half that of the KVIC model, and brought bio-gas technology within the reach of even the poorer sections

of the population. The word Deenbandu means friend of the poor. The cost reduction has been achieved through minimization of the surface area by joining the segments of two spheres of different diameters at their bases. The structure acts as digester, and pressure is exerted on the slurry again which is pushed into a displacement chamber. Once the gas is drawn out from the outlet, the slurry again enters the digester.

Khoiyangbam et al., (2004) had reported that annual contribution per fixed dome biogas plant (capacity $2m^3$) to the global methane budget in plain region of northern India was 53.2kg as compared to 22.3 kg of hilly area due to the difference in the ambient temperature under the two climatic conditions of hills and plain regions affects CH_4 flux. Kumar *et al.,* (2004) had made qualitative assessment of the methane emission data at Okhla landfill site in Delhi, India. They pointed out the shortcomings in Indian sanitary landfill management and record keeping which includes constraints in data collection, municipalities do not maintain the solid waste data due to lack of awareness, small financial budget and low priority. Singh and Sooch, 2004 had compared the economics of family size biogas plants i.e with capacity from 1 to $6m^3$ of three prevalent models like KVIC, Janta and Deenbandu. Comparison of the economics revealed that the cost of installation and annual operational cost of each capacity were higher for KVIC model followed by Janta and then the Deenbandhu model.

2.4 Microbiology of Anaerobic Digesters

Anaerobic digestion is a process in which organic matter is degraded by a consortium of microorganisms in the absence of oxygen to produce a mixture of methane and carbon dioxide called biogas. In the anaerobic digestion process different types of bacteria degrade the organic matter successively in a multistep process. The relative abundance of bacteria within an anaerobic digester often is greater than 10^6 cells per millilitre. This population consists of saccharolytic bacteria (10^8cells/ml), proteolytic bacteria (10^6cells/ml), lipolytic bacteria (10^5cells/ml), and methane forming bacteria (10^8cells/ml) (Geradi, 2003).

2.4.1 Stages in Anaerobic Digestion

The anaerobic process of complex organic polymers is divided into four interrelated steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis.

2.4.1.1 Hydrolysis

Hydrolysis is a reaction that breaks down the complex organic molecules such as polysaccharides into soluble monomers, proteins and lipids. This reaction is catalysed by enzymes like cellulose, protease and lipase excreted from the hydrolytic and fermentative bacteria. End products of this reaction are soluble sugars, amino acids, glycerol and long chain carboxylic acids (Ralph and Dong, 2010). Hydrolysis is a rather slow and energy consuming process and is normally considered as the overall rate limiting step for the complete anaerobic digestion of complex polymers (Gallert and Winter, 1999).

2.4.1.2 Acidogenesis

In the acid forming stage, soluble compounds produced through hydrolysis are degraded by a large diversity of facultative anaerobes and strict anaerobes through number of fermentative processes. The degradation of these compounds results in the production of carbon dioxide, hydrogen gas, alcohols, organic acids, some organic nitrogen compounds and organic sulphur

compounds. The most important of the organic acids is acetate since it can be used directly as a substrate by methanogenic bacteria. Acidogens have notably high growth rates compared to the methanogens and can survive extreme conditions such as low pH, high temperature and high OLRs (Ahring *et al*., 2001).

2.4.1.3 Acetogenesis

In this step low molecular weight volatile fatty acids are converted into acetate, hydrogen gas and carbon dioxide by acetogenic bacteria. This conversion process can only be thermodynamically favoured if the partial hydrogen pressure is kept low (Gerardi, 2003).

2.4.1.4 Methanogenesis

Finally, methane gas is produced by methane forming bacteria. Methane is formed around 66% from acetate decarboxylation proceeded by acetoclastic methanogenic bacteria and 34% from carbon dioxide reduction by hydrogen, catalysed by hydrogen utilizing methanogenic bacteria.

2.4.2 Syntrophic acetate oxidizing bacteria

Acetate is the main precursor for methane production during anaerobic digestion of organic matter. The degradation of acetate proceeds through two pathways: acetate cleavage by aceticlastic methanogens, being carried out by two genera of methanogens *Methanosarcina* and *Methanosaeta* (Hattori, 2008) and syntrophic acetate oxidation. The second pathway comprises a two-step reaction in which acetate is first oxidized to hydrogen and carbon dioxide and, these products, are subsequently converted to methane. This reaction is carried out by acetate oxidizing bacteria (*Clostridium* spp) in a syntrophic association with hydrogenotrophic methanogens of the order *Methanobacteriales* or *Methanomicrobiales* (Karakashev *et al.*, 2006). The decomposition pathway of acetate is known to be affected by the temperature, concentrations of ammonia and amino acids, the types of reactors and organic loading rate (Hattori, 2008; Hao *et al.*, 2011; Sasaki *et al.*, 2011).

2.4.3 Acetate Forming Bacteria

Acetate forming bacteria grow in a symbiotic relationship with methane forming bacteria. Acetate serves as a substrate for methane forming bacteria. When acetate forming bacteria produce acetate, hydrogen also is produced. If the hydrogen accumulates and significant hydrogen pressure occurs, the pressure results in termination of activity of acetate forming bacteria and loss of acetate production. However, methane forming bacteria utilize hydrogen in the production of methane and significant hydrogen pressure does not occur. Acetate forming bacteria are obligate hydrogen producers and survive only at very low concentration of hydrogen in the environment. They can only survive if their metabolic waste hydrogen is continuously removed. This is achieved because of their symbiotic relationship with hydrogen utilizing bacteria or methane forming bacteria.

2.4.4 Methane Forming Archaea

The methanogens are grouped in the domain Archaea. Hydrogen consuming methane production results in greater energy gains for methanogens than acetate degradation (Von Stockar *et al.*, 2006). In this reaction, hydrogen and carbon dioxide are converted into methane; therefore, entropy decreases, and heat is liberated. In contrast, acetate oxidation is entropy driven reaction in anaerobic cultures (increasing entropy). It exports the excess entropy by turning one molecule in an aqueous state into two gaseous molecules methane and carbon dioxide, which increases entropy considerably (Vlyssides *et al*., 2008). Although methane production using hydrogen is the more effective process of energy capture by methanogens, less than 30% of the methane produced in an anaerobic digester occurs by this method due to the limited supply of hydrogen in an anaerobic digester (Gerardi, 2003).

Although anaerobic digestion can be considered to take place in four stages all reactions occur simultaneously and are interdependent.

2.5 Important parameters in anaerobic digestion of solid waste

2.5.1 pH

The pH of the digester is an important indicator of the performance and the stability of an anaerobic digester. The pH level changes in response to biological conversions during the different processes of anaerobic digestion. A stable pH indicates system equilibrium and digester stability. Many aspects of the complex microbial metabolism are greatly influenced by pH variations in the digester. Although acceptable enzymatic activity of acid forming bacteria can occur at pH 5.0, methanogenesis proceeds at a high rate only when the pH is maintained in the neutral range. Most anaerobic bacteria, including methane-forming bacteria, perform well within a pH range of 6.8 to 7.2. (Gerardi, 2003). Zhang *et al.,* (2007) reported that anaerobic digestion of kitchen wastes with controlled pH value at 7.0 resulted in a relatively high rate of hydrolysis and acetogenesis with about 86% of total organic carbon and 82% of chemical oxygen demand were solubilized.

The pH in an anaerobic digester initially will decrease with the production of volatile acids. However, as methane-forming bacteria consume the volatile acids and alkalinity is produced, the pH of the digester increases and then stabilizes. At hydraulic retention time of more than five days, the methane-forming bacteria begin to rapidly consume the volatile acids. In a properly operating anaerobic digester a pH between 6.8 and 7.2 occurs as volatile acids are converted to methane and carbon dioxide (CO_2). The pH of an anaerobic digester is significantly affected by the carbon dioxide content of the biogas.

Digester stability is enhanced by a high alkalinity concentration. The composition and concentration of the feed sludge directly influence the alkalinity of the digester. For example, large quantities of proteinaceous wastes transferred to the anaerobic digester are associated with relatively high concentrations of alkalinity. The alkalinity is the result of the release of amino groups ($–NH_2$) and production of ammonia (NH_3) as the proteinaceous wastes are

degraded. Alkalinity is present primarily in the form of bicarbonates that are in equilibrium with carbon dioxide in the biogas at a given pH.

If the feed sludge to the anaerobic digester does not contain alkali compounds or precursors of alkali compounds, alkalinity must be added to the digester to maintain stable and acceptable values for alkalinity and pH. The quantity of alkalinity to be added should be based on the anticipated organic acid production capacity of the sludge feed (1 g of volatile acids per gram of volatile solids). Also, if the rate of acid production exceeds the rate of methane production, alkalinity must be added in the form of bicarbonates.

2.5.2 Substrate characteristics

The characterization of substrate is a key factor for the design and optimization of waste treatment and disposal methods. The physical and chemical characteristics of the organic wastes are important information for designing and operating anaerobic digesters, because they effect biogas production and process stability during anaerobic digestion. They include, but not limited to, moisture content, volatile solids content, nutrients contents, particle size and biodegradability. The results of the anaerobic digestion tests showed that food waste had average methane yields of 435 mL/gVS after 28 days of digestion at 50 ±2°C (Zhang *et al*., 2007). The methane yield accounted for 53% of the biogas produced. Sambo *et al.,* (1995) presented the results of a series of studies in which the effect of temperatures, pH, carbon-nitrogen ratio and retention time on biogas production from cow dung were investigated.

The most common data obtained on wastes is the amount of organic matter in terms of volatile solids and the biochemical methane potential. It is known, however, that the anaerobic biodegradability of organic matter depends on its composition, and that the amount of methane produced depends on the biochemical nature of the waste (Buffiere *et al.,* 2006). For instance carbohydrates, proteins and fats show different methane production rates (Angelidaki and sanders, 2004). Among these lipids are the most significant substances in the

anaerobic digestion, since the methane yield from lipids is higher than from most other organic materials. For example, the theoretical gas yield of glyceride trioleate is 1.4m^3 per kilogram of oil with a methane content of 70% (Angelidaki *et al.,* 1990). Although organic waste with a high content of lipids is an attractive substrate for biogas production (Neves *et al.,* 2008) reported that the low hydrolysis rate constants were obtained in the assays fed with kitchen waste that contained an excess of lipids. This was presumably due to a synergistic effect on the degradation of the other components since lipids adsorb onto solid surfaces and may delay the hydrolysis process by reducing the accessibility of enzyme attack.

The composition of the waste in terms of lignocellulosic compounds also influences the biodegradability (Owen *et al.,* 1979; Chynoweth *et al.,* 1993; Cho *et al.,* 1995; Angelidaki and Sanders, 2004; Hansen *et al.,* 2004; Buffiere *et al,* 2006; Gunaseelan, 2007). The composition of waste also determines the relative amounts of organic carbon and nitrogen present in the waste substrate (C/N ratio). A solid waste substrate with high C/N ratio is not suitable for bacterial growth due to deficiency of nitrogen. As a result the gas production rate and solid degradability will be low. On the other hand, if the C/N ratio is very low, the degradation process leads to ammonia accumulation which is toxic to the bacteria (Hartmann and Ahring, 2006). Kayhanian and Hardy, (1994) found that a C/N ratio within the range of 25-30 is considered to be optimum for an anaerobic digester.

The particle size has a significant role in anaerobic digestion of solid waste, especially during hydrolysis since a smaller particle size provides a greater area for enzymatic attack (Hartmann and Ahring, 2006). The increase of the average particle size in anaerobic digestion of food waste was reported to decrease the maximum substrate utilization rate coefficient (Kim *et al.,* 2000).

2.5.3 Temperature

Temperature is one of the most important factors affecting microbial activity in anaerobic digester, and methane production is strongly temperature dependent. Temperature determines the rate of an anaerobic degradation processes particularly the rates of hydrolysis and methanogenesis. There are three widely known and established temperature ranges of operation: psychrophilic (15-20^{o}C), mesophilic (30-40^{o}C) and thermophilic (50-60^{o}C). When selecting the temperature range, it should be kept constant as much as possible. In thermophilic range (50-60°C) fluctuations as low as ±2°C can result in 30% less biogas production (Zupancic and Jemec, 2010). Therefore it is advised that temperature fluctuations in thermophilic range should be no more than ±1°C. In mesophilic range the microorganisms are less sensitive; therefore fluctuations of ±3°C can be tolerated. With increasing temperature the reaction rate of anaerobic digestion strongly increases, thus promoting application of higher organic loading rate without affecting the organic removal efficiency (Desai *et al.,* 1994; Chae *et al.,* 2007). Using palm oil mill effluent as the substrate, Choorit and Wisarnwan, (2007) demonstrated that when the digester was operated at thermophilic temperature (55^{o}C), higher OLR is possible than that of mesophilic (17.01 against 12.25 gCOD/l/d) and the methane productivity was also higher (4.66 against 3.73 l/l/d). A similar study by Chae *et al.,* (2007) indicated that higher temperature of 35^{o}C led to the highest methane yield as compared to 30^{o}C and 25^{o}C although the methane contents only changed slightly (Chae *et al.,* 2007). Using cheese whey, poultry waste and cattle dung as substrates, Desai *et al.,* 1994 showed that when the temperature was increased from 20^{o}C to 40^{o}C and finally to 60^{o}C, the gas production as well as methane percentage in biogas increased.

Although the thermophilic anaerobic process could increase the rate of reaction, the yield of methane that could be achieved over the specified organic amount is the same regardless of the mesophilic or thermophilic conditions (0.25 kg CH_4/kg COD removed or 0.35 m^3 CH_4/kg

COD removed (at standard temperature and pressure i.e 0^{o}C, 1 atm.). Thermophilic process offer faster kinetics, higher methane production rates and pathogen removal. However, it is more sensitive to toxic substance and changes in operational parameters (Mata Alvarez, 2002). Furthermore, biomass washout that could lead to volatile fatty acids accumulation and methnogenesis inhibition could also occur if the thermophilic temperature could not be controlled (Poh and Chong, 2009). As a result, in tropical regions, mesophilic temperatures are the preferred choice for anaerobic treatment (Yacob *et al.,* 2005; Sulaiman *et al.,* 2009). Mesophilic bacteria are supposed to be more robust and can tolerate greater changes in the environmental parameters, including temperature. Although it requires longer retention time, the stability of the mesophilic process makes it more popular in current anaerobic facilities (Zaher *et al.,* 2007).

2.5.4 Nutrients

A balanced availability of nutrients for the growth of the microorganisms in biogas digesters is important for the process performance, i.e stability and substrate utilization (Takashima and Speece, 1989). Apart from a balance among the macronutrients carbon, nitrogen and phosphorus the availability of certain trace elements has also been shown to strongly impact the biogas production. Often, additions of a single or combinations of trace elements to laboratory scale processes have improved the performance, with a faster turnover of substrate and lower levels of VFA. Positive effects have been reported both on the overall degradation process and on specific substrates such as acetate, propionate or methanol (Pobeheim *et al.,* 2010). However combinations of several trace elements have shown both synergistic and antagonistic effects. Murray and Van Dan Berg, (1981) found that cobalt combined with Nickel increased the turnover of acetate more than expected when compared with

experiments where these metals were added individually. In addition, molybdenum was shown to enhance reactor performance only in combination with nickel and cobalt.

Kim *et al.,* (2002) reported that organic loading is an important factor when evaluating the trace element additions on a specific process. Furthermore, the main elements studied in relation to methane production efficiency are iron, nickel and cobalt, while reports on selenium, tungsten and other trace elements are scarce. In most cases, the effect of trace element addition has been assessed as process performance like methane production, volatile solid reduction, levels of volatile fatty acids. These parameters have, in some studies, been linked to total methanogenic activity tests (Patidar and Tare, 2006), specific methanogenic activity (Zandvort *et al.,* 2006) and maximum potential of acetate utilization (Zitomer *et al.,* 2008). Fermoso *et al.,* (2008) has linked process parameters to microbial community compositions. These authors investigated the effect of cobalt deprivation on the microbial community of a methanol fed up flow anaerobic sludge blanket reactor using specific methanogenic activity. Feng *et al.,* (2010) studied the effects of various concentrations of trace elements on biogas production and bacterial and archeal community composition in reactors fed with food industrial wastes at a final organic loading rate of 4.0 g VS/l/day.

Specific trace metals such as cobalt, nickel, tungsten or molybdenum serve as cofactors in enzymes which are involved in the biochemistry of methane formation (Zandvoort *et al.,* 2006). Apart from the enzymatic functions of the micronutrients, there also exist non enzymatic functions such as electron transfer in microbial respiration processes acceptors (Zandvoort *et al.,* 2006). Metal ions or micronutrients in this case are analogous to oxygen in aerobic respiration. Usually, the oxidation of organic matter is coupled to metal reduction and this can be energy yielding to the anaerobic bacteria. Recently the effect of trace elements on mesophilic anaerobic digestion of food waste was studied using inoculum of different origins (Facchin *et al.,* 2013). Zhang *et al.,* (2013) has reported enhancement in process performance

by supplementing trace elements during continuous anaerobic digestion of food waste. Trace metal requirements for continuous methane fermentation at thermophilic temperature has been reported by Qiang *et al.,* (2013).

2.5.5 Mixing

Mixing facilitates the contact between bacteria/enzymes and substrates preventing the accumulation of substrates and intermediates and guarantee homogenous conditions in the assays vessels (Angelidaki *et al.,* 2009). It also prevents the thermal stratification and the formation of a surface crust/scum build up in an anaerobic reactor (Karim *et al.,* 2005; Meroney and Colorado, 2009). Furthermore, mixing ensures that solids remain in suspension avoiding formation of dead zones by sedimentation of sand or heavy solid particles. Mixing also enables the particle size reduction as digestion progresses and the release of produced biogas from the digester contents (Kaparaju *et al.,* 2007). The effect of mixing duration and intensity on the performance of anaerobic digestion varies considerably. Stroot *et al.,* (2001) reported that minimal mixing resulted in excellent performance of high solids digestion of OFMSW with higher gas production rates and specific gas production. Minimally mixed solid waste presumably resulted in slower hydrolysis and acidogenesis, allowing syntrophs and methanogens to consume the fermentation products and by avoiding inhibition through accumulation of these compounds. Vigorous and continuous mixing was reported to be inhibitory at high organic loading rates probably due to the disruption of syntrophic relationships.

The mixing strategy can be performed in many ways, including continuous mixing, intermittent mixing and minimal mixing (Kaparaju *et al.,* 2007). According to Appels *et al.,* (2008) mixing can be performed through several means such as mechanical mixers, recirculation of slurry (digesting sludge), or by injection of the produced biogas. Mechanical

mixing systems generally use low speed flat blade turbines and are most suited for digesters with fixed covers. The digesting sludge is transported by the rotating impellers, thereby mixing the content of the digestion tank. Slurry recirculation is provided by centrifugal pumps, generally set up in an internal or external shaft tube to support vertical mixing. Slurry recirculation is performed by withdrawing the digesting sludge from the centre of the digester. The sludge is then pumped through external heat exchangers, where the digested sludge is blended with the raw sludge and heated to the desired temperature. It is then pumped back in the digestion tank through nozzles at the base of the digester or at the top to break the scum layer. The disadvantage of this method is that the flow rate in the recirculation should be very large to ensure a complete mixing thus the energy required is high. Biogas recirculation is a successful method of mixing the digester content and avoids the build-up of scum.

2.5.6 Inhibitions

Inhibition is usually indicated by a decrease in the microbial population and methane production. A wide variety of substances have been reported to be inhibitory to the anaerobic digestion processes. These kinds of substances can be found as components of the feeding substrate or as by-products of the metabolic activities of bacterial consortium in the digester. A material may be judged as inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth. Significant differences in inhibition or toxicity levels have been reported for most substances because of the natural complexity of the anaerobic digestion process and various biochemical mechanisms that affect inhibition, such as antagonism, synergism and acclimation (Chen *et al.*, 2008). Antagonism is defined as a reduction of toxic effect of one substance by the presence of another, whereas synergism is an increase in the toxic effect of one substance by the presence of another. Acclimation is the

ability of microorganisms to rearrange their metabolic resources to overcome the metabolic block produced by the inhibitory or toxic substances when the concentrations of these substances are slowly increased within the environment.

2.5.6.1 Ammonia

Ammonia is produced by the biological degradation of the nitrogenous matter, mostly in the form of proteins and urea (Kayhanian, 1999). Several mechanisms for ammonia inhibition have been proposed, such as a change in the intracellular pH, increase of maintenance energy requirement and inhibition of a specific enzyme reaction (Whittmann *et al.*, 1995). Ammonium ion (NH_4^+) and free ammonia (NH_3) are the two principal forms of inorganic ammonia nitrogen in aqueous solution. Free ammonia (1700 mg/l) is the most toxic because it can pass through a cell membrane, causing a proton imbalance and potassium deficiency (Sung and Liu, 2003). Ionic ammonia is less toxic; a concentration around 5000 mg/l affects acidogens and decreases the activity of methanogens by 50% (Sung and Liu, 2003). An increase in pH will result in a higher toxicity level due to a higher ratio of free ammonia to its ionized form. It is generally believed that ammonia concentrations below 200 mg/l are beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms (Liu and Sang, 2002). The presence of other ions, such as Na^+, K^+, Ca^+ and $Mg^{2+,}$ were found to be antagonistic to ammonia inhibitions (Hendriksen and Ahring, 1991).

2.5.6.2 Sulfide

In anaerobic reactor, sulphate is reduced to sulphide by the sulphate reducing bacteria. Sulphate reduction is performed by two major groups of sulphate reducing bacteria including incomplete oxidizers, which reduce compounds such as lactate to acetate and acetate to carbon dioxide, and complete oxidizers, which completely convert acetate to CO_2 and $HCO^-_{3.}$

Two stages of inhibition exist as a result of sulphate reduction. Primary inhibition is due to competition for common organic and inorganic substrates from sulphate reducing bacteria, which suppresses methane production. Secondary inhibition results from the toxicity of sulphide to various bacteria groups (Oude Elferink *et al.*, 1994).

2.5.6.3 Light metal ions

The light metal ions including sodium, potassium, calcium and magnesium are commonly present in the digestate of anaerobic digesters. They may be produced by the degradation of organic matter in the feeding substrate or by chemicals addition for pH adjustment. Moderate concentrations of these ions are needed to stimulate microbial growth, however excessive amounts will slow down growth, and even higher concentrations can cause severe inhibition or toxicity. Toxicity due to salt is primarily associated with bacterial cells dehydration due to osmotic pressure (Nayano *et al.*, 2010). Although the cations of salts in solution must always be associated with the anions, the toxic action of salts was found to be predominantly determined by the cation. The role of anions was relatively minor and largely associated with their effect on properties such as the pH of the media.

2.5.6.4 Heavy metal ions

The presence of heavy metals in trace concentration will stimulate the growth of anaerobic digesters. However, unlike other toxic substances, heavy metals are not biodegradable and can accumulate to potentially toxic concentrations. An extensive study on the performance of anaerobic digester found that heavy metal toxicity is one of the major causes of anaerobic digester upset or failure (Chen *et al.*, 2008). The toxic effect of heavy metals is attributed to their ability to inactivate a wide range of enzyme function and structures by binding of the

metals with thiol and other groups on protein molecules or by replacing naturally occurring metals in prosthetic groups of enzymes.

2.5.7 Hydraulic Retention Time and Organic Loading Rate

Retention time, in the anaerobic reactors, refers to the time that a certain substrate resides in a digester. It is determined by the average time needed for decomposition of the organic material, as measured by the chemical oxygen demand of the influent and the effluent material. The longer the substrate is kept under proper reaction conditions, the more complete will be its degradation. However, the rate of the reaction decreases with longer residence time, indicating that there is an optimal retention time that will achieve the benefits of digestion in a cost effective way (Vishwanath *et al.,* 1991). In a digester with continuous mixing, the contents of the reactor have a relative uniform retention time. In this system, the minimum HRT is dictated by the growth rate of the slowest growing, essential microorganisms of the anaerobic bacterial community. If the HRT is shorter, the system will fail due to washout of the slowest growing microorganisms that are necessary for the anaerobic process (Zaher *et al*., 2007). Shortening the HRT consequently reduces the size of the digester, resulting in capital cost savings. Furthermore, a shorter HRT yields a higher biogas production rate, but less efficient degradation of organic matter, associated with less process stability.

Hartmann and Ahring, (2006) compiled the reports from other researchers and found that the HRT of anaerobic digesters treating solid wastes varied from 3 to 55 days, depending on the type of waste, operational temperature, process stages and configuration of the digesters. The HRT for dry anaerobic digestion ranges between 14 and 30 days and for wet anaerobic processes it can be as low as 3 days. Salminen and Rintala, (2002), however, reported even a

longer retention time of 50-100 days for a digester treating solid waste from poultry slaughterhouse.

Furthermore retention time in the anaerobic digestion system depends on process temperature. Mesophilic digesters have longer retention time (10-40 days) then thermophilic digesters. Commonly used method for shortening the residence time in anaerobic reactors is mixing the digesters.

The organic loading rate (OLR) is defined as the amount of organic matter expressed in terms of volatile solids or chemical oxygen demand that must be treated by a certain volume of anaerobic digester in a certain period of time. The potential danger of a rapid increase in the organic loading rate would be that the hydrolysis and acidogenic bacteria would produce intermediary products rapidly. Since the multiplication time of methanogenic bacteria is slower, they would not be able to consume the fatty acids at the same rate. The accumulation of fatty acids will lead to a pH drop and affect the activity of methanogenic bacteria, causing the digester failure.

2.6 Types of anaerobic reactors

Anaerobic reactors or processes of solid waste can be distinguished into several types, based on the feeding mode (batch mode and continuous mode: single stage, two stages) and based on the moisture content of the substrate (wet or dry digestion). Furthermore with those basic types, the anaerobic reactors can be arranged according to the digestion process temperature (mesophilic or thermophilic) and the shape of the reactors (vertical or horizontal).

2.6.1 Batch and continuous feeding systems

There are generally two feeding modes in anaerobic digestion of solid waste: the batch system and the continuous system. In the batch system, digesters are filled once with fresh feedstock, with or without addition of inoculum, and sealed for the complete retention time,

after which it is opened and the effluent is removed. In the continuous system, fresh feedstock is continuously fed to the digester and an equal amount of digested material is withdrawn. Although batch systems have not succeeded in taking a substantial market share, especially in more developed countries, however the specific features of batch processes such as simple design and process control, robustness towards coarse and heavy contaminants, and lower investment cost make them particularly attractive for developing countries (Mata Alvarez, 2003). As discussed previously, the anaerobic digestion of organic wastes is accomplished by a series of biochemical processes. These processes can be separated into two main stages: the first stage where hydrolysis, acidification and liquefaction take place and the second stage where acetate, hydrogen and carbon dioxide are converted into methane. In one stage system, all these reactions take place simultaneously in a single reactor, while in two or multi stage system, the reactions take place sequentially in at least two reactors.

The major drawback of single stage digester systems is that these processes are required to proceed under the same operating conditions despite differences in growth rates and optimal pH of the microbial groups involved in each step. This is the reason why single stage systems are more easily upset compared to multi stage systems. This disadvantage is substantial especially in the case of substrates where degradation is limited by methanogenesis rather than by hydrolysis, e.g. cellulose poor kitchen wastes. These wastes, being very rapidly acidified, tend to inhibit the methanogenesis when the feedstock is not adequately mixed and buffered (Gerardi, 2003).

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; de Baere, 2000). 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 (de Baere, 2000; Vandevivere *et al.*, 2002).

2.6.2 Wet and dry anaerobic digestion

Anaerobic digestion processes can be termed as "wet" and "dry" digestions depending on the total solids concentration of the feed substrate. Anaerobic digestion is defined as a wet process if the total solids concentration of the substrate is less than 15% and as a dry process if the concentration reaches 20-40% (Lissens *et al* ., 2001). In wet digestion processes, the solid waste has to be conditioned to the appropriate solids concentration by adding process water either by recirculation of the liquid effluent fraction or by co-digestion with a liquid waste. The latter is an attractive method to combine several waste streams like sewage sludge or manure and OFMSW (Luning *et al.,* 2003, Hartmann and Ahring, 2006). Reactors used in wet digestion processes generally are referred to as continuous stirred tank reactors (CSTR), with application of mechanical mixers or a combination of mechanical mixing and biogas injection (Banks and Stentiford, 2007). The application of a wet digestion process offers several advantages such as dilution of inhibitory substances by process water and requirement of less sophisticated mechanical equipment. However, disadvantages, such as complicated pre-treatment, high consumption of water and energy for heating and the reduction of

working volume due to sedimentation of inert materials have to be taken into account (Vandevivere *et al*., 2002; Banks and Stentiford, 2007).

