ANAEROBIC DIGESTION OF PULP AND PAPER MILL SLUDGE

A thesis submitted

in partial fulfillment of the requirement for the degree of

Doctor of Philosophy

by

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(Veluchamy C)

Abstract

Anaerobic digestion (AD) of pulp and paper mill sludge (PPMS) was studied with and without pretreatment, in biochemical methane potential (BMP) assay, batch reactor and with a semi-continuous lab scale anaerobic reactor. Initially, AD of PPMS without pretreatment (as control) was carried out by using cow dung as inoculum in a BMP test. The results indicated that PPMS has a high potential for energy recovery in the form of biogas. Based on the experimental result on gas production, volatile solids (VS) reduction, F/M ratio 2.0 was perceived as best and achieved higher methane production (264±5 mL CH_4/g of VS degraded). As PPMS contain inherent recalcitrant characteristics of lignocellulose content, turns hydrolysis step into a rate-limiting stage AD.

Therefore, further experiment was carried out to overcome the hydrolysis step, different pretreatment such as thermal (autoclave, hot air oven, hot water bath and microwave oven), electrohydrolysis (direct current) and biological (isolated bacterial strain) pretreatments on PPMS were studied. The chemical and instrumental (FT-IR, XRD, FESEM) analyses exposed that all the pretreatment methods have shown improvement in solubilization. Among the pretreatments studied, in thermal pretreatment-hot air oven (80^{o}C for 90 min), in electrohydrolysis (15 V for 45 min), in biological pretreatment (*Bacillus mojavensis* (CDb1)) showed the highest impact on sludge solubilization. Further, screened pretreatment (in the previous experiment) were tested for enhanced methane production in BMP assay. The result revealed that the specific methane production potential was increased from 264±5 to 303±4 mL of CH_4/g VS degraded (for thermal pretreatment), 301±3 mL of CH_4/g VS degraded (for electrohydrolysis pretreatment), and 295±3 mL of CH_4/g VS degraded (for biological pretreatment). A net energy of 8,735 kJ was gained after thermal pretreatment and 13,224 kJ was gained after electohydrolysis pretreatment. In addition to that three kinetic models were studied. Among that modified Gompertz and logistic function models represents and reproduce the experimental data, while earlier has better fit.

Designed anaerobic auger plug flow reactor (AAPFR) experimental studies were carried out in two places: one at India, IITG (Environmental lab) campus and another one at Canada, University of Guelph Ridgetown Campus. At India, the AAPFR was operated for 75 d with thermal pretreatment (30 d) and without pretreatment (30 d) at 21 d HRT with specified OLR (6.3 kg VS/m^3/d). The CH_4 yield obtained from the continuous study was not significantly different from the BMP and batch study, and experimental CH_4 yield was an equal to 310 mL CH_4/g of VS degraded in AAPFR operation. At Canada, study was majorly emphasized on the effect of increasing organic loading rate (OLR) on the CH_4 production in long-term experiments (130 d) in corn silage (lignocellulose material). The increase in biogas production was observed with an increase in OLR. In addition to this, increase in OLR resulted in a decrease in CH_4 content and increase in H_2S concentrations. However, the reactor showed a stable operation at an OLR 6.5 kg/m^3/d. The reactor lost its stability at an OLR 8.8 kg/m^3/d, which was apparent by decrease in biogas yield and its CH_4 content.

Further, a development of mathematical modeling on a mass diffusion model on the effect of moisture content (MC) for the solid-state anaerobic digestion (SS-AD) was carried out. This model proposed that the decreased MC causes augmented mass diffusion resistance by the accumulation of

hydrolytic product and lead to the reduced methane gas production. According to this hypothesis, a new SS-AD model was developed based on mass diffusion limitation and hydrolysis inhibition.

Keywords: Pulp and paper mill sludge, anaerobic digestion, pretreatment, lignocellulose waste, batch and semi-continuous experiment, anaerobic auger plug flow anaerobic reactor, mathematical modeling.

List of abbreviations

PPMS	Pulp and Paper Mill Sludge
AD	Anaerobic Digestion
BMP	Biochemical Methane Potential
sCOD	Soluble Chemical Oxygen Demand
CPCB	Central Pollution Control Board
BOD	Biochemical Oxygen Demand
WWT	Wastewater treatment
ETP	Effluent treatment plant
SMA	Specific methanogenic activity
TMY	Theoretical methane yield
EMY	Experimental methane yield
SMY	Specific methane yield
MC	Moisture Content
SS-AD	Solid State Anaerobic Digestion
LS-AD	Liquid State Anaerobic Digestion
ADM1	Anaerobic Digestion Model No.1
F/M	Food to Microorganisms
VS	Volatile Solids
TS	Total Solids
DC	Direct Current
CFU	Colony Forming Unit
NaOH	Sodium Hydroxide
TKN	Total Kjeldahl Nitrogen
VFA	Volatile Fatty Acids
OLR	Organic Loading Rate
AAPFR	Anaerobic Auger Plug Flow Reactor
IITG	Indian Institute of Technology Guwahati
UoG	University of Guelph
CARES	Centre for Agricultural Renewable Energy and Sustainability
NDF	Neutral Detergent Fiber
ADF	Acid Detergent Fiber
FESEM	Field Emission Scanning Electron Microscopy
FT-IR	Fourier Transform Infrared
XRD	X-ray Diffractograms
MSW	Municipal Solid Waste
WAS	Waste Activated Sludge
PS	Primary Sludge
MGWL	Monosodium Glutamate Waste Liquor
FOS/TAC	Ratio of volatile organic acid to alkaline buffer capacity

Notations

CH_4	Methane
CO_2	Carbon dioxide
H_2	Hydrogen
$CaCO_3$	Calcium carbonate
CH_3COOH	Acetic acid
M	Potential methane production
R_m	Maximum methane production rate
λ	Lag phase time
R_h	Hydrolysis rate of sugar
R_i	Reduction rate due to inhibition of sugar
k_h	First-Order hydrolysis rate coefficient
k_i	Inhibition coefficient of hydrolysis
X_F	Volatile substrate concentration
S	Sugar concentration in the substrate layer
R_u	Utilization rate of sugar
K_s	Half saturation coefficient
μ_{max}	Maximum specific growth rate
$Y_{\lambda M/S'}$	Microbial growth yield
X_M	Volatile solid concentration in inoculum
R_d	Diffusion rate of sugar
D_e	Effective mass diffusion coefficient of sugars
L	Substrate layer thickness
A	Surface area of microflora
V	Volume of substrate layer
S	Concentration of sugar in upstream substrate layer
S’	Concentration of sugar in downstream microflora surface
D	Glucose diffusion coefficient
α_F	Organic fraction in substrate
α_M	Organic fraction in inoculum
n	F/M ratio
X_{MC}	Moisture content present in the reactor
Y_t	Cumulative methane production
M_{max}	Maximum methane production potential
R_{max}	Maximum rate of methane production
t	Digestion time
$Y_{\Delta CH_4/\Delta S}$	Methane yield coefficient
X_F^0	Initial substrate concentration
X_M^0	Initial inoculum concentration

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"Sooner or later, we will have to recognise that the Earth has rights, too, to live without pollution. What mankind must know is that human beings cannot live without Mother Earth, but the planet can live without humans."

Evo Morales

Chapter 1

INTRODUCTION

This chapter consists of a brief discussion about pulp and paper mill sludge problems, its treatment, and management. The chapter also deals with the major objectives, need of the study, scope of thesis and finally thesis organization.

1.1 OVERVIEW

The pulp and paper industry is one of the most polluting industrial sectors in the world. It is mostly depicted as energy and water intensive (Pokhrel and Viraraghavan, 2004). The global pulp and paper waste and wastewater treatment market is expected to increase by 60 % between 2012 and 2020. There are more than 7745 mills producing 192 million tonnes of pulp and many thousands of other types of pulp and paper mills in the world (RISI, 2015). According to the Central Pollution Control Board (CPCB) and Ministry of Environment and Forest, Government of India, has identified the pulp and paper industry as the most polluted industry in India (Naidu, 2013). Approximately 905.8 million m^3/year fresh water is consumed (60 m^3/tonne of product) and 695.7 million m^3/year effluents is produced by the production of paper. The industry discards vast amounts of sludge that has been generated during wastewater treatment (WWT).