The reactors used in dry anaerobic digestion processes generally do not apply mechanical mixers and may use biogas injection to perform mixing of the digester content (Luning *et al*., 2003). However, using this technique, complete mixing of the digestate is almost impossible; thus, the ideal contact of microorganisms and substrate cannot be guaranteed. As a consequence, individual processes may run in different parts of the reactor, which limits an optimal co-operation of the microbial groups involved in the digestion process (Hartmann and Ahring, 2006). Thus, the digesters used in dry anaerobic digestion can be considered as plug flow reactors. Dry anaerobic digestion offers less complicated pre-treatments and higher loading rate (10 kg VS.m^3.d.1 or more). However, the systems require more sophisticated mechanical equipment (Lissens *et al*., 2001) and less possibility to dilute the inhibitory substances (Vandevivere *et al.,* 2002). In general, both anaerobic digestion processes can be considered a proven technology for the treatment of organic solid waste. Biogas yield as such is however of very little use because it is much more dependent on waste composition than on process performance. For example, the methane yield in one full-scale plant varied between 170 and 320 Nm^3 CH_4/kg VS fed (40 and 75 % VS reduction) during the summer and winter months, respectively, as a result of the higher proportion of garden waste during summer months (Saint Joly *et al.,* 1999). Garden wastes are indeed known to yield much less biogas, relative to kitchen wastes, due to the higher proportion of poorly degradable lignocellulosic fibres. Similarly, Pavan *et al.,* (1998), using the same reactor configuration, observed a two-fold larger volatile solid reduction with source-separated biowaste relative to mechanically-sorted OFMSW. Luning *et al.*, (2003) reported that biogas production figures of the wet digestion process (Waasa process) and the dry digestion process (Valorga process) were identical. The wet process produced more wastewater; however, this was compensated by a

smaller amount of digestate to be disposed of and the separation of inert materials suitable for recycling. De Baere and Mattheews, (2008) reported that although the applications of both systems have continued to increase in total capacity, dry digestion systems have been dominant since the beginning of the 1990's. In 2008, dry anaerobic digestion provided almost 54% of the capacity while the rest applied wet anaerobic digestion.

2.7 Process Enhancement

In recent years, significant efforts has been dedicated in finding ways of improving the performance of digesters treating different wastes, especially solid wastes because of the obvious link between successful pre-treatments and improved yields (Mata Alvarez *et al.*, 2000). Many studies have been conducted regarding almost every aspect of anaerobic digestion of solid waste which is useful for process improvement. The aspects of process enhancement include co-digestion with other wastes, pretreatment of substrate and use of microbial stimulants.

2.7.1 Co-digestion

Co-digestion of food waste with other types of waste is an interesting alternative to improve biogas production and to obtain a more stable process. The use of a co-substrate improves the biogas yields due to positive synergisms established in the digestion medium and supply of missing nutrients by the co-substrates. Various types of solid wastes streams such as sewage sludge, cattle manure and organic industrial waste has been used as co-substrate for anaerobic digestion of food waste. Studies on co-digestion of food waste with other wastes streams, such sewage sludge (Kim *et al.*, 2003; Heo *et al.*, 2003; Kim *et al.*, 2011), cattle dung (Li *et al.*, 2009), dairy manure (Li *et al.*, 2010; El Mashad *et al.*, 2010) and press water (Nayano *et al* 2010). Co-digestion with sewage sludge will improve the characteristics of food waste including its content of micro and macro nutrients leading to a better C/N ratio.

2.7.2 Pretreatments

Food waste contains variable type and amount of organic matter whose behavior in digester depends on the biodegradation of organic pools characterized by different methane production rates. Although food waste has been regarded as readily biodegradable because of its high volatile fraction as 90% of total solids, its hydrolysis reaction is still a rate limiting step (Kim *et al.,* 2006). Enhancement of the hydrolytic reaction during anaerobic digestion could shorten the hydraulic retention time and thus improve the economics of the process. During recent years, various studies have been conducted on pre-treatment of food waste, such as mechanical and sonication (Hidalgo D *et al.,* 2012), sonication at lower frequencies (37 KH_2) enhanced methane production by 10-47 % for agro waste, Thermal (Wang *et al.,* 2006; J. Ma *et al.,* 2011), thermal pretreatment increased the content of methanogens thus increasing the rate of anaerobic digestion, acid addition increased the methane yield with about 50% indicating increase of microbial ability to utilize organic material for biogas production (Karlsson and Ejlertsson, 2012), alkaline treatment improved solubilization of food waste but no significant increase in methane production (Eom *et al.,* 2009) and enzymatic (Rintala *et al.,* 1994; Kim *et al.,* 2006; Moon and Song *et al.,* 2011). Enzyme additions increased the specific methanogenic activity, but methane yield was not improved in batch assays and reactor studies. Kim *et al.,* 2006 reported that enzyme additions increased volatile solid reduction efficiency for food waste. It has improved soluble COD removal efficiency.

2.7.3 Microbial Stimulants

Singh *et al.,* (2001) evaluated the effect of microbial stimulants (Aquasan and Teresan) on biogas yields from cattle dung and combined residues of cattle dung and kitchen waste respectively. In a bench scale study (1:1 dry matter) the addition of Teresan at 10ppm concentration produced 34.8% more gas than the uninoculated mixture at 15% TS concentration. Tests carried with a formulation Teresan in India increased the saprophytic bacterial population significantly in farm yard manure amended soil and enhanced the growth and yield of the treated crops (Kumar, 1994). Aquasan is a highly effective plant extract material derived from Yucca plant having the same composition as Teresan but with added urease. Both of these materials work directly on the microbial population restricting the odour emission by enzyme interference and speeding up digestion by stimulating the bacterial metabolism.

2.8 Gaps in existing Research

It is clear from the literature review that even though a lot of research has been done in the field of anaerobic digestion of different organic matter, experimental data on the biomethane potential of cooked food waste and combined cooked and raw kitchen wastes are limited with respect to Indian context.

Extensive process characterization has been carried out on energy crops and manures, and this could usefully be extended to include food wastes. These studies differ by the selection of operating parameters and thus research at laboratory scale is necessary for determining the operational parameters at full scale, also taking into account that anaerobic digestion of food waste has to face the high heterogeneity that eventually determines waste composition. A pilot scale study will be carried out to validate the results of laboratory scale reactors.

Anaerobic digestion of cattle dung has been the commercial biogas energy production technique for many years in India. For application of this technology to other wastes such as food wastes it is necessary to determine the optimal operating conditions which will increase biogas production.

3 *Characterization of food waste and the role of individual organic fractions of food wastes on anaerobic digestion*

Food waste is the single largest component of the waste stream by weight. Food waste alone constitutes about 30-40% of the total municipal solid waste generated in India (Kashyap *et al.,* 2003). Our institute's cafeteria caters around 2400 students/day. In this process, the catering establishment produces approximately 50 kg of uncooked food and 700-800 kgs of cooked food as waste. The food waste includes uneaten food and food preparation leftovers. Due to the relatively high moisture content of food waste, bioconversion technologies such as anaerobic digestion (AD) are more suitable compared to thermochemical conversion technologies such as combustion and gasification. Importantly, AD also prefers cooked and oily food waste to be digested whereas composting does not (Curry and Pillay, 2012).

Food wastes contain variable type and amounts of organic matter whose behavior in digesters depends on the biodegradation of organic pools characterized by different methane production rates. These pools need to be identified and their availability for decomposition determined in order to predict the biogas production.

The stepwise Van Soest chemical digestion (Van Soest and Wine, 1967) has been used to characterize the composition of crop residues (Trinsoutrot *et al*., 2000; Henriksen *et al.*, 2007) and organic fraction of solid wastes (Buffiere *et al.,* 2006) (Bruun *et al.,* 2005; Corbeels *et al.,* 1999; Pansu and Thuriès 2003; Trinsoutrot *et al.,* 2000). Few studies in soil biodegradation have investigated the biodegradation of Van Soest fractions and their impact on the global process. In soil degradation, carbon mineralization was best correlated with the neutral detergent fiber (NDF) (Kyvsgaard *et al.*, 2000) and the decomposition of pig slurries during the initial stages of biodegradation was significantly correlated with the Van Soest-soluble fraction (Morvan and Nicolardot, 2009).

To understand the relative contribution of the different fractions to the biodegradation of food wastes in digesters, we studied anaerobic biodegradation of neutral detergent fiber fraction and compared it to the one of raw food wastes.

3.1 MATERIALS AND METHODS

3.1.1 Food waste collection and analyses

The food waste was collected as source separated waste from the Institute's mess. To gain an understanding of the compositional variability and amount of food waste, daily and weekly sampling was performed. The food waste composition and variability of total solids was studied for a period of one month.

3.1.2 Waste stream sorting

The food wastes from our Institute's mess were separated and sorted according to their categories (cooked pulses, cooked rice, cooked vegetables and raw vegetables etc). The distribution of each category was expressed in terms of kilograms.

3.1.3 Biochemical Characterization

Total solids (TS) and volatile solids (VS) were determined according to standard methods (APHA, 1998) by drying of the substrate at 105°C (24h) and incineration at 550°C (2h). The carbohydrates were measured by the Anthrone method (Dreywood, 1946). Concentrations are expressed in glucose equivalent. The lipids were measured by accelerated solvent extraction (ASE) (ASE 200, Dionex), using petroleum ether. VFA concentrations were measured in the soluble fraction by gas chromatography (GC 800, Fisons Instruments). Soluble fractions were obtained after centrifugation (BECKMAN, 14 000*g*, 15 min). Chemical oxygen demand (COD) was determined on soluble and solid fractions, and measured by a colorimetric method using Hach 0-1500 mg/L vials. In addition, soluble and particulate total organic carbon was measured by combustion (680°C) catalytic oxidation/NDIR (Non-dispersive infrared gas analyzer) method using a Shimadzu TOC-VCSN analyzer.

3.1.4 Fractionation

The oven dried substrates (105^{o}C) were fractionated using the method proposed by Van Soest (Van Soest, 1963; Van Soest and Wine, 1967) and the content of cellulose; hemicellulose and lignin fractions in the substrate were measured. The sequential fractionation procedure was performed using a Fibre bag system (Gerhardt Germany) for six samples with the following steps:

(1) the soluble compounds were obtained by extraction with neutral detergent (30 g/L $C_{12}H_{25}Na0_4S$; 18.61 g/L $C_{10}H_{14}N_2Na_2O_8$, $2H_2O$; 6.81 g/L $Na_2B_4O_7$, $10H_2O$; 4.56 g/L Na_2HPO_4; 10 mL/L $C_6H_{14}O_4$) at 100°C for 60 min. The residual fraction obtained was called NDF for Neutral Detergent Fiber.

(2) the hemicellulose compounds were extracted from the NDF fraction by acid detergent (20 g/L $C_{19}H_{42}NBr$; 26.7 mL/L H_2SO_4, 95–97%) for 60 min at 100°C. The residual fraction obtained was called ADF for Acid Detergent Fiber fraction.

(3) the cellulose compounds were extracted from the ADF fraction for 3 h with H_2SO_4 (72%). The remaining fraction corresponds to lignin and was called ADL fraction for Acid Detergent Lignin fraction.

At the end of each step, the extracted samples were washed with deionized water and oven-dried at 105°C and combusted at 550°C to determine VS content while some quantity of sample were kept aside and used for BMP measurements.

Van Soest fractionation was used in this study to characterize food residues. The fractions obtained were therefore called in this case SOLUBLE-like, HEMICELLULOSE-like, CELLULOSE-like and LIGNIN-like fractions, and the fractions were named SOL, HEM, CELL and LIGN fractions.

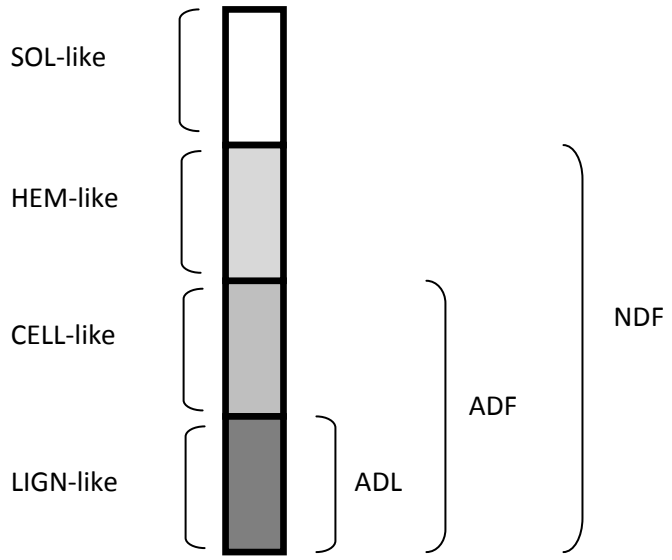

Figure 3.1

Van Soest fractionation principle. The sequential fractionation procedure was performed using a Fibrebag system (Gerhardt Germany). Van Soest fractionation was used in this study to characterize food residues and not crop residues like it was originally designed for. The fractions obtained were therefore called in this case SOLUBLE-like, HEMICELLULOSE-like, CELLULOSE-like and LIGNIN-like fractions. The fractions were named SOL, HEM, CELL and LIGN fractions. ADL : Acid Detergent Lignin ADF : Acid Detergent Fibre. NDF : Neutral Detergent Fibre.

3.1.5 Biogas composition

The biogas composition was determined by gas chromatography (Varian CP-4900 micro GC) equipped with a Molsieve 5A PLOT and Haye Sep A column and with helium as the carrier gas. Detection was performed with a thermal conductivity detector (TCD). Calibration allowed the measurement of CO_2, CH_4, H_2, O_2 and H_2S.

Biochemical methane potential (BMP) in flasks

3.1.6 BMP of raw wastes

Anaerobic digestion experiments to measure the biochemical methane potential (BMP) were carried out adapting the protocol originally proposed by Chynoweth *et al.*, (1993); Angelidaki and Sanders, (2004). The inoculum used for the studies originated from an up flow anaerobic sludge blanket (UASB) reactor treating sugar industry wastewater. The experiments were carried out in 500 mL serum bottles with rubber caps. The inoculum to substrate ratio (in VS) was 2. The bottles were filled up to 300 mL with distilled water and flushed with N_2 before the experiment. The bottles were incubated at 35°C. A blank test and a test with a fully-biodegradable compound (ethanol) were carried out during 33 days to determine endogenous activity and inoculum activity, respectively. All the experiments were carried out in triplicate and the results were expressed as average (with standard deviations). Biogas volume was measured with water displacement method. Biogas volume and composition were measured every two days at the beginning of the experiment and then every four days until the end of the experiment. Methane production was calculated by dividing the corrected methane volume (standard conditions of temperature and pressure: 0°C, 1atm) by the weight of sample (VS) added to each bottle.

3.1.7 BMP of Van Soest fractions

The biochemical methane potential of the NDF fractions was determined as previously described taking the dried extracts as substrates.

Expression of results

BMP values (raw wastes and fractions) were expressed as mL CH_4 (STP)/g_{VS} of raw wastes. The BMP of NDF fractions were also expressed as mL CH_4/g_{VS_NDF}. The relative contribution of NDF fractions to BMP of raw substrates were calculated as follows:

BMP_NDF_{raw} = BMP_NDF x F_NDF (**Equation 1)**

where BMP_NDF is the BMP of NDF (ml CH_4/g_{VS_NDF}), and F_NDF is the fraction of organic VS of raw wastes present in NDF fraction. BMP_NDF_{raw} is expressed in $mlCH_4/g_{VS}$ of raw substrate.

The BMP of SOL fraction was expressed as mL CH_4/g_{VS_SOL} and mL CH_4/g_{VS_raw} calculated as follows:

BMP_SOL_{raw} = (BMP- $BMP\_NDF_{raw)}$ **(Equation 2)**

BMP_SOL = BMP_SOL_{raw} / (1-F_NDF) **(Equation 3)**

where BMP_SOL is the BMP of SOL (mL CH_4/g_{VS_SOL}) and BMP_SOL_{raw} is the BMP of SOL (mL CH_4/g_{VS_raw}).

3.1.8 Batch reactors

Two double walled reactors of 3L effective volume were used in this work. Temperature was maintained at 35°C by a water recirculation. Mixing was done by magnetic stirring. The biogas production volume was measured on-line by a home-made gas counter using water displacement method. The "Modular SPC" software, developed at the Institut National de la Recherche Agronomique – Narbonne laboratory, was used for data acquisition.

Two series of experimentations were carried out at the same time, each one with two reactors. In the first series, the reactors were fed with cooked dals (CD) and cooked vegetables (CV) while in the second one, they were fed with cooked rice (CR) and mixed food wastes (MFW). Both reactors were seeded at a volatile suspended solids (VSS) concentration of 15g/L with anaerobic sludge, taken from the outlet of an upflow anaerobic sludge blanket (UASB) reactor treating sugar wastewater. Series 1 and 2 lasted respectively 412 and 563 hours during which 1g VS of food waste was added per liter and per batch.

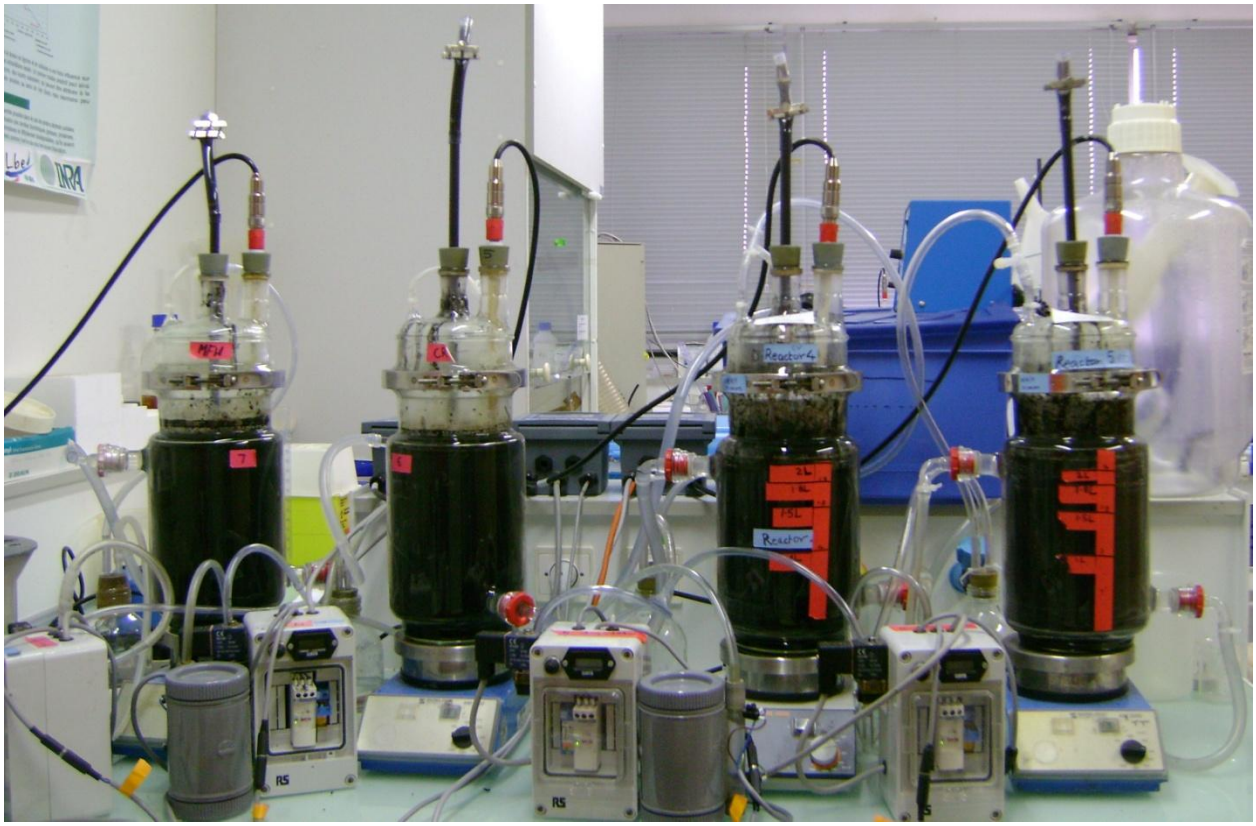

Figure 3.2 Laboratory Scale reactors used for biochemical methane potential assays. The four reactors in parallel with an effective volume of 3L each. The temperature is maintained at 35^{o}C by water circulation in a water jacket. The biogas was collected continuously and the volume produced measured with an electromagnetic volumetric gas counter (based on the principle of water displacement).

3.2 RESULTS AND DISCUSSION

This study was carried out in the context of Indian canteen food wastes valorization. Canteen food wastes contain variable type and amounts of organic matter. The main focus of this study was the major constituents of canteen food wastes. Their behavior in digesters depends on the biodegradation of organic pools characterized by different biogas production rates. These pools needs to be identified and their contribution to methane production determined in order to predict their anaerobic biodegradation. To better understand the relative contribution of the different pools to methane production from food residues, a Van Soest fractionation was performed and the methane potentials of the residual fractions were measured in flasks. The behavior of the raw wastes in flasks and batch reactors was also investigated.

3.2.1 Characterization of food wastes

Waste stream sorting

The food waste were separated and sorted according to the following categories: fruits, vegetables, cereals etc. The quantity (in kilograms) of each waste category was also estimated and represented in Figure 3.3. Raw vegetables represented the main quantity of wastes (31.7%), followed by cooked rice (16.6%), cooked vegetables (16.3%), and cooked dals (9.9%).

This study focuses on the cooked wastes. Indeed, even if raw vegetables represent the major fraction of the canteen wastes, raw vegetables biodegradability data were well reported in literature (Gunaseelan, 2004; Gunaseelan, 2007). On the contrary, cooked wastes are specific to this canteen and needed further investigation. Canteen food wastes are not homogeneous in their day to day composition hence it is very difficult to obtain duplicate and representative samples. Taking a representative mixture of the more abundant Indian canteen food wastes enables to have a model of food wastes which could be treated by anaerobic digestion.

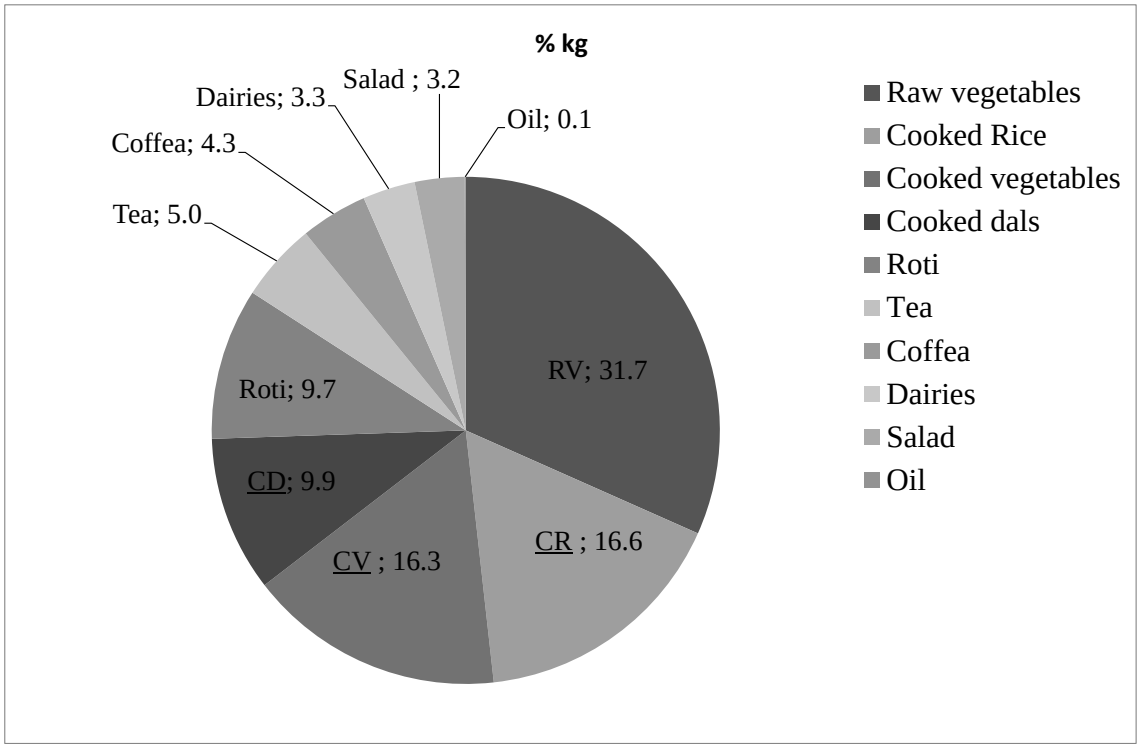

Figure 3.3: Distribution of food waste quantities in the Institute Mess.

3.2.2 Biochemical characterization

The three major substrates studied were: cooked rice (CR), cooked vegetables (CV), cooked dals (CD). Another fraction (mixed food wastes: MFW) was created by mixing the previous wastes in the following proportions in weight: 39% CR, 38% CV and 23% CD (56%; 21% and 23% on VS basis respectively) corresponding to the proportion observed in the Indian canteen wastes (Figure 3.3). The biochemical composition of the wastes was analyzed in terms of total solids (TS), volatile solids (VS) which are assimilated to organic matter, total carbon, chemical oxygen demand, sugars, lipids and fiber contents. As the considered food wastes contain no meat, fish or milk, proteins analysis was not made. The fiber analysis is obtained from Van Soest fractionation. The organic content of the wastes was separated in SOL, HEM, CEL and LIGN fractions. The results are presented in Table 3.1

CV contains the highest amount of carbon (53%), lipids (18%) and soluble COD. The high lipid content in CV can be explained by the fact that CV is cooked in oil. Their composition was therefore different from the one of raw vegetables which contains generally a low amount of lipids.

CR has the highest concentration of volatile solids (38%) and sugars (22%), which is consistent with the composition of this substrate rich in starch. The low level of total carbohydrates of this substrate, usually around 80% (Kouakou *et al.,* 2008), could be due to compounds formed during total carbohydrates analysis that can interfere with the colorimetric technique used during the analytical method. CR does not contain lipids and its soluble COD content is low. CR was only boiled: no oil was added during the cooking process, explaining the absence of lipids in this category.

CD contains some lipids (16%) due to its cooking with the addition of fat substances. The soluble COD values tendency (CV > CD > CR) followed the one of SOL fraction showing the coherence of the soluble fraction analysis for this kind of substrates. SOL fraction was

higher than sCOD because of the solvent used for extraction. Indeed, SOL fraction was obtained after neutral detergent extraction whereas sCOD was obtained after water extraction and centrifugation.

The biochemical parameters of MFW equaled the weighed sum of each waste parameter. The measured values differed from the theoretical one at less than 5% except for sCOD and lipids which differed from theoretical value at 20% and 40% respectively. This is probably due to the mixing process for MFW.

Table 3.1 : Biochemical characterization of substrates

Substrate	CR	CV	CD	MFW
Total Solids (TS) g_{TS}/g_{raw}	0.39	0.15	0.27	0.27
Volatile Solids (VS) g_{VS}/g_{raw}	0.38	0.14	0.26	0.26
VS/TS (%)	97	93	96	96
Total Organic Carbon (TOC)- g_C/g_{VS}	0.43	0.53	0.47	0.44
Soluble Chemical Oxygen Demand-g_{SCOD}/g_{VS}	0.05	0.35	0.11	0.10
Sugars-g glucose/g_{VS}	0.22	0.18	0.13	0.20
Lipids-g/g_{VS}	0	0.18	0.16	0.11
Soluble -g/g_{vs}	0.10	0.58	0.39	0.29
Hemicellulose– g/g_{vs}	0.87	0.23	0.51	0.63
Cellulose– g/g_{vs}	0.01	0.14	0.10	0.06
Lignin– g/g_{vs}	0.02	0.05	0	0.02

3.2.3 Biochemical fractionation

Concerning the fractionation, it is worth noticing that the lignocellulosic content (CELL+ LIGN) of each food waste is low. The maximum value was observed with CV (19% of VS content). The lignocellulosic content of these wastes is understandable by the inherent nature of the vegetables and the presence of plant cell wall in this type of residues. CR contains the smallest SOL fraction and the highest HEM fraction. It results from the fact that rice, which contains a large amount of starch, was not pretreated with amylase before Van Soest fractionation. Thus, starch could be counted as HEM fraction. CD as a leguminous is constituted in majority of SOL and HEM fraction.

The biochemical fractionation parameters of MFW are in adequacy with the weighed sum of each waste parameter at around 5% (except for SOL fraction: 10%).

3.2.4 Biodegradability of wastes

The biochemical methane potential of raw substrates and of the NDF fractions, were performed in the same conditions as raw substrates.

3.2.5 BMP of raw substrates

The BMP reveals that CV was more biodegradable substrate with 389 ±7 mL $CH_4/g_{VS,}$ the second was CD with 318 ± 8 mL CH_4/g_{VS}, and CR produced around 234 ± 11 ml CH_4/g_{VS}. The reconstituted mixture of the three wastes had a measured BMP of 277 ± 9 ml CH_4/g_{VS} while the BMP calculated considering the contribution of each category and their individual BMP is 281ml CH_4/g_{VS}. This result shows that the BMP measured for the mixture of all the wastes is the sum of the individual contribution of the three wastes and that no synergetic effect was therefore observed.

CR does not contain lipids. This aspect has an important impact on biodegradation since the

BMP of CR was the lowest with only 234mL CH_4/g_{VS}. BMP of boiled rice have been reported by Cho et al., (1995) at 294ml CH_4/g_{VS}. These values are quite low for waste with small lignocellulosic content and are close to BMP of fruit and vegetables peels (Gunaseelan, 2007) or lignocellulosic residues (Dumas et al.,;Bauer *et al.*, 2009).

CV contain higher amount of CELL and LIGN fraction (19%) than the other wastes. This value is in adequacy with Van Soest fractionation of fruit and vegetables wastes reported in literature (Buffiere *et al.,* 2006; Gunaseelan, 1997; Gunaseelan, 2004; Gunaseelan, 2007). However, this lignocellulosic content is low compared to lignocellulosic compounds like straw for example (Bauer *et al.*, 2009). CV also has a higher concentration of lipids than the other substrates. Lipids are known to have a high methane potential yield for example between 1010-1020 $mlCH_4/g_{VS}$ for oleic and stearic acid (Davidsson *et al.*, 2008). Low lignocellulosic content and high lipids concentration can explain the high BMP value of CV.

3.2.6 BMP of substrate fractions

The BMP of the NDF fractions was measured and expressed as ml $CH_4/g_{VS\ \_NDF}$ and as ml $CH_4/g_{VS\_\ raw}$ .The BMP of ADF and ADL fraction were also measured but were negligible or equal to zero and therefore not presented. The BMP of NDF fractions are presented in (Table 3.2) and compared to the ones of raw substrates.

The highest BMP of NDF residue was obtained with CV (182± 10 ml $CH_4/g_{VS\ \_NDF}$) indicating that this residual fraction is the most biodegradable. The other BMP of NDF fraction equaled 149±31 (CR), 120±12 (CD), and finally 93±5 ml $CH_4/g_{VS\ \_NDF}$ for MFW. These biodegradabilities are quite low. The BMP_NDF of MFW was surprisingly the lowest one and do not respect the weighed sum of BMP_NDF of the other wastes. This result can be explained by the fact that the soluble compounds in the mixture behave differently than the sum of all the soluble compounds taken separately in each waste.

According to the VS content of SOL fraction, it was possible to estimate the methane production of SOL fraction for $1g_{VS\_SOL}$ (Equation 3). These values are quite high indicating the high biodegradability of these fractions. The molecules solubilized during first Van Soest extraction have quite the same BMP values (Table). The behaviors of the SOL fractions during anaerobic digestion do not differ from one foodwaste to the other. It would be interesting to have a deeper characterization of the molecules consisting the SOL fraction in order to understand why the biodegradability's were quite similar and to explain more in details what occurred during anaerobic digestion of the various wastes.

Calculating the BMP of SOL fraction per g_{VS} of raw substrate, two categories could be observed: CR with a NDF methane production of 114±48 mL CH_4/g_{VS_raw}, and the other wastes with quite the same values of methane production: 77±68 (CV), 73±23 (CD), 62±7 mL CH_4/g_{VS_raw} (MFW). A comparison of these methane production values with the BMP of raw substrates reveals that the lower contribution of SOL fraction to BMP was observed for CR with only 50%. The fact that the SOL contribution of CR was smaller could be explained by the low quantity of SOL fraction in this waste than in the other ones. Indeed, CR contains an important part of starch which is known to be enclosed in a matrix of fiber and protein affecting the digestibility and the physicochemical properties (Ovando-Martínez *et al.*, 2011). The fractionation of CR was performed in the same way as for the other substrates, enabling a correct comparison of their behavior. However, Van Soest fractionation could be performed after an amylase pretreatment in order to solubilize protein and favor starch availability.

It is also important to note that the VS content of NDF fractions could be overestimated, due to neutral detergent chemicals that may be present in residues even after the rinsing process. This overestimation of VS content in NDF fraction can have an important impact on the calculated BMP of SOL fraction which could be overestimated.

According to biochemical and Van Soest fractionation and also with respect to BMP of raw

wastes and of their Van Soest fractions, the food wastes could be classified in two categories:

- substrates with low soluble compounds (sCOD, SOL) and BMP value: cooked Rice
- substrates with high soluble concentrations and BMP values: cooked Vegetables, cooked dals and mix food waste.

Table 3.2: Biochemical methane potential of NDF fractions, SOL fractions for the four wastes studied

Substrate	CR	CV	CD	MFW
BMP-ml CH_4/g_{VS_raw}	234±11	389±7	318±8	277±9
BMP_NDF ml CH_4/g_{VS_NDF}	149±31	182±10	120±12	93±5
F_NDF g_{VS_NDF}/g_{V_raw}	0.77	0.42	0.60	0.66
BMP_NDF_{raw} ml CH_4/g_{VS_raw} (obtained by calculation)	114±48	77±68	73±23	62±7
BMP_SOL ml CH_4/g_{VS_SOL} (obtained by calculation)	517	538	615	634
BMP_SOL_{raw} ml CH_4/g_{VS_raw} (obtained by calculation)	118	312	245	215
SOL fraction contribution to BMP (%)	50	80	77	77

3.2.7 Biodegradation in batch reactors

Four anaerobic digesters were operated in parallel to study the biodegradation of food wastes for a longer period of time. A large concentration of sludge was used as inoculum, in order to minimize biomass growth and shorten the initial start-up period (Jensen *et al.,* 2009). Each reactor was operated in batch mode for two months. The reactors were fed with $1g_{VS}$/L/ batch. At the beginning of each batch, i.e. at the end of the previous one, biochemical parameters were measured such as TS, VS, VSS, sCOD and VFA. The biogas production was monitored continuously during the batches in order to quantify the biogas yield. A batch was considered as over when the biogas production returned to a low level (around 6mL/h), corresponding to the endogenous production as determined at the beginning of the experiment during batches fed with ethanol as the sole source of carbon.

3.2.8 Biogas production rate (BPR)

The Biogas Production Rate (BPR) was measured online and expressed in mL/h. For all the substrates; the first batch lasted around 150h and was longer than the subsequent batches (around 80h). An example of the first and the last batch with cooked pulses is presented in figure 3.4. During the first batch, BPR started to increase only after a lag phase of about 20 h. Before 20h, a low BPR was monitored indicating very low metabolic activity of the biomass. Then BPR was roughly constant at an average value of 15 mL biogas/h for the resting 110 hours. At 150 h, the reactor was fed again even if the batch was not fully completed. The shape of the BPR curve obtained during last batch differed greatly from that of first batch. For the last batch, the BPR started to increase after 5 hours and reach its maximum after 15 hours. BPR was maximum at 50 ml biogas/h for 10h and then decreased rapidly within the following hours. In the last batch, (i) the maximum BPR was recorded at the beginning of the batch and lasted for a few hours; (ii) it was followed by a quick decrease of the BPR

indicating the exhaustion of the rapidly biodegradable material; (iii) thereafter, the BPR was low and decreased slowly, indicating the metabolization of slowly biodegradable organic matter. Acclimatization was noted as (i) the duration of the batches is shortened (ii) the maximum BPR is increasing with time.

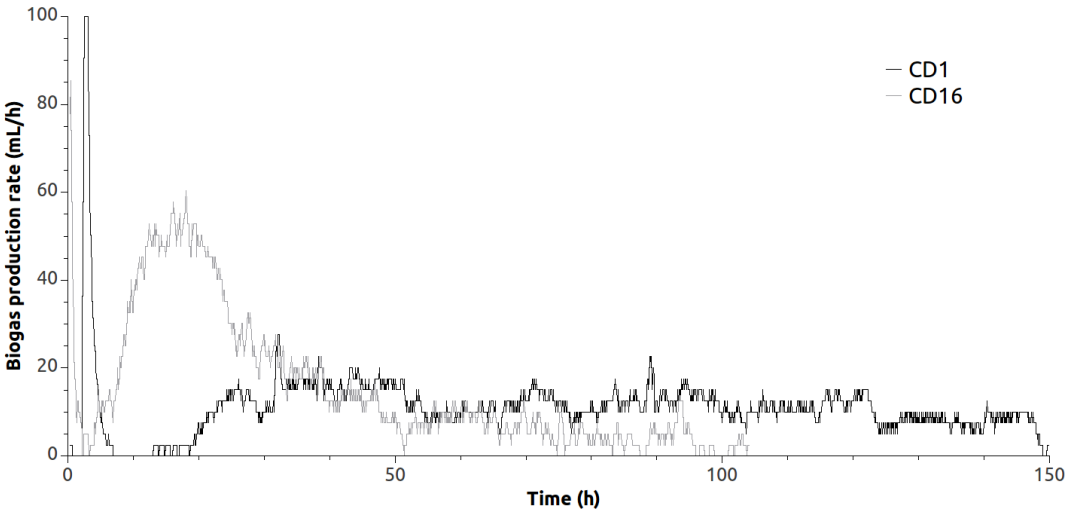

Figure 3.4: Comparison of Biogas Production Rate (BPR) in the first batch (black line) and last batch (grey line) for Cooked Dals (CD). CD1: Cooked dals 1st batch. CD 16: Cooked dals 16th batch.

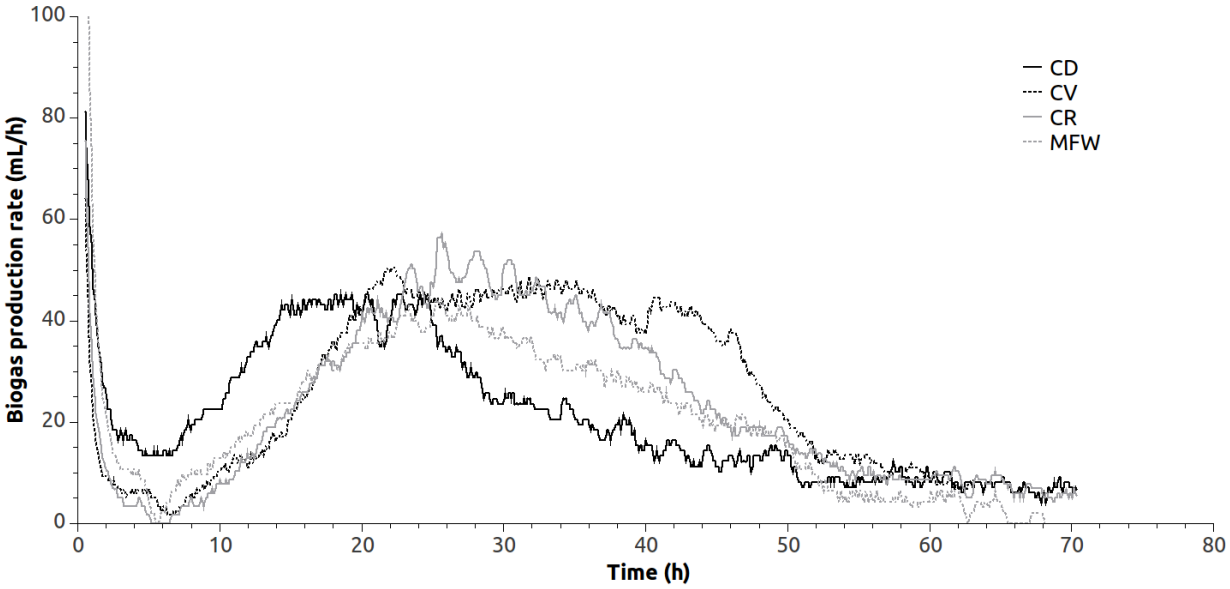

Figure 3.5 presents the BPR of the typical batches obtained with four substrates after the acclimatization period. CD: Cooked dals. CV: Cooked vegetables. CR: Cooked rice. MFW: Mixed foodwaste.

The BPR had a general tendency which was rather close for all the substrates. Indeed, the BPRs increased after 5 hours and reached their maximum (45 and 55 ml/h) between 15 and 25 hours after the feed, then the BPR was maintained at a plateau for some hours (10 to 20 hours). At 60h, all the BPR were 6L/h or less.

Despite this global biodegradation rate tendency, some small differences could be noted. Particularly, in the case of CD, the BPR seems to increase quickly after 5h and to decrease before 30 h showing the higher rapidly biodegradable part of this substrate. Concerning CV, the plateau seems to take longer time showing that the high biodegradable fraction of this substrate takes time to be fully degraded. The fact that CV contains a high part of oil could explain this tendency.

As the different substrates behave almost in the same way in reactors, the mixture was neither detrimental nor synergistic to BPR.

3.2.9 Total methane production

Considering the endogenous BPR at 6 mL biogas/h, the total endogenous production after 60h of operation accounts for 360 mL of biogas which should be removed from the total volume produced. Considering the total biogas production of each waste and a global methane composition in biogas of 55% the maximum methane production could be calculated. The latter was 285, 366, 324 and 300 ml CH_4/g_{VS} respectively for CD, CV, CR and MFW. These results are in adequacy with the BMP tests values for CV (389 ml CH_4/g_{VS} in BMP and 366 mL CH_4/g_{VS} in reactor) and MFW (277 mL CH_4/g_{VS} in BMP and 300 ml CH_4/g_{VS} in reactor) but are different for CD (318 ml CH_4/g_{VS} in BMP and 285 ml CH_4/g_{VS} in reactor) and particularly CR (234 ml CH_4/g_{VS} in BMP and 324 ml CH_4/g_{VS} in reactor). These results raise the fact that the data obtained in BMP should be considered carefully. BMP results can be used to compare substrates or physical/chemical conditions at the same time, but should not be taken as the real methane production that a substrate can produce in a reactor. For this reason, batch experiments in reactors are more appropriate.

Table 3.3: Maximum value of BPR and the number of hour when it was reached. Maximum methane production at the end of batch (with a mean methane composition in biogas of 55%).

	CR	CV	CD	MFW
BPR_{max} (%)	55	50	45	45
Number of hour at BPR_{max} (ml CH4/gVS)	26	23	14	25
Methane production (mL CH_4/g_{VS})	324	366	285	300

4 *Pilot scale and microbial diversity studies of anaerobic digestion of food waste in a horizontal plug flow reactor*

In light of rapidly rising costs associated with energy supply, waste disposal and increasing concerns with environmental quality degradation, conversion of food wastes to energy is more an economically viable solution (Zhang, 2007; Nikolausz *et al.*, 2013). In fact, due to the relatively high moisture content of food waste, bioconversion technologies such as anaerobic digestion (AD) are more suitable compared to thermochemical conversion technologies such as combustion and gasification. Because of the decrease of fossil energy resources and of the production of regenerative CH_4 combined with the low energy demand of anaerobic digestion, the energy balance is positive in the anaerobic process.

The study of microbial communities represents another growing area of research in the field of anaerobic digestion. A deeper knowledge in microbial dynamics could provide information for predicting system performance under a given set of conditions, or help in designing engineered systems to foster the development of specific communities (Briones and Raskin, 2003). Process stability is a key issue in AD of wastes due to the fluctuating substrate quality and quantity which requires a high adaptability of the microbial communities. It is well known, that methanogens are sensitive to process imbalances due to the lack of functional redundancy, while bacterial members of the anaerobic digestion process are more diverse and less prone to inhibitions. In biogas processes, high ammonia concentrations have been shown to be an important factor for influencing the main pathways for methane formation (Schnurer *et al.*, 2008, Westerholm *et al.*, 2011).

Anaerobic digesters harbour complex and highly dynamic microbial communities, and information on the specific importance of particular phylogenetic and functional groups for the overall efficiency and stability of the biogas synthesis is limited. Thus, comprehensive understanding of the microbial community structure and activity of various members under

changing process conditions is a prerequisite for process control and optimization. The introduction of cultivation-independent studies to microbiology has converted our previous view on species diversity. It has been proven that most of the bacteria present in various ecosystems (from the human intestine to deep-sea sediments) are currently uncultivated and are known via their genomic material (Achtman and Wagner, 2008). To explore the "whole" bacterial diversity of a particular environment is labour intensive, time-consuming and expensive both through traditional cloning or applying next generation sequencing tools (Metzker, 2010). This is especially true if the aim of the study is to characterize numerous samples, e.g. to resolve spatial or temporal changes in microbial communities. Therefore community fingerprinting techniques, that allow rapid and cost-effective profiling and comparison of microbial communities, are extremely important in current research. The most commonly used methods are denaturing gradient gel electrophoresis (DGGE, Muyzer *et al.*, 1993) and terminal restriction fragment length polymorphism (T-RFLP, Liu *et al.*, 1997).

In the present work a 60 m^3 horizontal plug flow reactor is set up inside our (BITS Pilani K. K. Birla Goa campus) institute's premises for anaerobic digestion of food wastes. The aim of this pilot plant was to evaluate and optimize the methane potential of solid wastes and to have a process for sustainable energy production for small communities. The performance of this digester was monitored for approximately two years and the microbial diversity with respect to methanogens was studied by T-RFLP technique for about a month (5 weeks). Methanogenic archaea was assessed by amplifying the *mcrA* gene, encoding the alpha subunit of methyl coenzyme-M reductase, a key enzyme of the methanogenesis. Detailed identification of the dominant members of the community was obtained by cloning and sequencing approach. Ammonia nitrogen is an important buffer in the anaerobic digestion process and an essential nutrient for the microorganisms. However, high concentrations can be toxic to the microorganisms mainly influencing the phase of methanogenesis and causing

operational failure of anaerobic digestors (Calli *et al.*, 2005). Therefore the effect of increasing concentrations of ammonia on microbial diversity of anaerobic digestion process was also studied.

4.1 MATERIALS AND METHODS

4.1.1 Digester running conditions

A pilot plant of horizontal plug flow reactor (Figure 4.1) (Indian Institute of Science model, Chanakya *et al.,* 2005) was constructed in November 2010 which has an operational volume of 60 m^3. The feed for this reactor was food waste from Institute's cafeteria catering to approximately 2400 student equivalent. Startup of the reactor was done by using cow dung as the inoculum. The characteristics of the food waste and the inoculum is given in Table 4.1. The reactor was initially fed with 500kgs of cow dung and left undisturbed for 6 days, followed by which addition of food waste was started and gradually increased until it reached its full capacity.

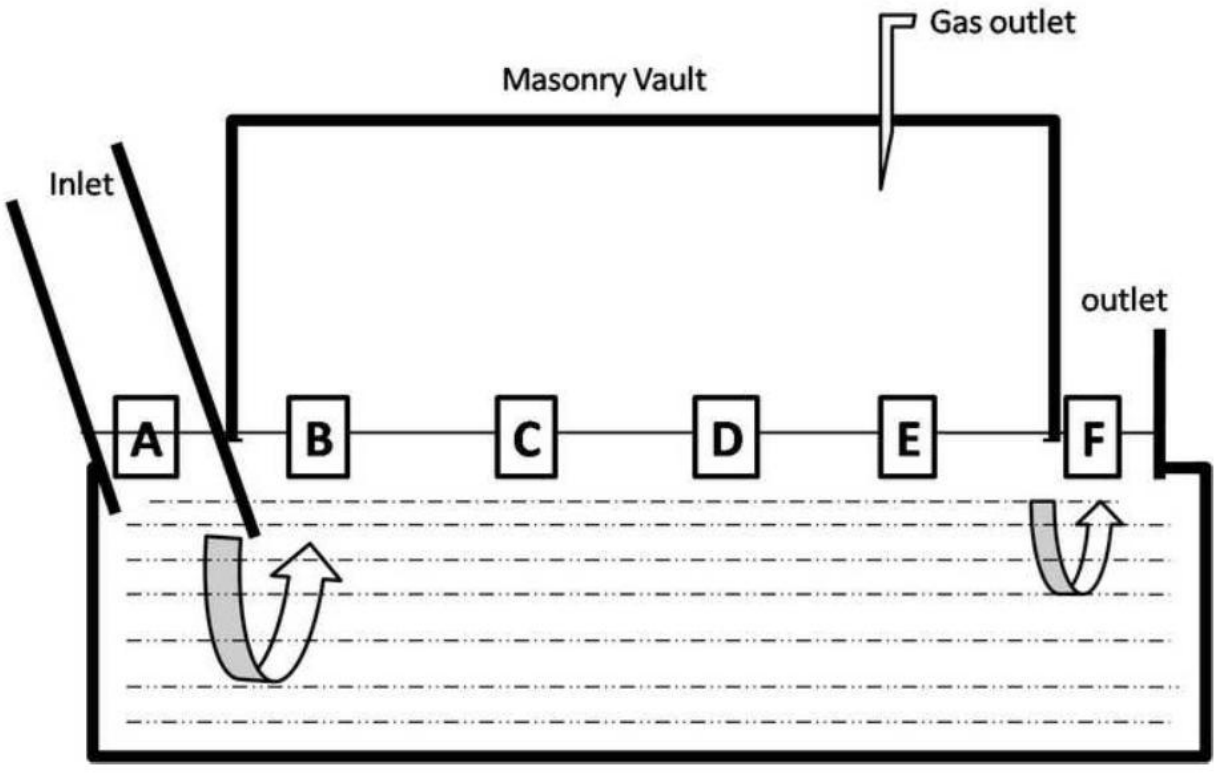

Figure 4.1: Schematic drawing of the Horizontal Plug flow Reactor (60m^3capacity, Indian Institute of Science model) for anaerobic digestion of food waste. A, B, C, D, E and F are six points from where samples were taken for microbial diversity studies.

Table 4.1 Characterization of food waste and inoculum

Parameters	Food waste	Inoculum (cow dung)
Moisture (%)	72.60	78.67
Total solids (%)	27.43	21.33
Volatile solids (%)	26.35	18.99
VS/TS (%)	0.96	0.89
COD (mg/L)	623.90	547
TKN (mg/L)	5.42	2.7

4.1.2 Total solids (TS) Estimation

For Total solids, a known amount of sample (food waste/cow dung) was transferred into a previously weighed crucible and dried at 105^{o}C for 24 h. The increase in weight over that of the empty crucible represents the total solids (APHA, 1998).

4.1.3 Volatile solids (VS) Estimation

For Volatile solids estimation, the dried sample obtained after TS estimation was ignited in a muffle furnace at 550 ^{o}C for 2 h. The weight lost on ignition represents the volatile solids (APHA, 1998).

4.1.4 Chemical oxygen Demand

The Chemical Oxygen Demand measurement is performed on fresh waste (food waste/cow dung). Prior to use the substrates, were ground in a blender to give a fraction with particle size less than 2 mm. The substrate (1g) was then suspended in 1L of distilled water, stirred on a magnetic stirrer for one hour, and the COD of the suspension is measured as described by Raposo *et al.,* (2008). Briefly, 10 ml of suspension was digested with Potassium dichromate

and concentrated sulphuric acid at 150^{o}C for 2 h in a COD Block Digestion Unit. After cooling the digestate is titrated against ferrous ammonium sulphate (0.5 N) using 1, 10-phenantroline as indicator.

4.1.5 Biogas Measurement

The biogas production from the pilot reactor was measured using mechanical flow meter (Siya Instruments, India) and the biogas from ammonia experiments conducted in 125ml serum vials was measured by water displacement set up (Singh *et al.*, 2001). A tube was used to connect the reactor with an inverted graduated measuring cylinder immersed in a 1000 ml beaker filled with water. Biogas produced was collected in the graduated cylinder connected with a water reservoir which allowed volumetric biogas measurements at atmospheric pressure.

4.1.6 Gas Composition

Gas samples were taken periodically for composition analysis by gas chromatography. The samples were analyzed with a gas chromatograph (GC-7610, Chemito) equipped with thermal conductivity detector. The carrier gas was hydrogen. The oven, injector and detector temperatures were 80, 150 and 250^{o} C respectively.

4.1.7 Extraction and purification of nucleic acids

After stabilization of the anaerobic digester i.e after two years of continuous operation, samples from different stages of the digester (Figure 1) were collected using a fountain gun of 500 ml capacity. Sampling for diversity studies was done for 5 weeks at the same OLR of 1.28 Kg VS/m^{3}/day. The samples were collected and used immediately for DNA extraction. Total DNA was extracted using QIAamp DNA stool mini kit (Qiagen). DNA quantity and

purity were determined photometrically using a Nanodrop® ND-1000 UV/Vis spectral photometer (PeqLab, Germany) and by agarose gel electrophoresis (Nikolausz *et al.*, 2013).

4.1.8 Polymerase Chain Reaction

PCR was carried out using the Taq PCR Master Mix Kit (Qiagen) in 25 µL final volume with 2 µL isolated DNA. The mcrA- specific forward primer (5′GGTGGTGTMGGDTTCACMCARTA-3′) and the reverse primer mcrA-rev (5-CGTTCATBGCGTAGTTVGGRTAGT-3′) were used for PCR amplification as described by Steinberg and Regan (2008). In case of PCR for subsequent T-RFLP analysis, the reverse primer was 5′-labeled with the phosphoramidite fluorochrome 6-carboxyfluorescein. PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized with UV excitation. PCR products were purified using a QIA quick PCR purification kit (Qiagen) and quantified photometrically using a NanoDrop® ND-1000 UV/Vis spectral photometer (PeqLab, Germany).

4.1.9 Terminal Fragment Length polymorphism (T-RFLP) analysis

Purified PCR products (10 ng per sample) were digested in a 10-µL reaction volume with 10 U of either the restriction endonuclease *HaeIII* or *MspI* (New England Biolabs, Schwalbach, Germany). Reactions were carried out at 37 °C overnight, and then samples were precipitated with 0.1 volume of 3 M sodium acetate (pH 5.5) and 2.5 volume of absolute ethanol. After washing in 70% ethanol, DNA pellets were dried and subsequently resuspended in 20 µL HiDi formamide containing 1.5 % (v/v) GeneScan-500 ROX standard (Applied Biosystems, Weiterstadt, Germany). Fluorescently labeled terminal restriction fragments (TRFs) were separated on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) with POP-7TM polymer, with two replicates for each restriction analysis to ensure reproducibility. The

GeneMapper V3.7 software (Applied Biosystems) was used to analyze the T-RFLP (Terminal Restriction Fragment Length Polymorphisms) chromatograms. Fluorescence signals of T-RFs in the size range of 50–500 bp were extracted to exclude potential primer peaks, and peaks with signal below 100 relative fluorescence units were also discarded from the analysis. Relative T-RF abundances were calculated by dividing the individual T-RF areas by the total peak areas (100%).

4.1.2.0 Cloning and sequence analysis

Cloning of purified non-labeled PCR products was carried out with QIAGEN PCR Cloning Kit (Qiagen). The same PCR protocol with mlas and mcrA-rev primers was used for the re-amplification of the inserts from selected clones as described before. PCR products were screened by T-RFLP analysis to select clones with inserts matching the predominant peaks of the community T-RFLP patterns. A simplified protocol was used for the T-RFLP screening of the clones by omitting the purification of PCR products and ethanol precipitation of the restriction enzyme digestion. Partial sequencing of purified PCR products was performed with one of the *mcrA*-specific primers or both of them using the Big Dye Terminator Ready Reaction Cycle Sequencing Kit 1.1 (Applied Biosystems) and an ABI PRISM 3130xl Genetic Analyzer. The BLASTN and BLASTX algorithms (Altschul *et al.* 1990) were used to search for similar sequences in the public databases.

4.1.2.1 Statistical Analysis

Each T-RF was assumed to be an individual OTU. T-RFs presence-absence was further used for the statistical analysis. The similarity between the sampling sites was analyzed using cluster analysis. A Dendrogram was constructed based on Bray-Curtis similarity distance measures with UPGMA (unweighted Pair Group Method with Arithmetic Mean) (Bray &

Curtis, 1957; Michener and Sokal, 1957). Diversity indexes describing species richness and species evenness indices were calculated using the T-RFs. The Shannon–Weaver index of diversity (*H*) (Shannon and Weaver, 1963), Simpson index of diversity (*D*) (Simpson, 1949) and the equitability index (*E) (Pielou, 1975)* were calculated using the following formula,

$H = -\Sigma (n_i/N) \log(n_i/N)$,

$D = 1 - \Sigma (n_i/N)^2$

$E = H/\log S$

where n_i is the frequency of each T-RF, S is the number of T-RFs (used to indicate the number of species) and N is the sum of all the surfaces for all T-RFs in a given sample (used as estimates of species abundance). The statistics was done using Paleontological statistics software (PAST) (http://folk.uio.no/ohammer/past/)

4.1.2.2 Role of ammonia

Samples were collected from the outlet of the reactor and incubated anaerobically at 37 ^{o}C till endogenous gas production was reached. Using this as the inoculum, laboratory microcosms in 125 serum vials was set up using 1mM acetate as the substrate at different concentrations of ammonia (0, 150, 300, 500, 750, 1000, 1500 and 2000 mg/l). Quantification of biogas and composition analysis was done after 24 hours and 48 hours. During the same period samples were withdrawn for DNA extraction. The change in the community structure was determined by T-RFLP as described above.

4.2 Results and Discussion

4.2.1 Performance of the biogas reactors

This reactor had been operated for more than 750 days with increasing organic loading rates (OLR). (Figure 4.2) shows the operational conditions of the reactor with respect to daily biogas production, and OLRs. The reactor was started with OLR of 0.32 kg VS/m^3/day and gradually increased to 1.28 kg VS/m^3/day. The minimum and maximum loadings based on actual volume were 0.16 and 1.60 kg VS m^{-3}. During summer (May till August) and winter (December) breaks for the Institute, the loadings were less. The average hydraulic retention time was 120 days based on the digester volume divided by the mass input on a wet weight basis (Banks *et al.*, 2011). The average total biogas production and the proportion of methane was 38m^3 and 50%.