The huge volume of pulp and paper mill sludge (PPMS) is produced from the physical and biological treatment of wastewater, varying from 0.3 to 1 m^3 of primary sludge per ton of paper produced (Priadi et al., 2014). Mahmood and Elliott (2006) have reported that the ratio between primary to bioslugde is about 70/30 in most of the Canadian pulp and paper industry. Conversely, this ratio can fluctuate among individual mills depending on the raw material usage (Bajpai, 2015). Some industry produces only primary sludge and no biosludge, and vice versa. Produced sludge contains 45-55 % organic matters with many types of nutrients including nitrogen, phosphorus, potassium, calcium, iron, magnesium, copper, silicon, zinc and manganese (Jackson et al., 2000). Ali and Sreekrishnan (2001) reported that pulp and paper mill effluent and its produced sludge have a high biochemical oxygen demand (BOD), chemical oxygen demand (COD), chlorinated compounds (measured as adsorbable organic halides, suspended solids (mainly fibres), fatty acids, tannins, resin acids, lignin and its derivatives, and sulfur compounds, etc. Pollutants such as tannins, lignin and resin acid, etc., can occur naturally from the wood extractives and other chlorinated compounds, phenols, dioxins, furans of the xenobiotic compound which would be formed later from the paper production processes steps. The high concentration of organic compounds renders biological treatment as anaerobic digestion processes possible. The traditional sludge management

methods including landfilling which produces leachate that contaminates ground and surface water, and incineration which emits greenhouse gases. Due to strict regulatory pressure and some constraints in many countries, landfilling and incineration have come under strong public opposition.

Although PPMS have been produced from the paper industry for many decades, specific legislations and regulations related to their handling and utilization have not been separately developed nor worked upon. Therefore, the regulations that have been devised for sludge from other industrial waste or municipal waste are usually used as the baseline for regulating the disposal and utilization of PPMS. Sludge from the wastewater treatment plant of the paper industry can be disposed generally (Fig. 1.1) by dumping on low-lying areas (pastureland within the vicinity of the mill), or on forestland, or landfilling, or some time used for incineration/combustion after drying. Landfilling is becoming increasingly difficult to implement because of the rapid shrinking of landfill space and public opposition to open a new landfill site (Bajpai, 2015). Van Ewijk and Stegemann (2016) reported that in many countries the cost of opening a new landfill is prohibitive due to legal restrictions and public opposition. Incineration, which is second next to a landfill in the least priority option, and suffers from its own drawbacks, such as dewatering which requires high investment, rising supplemental fuel costs, high capital costs and air pollution concerns. This has forced the industry to look for alternative disposal options. With the demand for energy growing worldwide, concerns of energy security and climate change on the rise, anaerobic digestion a versatile technology for renewable energy generation through non-oxidative metabolism that provides a potential alternative for managing PPMS. Thus, anaerobic digestion has become an alternative energy source to augment, and to supplement fossil fuel at an industrial level. In addition, anaerobic digestion has many advantages such as low sludge production and chemical consumption, requirements of land is small due to smaller reactors, effective sludge stabilization and energy production in the form of methane. The potential application of anaerobic digestion process has been further developed by different pretreatment methods and its untapped potential is still large because non-fossil fuels constitute only 3 % of the total vehicular fuels in 2005 (Mata-Alvarez, 2002). Anaerobic digestion is a microbial mediated process in which the organic materials are metabolized by the diverse group of microorganisms to methane and carbon dioxide in the absence of an oxygen environment.

Anaerobic digestion has a unique and integrative potential, simultaneously act as waste treatment and resource management. Rittmann and McCarty (2012) have reported that anaerobic digestion uses diverse groups of naturally available bacteria and archaea to do biodegradation, and produce renewable energy from discarded organic materials. Anaerobic digestion process takes place by the various groups of microorganisms with a series of interdependent metabolic stages hydrolysis, acidogenesis, acetogenesis, and methanogenesis for the degradation of organic complex material to useful energy source as the biogas (Metcalf et al., 2003; Elliott and Mahmood, 2007). Biogas as a renewable energy source is obtained from the anaerobic digestion process could be used for heating the digester or lighting in industry. The remaining unrecovered organic material and nutrients retained in the effluent (digestate) might be returned to the agricultural land by adopting proper post treatments.