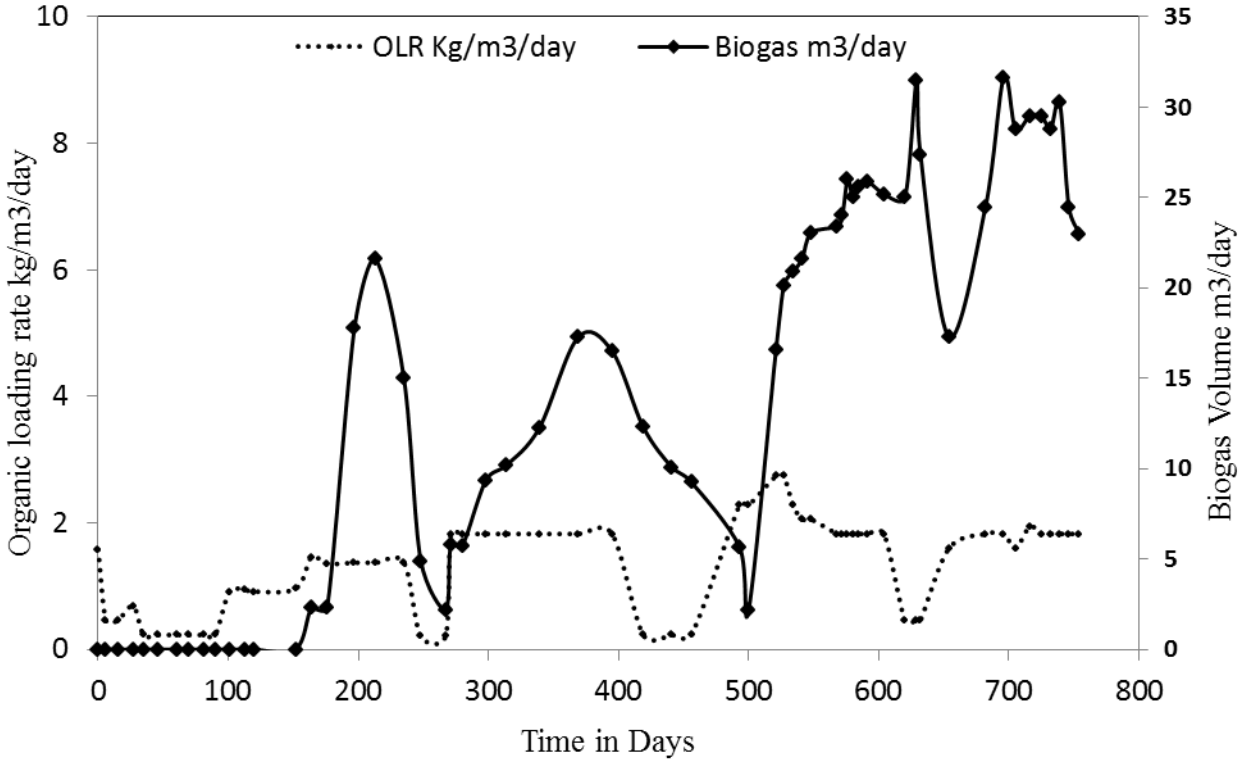

Figure 4.2. The biogas production rate and organic loading rate (OLR) of the reactor operated for 750 days.

Digestate Characteristics

The values for TS and VS content of digestate during the study period are shown in Figure 4.3. The average solid content was TS 4.15% and VS 3.18%. There are some variations in the TS and VS content of individual samples of digestate but the VS: TS ratio remained almost constant Figure 4.4. The average digester pH in the study period was 6.03 at the inlet and 7.2 at the outlet Figure 4.5.

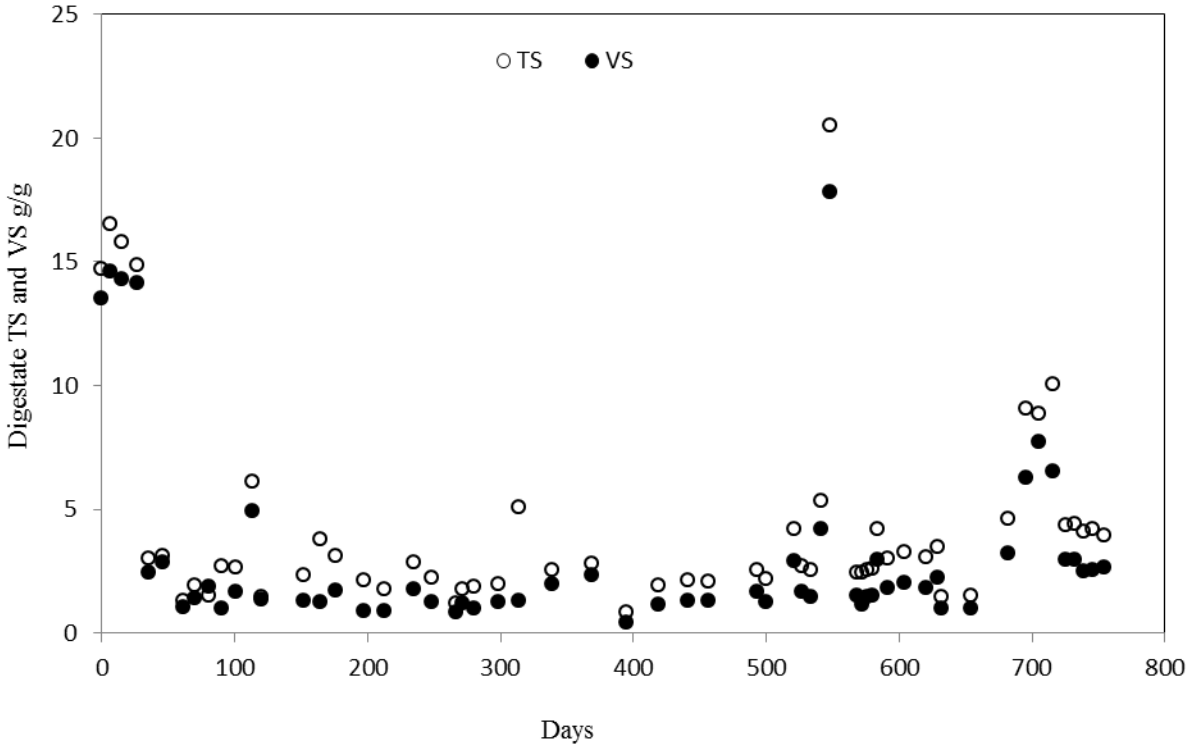

Figure 4.3. Total solids (TS) and Volatile solids (VS) content of the Digestate. The reactor was operated for 750 days. Digested samples were collected from the outlet of the reactor.

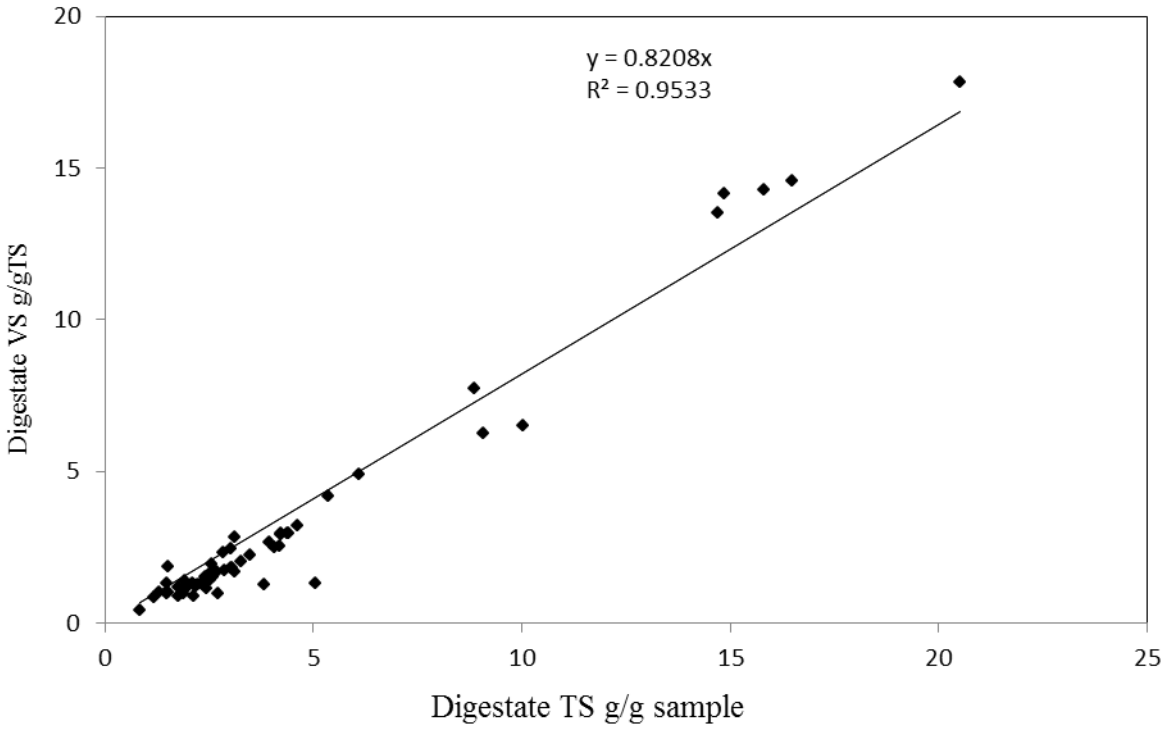

Figure 4.4 The Volatile solids: Total solids (VS: TS) ratio of digestate.

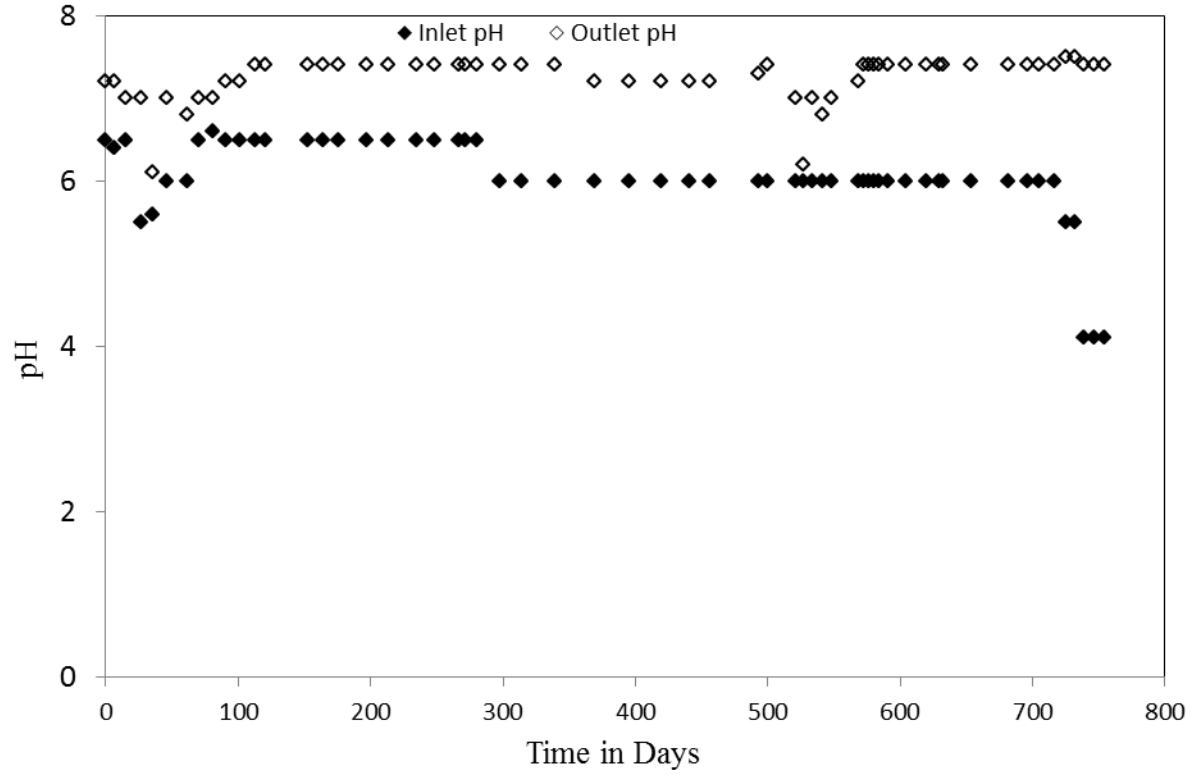

Figure 4.5: pH of the horizontal plug flow reactor at the inlet and outlet of the reactor during the study period

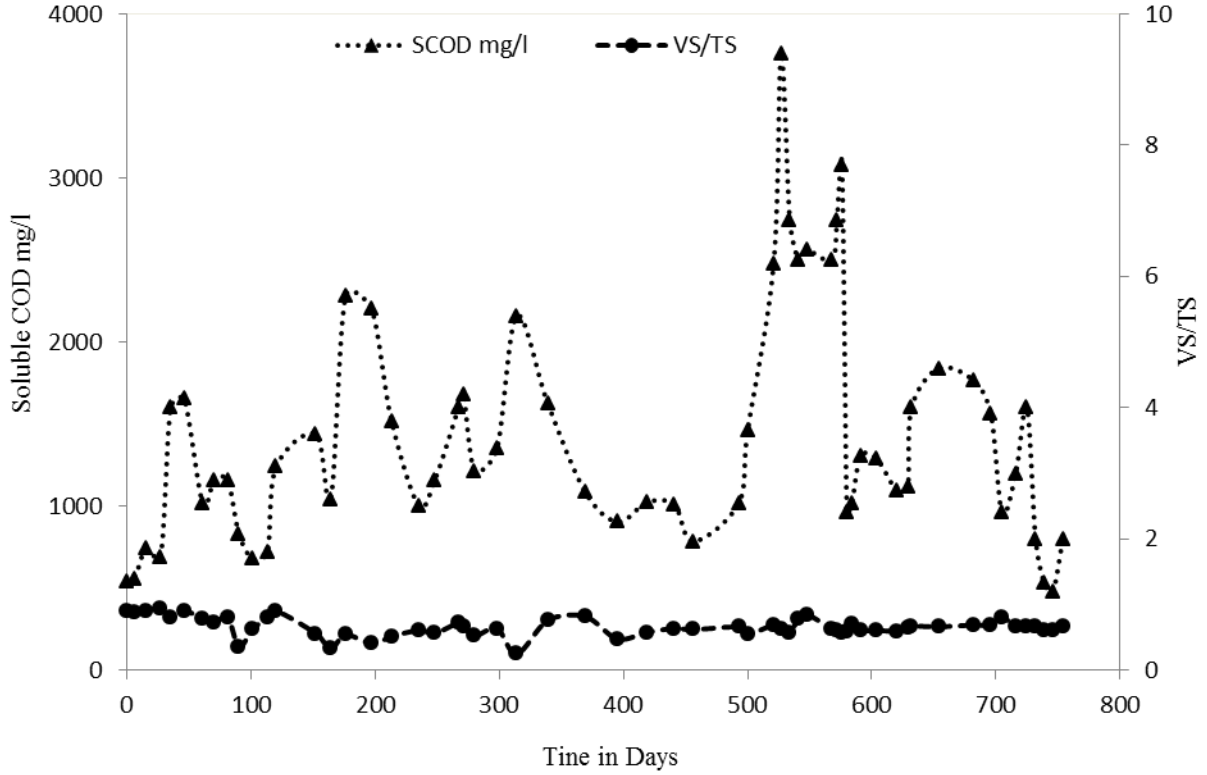

Figure 4.6 The soluble chemical oxygen demand and VS/TS ratio of the reactor.

In anaerobic reactors, weekly analysis of the digestate indicates the condition of the substrate within the reactor. The total organic carbon of the digestate was monitored by chemical oxygen demand (COD) measurements. Figure 4.6 indicates the COD concentrations in anaerobic digestate during the study. The total organic and inorganic matter in the digestate is represented by the total solids determinations as shown in Figure 4.3. Decrease in total solids in the digestate as the study progressed is due to destruction of the volatile solids. At the intial phase of digestion, the volatile fractions represented 80-90% which was reduced to 50-60% at the end of digestion process. The average digester solids content was TS 4.15% and VS 3.18%. There was some variation in the TS and VS content of individual samples of digestate but the VS: TS ratio remained almost constant. Figure 4.5 depicts the pH of the reactor. The average digester pH in the study period was 6.03 at the inlet and 7.2 at the outlet.

4.2.3 Community composition and activity of methanogens in horizontal plug flow reactor

Figure 4.7 illustrates the results of the T RFLP with restriction enzyme *MspI for mcrA* gene. Similar results were obtained with restriction enzyme *HaeIII*. T-RFs contributing atleast 1% of the total T-RF peak area were considered. A total of 53 operational taxonomic units (OTUs) were distinguished with the restriction enzyme *MspI*. The taxonomic affiliation of the predominant methanogens was determined by sequence analysis of *mcrA* clone libraries. Out of 89 clones for mcrA gene, 22 clones were selected for partial sequencing. According to these sequence data, 5 OTUs which were dominant were identified. Hydrogenotrophic methanogens were predominantly represented by a phylotype affiliated to the genus *Methanoculleus* at two different T-RFs of 175.7 and 57.31. *Methanoculleus* is a hydrogenotroph i.e it utilizes H_2/CO_2 and formate as methanogenic substrates and requires acetate for growth (Demirel and Scherer, 2008). The relative T-Rf abundance of 175.7 band was 49% at the inlet and 24% at the outlet and the relative T-RF abundance of 57.31 band was 21% at the inlet and 6% at the outlet. The relative T-RF band 464.33 representing *Methanotorris* was 5.6% at the inlet and 35% at the outlet. The relative T-RF band 470.89 representing Methanobacterium was around 8% at the inlet and outlet. The relative T-RF band 167.18 representing *Methanosarcinaceae* which convert acetate to methane gas during growth and can also utilize other organics as substrate was 7% at the inlet and 13% at the outlet. *Methanosarcinaceae* which is less represented compared to other T-RFs has a much lower substrate affinity with a higher maximum substrate utilization rate and it would tend to predominate at high acetate concentration further emphasizing hydrogenotrophic pathway of methanogenesis to be predmoniant (Yu *et al.,* 2005). The low percentage of *Methanosarcinaceae* is also reported in other studies as well that H_2 utilizing pathway was the main and common route for methanogenesis in anaerobic digestion (Kim *et al.,* 2013,

Barret *et al.*, 2012). The occurrence of methanogens at every stage of the reactor is due to the fact that the effluent is recycled every day so as to retain the methanogens inside the reactor. Cluster analysis of T-RFs indicated relatedness at different stages of the reactor (Figure 7). Distinct clustering pattern was observed among the samples belonging to inlet, middle compartment and outlet of the reactor. T-RFs at the regions A and B are closely clustered and T-RFs C, D, E and F are together. Diversity indices like Shannon Weaver diversity index (H), Simpson index (D) and Equitability index (E) describing various aspects of ecosystem was determined (Table 2). Shannon –Weaver index (H) describes the diversity of phylotypes in the samples studied, whereas Simpson index (D) gives us an insight about the abundance of phylotypes. Equitability index (E) describes the evenness in the distribution of phylotypes. All samples showed a high diversity index ranging from 1.41 – 1.95 for Shannon Weaver index, 0.63 – 0.78 for Simpson index but low equitability index values i.e 0.21 – 0.27. Thus the above values indicate high diversity but less evenness.

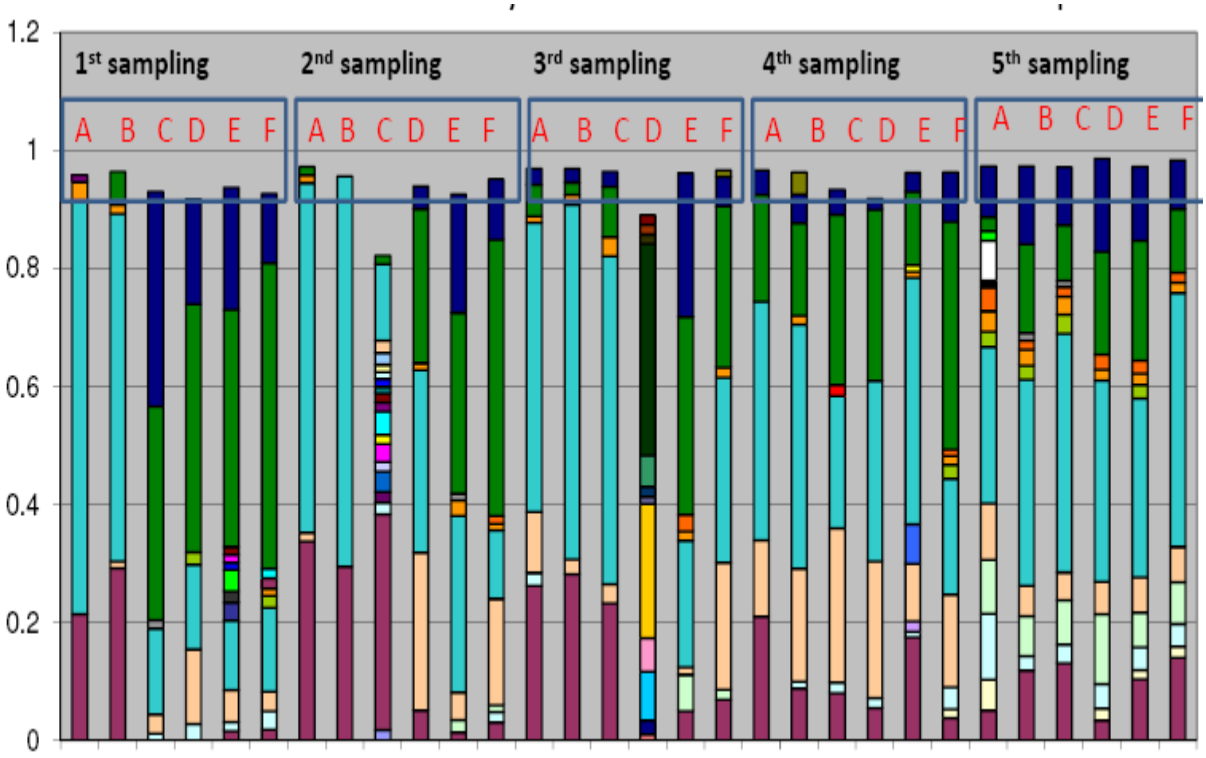

Figure 4.7a Relative abundance of methanogens based on the diversity of *mcrA* genes obtained at different positions of the horizontal plug flow reactor. Sample designations start with the sampling number followed by position of sampling and M stands for *MspI* restriction enzyme.

	Length (bp)	Organism
	175.70	*Methanoculleus sp.*
	57.31	*Methanoculleus sp.*
	464.33	*Methanotorris sp.*
	470.89	*Methanobacterium sp.*
	167.18	Methanosarcinaceae

Identification of TRFLP fragments after cloning and sequencing

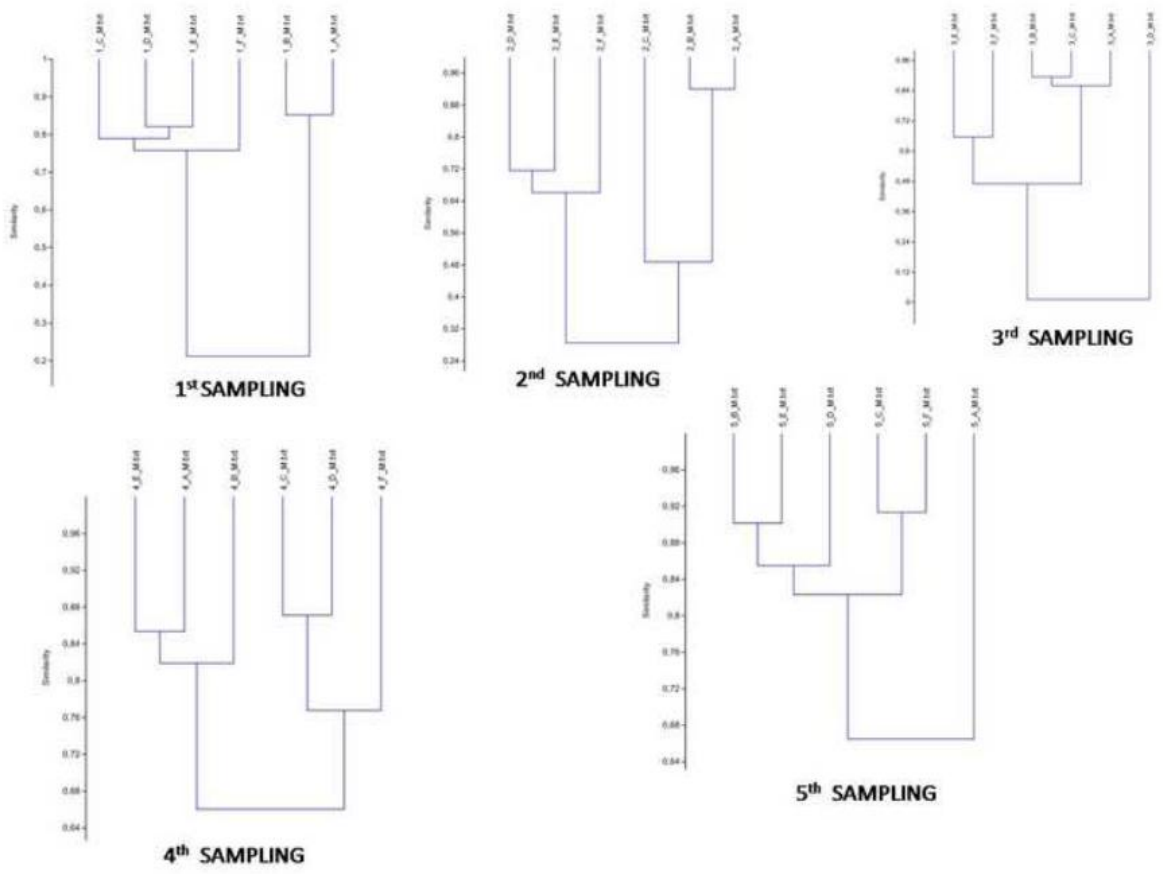

Figure 4.7b Cluster analysis of methanogens based on the diversity of *mcrA* genes obtained at different positions of the horizontal plug flow reactor. The software used for the study are BLASTIN and BLASTX

Table 4.2: Average diversity indices of anaerobic digestion of food waste in a horizontal plug flow reactor sampled at 6 different positions (A-F).

Diversity indices	A	B	C	D	E	F
No of Taxa	21	21	32	33	27	26
Simpson index	0.66	0.63	0.75	0.78	0.78	0.75
Shannon Weaver index	1.51	1.41	1.96	1.93	1.95	1.84
Evenness	0.25	0.21	0.25	0.25	0.27	0.26

4.2.4 Effect of ammonia

Table 4.3 shows amount of biogas and methane produced when inoculum from food waste based horizontal plug flow reactor was incubated at different concentrations of ammonia. Relative abundance of various functional groups of methanogens based on the diversity of *mcrA* genes in the presence of different concentrations of ammonia at 24h and 48h in comparison with inoculum after endogenous respiration is shown in Figure 4.8a and Figure 4.8b respectively. Cluster analysis of OTU distributions from TRFLP analysis is shown in Figure 4.9a and Figure 4.9b respectively. There is no significant difference in the amount of biogas produced or in the microbial diversity at varying concentrations of ammonia.

Table 4.3: Biogas and methane production during anaerobic digestion of food waste in serum vials at different concentrations of ammonia.

Ammonia Concentration mg/L	Biogas (ml/day)		Methane %	
	24 h	48 h	24 h	48 h
0	96	103	29.5	26.6
150	107	116	38.9	46.4
300	111	115	41	46.4
500	108	117	43.6	46.3
750	112	119	45	49.49
1000	109	121	44	47.44
1500	107	116	44.5	48.18
2000	118	125	46	47.1

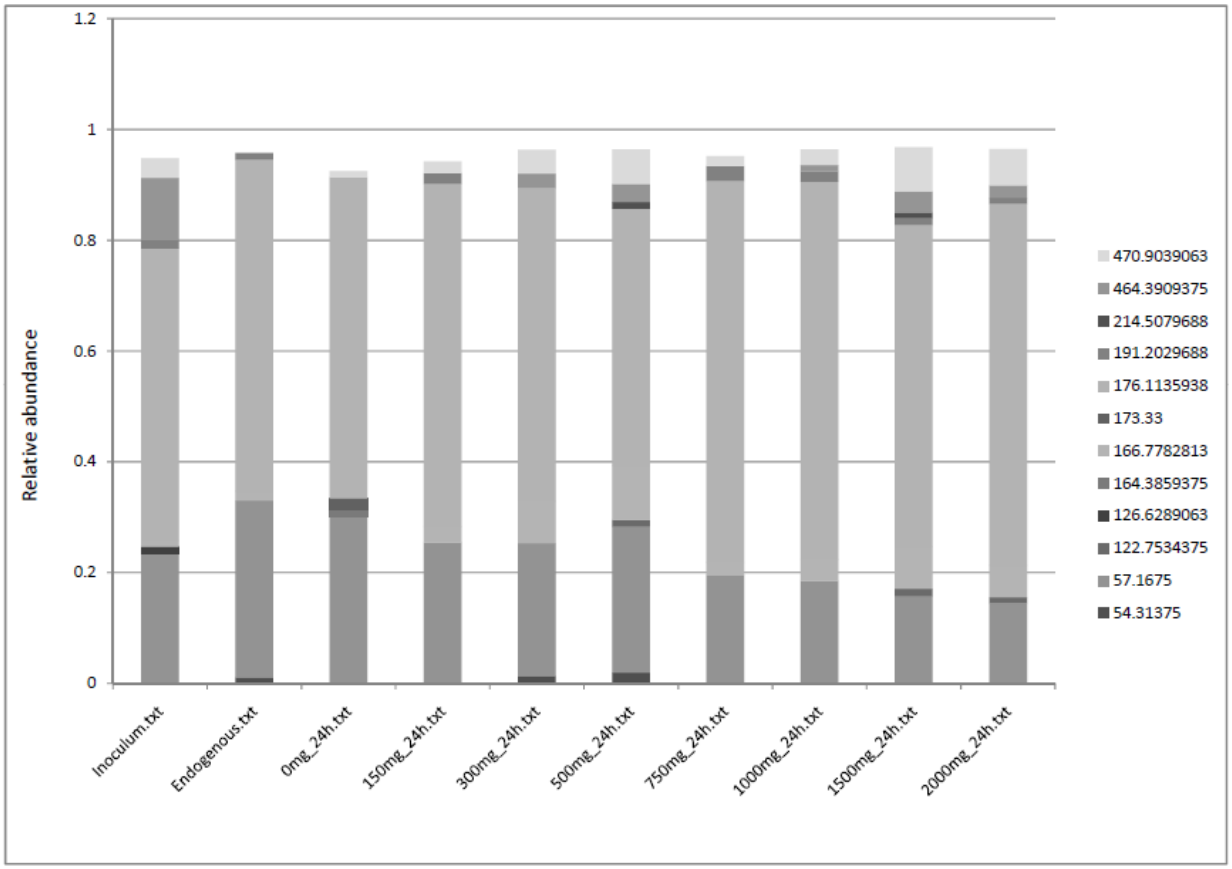

Figure 4.8a Relative abundance of methanogens based on the diversity of *mcrA* genes obtained in the presence of different concentrations of ammonia during 24hours incubation.

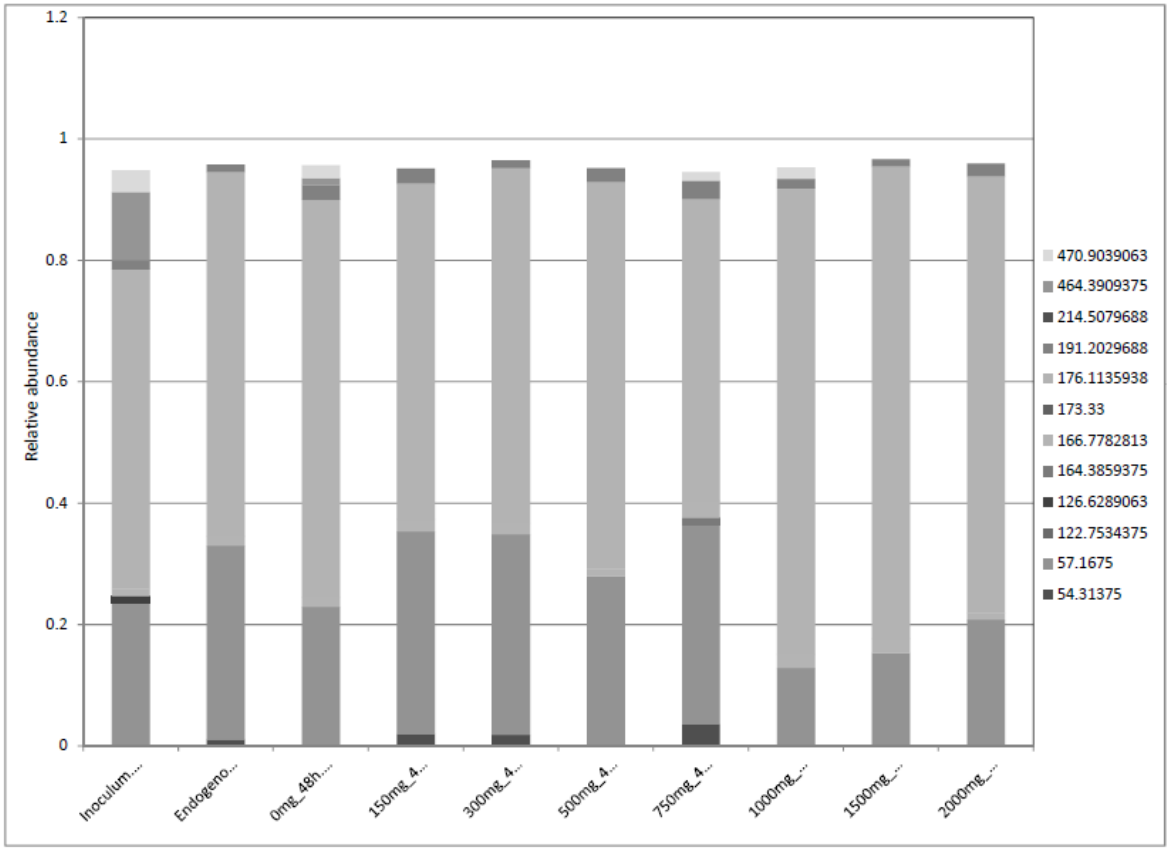

Figure 4.8b Relative abundance of methanogens based on the diversity of *mcrA* genes obtained in the presence of different concentrations of ammonia during 48 hours incubation.

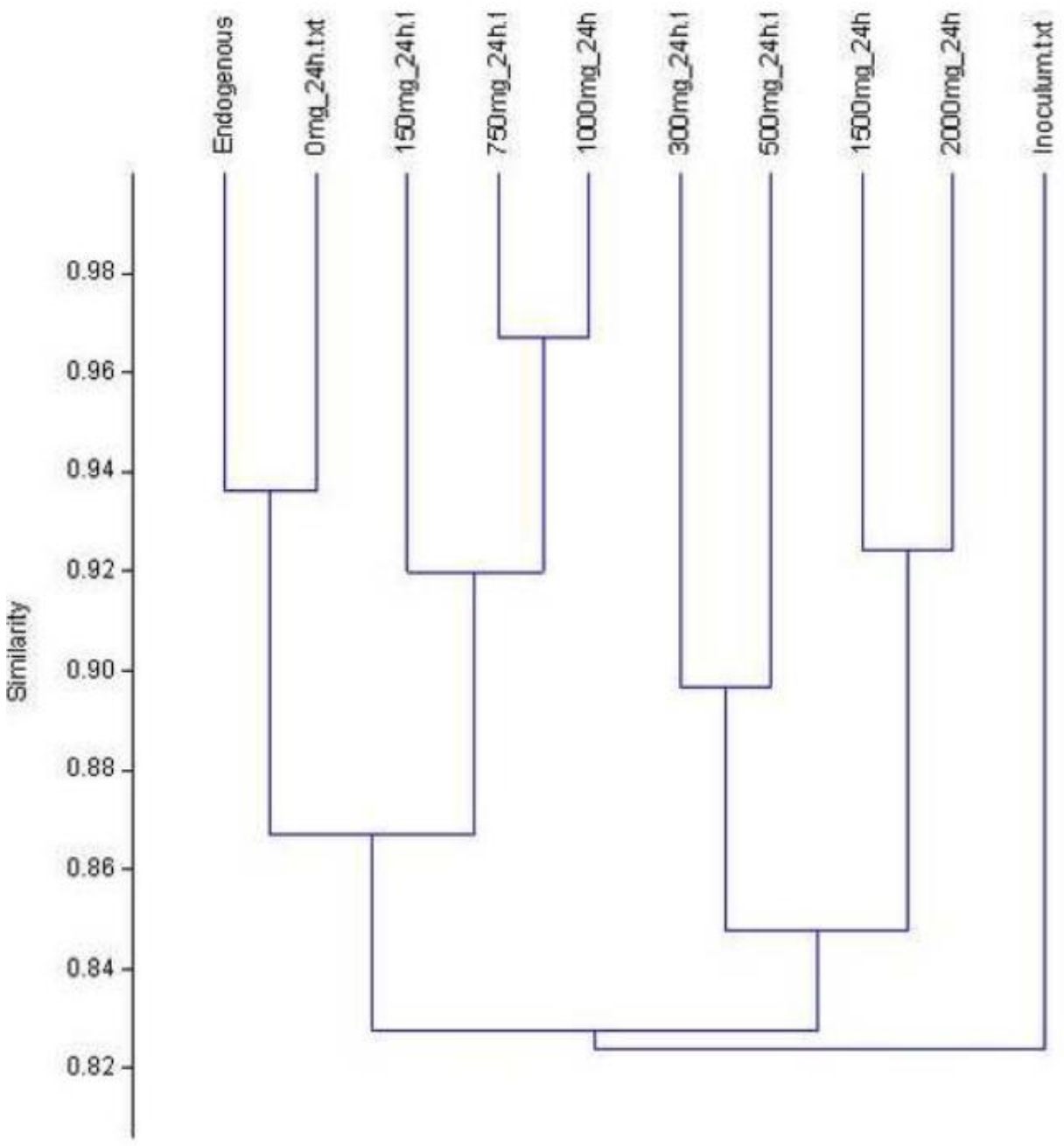

Figure 4.9a: Cluster analysis of methanogens based on the diversity of *mcrA* genes obtained in the presence of different concentrations of ammonia during 24 hours incubation. The X axis indicates the relative abundance of various methanogans and Y axis indicates the different concentrations of ammonia (mg/l)

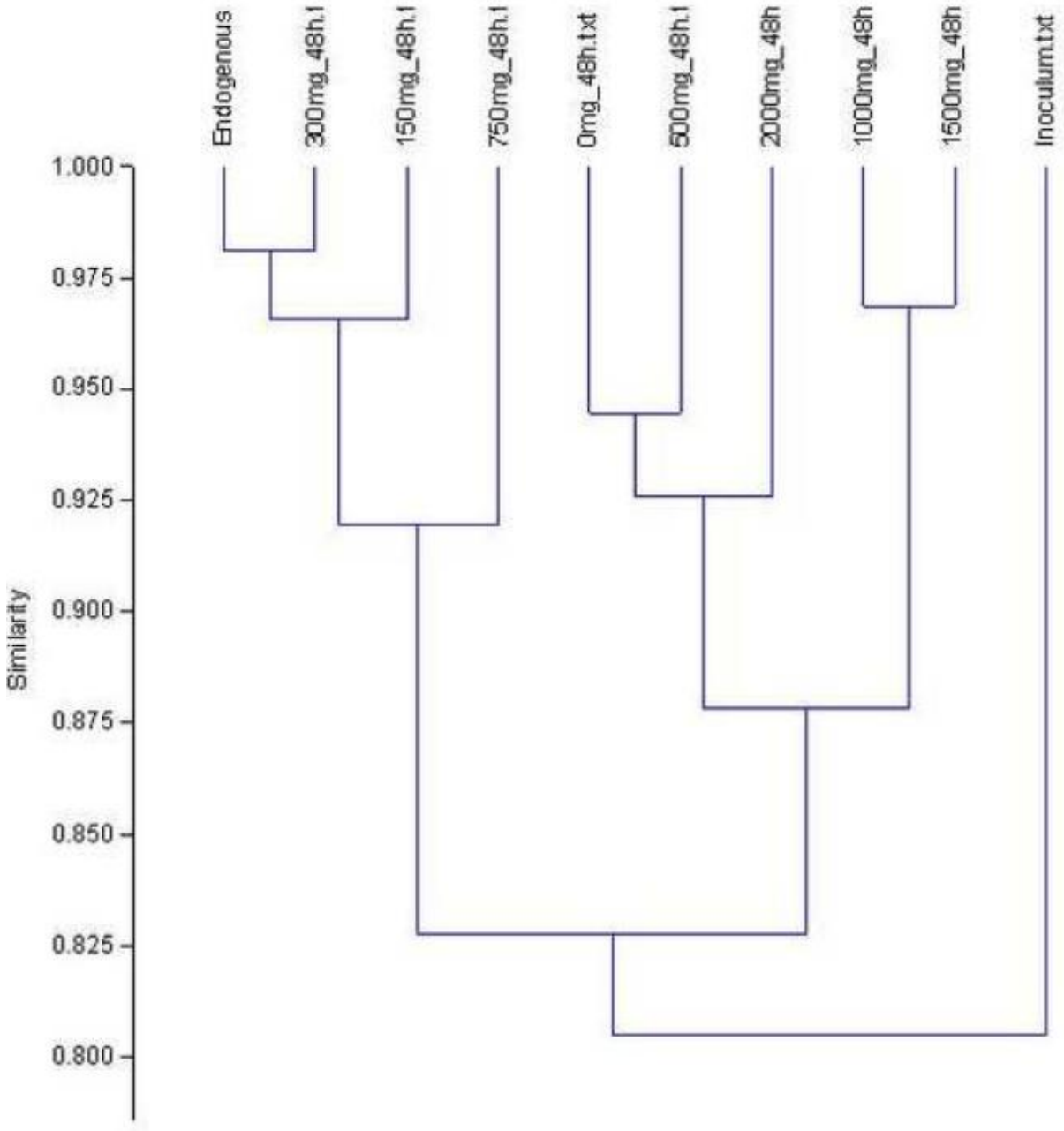

Figure 4.9b Cluster analysis of methanogens based on the diversity of *mcrA* genes obtained in the presence of different concentrations of ammonia during 48 hours incubation. The X axis indicates the relative abundance of various methanogans and Y axis indicates the different concentrations of ammonia (mg/l).

5 *Effect of pre-treatment on undigested food waste fractions*

Food waste is characterized by their high organic content, most of it being composed of easily biodegradable compounds carbohydrates, proteins and in some cases, small amount of lipids. The anaerobic biodegradability of organic matter depends on its composition and the amount of methane produced depends on the biochemical nature of the waste (Buffiere *et al.,* 2006). For instance carbohydrates, proteins and fats show different methane production rates (Angelidaki and sanders, 2004).

Although food waste has been regarded as readily biodegradable because of its high volatile fraction (90 % of total solids), its hydrolysis reaction is still a rate limiting step (Kim *et al.,* 2006). Enhancement of the hydrolytic reaction during anaerobic digestion could shorten the hydraulic retention time and thus improve the economics of the process. During recent years, various studies have been conducted on pre-treatment of food waste, such as mechanical and sonication (Hidalgo D *et al.,* 2012), thermal (Wang *et al.,* 2006; J. Ma *et al.,* 2011), acid (Karlsson and Ejlertsson, 2012), alkaline (Eom *et al.,* 2009) and enzymatic (Rintala *et al.,* 1994; Kim *et al.,* 2006; Moon and Song *et al.,* 2011).

The commonly seen undigested solid fractions in the outlet of the pilot scale anaerobic digester set up in the Institute's premises are cottage cheese, whole potatoes and whole eggs. Studies have shown that digestate can still contain a high biogas potential, mainly as a consequence of residual and undigested volatile solids (Hansen *et al.,* 2006). Digested solid fraction, with its biogas and methane potential could be used as a substrate for anaerobic digestion (Menardo *et al.,* 2011). Utilizing digested; separated solid fraction in this manner would capture residual methane and consequently, could reduce GHG emissions (Amon *et al.,* 2006).

An attempt has been made in this work by conducting lab scale studies to improve anaerobic digestion by pre-treating cottage cheese using thermal, chemical, thermo-chemical or enzymatic methods. These pre-treatments could improve the waste stabilization and methane production but their application should be proved to be commercially viable in relation to the additional processing costs. (Esposito *et al.*, 2012). Due to high fat and protein content, cottage cheese can be considered as a good substrate for anaerobic digestion process. For enzymatic treatment we have used enzymes from extreme halophiles as extremozymes are more stable in harsh conditions.

5.1 MATERIALS AND METHODS

5.1.1 Screening of Haloarchaea for enzyme production

The first stage in this study was screening of extreme halophiles isolated from solar saltern for production of protease and lipase. The cultures were grown on NTYE (NaCl, Tryptone, and Yeast Extract) medium. The culture medium for enzyme production was composed of: (w/v) NaCl 200g/l, $MgCl_2.6H_2O$ 13g/l, KCl 4g/l, $CaCl_2.H_2O$ 1g/l, $NaHCO_3$ 0.2 g/l, NH_4Cl 2g/l, $FeCl_3.6H_2O$ 0.005g/l, KH_2PO_4 0.5g/l, pH 7.0 Inoculum density 2% (v/v). For production of protease skim milk was used as substrate and for lipase olive oil was used as substrate.

5.1.2 Screening Method

The organisms were allowed to grow on agar plates containing substrate for particular enzymes i.e. skim milk for protease and olive oil for lipase and incubated at room temperature. The enzymes used in this study and their reported activities were: protease 125 units/ml. Lipase 150 units/ml. The activity of protease was checked with skim milk as substrate and activity of lipase was checked with olive oil as substrate. For measuring protease and lipase activity the raw enzyme used for the assay was isolated from the culture broth following separation of cells. The culture medium was centrifuged at 8,000 rpm for 20 minutes at 4^oC and the cell free supernatant was used as the source of enzyme.

5.1.3 Concentration of the cell free supernatant (CFS)

The cell-free supernatant (CFS) of the isolates obtained as described above was subjected to ultrafiltration. Briefly, 50 ml of the CFS obtained was concentrated 10 times (i.e. 5 ml) of its original volume using nominal molecular weight cut-off (NMWCO) 3.0-kDa cellulose membrane in a stirred ultrafiltration cell (Model 8050, Millipore, U.S.A.).

5.1.4 Substrate

Cottage cheese separated from the undigested solid fraction of food waste was used as the substrate. The cottage cheese was collected from the outlet of the horizontal plug flow reactor treating food waste.

5.1.5 Pre-treatments

5.1.5.1 Thermal: The substrate was autoclaved at 15 psi and 120^{o}C for 20 minutes.

5.1.5.2 Chemical: The substrate was treated with sodium hydroxide 0.5 M.

5.1.5.3 Thermo-chemical: The substrate was first autoclaved at 15 psi and 120^{o}C for 20 minutes and then 0.5 M sodium hydroxide was added.

5.1.5.4 Enzymatic: First, the single enzyme hydrolysis of cottage cheese using protease and lipase was carried out at different concentrations from 0.02 to 0.5% (v/v) with each enzyme. Secondly, three levels of mixed enzyme ratio were tested with 0.04 % of mixed enzyme ratio: 1:1, 1:2 and 2:1 of protease and lipase respectively to determine the optimal enzyme mixture ratio.

5.1.6 Biochemical Methane potential assays of pre-treated Cottage Cheese

Methane potential was determined in batch assays as described by Owens and Chynoweth, 1993; Hansen *et al.,* 2004 and Angelidaki *et al.,* 2009. The inoculum for batch assays was the effluent from a horizontal plug flow reactor treating food waste. The reactors were supplemented with nutrients, trace elements and bicarbonate. Finally, the reactors were made up to the working volume 0.1L with distilled water and the headspace was flushed with nitrogen. A control without substrate was also set up to account for the endogenous biogas produced from the inoculum. All the experiments were carried out in duplicates. The reactors were shaken manually once a day. Biogas production was measured using water displacement technique. Gas samples were taken periodically for composition analysis by gas

chromatography using hydrogen as carrier gas. The calculated biogas production is also corrected for blank biogas production.

5.1.1.7 Analysis

Total solids and volatile solids were measured in accordance with standard methods (APHA, 1998). The chemical oxygen demand measurement is performed on fresh waste. Prior to use the substrates were ground in a blender to give a fraction with particle size less than 2 mm. The substrate (1 g) is then suspended in 1L ml of distilled water, stirred on a magnetic stirrer for one hour, and the COD of the suspension is measured as described by Rapaso *et al.,* (2008). Gas samples were taken periodically for composition analysis. The samples were analysed with a gas chromatograph (GC-7610, Chemito) equipped with thermal conductivity detector. The carrier gas was hydrogen. The oven, injector and detector temperatures were 60, 150 and 180^{o}C respectively.

5.2 RESULTS AND DISCUSSION

5.2.1 Screening of protease and lipase

Four haloarchaeal strains were selected and screened for production of lipase and protease enzyme. It was found that BK-11 and BK-20 were shown to produce larger zone of clearance (Table 5.1). These two strains were selected for further work.

Table 5.1 Extremely halophilic archeal strains producing protease and lipase

Archaeal strain	Protease	Lipase
BK6	-	-
BK7	-	-
BBK1	+	-
BK11	+	+ +
BK18	+	+
BK19	+	+
BK20	+ +	+
BK3	-	-

Table 5.2 Characteristics of the Substrate

The main characteristics of cottage cheese used in the experiment are reported in table (2).

Parameters	
TS	46.74%
VS	46.09%
VS/TS	0.96%
SCOD	860 mg/l
Fats	25-27%
Proteins	17-18%
Moisture	53.26%
pH	5.5

5.2.3 Effect of pre-treatment on solubilisation of cottage cheese

Four different pre-treatments (thermal, chemical, thermo-chemical and enzymatic) were used in order to hydrolyse cottage cheese. The soluble COD of cottage cheese increased with each pre-treatment compared to the untreated sample. The most effective pre-treatments were thermal and enzymatic. Chemical and thermo-chemical were less effective in terms of solubilisation. Cottage cheese consists of 25-27% of fats. Sodium hydroxide has been reported more efficient in hydrolysing proteins and carbohydrates than lipids (Luste *et al.,* 2009) which could explain lesser solubilisation of cottage cheese with alkali treatment. Biological and physiochemical pre-treatments promote the substrate hydrolysis, breaking down the polymer chain into soluble components (Vavilin *et al.,* 2007). For enzymatic pre-treatments an increase in soluble COD was observed with increasing concentrations of enzymes. Pre-treatment with protease showed higher solubilisation percentage in comparison with lipase (Figure 5.1).

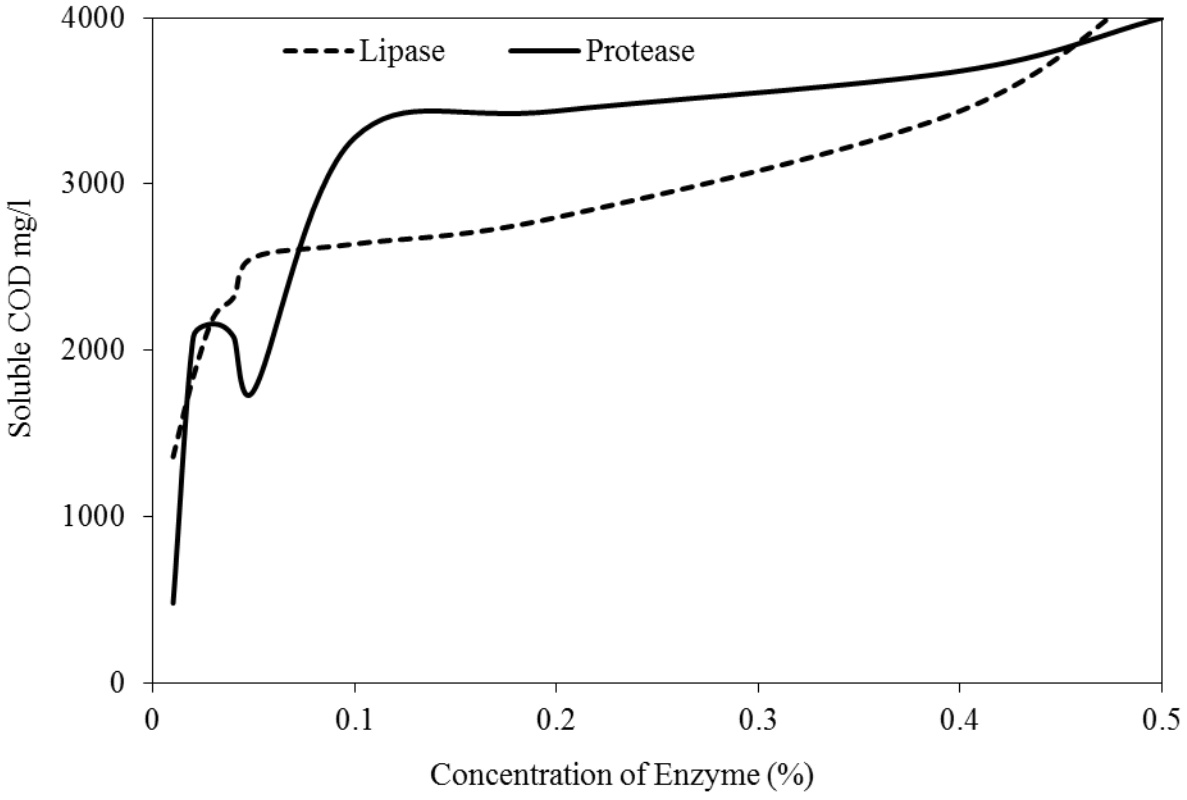

Figure 5.1 Solubilisation of cottage cheese during enzymatic pre-treatment using lipase and protease

5.2.4 Biogas Production and Methane yield during Pre-treatments

For the different pre-treatments studied, biogas production started immediately with thermal pre-treatment, but thermo-chemical and chemical pre-treatment showed a lag phase of around 15 days. The highest cumulative biogas production was obtained in thermo chemical pre-treatment; 615 ml/g VS followed by thermal treatment 602 ml/g VS (Figure 5.2). The lowest production, 410 ml/g VS was observed in chemical treatment. Thermal pre-treatment is capable of speeding the hydrolytic phase of anaerobic digestion favoring organic molecule degradation and to accelerate the bacterial metabolic process (Kaparaju and Rintala, 2005). Alkaline pre-treatment generally requires longer reaction times compared to other pre-treatment methods (Chandra *et al.*, 2012). The highest methane yield was obtained with thermo-chemical pre-treatment 441ml/g VS followed by thermal alone (357 ml/g VS)

whereas lower yield was obtained in chemical pre-treatment 293 ml/g VS added (Figure 5.3). Though thermo-chemical pre-treatment of cottage cheese showed high methane potential (441 ml/g VS added), but the methane production started after a lag phase of 2-15 days most likely due to inhibitory compounds of alkali treatment. A disadvantage of alkaline pre-treatment is the generation of irrecoverable salts and/ or the incorporation of salts into the substrate during pre-treatment reactions (Yi *et al.*, 2009).

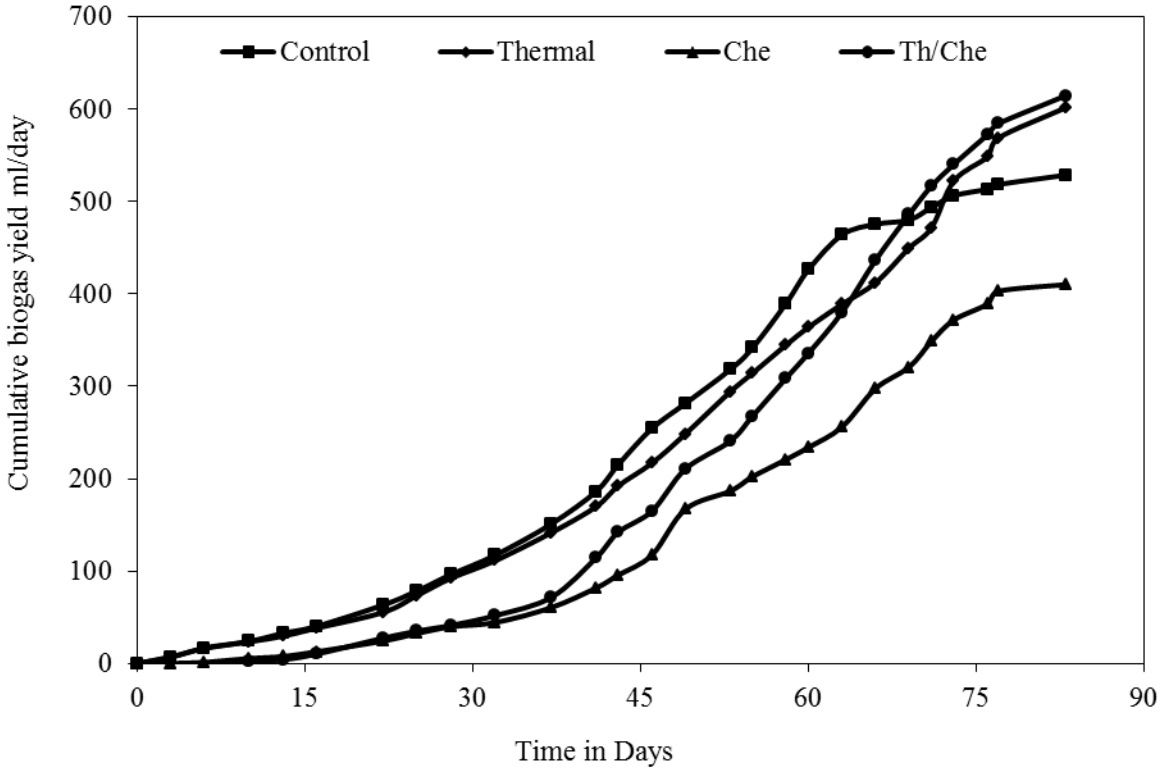

Figure 5.2 Cumulated biogas yield for cottage cheese with different pretreatments and without treatment (control).