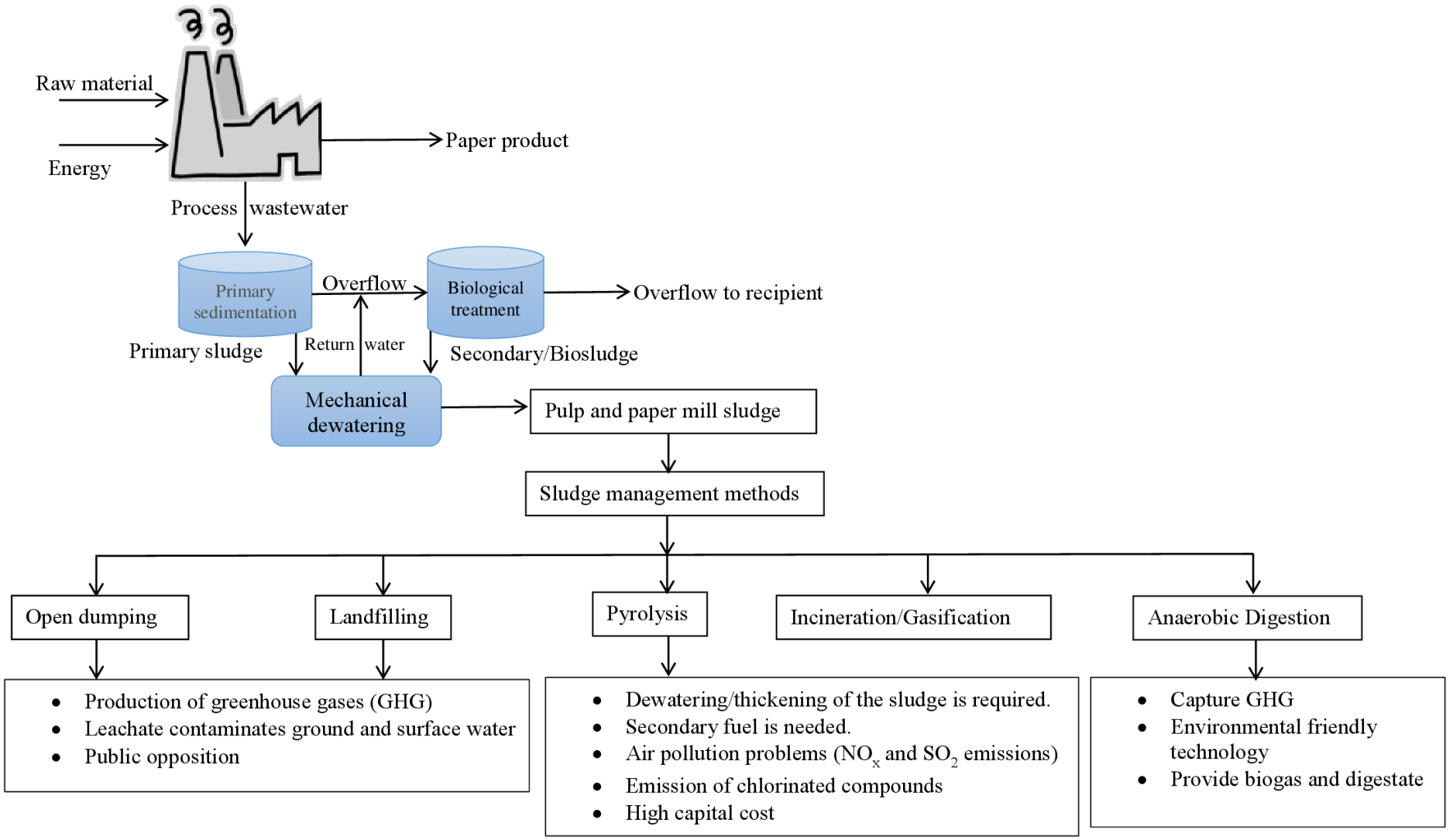

Fig. 1.1. Schematic diagrams of the sludge production process and its management/treatment process

Anaerobic digestion scores better option than any other treatment available currently for the ecological balance in terms of global warming (Mata-Alvarez et al., 2000). Unlike anaerobic digestion of wastewater, anaerobic digestion of PPMS (both primary and biosludge) is still in its infancy (Meyer and Edwards, 2014). This is primarily due to the inherent recalcitrant characteristics of lignocellulose content extant in the PPMS, which makes the hydrolysis stage as a rate-limiting step in anaerobic digestion process. Lignocellulose material consist of three biopolymers: cellulose, hemicellulose, and lignin, which together form a rigid and complex structure (Zheng et al., 2014).