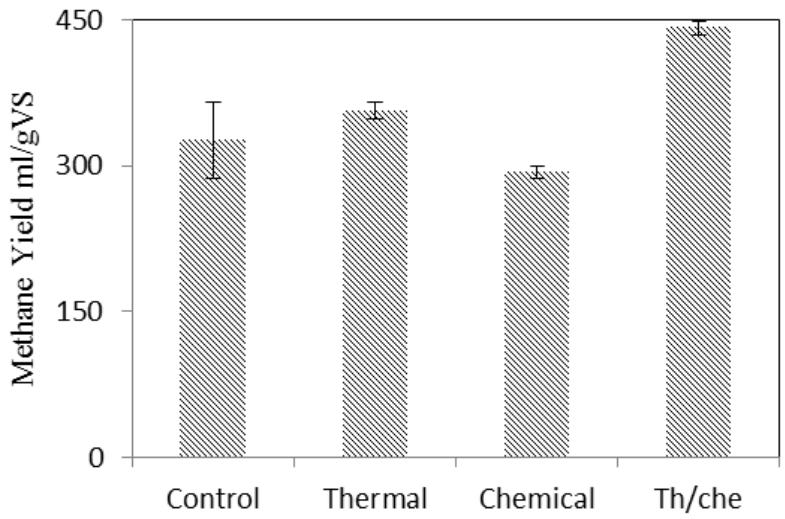

Figure 5.3 Cumulated methane yield of Cottage cheese without pretreatment (control) and with different pretreatments.

5.1.2.5 Single enzyme treatment (Lipase)

In single enzyme treatment with lipase, biogas production in control, 0.08%, 0.1% and 0.2% evolved similarly, starting to decrease from day 25 on and reaching the plateau after 40 days (Figure 5.4), whereas 0.02%, 0.04% and 0.06% reached maximum biogas production faster, after approximately 20 days. With lowest enzyme addition there was a significantly higher yield than for the experiments without enzyme additions. The highest enzyme dosages decreased methane yield from cottage cheese. The increase in soluble COD from pre-treatment may not be inhibitory but increasing the organic loading to the methanogens and overloading the anaerobic digester can be inhibitory (Carlsson *et al.*, 2012). The maximum biogas production of 623 ml/g VS was observed at 0.06 %. The addition of enzymes gave a slight increase in the initial methane production rate for different concentrations of enzymes compared to the control Figure 5.6. The average methane production was 335 ml/gVS. The addition of enzymes in anaerobic digesters treating food processing waste resulted in

improved digestion and biogas production (Parawira, 2011). Cottage cheese is a product of dairy industries, it has been reported that lipases are very promising alternative for degrading lipid rich wastewater generated by dairy and slaughter house industries (Mendes *et al.,* 2006).

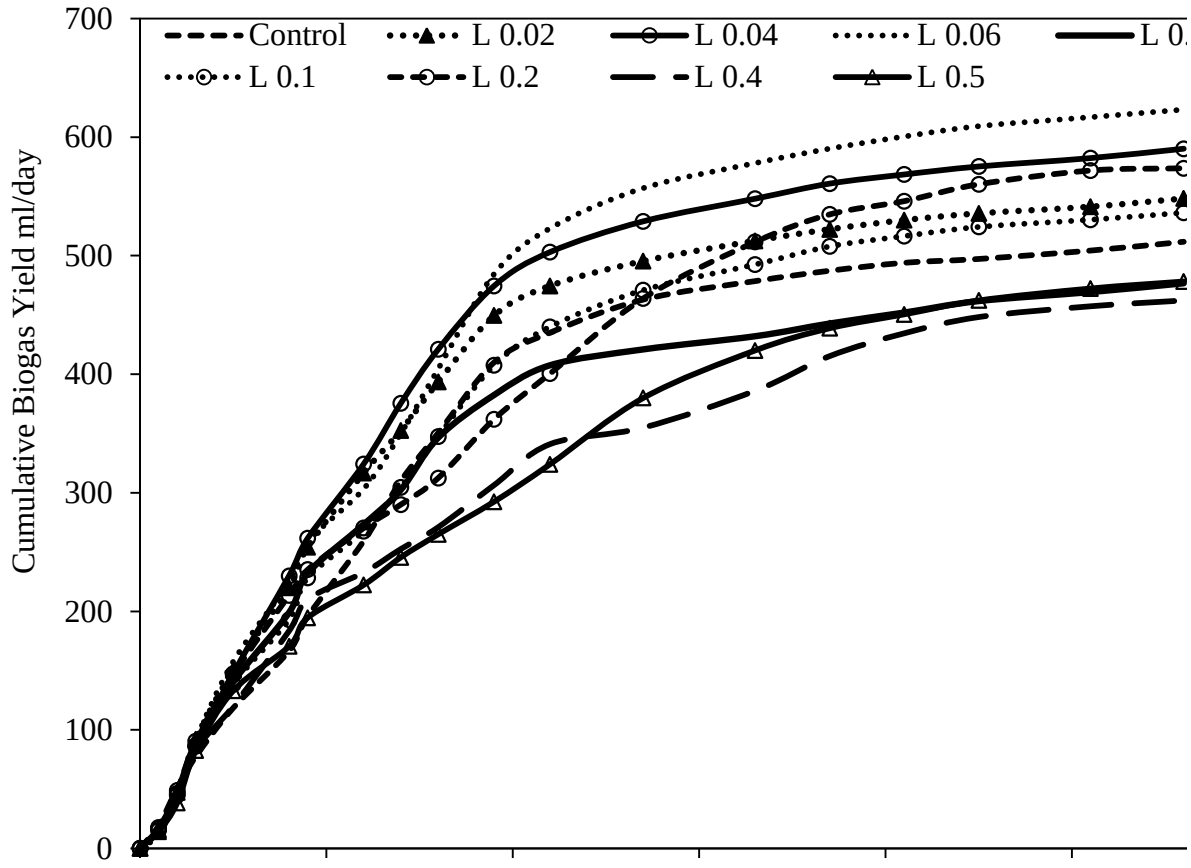

Figure 5.4 Cumulated biogas yield for cottage cheese pretreated with different concentrations of lipase.

5.1.2.6 Single enzyme treatment (Protease)

In single enzyme treatment with protease, biogas production in control, 0.04% and 0.06% evolved similarly, starting to decrease from day 25 on and reaching the plateau after 40 days (Figure 5.5), whereas 0.08% to 0.5% reached maximum biogas production faster, after approximately 20 days. In case of protease enzyme an increase in concentration of the enzyme gave higher biogas yield. However, the average methane production was 328

ml/gVS which is slightly lower than that obtained in single enzyme treatment with lipase (Figure 5.6).

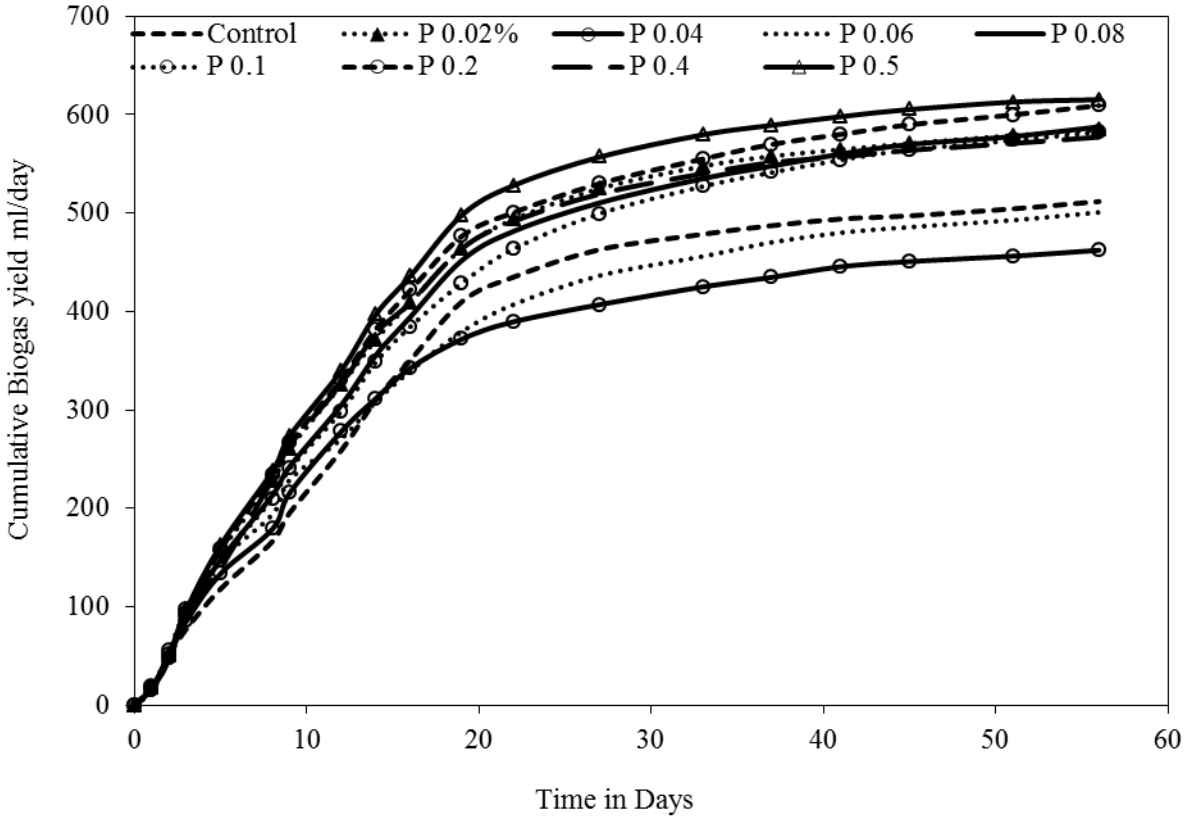

Figure 5.5 Cumulated biogas yield for cottage cheese pretreated with different concentrations of protease.

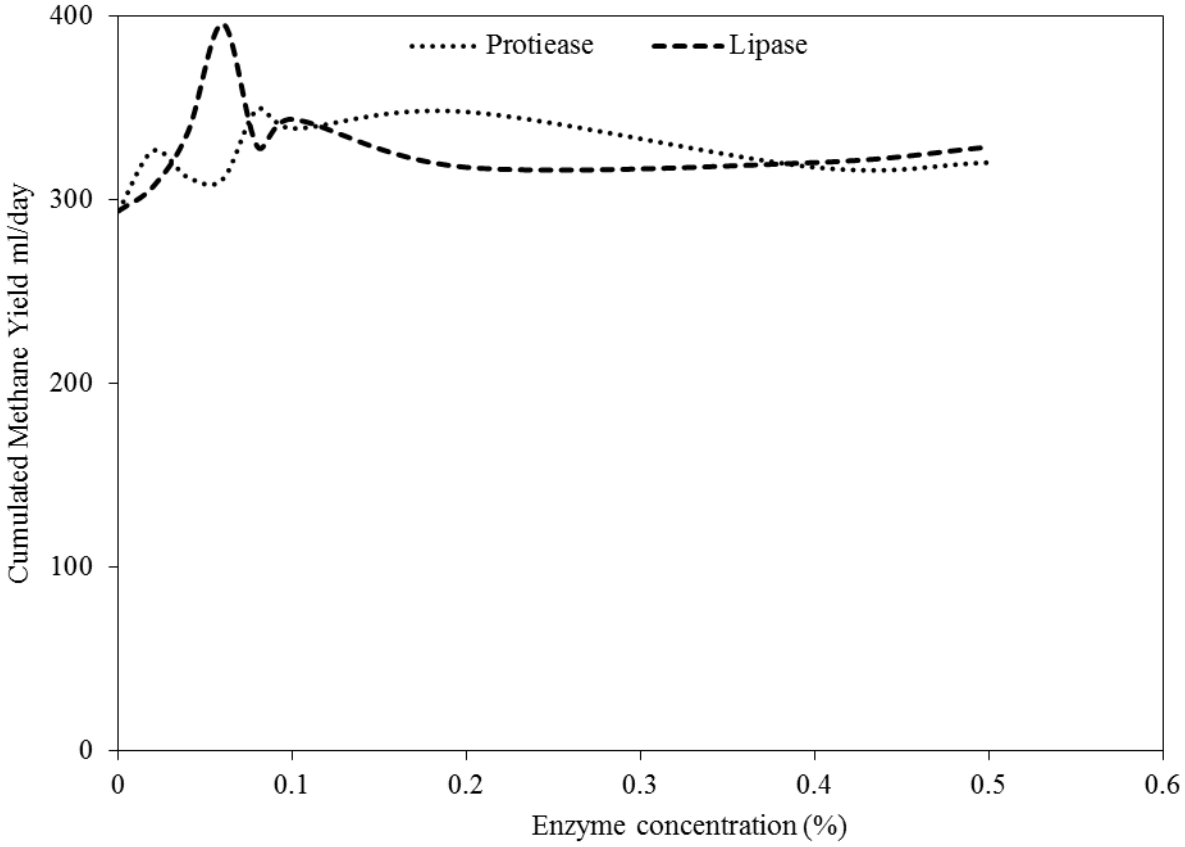

Figure 5.6 Cumulated methane yield for cottage cheese at pretreated with different concentrations of lipase and protease.

5.1.2.7 Mixed Enzyme pre-treatment

For mixed enzyme pre-treatment two enzyme combinations with three different ratios 1:1, 1:2 and 2:1 (Lipase: Protease) were investigated using an equivalent dosage of 0.04 % (v/v), to evaluate the effect of mixed enzyme ratio on methane production. The methane yield observed was 526, 571 and 539 ml/gVS CH_4 at 1:1, 1:2 and 2:1 Lipase: Protease respectively. For all three mixed enzyme ratios methane production was higher than those of single enzyme treatments. Though different ratios of enzyme additions did not show much significance but the biogas production rate and biogas yield were higher compared with the control (Figure 5.7). As expected from single enzyme pre-treatment results 1:2 ratio of lipase: protease showed higher methane production in comparison with 1:1 and 2:1 ratio.

In single enzyme pre-treatments, we observed that the average methane production was similar that is 335 ml and 328 ml for lipase and protease respectively (Figure 5.8). However, in the case of mixed enzyme system, pre-treatment at 1:2 ratio of lipase:protiease showed higher methane production than the 1:1 and 2:1 ratio. As discussed earlier, increase in soluble COD, can increase the organic loading to the methanogens and overload the anaerobic process. Luste *et al.,* 2009 have reported inhibitions at higher concentrations of enzymes for meat processing waste.

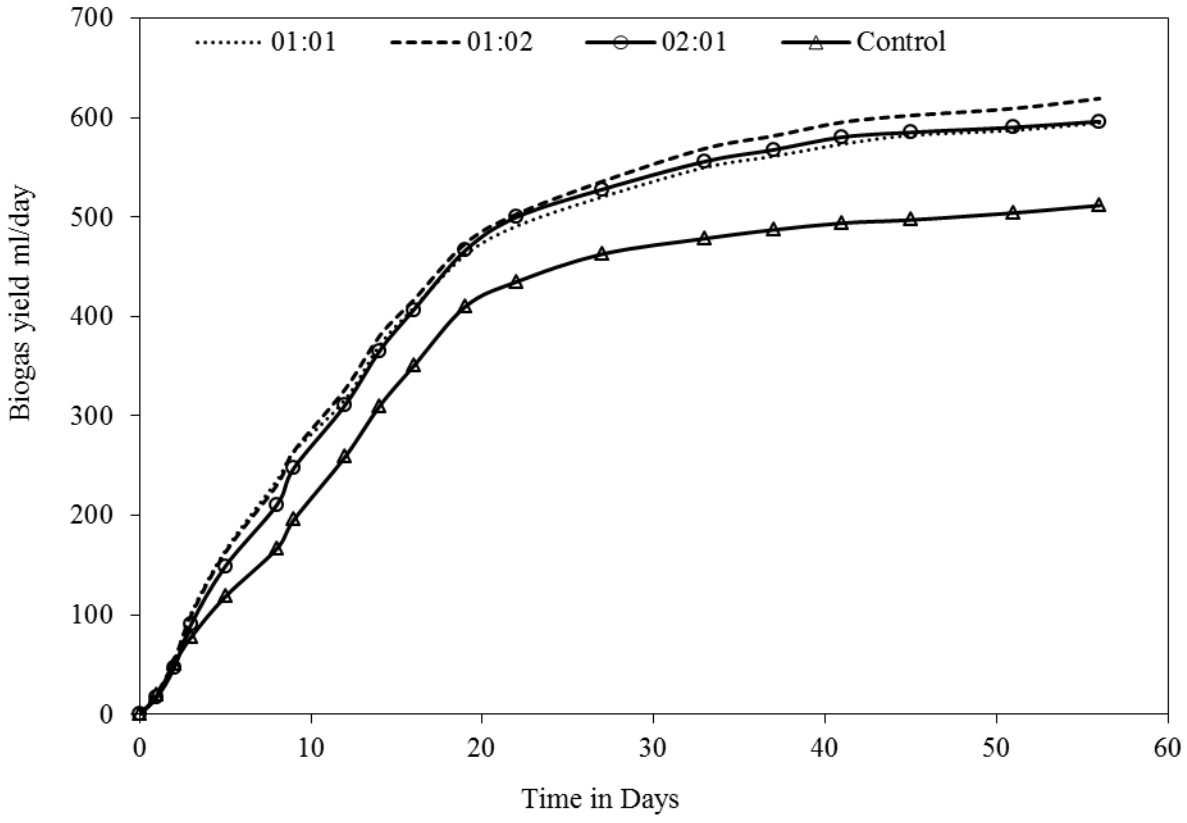

Figure 5.7 The effect of enzyme mixture addition on biogas yield from cottage cheese at different mixed enzyme ratio.

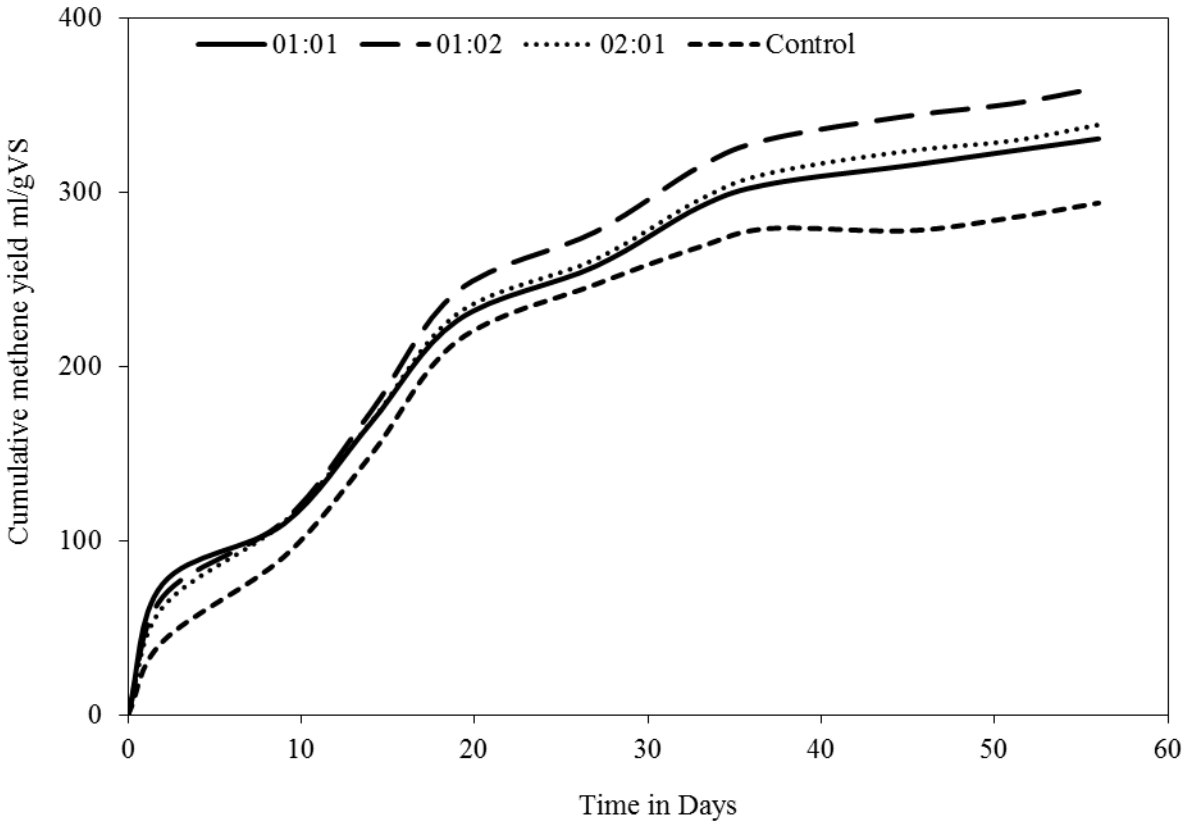

Figure 5.8 The effect of the enzyme mixture addition on methane production from cottage cheese at different mixed enzyme ratios.

6 *Effect of microbial stimulants on anaerobic digestion of food waste*

The physical and chemical characteristics of organic wastes like pH, moisture content, total solids, volatile solids, particle size and biodegradability play an important role in anaerobic digestion process. Zhang *et al.*, (2007) determined the average methane yield of food waste at 6.8 and 10.5 g VS/L were 425 ml and 445ml/g VS/L after 28 days of digestion. There are several technologies that have been used to increase methane generation. Possible techniques include pretreatment of the feedstock with heat, ultrasonic devices, or impact grinding; microbial stimulants; or co-digestion with other wastes. It has been reported by Singh *et al.*, (2001) that the use of microbial additives with food waste increased gas production by 16%. Plant secondary metabolites namely caffeine and saponin which acts like metabolic stimulants are used in this study to see the effects on the anaerobic digestion of food waste. Plants produce various secondary compounds mainly for protecting themselves from insects, animals, fungi or bacteria. Besides tannins, saponins are the most widely occurring compounds. Few studies were conducted to evaluate the potential of secondary plant constituents as natural agents to manipulate rumen fermentation with mixed results (Wallace *et al.*, 1994; Sliwinski *et al.*, 2002; Wina *et al.*, 2005). As the processes that take place in rumens is similar to anaerobic digestion, we studied the effect of saponin on anaerobic digestion. We also selected caffeine for our study as it is the most powerful and addictive stimulant and it is found in coffee spent ground apart from tannins and polyphenols whose properties have not been utilized properly (Pandey *et al.*, 2000).

6.1 MATERIALS AND METHODS

6.1.1 Food waste collection and analyses

The food waste was collected as source separated waste from the Institute's cafetaria. To gain an understanding of the compositional variability and amount of food waste, daily and weekly sampling was performed. The food waste composition and variability of total solids was studied for a period of one month.

6.1.2 Anaerobic Digestion Tests

The biodegradability of food waste from the institute mess was determined using batch anaerobic digestion (AD) tests. The initial tests were to optimize the concentration of total solids. Samples used for this study were the composite samples which were prepared by mixing all food waste samples and then taking representative samples from the mixture. The representative samples were shredded in an electric blender and then used for AD tests. The AD tests were carried out in 0.5 L bottles at varying initial total solids loadings (5%, 8%, 9%, 11% and 14%) each in duplicate. Cow dung was used as inoculum. The mixing ratio of food waste to cow dung was 80:20. After the inoculum and food waste were added, each reactor was filled up to 400 ml with distilled water. The pH value was adjusted to 7.2 using 3N NaOH. The reactors were tightly closed with a screw cap. To ensure anaerobic conditions, the headspace was purged with N_2 gas. Each reactor was manually mixed twice a day and the pH was maintained at 7.2 using 3N NaOH.

6.1.3 Anaerobic Digestion Tests using Microbial Stimulant

From the tests described previously, 8% TS content was found to be optimum for biogas production. To test the effect of plant secondary metabolite caffeine, similar batch tests as described before were set up at 8% TS content. One set of digestion served as control and the other set were spiked with 100 ppm of caffeine on the 4^{th} day and 6^{th} day of incubation. In another experiment, the effect of different concentrations of plant secondary metabolites like caffeine and saponin on biogas production from food waste was studied at 8% TS content in 0.5 L reactors. The different concentrations of caffeine and saponin used were 50, 100, 150 ppm. The effect of saponin on biogas production was also studied at 25 ppm. In all the above mentioned experiments, the biogas production was measured by water displacement set up (Singh *et al.*, 2001).

6.1.4 Critical Micelle Concentration (CMC) of saponin

CMC value for saponin was determined by diluting the saponin until reaching the critical Micelle concentration (CMC) which was determined by plotting the surface tension as a function of saponin concentration and then the surface tension at that point wherein there is no further decrease in surface tension is called as CMC (Kim *et al.*, 2000).

6.1.5 Substrate analysis

Aliquots of fresh and digested samples were drawn from each set of experiments, and analyzed for total solids and total Kjeldahl nitrogen according to the standards methods of American public health association (APHA, 1998). Chemical oxygen demand (COD) was determined as described by Raposo *et al.*, (2008).

6.1.6 Scanning Electron Microscopy (SEM)

Fixation and dehydration of anaerobic samples were done according to Wu *et al.*, (2001). The dehydrated samples were dried using the critical point drier (Lab companion HP-300 drier) and then coated with platinum by an ion sputter JEOL JFC -1600 Autobine coater. Finally the platinum coated samples were observed in a JEOL JSM-6360 LV Scanning Electron Microscope with an accelerating voltage of 20 KV. The microscopic observation was carried out and photographed extensively to make sure that the predominant microbial population was obtained.

6.2 RESULTS AND DISCUSSION

6.2.1 Anaerobic Digestion tests

Anaerobic experiments were set at varying total solids concentrations of 5%, 8%, 9%, 11% and 14%. Among all the different TS concentrations 8% TS yielded more biogas and 11% and 14% did not yield biogas production which indicates that dry digestion was not possible with respect to our case. The biogas production rate and biogas yield during the digestion of food waste are shown in Figure 6.1.The maximum biogas yield of 573 ml/g TS was found at 8% of TS concentration with 18 days of digestion whereas, at 9% TS 267ml of biogas was produced. At 8% and 9% TS gas production increased until day 5. At 5% TS, biogas production was relatively low during the first three days of digestion, increased to reach peak on the fifth day of digestion and then declined again.

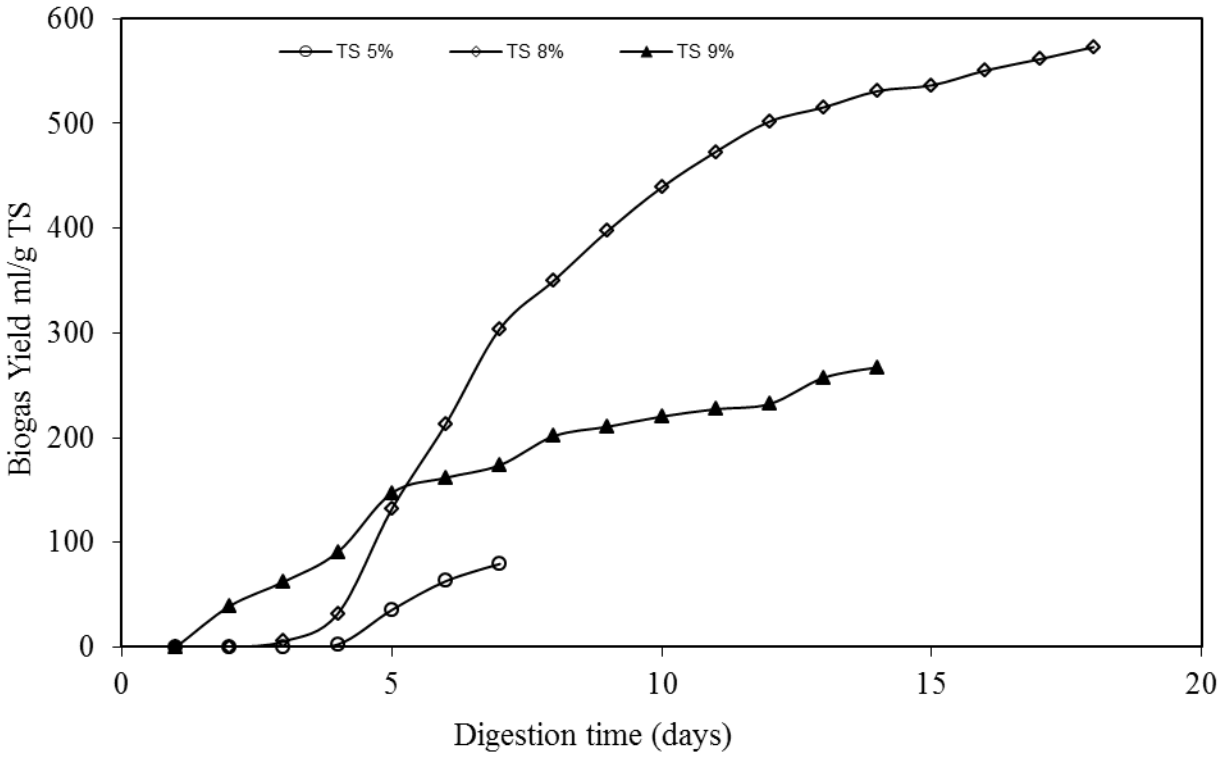

Figure 6.1 Daily biogas yields during anaerobic digestion of food waste at different total solids concentrations.

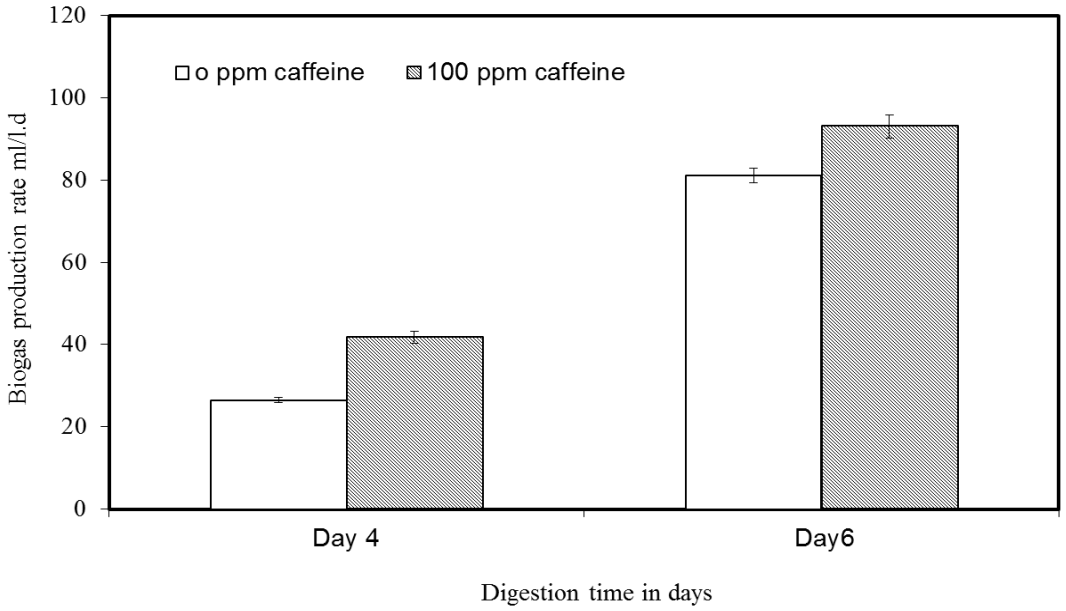

Figure 6.2 The effect of addition of Caffeine on biogas production rate at Day 4 and Day 6 during anaerobic digestion of food waste at total solids concentration of 8%.

6.2.3 Anaerobic Digestion tests using microbial stimulants

In the second experiment, the effect of microbial stimulant, caffeine (100 ppm) was evaluated on food waste and cattle dung mixture (80:20) at total solid concentration of 8%. The biogas yield and biogas production rate from food waste spiked with caffeine are shown in Figure 6.2. It was observed that the addition of single dose of caffeine at the rate of 100 ppm on the fourth day of incubation resulted in increased gas yield 42 ml/g TS in comparison with control 26 ml/g TS. To confirm the effect of caffeine we spiked with additional 100 ppm of caffeine on the 6^{th} day. After the second spiking it was observed that biogas production increased by 16% in comparison with the control.

Further biogas production was also evaluated using different concentrations of caffeine (50, 100 and 150 ppm). The addition of caffeine at the rate of 50, 100, 150 ppm to the food waste cow dung mixture on the first day of addition resulted in biogas production on day two itself

with all the three concentrations of caffeine whereas, control (0 ppm caffeine) started biogas production on day four. The biogas production of food waste was found at an average of 367.8 ml/g TS at all the three concentrations of caffeine. The maximum biogas production of 408.5 ml/g TS was found at 100 ppm caffeine whereas, 50 ppm and 100 ppm caffeine produced 359 ml and 336 ml in comparison with the control which showed 182 ml/gTS (Figure 6.3). This is the first study of effect of caffeine as stimulants in anaerobic environments.

Caffeine is known to induce laccase enzyme and sclerotization in *Rhizoctonia solani, Pseudomonas fluoresens* interactions due to the triggering of calcium or heat shock signaling pathways by bacterial metabolites as shown by Rollin and Dickman, (1998). Crowe and Olsson, (1998) found that laccase enzyme was induced 5 fold during caffeine driven sclerotization in *R. solani*. Ogunseitan, (2002) reported caffeine induced production of haem associated peroxidase activity in *Pseudomonas putida* ATCC 700097. Various studies had shown that caffeine is known to induce different enzymes but phosphodiesterase enzyme is inhibited by caffeine, the stimulatory effect of this plant secondary metabolite being the result of raised cAMP levels. cAMP, the cell signaling molecule is a general activator of cell activity.

Contradicting reports are available on the effect of saponins on methanogenesis. Guo *et al.,* (2008) reported inhibition of methanogenesis in the presence of saponin probably due to interference with interspecies hydrogen transfer between the protozoa and associated methanogens. Hiroki *et al.,* (2004) reported that the addition of saponin was effective for improving anaerobic digestion especially increasing the acidogenesis rate. In our studies addition of saponin inhibited biogas production. As Quillaya saponin used in our study is used as a biosurfactant in the biodegradation of hydrocarbons (Pijanowska *et al.,* 2007), we measured the surface tension of saponin at different concentrations to calculate critical

Micelle Concentration (Figure 6.4). The CMC (Critical Micelle Concentration) for saponin was found to be 6 ppm and surfactants above CMC usually inhibits bacterial growth. This might be the reason in our case to observe inhibition of biogas production in the presence of saponin.

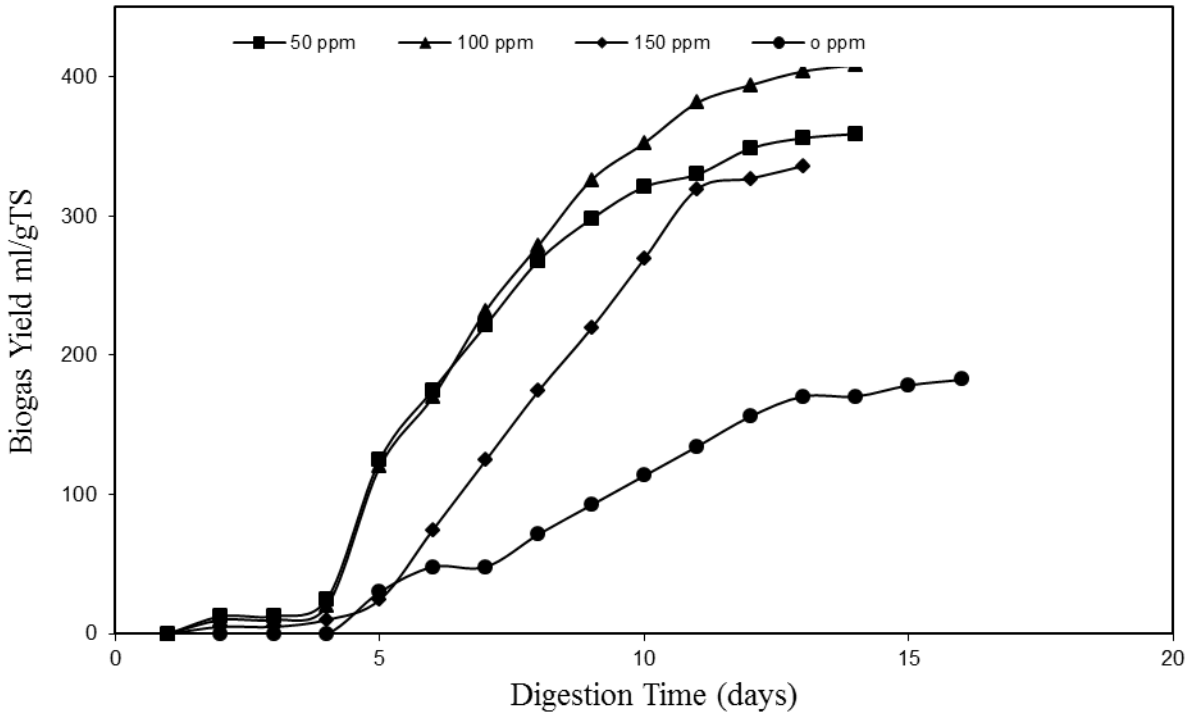

Figure 6.3 Effect of different concentrations of caffeine on biogas production during anaerobic digestion of food waste at total solid concentration of 8%.

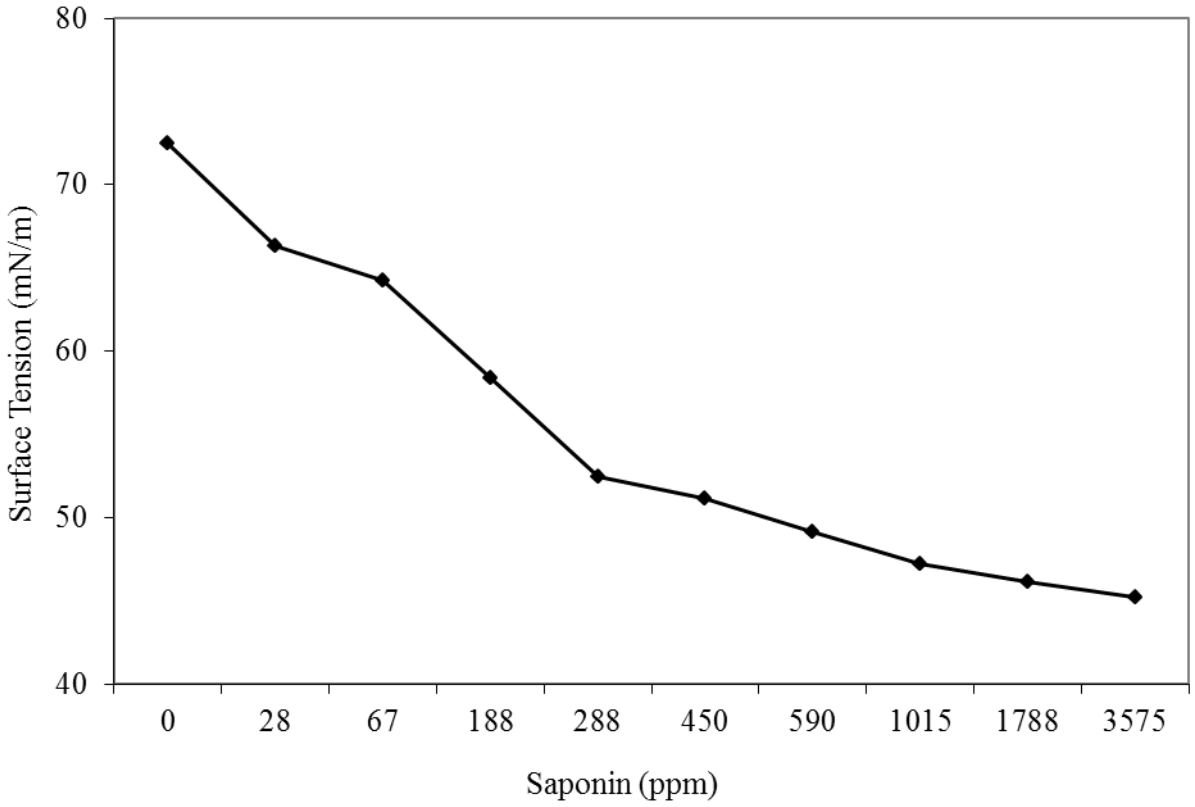

Figure 6.4 Critical Micelle concentration of saponin. CMC value for saponin was determined by diluting the saponin until reaching the critical Micelle concentration (CMC) which was determined by plotting the surface tension as a function of saponin concentration and then the surface tension at that point wherein there is no further decrease in surface tension is called as CMC.

6.2.4 Scanning Electron Microscopy (SEM)

Anaerobic samples were observed with SEM. SEM was used to observe the main cellular morphology during anaerobic digestion in the presence of caffeine. Figure 6.5 showed the diversity of morphotypes found in the anaerobic digestion with rod like morphologies that could correspond to *Methanobacterium* and *Methanobrevibacter* (Yang *et al.*, 2008).

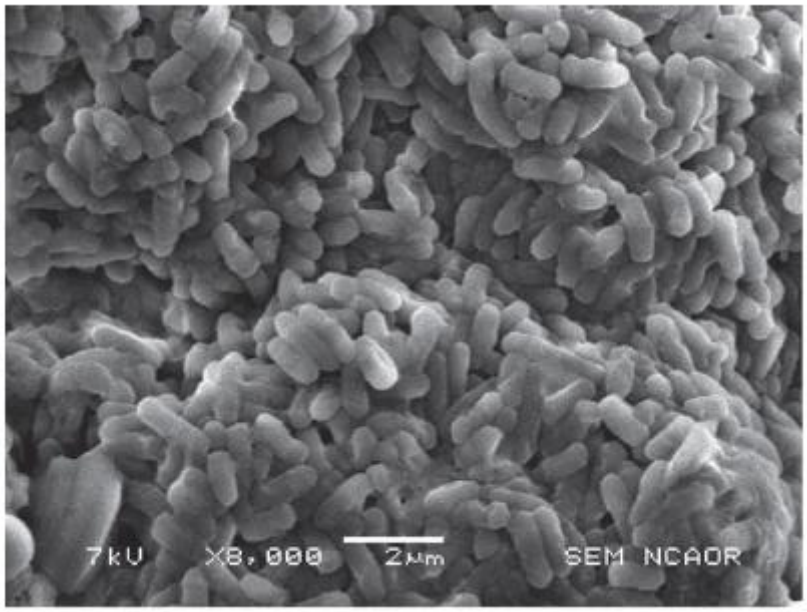

Figure 6.5 Scanning Electron Microscopy of anaerobically digested food waste. The diversity of morphotypes found in the anaerobic digestion with rod like morphologies that could correspond to *Methanobacterium* and *Methanobrevibacter*

7 *Biochemical methane potential of agro wastes*

Apart from food waste, agro wastes and energy crops represent an important source of biomass that can serve as a substrate in anaerobic digestion, resulting in the production of renewable energy. This could substitute fossil fuel-derived energy and reduce environmental impact including global warming and acid rain (Chynoweth *et al.*, 2001). The wastes selected for our study are coconut oil cake (residue obtained after oil extraction), cashew apple waste (crushed apple waste obtained after extraction of "Feni" – "Feni" is a form of liquor i.e., distilled from the pure fermented juice of cashew apple without addition of spirit), and grass from lawn cuttings.

The direct comparison of bio-methane production from different feedstock is difficult as performance data for specific types are often produced under a wide variety of experimental conditions e.g. mixing regime, temperature, total solids, volatile solids and hydraulic retention time. For this reason, it is better to compare feedstock by their ultimate methane yield determined by the biochemical methane potential (BMP) assay (Owens and Chynoweth 1993; Hansen *et al.*, 2004; Angelidaki *et al.*, 2009). Chynoweth *et al*., (2001) studied the biochemical methane potential of a variety of feedstocks. Gunaseelan, (2004) reported the ultimate methane yields of several fractions of fruit and vegetable solid wastes, sorghum and napiergrass using the BMP assay. Cavinato *et al*., 2010 studied the co-digestion of cattle manure with agro- wastes and energy crops. Similar studies on biomethanization of energy crops were reported by Demirel and Scherer, (2009). The purpose of this work was to study the anaerobic digestion of three different substrates namely coconut oil cake, cashew apple waste, and grass cuttings in order to evaluate the potential of anaerobic digestion as an alternative to the conventional solution like composting and incineration while reducing the energy consumption of fossils fuels.

7.1 Materials and Methods

7.1.1 Feedstocks

Coconut oil cake and cashew apple waste were collected from local industries in and around Goa. Grass cuttings were from the lawns of our institute campus (BITS-Pilani, K. K Birla Goa Campus). Cashew apple waste was collected from a cashew plantation located in Sancoale, Goa. Coconut oil cake was collected from a factory extracting coconut oil located in Cansaulim, Goa. And grass clippings were from the lawns of our institute campus.

Prior to use, the substrates were ground in a blender to give a fraction with particle size less than 2 mm and stored at 4^{o}C until use.

7.1.2 Inoculum

Cow dung was used as inoculum with total solid content of 20g/l. The inoculum was pre incubated in order to deplete the residual biodegradable organic material present in it.

7.1.3 Analytical methods

7.1.3.1 Total solids (TS) Estimation

For total solids, a known amount of sample was transferred into a previously weighed crucible and dried at 105^{o}C for 24 h. The increase in weight over that of the empty crucible represents the total solids (APHA, 1998).

7.1.3.2 Volatile solids (VS) Estimation

For volatile solids estimation, the dried sample obtained after TS estimation was ignited in a muffle furnace at 550 ^{o} C for 2 h. The weight lost on ignition represents the volatile solids (APHA, 1998).

7.1.3.3 Chemical oxygen Demand

The Chemical oxygen demand measurement is performed on fresh waste. Prior to use, the substrates were ground in a blender to give a fraction with particle size less than 2 mm. The substrate (1 g) is then suspended in 1L of distilled water, stirred on a magnetic stirrer for one hour, and the COD of the suspension is measured as described by Rapaso *et al.,* (2008). Briefly, 10 ml of suspension was digested with Potassium dichromate and concentrated sulphuric acid at 150^{o}C for 2 h in a COD Block Digestion Unit. After cooling the digestate is titrated against ferrous ammonium sulphate (0.5 N) using 1, 10-phenantroline as indicator.

7.1.3.4 Biochemical Methane Potential Assays

The Biochemical methane potential (BMP) of the substrates was performed according to (Hansen *et al.,* 2004; Angelidaki *et al.,* 2009). The methane potential of the substrates was determined over the following range of VS, i.e. 3.0, 3.5, 4.0, 4.5 and 5.0 g VS/l. The reactors were supplemented with nutrients, trace elements and bicarbonate. Finally, the reactors were made up to the working volume 0.1L with distilled water, and the headspace was flushed with nitrogen. A control without substrate was also set up to account for the endogenous biogas produced from the inoculum. All the experiments were carried out in triplicates. The bottles were shaken manually once a day. Biogas production was measured using water displacement technique. Gas samples were taken periodically for composition analysis by gas chromatography using hydrogen as carrier gas. The calculated biogas production is also corrected for blank biogas production.

1ml macronutrient, 0.8 ml micronutrients, and 5 ml of buffer from the stock solutions were added (Table 6.1).

Table 6.1 COMPOSITION OF SOLUTIONS FOR BIOCHEMICAL METHANE ASSAYS

Macronutrients	(g/L)
NH_4Cl	26.6
KH_2PO_4	10
$MgCl_2$, $6H_20$	6
$CaCl_2$, $2H_20$	3
Micronutrients	
$FeCl_2$, $4H_2O$	2
$CoCl_2$, $6H_2O$	0.5
$MnCl_2$, $4H_2O$	0.1
$NiCl_2$, $6H_2O$	0.1
$ZnCl_2$	0.05
H_3BO_3	0.05
Na_2SeO_3	0.05
$CuCl_2$, $2H_2O$	0.04
Na_2MoO_4, $2H_20$	0.01
Buffer	
$NaHCO_3$	50

7.1.4 Biogas Measurement

The biogas production was measured by water displacement set up (Singh *et al.*, 2001). A tube was used to connect the reactor with an inverted 250 ml graduated measuring cylinder immersed in a 1000 ml beaker filled with water. Biogas produced was collected in the graduated cylinder connected with a water reservoir which allowed volumetric biogas measurements at atmospheric pressure.

7.1.5 Gas Composition

Gas samples were taken periodically for composition analysis by gas chromatography. The samples were analysed with a gas chromatograph (GC-7610, Chemito) equipped with thermal conductivity detector. The carrier gas was hydrogen. The oven, injector and detector temperatures were 80, 150 and 250^{o}C, respectively.

7.1.6 Batch Reactors

A double walled reactor of 3L effective volume was used in this study. Temperature was maintained at 35°C by a water recirculation. Mixing was done by magnetic stirring.

Series of experiments were carried out at the same time. The reactor was seeded at a volatile solids (VS) concentration of 3.0 g/L with anaerobic sludge, taken from the outlet of an anaerobic pilot reactor treating food waste.

7.2 Results and Discussion

7.2.1 Selection of Feedstocks

7.2.1.1 Coconut oil Cake

India is the third largest coconut producing country in the world with a cultivation area of about 1.78 million hectares (Moorty and Vishwanathan, 2009). In a conventional edible oil manufacturing mill, oil is extracted from dried copra (matured coconut Kernel) leaving behind a protein and lignocellulosic rich oil cake.

The advantage of using oil cakes as a substrate for biogas production is their cheaper availability throughout the year. Moreover, with increasing emphasis on cost reduction of industrial processes and value addition to agro-industrial residues, utilization of oil cakes as an energy source seems to be promising because of their higher energetic value. Typically coconut oil cake was used as cattle feed, but the dominant factor that affected coconut oil cake market was the situation of other oil cakes (meal), especially, soybean meal and sunflower meal. Also the reason for looking for alternative use of coconut oil cake rather than as cattle feed is the introduction of attractive incentives for green fodder cultivation by Government of Goa, India. High incentives are given by the local government, which is incentive under Perennial Fodder Cultivation at 370 USD per hectare area of land for the 1st year, 185 USD each per hectare area of land for 2nd and 3rd years. This has resulted in decline in demand for coconut oil cake as cattle feed in Goa, India.

7.2.1.2 Cashew Apple waste

Cashew is an important cash crop grown in India, on an area of 820,000 hectares which produced 539, 000 tons of raw nuts in 2004-2005. The fruit consists of mainly the nuts containing an embryo (Kernel) and a false fruit commonly called cashew apple. The nuts represent only 10 % of the total fruit weight, and large amount of cashew apples are lost in the field after nut removal (Nagaraja and Bhuvaneshwasri, 2007). Although cashew apples

can be consumed as juice, jams, and other food stuffs, the cashew tree cultivation is an agricultural activity directed at the production of the cashew nuts. Due to its large availability and low cost, cashew apple has been studied as a substrate for fermentative and enzymatic processes for several applications (Honorato and Rodrigues, 2010; Fontes *et al.,* 2009; Chagas *et al.*, 2007; Rodrigues *et al.,* 2007 and Honorat *et al.*, 2007). As such, cashew apple is considered an agricultural residue rich in reducing sugars (fructose and glucose), vitamins, minerals and some amino acids (Silveria *et al.,* 2010) and can be a suitable low cost substrate for anaerobic digestion studies.

7.2.1.3 Grass

Energy production from lignocellulosic biomass will be a major alternative to conventional energy sources. The efficient conversion of plant biomass to biogas remains a challenge because of the presence of recalcitrant and insoluble starting materials (Yang *et al.,* 2009).

The characteristics of the different substrates selected are shown below in Table 6.2

Table 6.2 Characteristics of substrates

Feedstock	TS %	VS %	VS/TS %	TCOD g/gVS	COD g/kg
Coconut oil cake	90.93	84	0.92	0.87	1.057
Cashew apple	21.8	21.2	0.97	0.9	1.077
Grass	56	53	0.95	0.4	2.3

The TCOD /VS ratio close to unity indicate better biogas potential and thus methanogenesis can be elucidated from COD/VS ratio (Angelidaki and Sanders, 2004).

7.2.2 Digestion of coconut oil cake

The daily biogas production rate showed multiple peak values over time (Figure 7.1). Multiple peaks indicate the presence of multiple substrates within coconut oil cake or solid matrix of the coconut oil cake, and as a result, additional cellulose becomes bioavailable (Eleazer *et al.*, 1997). Coconut oil cake is rich in lignocellulosic content and protein (Moorty and Vishwanathan, 2009). Biogas production increased until 30^{th} day, and then there was slight increase until 60^{th} day. There was an initial lag phase for 5 days. The average biogas production was found to be 451 and 662 ml/gVS at 4 and 4.5gVS/l respectively (Figure7.2). The percentage of methane in the biogas produced from coconut oil cake was found to be 55% which corresponds to 383 ml CH_4/gVS and 277 ml CH_4/g VS added respectively. At lower 3.0 and 3.5 g VS/l as well as higher concentration of volatile solids (5gVS/l) the reactors did not recover from the initial lag phase, and no significant amount of biogas was produced. Increasing the volatile solid loading rate decreased biogas production; this could be due to accumulation of intermediates as reported by Hansen *et al.,* (2004) for waste rich in fats and lipids. Coconut oil cake contains about 5-6% of oil in it even after extraction as the extraction of oil is only by mechanical extraction.

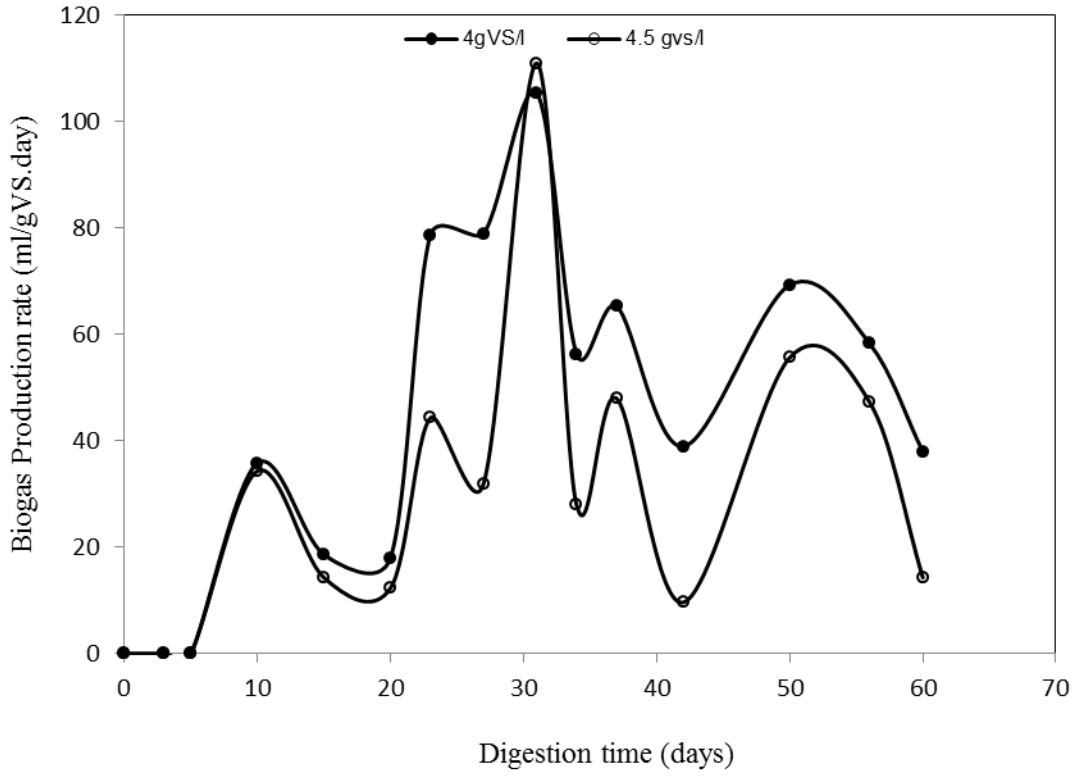

Figure 7.1 Daily biogas production rates for coconut oil cake at different volatile solid concentrations

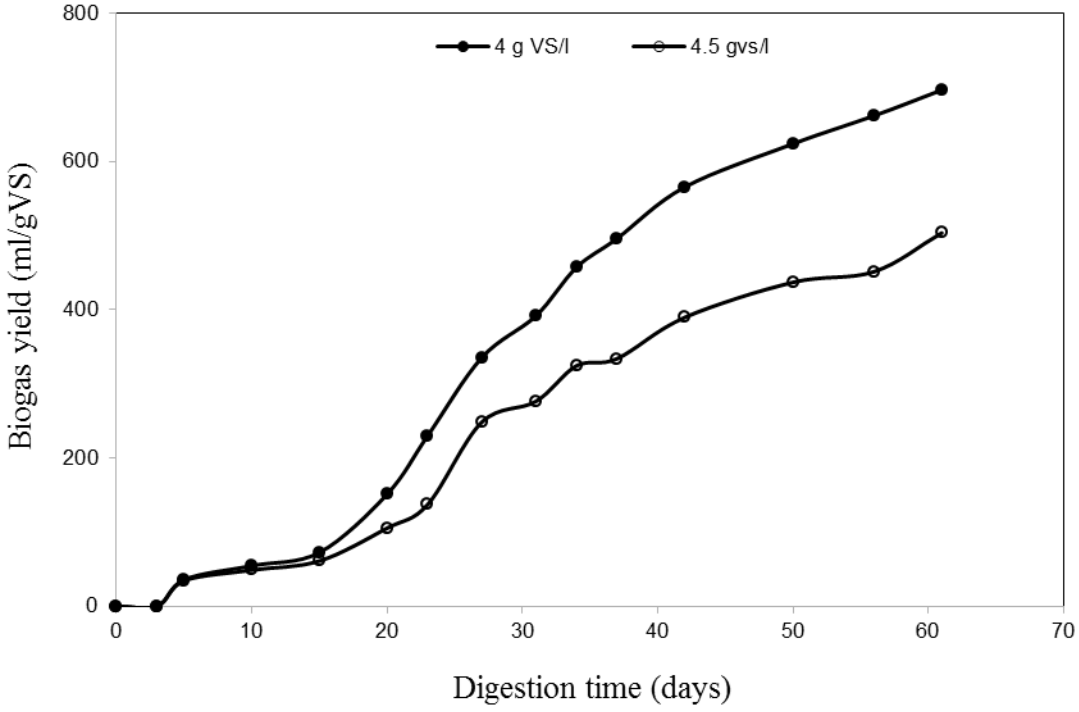

Figure 7.2 Biogas yield for coconut oil cake at different volatile solid concentrations

7.2.3 Digestion of Cashew apple

The biogas production started from the first day, but declined rapidly with no biogas produced at the end of 14 days of digestion (Figure 7.3 and Figure 7.4). The pH values in the digesters at the end of digestion time were below 6.0. Fruit and vegetable wastes tend to have low total solids and high volatile solids. The VS/TS ratio for cashew apple was very high 0.97gVS/gTS. The rapid hydrolysis of the feedstocks might have led to acidification and consequent inhibition of methanogenesis, which is a major limitation of one-stage anaerobic digestion system (Ward *et al.*, 2008).

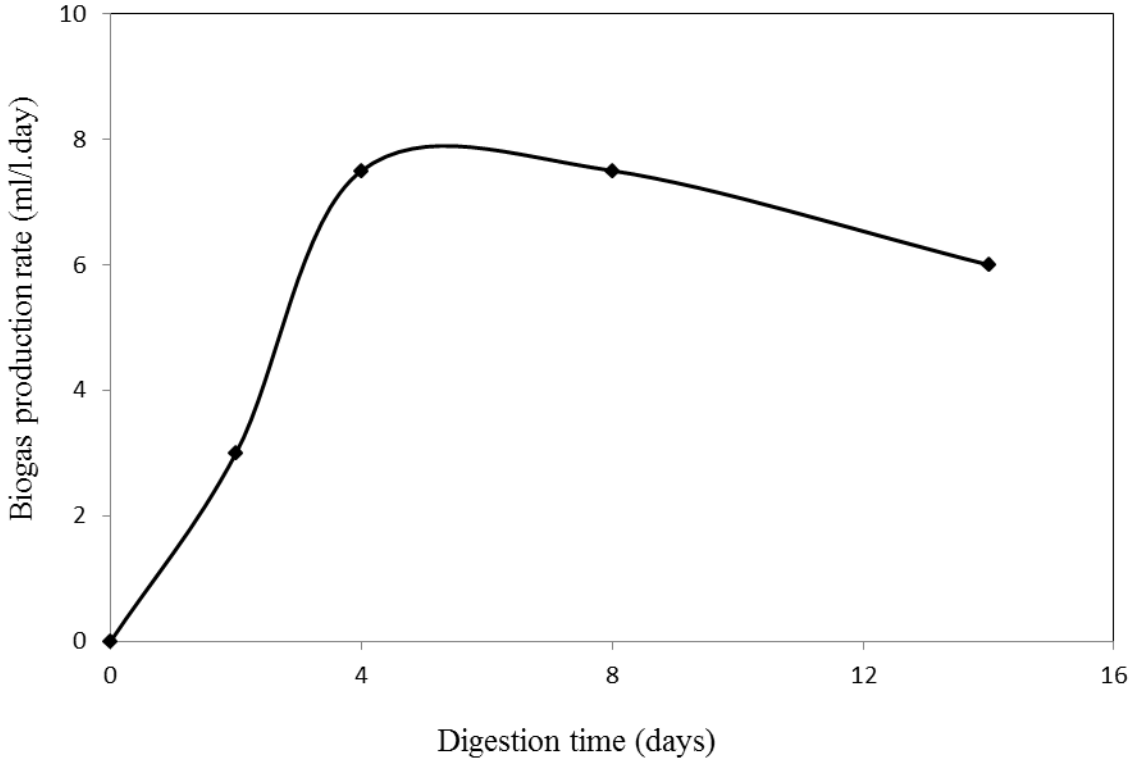

Figure 7.3 Biogas production rate for cashew apple waste.

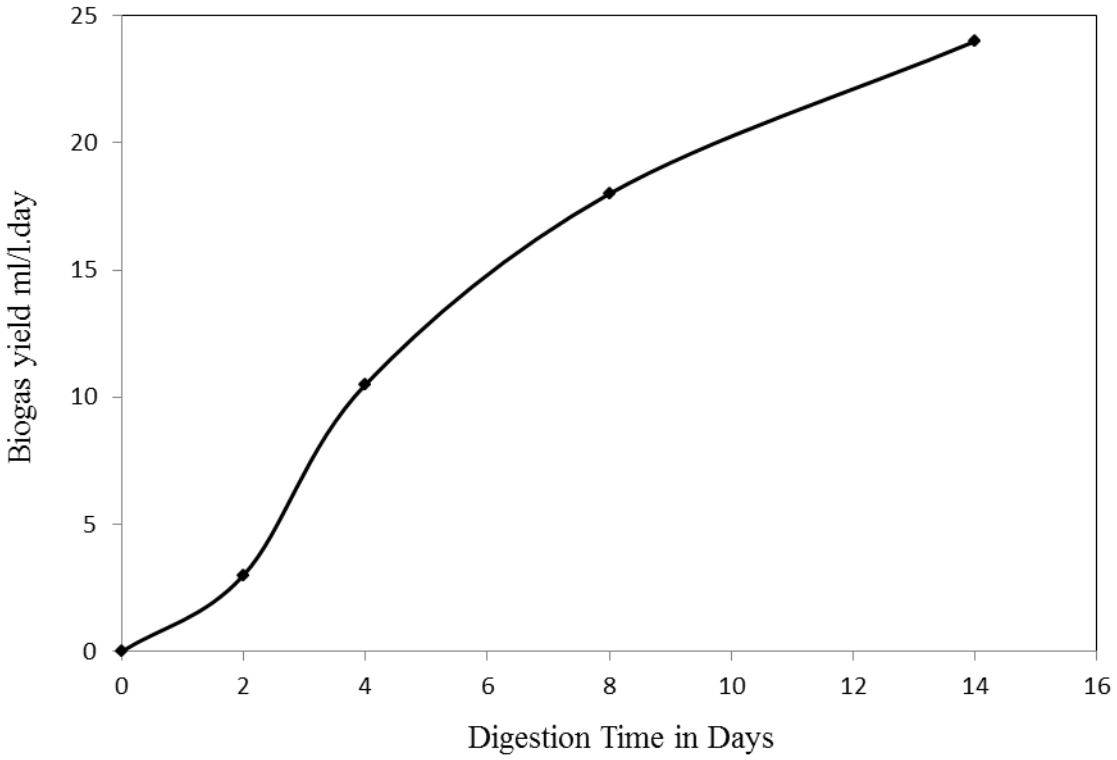

Figure 7.4 Biogas yield for cashew apple waste.

7.2.4 Two stage studies for Cashew apple waste

The substrate, that is cashew apple waste, was kept in the hydrolytic stage in reactors at different loading rates by following the pH which indicates acidification of the reactor. Hydrolysis stabilized at pH 5.5 for OLR of 3.5-4.0 g VS/l after 15 days. After 15 days the pH was adjusted to 7.2 so as to start the methanogenesis.

Maximum biogas 132 and 140 ml/gVS was produced at OLR of 3.5 and 4 gVS/l (Figure 7.5). The methane content of the biogas was 46% and 46.2% which corresponds to 60.7 ml and 64.6 ml at 3.5 and 4.0 gVS/l respectively at the end of 25 days of digestion.

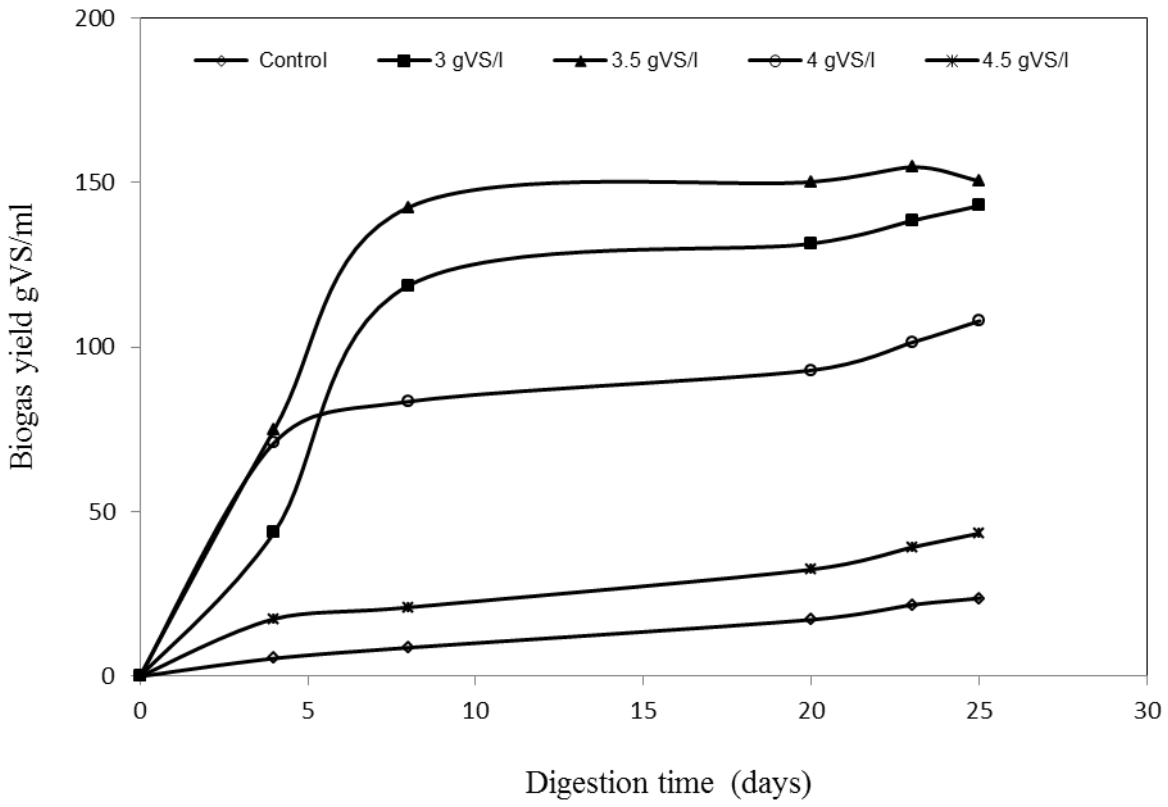

Figure 7.5 Biogas yield for cashew apple waste in a two-stage reactor at different volatile solid concentrations.

7.2.5 Digestion of Grass

The daily production rates and average biogas yield for digestion of grass waste are shown in Figure 7.6 and 7.7. The daily biogas production rate showed multiple peak value overtime. Multiple peaks are due to presence of multiple substrates within a single component as explained earlier. The daily biogas production rate was highly variable during the 25 days of digestion and then stabilized until the end of the experiment. As shown in Figure 7.6 and Figure 7.7 the biogas yield increased with increase in volatile solid rates. Volatile Solids analysis determines the total amount of organic matter in a substrate. With increasing VS, the amount of organic matter being added to the digester or the reactor increases and hence contributing to increasing methane yield per VS with increasing VS loading. The average biogas yield was 391, 490, 501, 557 and 651 ml/g VS respectively. The percentage of methane in the biogas was found to be 51% which corresponds to 199, 250, 256, 284 and 332 $mlCH_4/gVS$. The results gave a biogas yield higher than that found in batch experiments with grass carried out by Liu *et al.*, (2009), who obtained a yield of 372 ml/g VS. Grass and leaves contain waxes and lipids that were not quantified. However, these compounds would not be as degradable as carbohydrates (Yang *et al.*, 2009). The percentage of methane in the biogas produced from grass was found to be 51%.

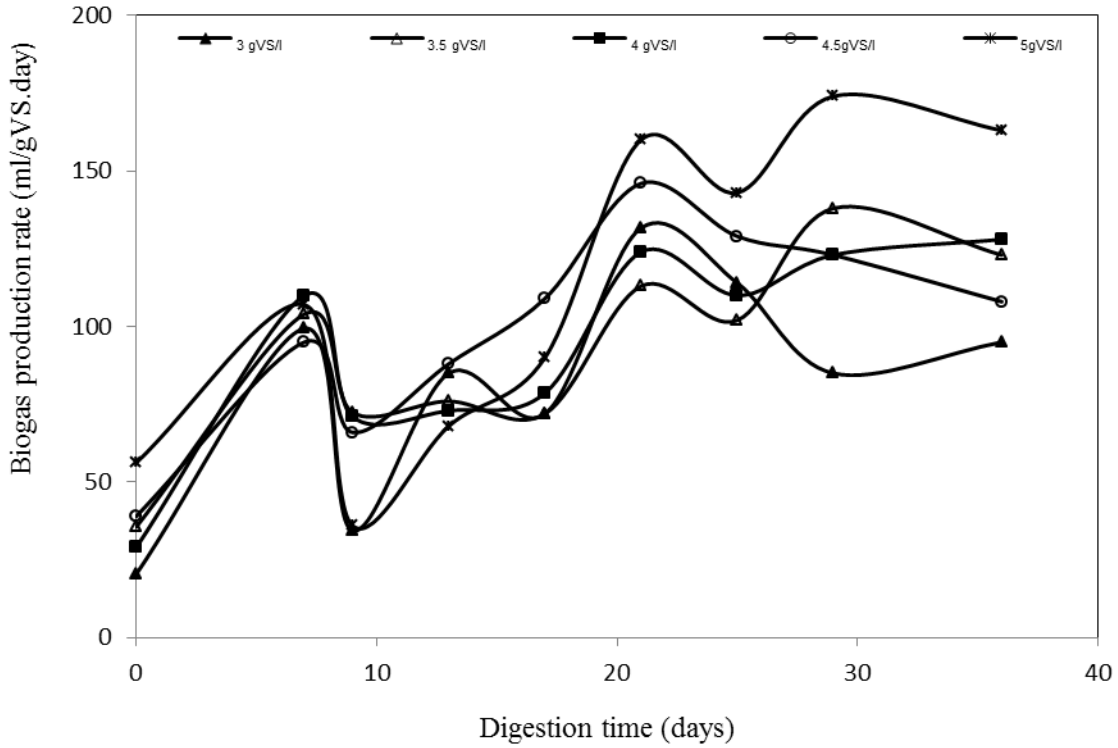

Figure 7.6 Biogas production rate for grass at different volatile solids concentrations.

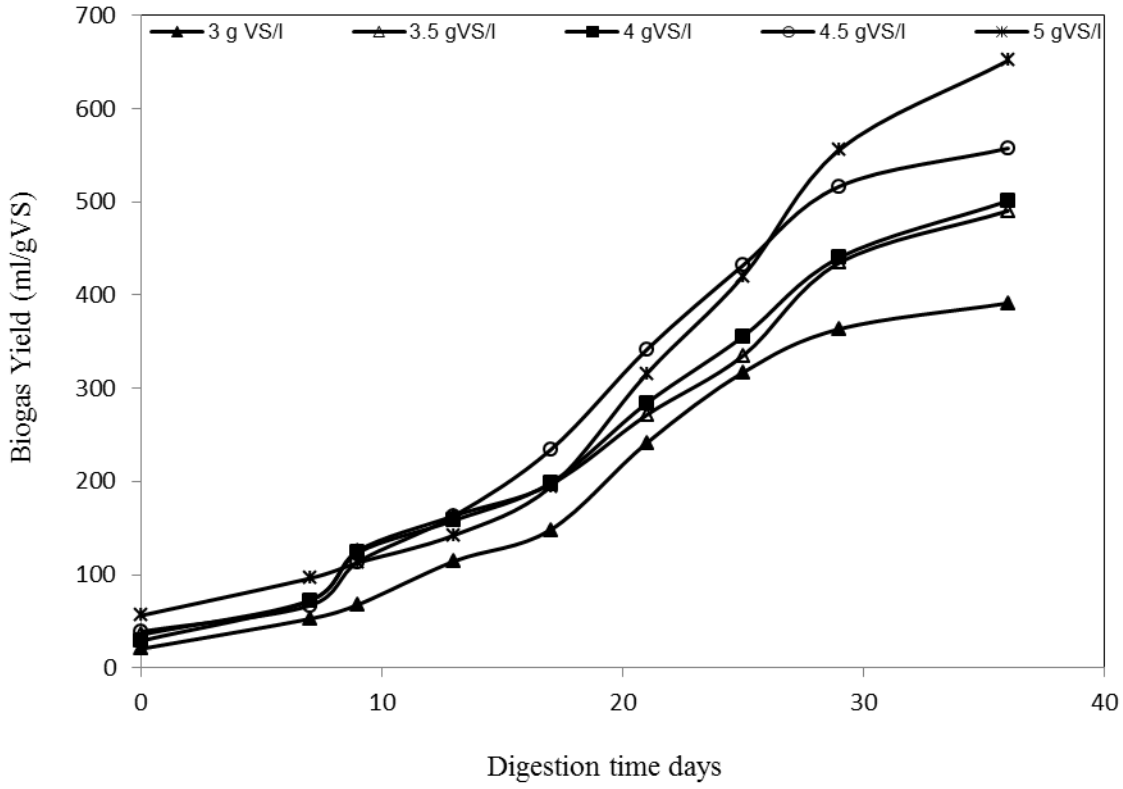

Figure 7.7 Biogas yield for grass at different volatile solid concentrations.

7.2.6 Fed Batch studies for Coconut oil Cake

Biogas Production

Based on the previous results, the anaerobic biodegradability of coconut oil cake was evaluated in fed batch mode in a 5L anaerobic reactor. The substrate was added at 4g VS/L/batch for all the batches. The volume of biogas produced was determined using water displacement method and results obtained are presented in (Figure 7.8) which represents the percentage of methane in the biogas produced with time during a typical batch. A significant lag phase was seen for all the batches during the start of each batch. The maximum biogas was produced between 5-13 days of digestion. The first cycle of fed batch was operated for 15 days after which there was no production of biogas. Following this, second batch of substrate addition was made. The reactor was operated for three batches with feeding after indication of endogenous respiration. On all three accounts of substrate addition, the maximum methane yield was 320 ml/g VS. The results indicate that there is no accumulation of inhibiting chemicals (Sharma *et al.,* 1999) when operated in fed batch mode. This aspect can be taken into account for designing pilot scale anaerobic digester for coconut oil cake.

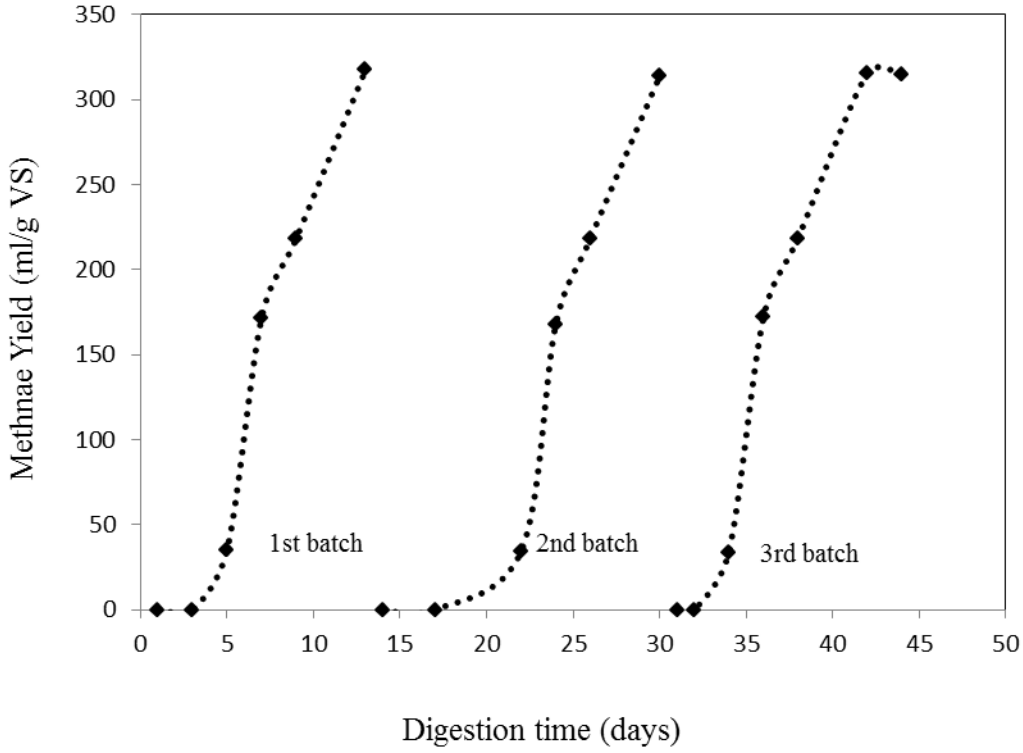

Figure 7.8 Methane yield of coconut oil cake for three sequential batches.

7.2.7 Methane Production potential

The maximum biodegradability (Bo) is given as m^3CH_4 produced per kg VS. The theoretical maximum methane production is 0.35 m^3CH_4/kg COD and one gram of VS for 'biological' sludge is normally considered equal to 1.4 g COD (Zeeman and Gerbens, 2004). Thus, the theoretical max methane production will be 0.49 m^3CH_4/kg VS. Thus, the Bo for the substrates tested i.e coconut oil cake, and grass cuttings except cashew apple were 0.36, and 0.33 m^3CH_4/kg VS respectively. As the methane production potential and the biodegradability of coconut oil cake was higher compared to grass clippings it was taken for fed batch studies.

The local oil mill which processes coconuts handles about 500 kg of dried coconut copra per day and after extraction of oil, about 200 kgs of coconut oil cake is available as waste residue whose market as cattle feed is on the decline. Based on the fed batch studies we will be able

to produce 320ml/g VS of methane or in other words 64000L/200kg VS or 6400L/200kg of raw coconut oil cake (@ 10% TS). Therefore this mill could produce 6.43 L of biogas per day which could replace firewood used for cooking for 6 families considering each family has 5- 6 people and do cooking thrice in a day. The amount of biogas thus produced will also replace 355 kg of CO_2/m^3 of biogas.

CONCLUSION

Food waste collected from the institute's mess was characterized for its potential as a substrate for anaerobic digestion. Raw vegetables represented the main quantity of wastes (31.7%), followed by cooked rice (16.6%), cooked vegetables (16.3%), and cooked dals (9.9%). In order to study the role of individual fractions of food waste on anaerobic digestion, the biochemical methane potential of their components and a mixture (mixed food waste) were measured. To understand the relative contribution of these fractions to methane production, a Van Soest fractionation was performed and methane potential of the residual fractions was measured in flasks. The behavior of raw wastes in flasks and batch reactors was also studied. The average methane yield for cooked rice, cooked vegetables, cooked dals and mixed food waste were 233, 390, 318 and 298 mlCH_4/gVS respectively after 33 days of digestion. The biochemical methane potential of cooked vegetables was the highest. Fractionation of substrates shows that high biochemical methane potential values could be attributed to the high content of lipids which has an important methane potential. Cooked rice has the lowest BMP value due to the high cellulose/hemicellulose fraction. Further, batch experiments in reactors; indicates the same global tendency of degradation for the four substrates. Cooked dals seemed to be more rapidly degraded than the other substrates. Mixing the food wastes was neither detrimental nor synergistic to biodegradation in anaerobic digestion process.

The study was followed by construction of a horizontal plug flow reactor for anaerobic digestion of food waste generated in the institute. The performance of this reactor was monitored for approximately two years and the microbial diversity with respect to methanogens was studied by T-RFLP technique for about a month. Identification of the dominant members of the community was obtained by cloning and sequencing approach.

Ammonia nitrogen is an important buffer in the anaerobic digestion process and an essential nutrient for the microorganisms. The effect of increasing concentration of ammonia on microbial diversity of anaerobic digestion was also studied. The decrease in total solids in the digestate as the study progressed is due to destruction of the volatile solids. At the initial phase of digestion, the volatile fractions represented 80-90% which was reduced to 50-60% at the end of digestion process. A total of 53 operating taxonomic units were distinguished with the restriction enzyme *MspI*. The 5 OTUs which were dominant were identified. *Methanoculleus* sp. was found to be the dominant strain in the reactor indicating hydrogenotrophic pathway of methanogenesis. No short-term effect of the varying concentrations of ammonia on the community structure was observed.

The digestate coming from the outlet of the plug flow reactor showed some undigested solid fraction, which were pretreated to enhance the biogas production. The pre-treatments studied (thermal, chemical, thermo-chemical and enzymatic) effectively hydrolyzed cottage cheese into soluble organic compounds. Enzymatic and thermo chemical pre-treatments were the most effective pre-treatments for cottage cheese. Chemical pre-treatment showed the poorest performance both in terms of solubilisation and biogas production. High temperature required for thermo chemical pre-treatment would likely raise the economic and energy dynamics of the process. Moreover, the enzymes were especially suitable for protein and lipid rich cottage cheese with low dose requirement. Cell free enzymes offer several advantages in the treatment of waste especially to reuse the separated solid fraction as a feedstock for methane production.

Further the effect of microbial stimulants on anaerobic digestion of food waste was studied. Addition of caffeine increased biogas production by 16% in comparison with the control. Caffeine which serves as a stimulant enhanced biogas production by potentially increasing

microbial activity. The use of such stimulants serves as a promising means for increasing biogas production.

Apart from food waste, agro waste residues were used as substrates for anaerobic digestion. For coconut oil cake biogas was produced only at 4 and 4.5 gVS/l. Coconut oil cake has a high potential for biogas production. However, they contain slowly biodegradable organic matter and the loading rates should remain low to avoid any accumulation of slowly biodegradable solids in the digesters. The potential of cashew apple for anaerobic digestion is very low. For cashew apple as a substrate, phase separation resulted in high process stability and significant biogas productivity compared with a single stage reactor.

FUTURE SCOPE OF THE WORK

Anaerobic digestion has been successfully operating in many parts of the world, but there are still problems and challenges in this technology that needs to be overcome. The future of research in anaerobic digestion, therefore, should concentrate on making it more efficient and cost effective. There are several areas in which research should focus, both for anaerobic digestion of food waste and anaerobic digestion in general. Basically waste management process includes source separation and waste composition. These should also take into consideration issues such as odor emission, sanitation and pest control.

An important area of research that will greatly impact urban environment is reducing the retention time and the size of the digester. Another area for future work is optimizing the environment for the bacteria inside the anaerobic digester. Process improvement includes use of enzymes that will catalyze biological reactions, developing other biocatalysts, improving inoculation concentrations, exploring new population of bacteria that are involved in this process and improving automated control technologies so that the digester can diagnose itself and respond to changing environment. The use of sensors for monitoring the various parameters of anaerobic digestion like pH, volatile fatty acids, temperature, and gas flow rate will help in enhancing the biogas generation process.

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LIST OF PUBLICATIONS

Vidhya Prabhudessai., Anasuya Ganguly, Srikanth Mutnuri (2009). Effect of caffeine and saponin on anaerobic digestion of foodwaste. *Annals of Microbiology* 59(4); 643-648.

Vidhya Prabhudessai, Anasuya Ganguly and Srikanth Mutnuri., (2013). Biochemical Methane potential of Agro wastes. *Journal of Energy.* Article id 350731.

APPENDIX I

LIST OF PRESENTATIONS

Microbial Diversity in a pilot plant scale horizontal plug flow reactor set up for anaerobic digestion of food waste. Vidhya Prabhudessai, Marcell Nikolausz and **Srikanth Mutnuri**. Poster Presentation at 2nd water research conference 20th-23rd January 2013, Singapore.

Role of organic fractions on anaerobic digestion of food wastes: an Indian Canteen study. Vidhya Prabhudessai, **ClaireDumas**, Srikanth Mutnuri, Jean Philippe Steyer and Michel Torrijos. Oral Presentation at 13th International Waste Management and Landfill Symposium 3rd -7th October 2011 Italy.

Anaerobic co-digestion of sewage and food waste. Vidhya Prabhudessai and **Srikanth Mutnuri**. Oral Presentation at International Conference on Biogas Microbiology 14th -16th September 2011 Leipzing, Germany.

Biochemical Methane Potential of agro wastes. . Vidhya Prabhudessai, **Pratham Arora** and Srikanth Mutnuri. Poster presentation at 3rd Asia pacific young water professionals conference 21st -24th November 2010. Singapore.

APPENDIX II

CURRICULUM VITAE OF VIDHYA PRABHUDESSAI

Ms. Vidhya Prabhudessai was born on 1st December 1984 in Vasco, Goa. She completed her Bachelor of Science in Botany from Carmel college of Arts and Science for women, Nuvem in 2005 and Master of Science in Botany from Goa University in 2007. She joined BITS Pilani Goa campus in 2008. She was a recipient of UGC fellowship from October 2008 to September 2011. She was a recipient of Indo French sandwich PhD scholarship for the year 2009-2010. As a part of this scholarship she has worked in INRA National Institute of Agronomic Research. She has two papers in International Journal.

APPENDIX III

CURRICULUM VITAE OF Dr. M. SRIKANTH

Dr. M. Srikanth was a recipient of DAAD-UGC Scholarship to complete his Doctoral Research at UFZ – Centre for Environmental Research, Germany and obtained his degree from Anna University Chennai in the year 2004. He joined BITS Pilani K.K Birla Goa Campus as a full time faculty by 2005. He worked as convener for two International Conferences in Environmental Biotechnology held in the year 2009 and 2011 at BITS Pilani K.K Birla Goa Campus. Dr. M. Srikanth conducted International Workshop on Bioremediation in association with Dr. Max Haggblom, Rutgers University USA for two weeks from January 4 – 16, 2010. He was principal investigator for four research projects funded by DST, DBT, UGC and GEDA and currently he has three projects funded by CSIR, DSTE and DBT. He has published twelve research papers in International Journals and written two Book Chapters. He received Helmholtz association's Junior Scientist Award and FEMS Young Scientist Award to participate in International conference on Environmental Biotechnology, Leipzig, Germany, 2006 and 14th International Biodeterioration and Biodegradation symposium Sicily, Italy, 2008 respectively. He had attended National and International Conferences to present his research work as Oral and Poster presentations. He has Research Collaborations with Scientists from IISc Bangalore, INRA France, UFZ Germany, GTZ-BMU Germany and Rutgers University USA. He is a Recipient of American Society for Microbiology & Indo US Science and Technology Forum (ASM IUSSTF) Indo US Research Professorship for October 2010.