Cellulose is the main component and consists of a linear polysaccharide polymer of cellobiose strongly linked by β-1, β-O-4 glycosidic linkages. The hydrogen bonds and vander Waals forces forms the interlinking of the cellulose chain, resulting in the high tensile strength of microfibrils (Ha et al., 1998). Cellulose microfibrils are attached to each other by hemicellulose, and covered by lignin complexes. These specialized and complicated structures make cellulose resistant to chemical and biological attacks. Hemicellulose polymers are random, amorphous, and branched heterogenic polysaccharides formed by hexoses, pentoses, and acids. Short and branched chains of hemicellulose combined with network of cellulose microfibrils interact with lignin leading to a rigid cellulose-hemicellulose-lignin matrix. The different form of inter-unit linkages, such as β-O-4, β-β, β-5, β-1, 4-O-5, etc. are combined to build the heterogeneous and highly cross-linked lignin component (Sasaki et al., 2013). Palmqvist and Hahn-Hägerdal (2000) reported that lignin plays the role of cement rigid three dimensional structure by forming the cross-linking between cellulose and hemicellulose. Microbial activity on degradation of lignocellulose material is so difficult because of the recalcitrant structure leads to a slow and incomplete hydrolysis and thereby results a longer solids retention time (large reactors), and overall lower degradation efficiency. Ultimately, this makes the treatment capital expensive too (Elliott and Mahmood, 2007). Thus, the hydrolysis stage forms the main bottleneck in anaerobic digestion of PPMS.

Over the last decade, different pretreatment techniques were used in anaerobic digestion. The majority of the literature reports, thermal pretreatment or combination of pretreatment (alkali-thermo or thermos-chemical) were proposed and tested majorly for municipal wastewater treatment plant sludge. Some literature reports that pretreatment on PPMS has increased the methane production for both mesophilic (43 to 145 %) (Saha et al., 2011) and thermophilic (4 to 58 %) anaerobic conditions (Gavala et al., 2003; Bayr and Rintala, 2012). Thermal processing, sonication, and mechanical integration have seemed to be the prominent technology for PPMS pretreatment. Results from the previous studies were conflicting. Either alone or a combination of ultrasonic, microwave oven and/or enzymatic pretreatment did not illustrate the substantial effect on the methane production from PPMS (Karlsson et al., 2011). Bayr et al. (2013) studied the biological pretreatment in a batch experiment by adding a mixture of accelerases (70 mg/g of VS) to the biosludge from the paper industry and reported that succeeding anaerobic digestion does not have any improvement. Nevertheless, enzymatic pretreatment might have the potential to escalate the sludge digestibility (Lin et al., 2009). On the antagonistic, Elliott and Mahmood (2012) recommend that PPMS could be more amenable to pretreatment because of the higher volatile fraction and reported that the residence time of sludge in a biogas reactor have substantially reduced from 15-25 d to 7 d.

Among the emerging pretreatment technologies prior to anaerobic digestion in order to enhance digestibility of sludge material with the increased rate of production of biogas/methane (Elliott and Mahmood, 2007; Meyer and Edwards, 2014). Some studies on PPMS has also proven by the increased rate of solubilisation at the hydrolysis stage and a higher reduction in volatile solid content after digestion. It is difficult to suggest which pretreatment techniques claims the utmost potential for PPMS. This is because the proportion and composition of sludge might vary based on raw material, pulping, and in the paper production process. Hence, pretreatment is necessary to brittle the bottleneck present in anaerobic digestion of sludge. Moreover, there was very limited literature that inspects the different pretreatment effects on PPMS. Anaerobic digestion of PPMS have been carried out majorly by BMP assay, with very few being bench scale. On analyzing the pretreatment literature, it was perceived that the pretreatment studies gained interest only in the last decade.

Hence, the objective of the present study was to treat the PPMS using anaerobic digestion. Different pretreatment techniques were studied to enhance the hydrolysis stage, followed by BMP with a different F/M ratio and batch study, to achieve high degradation and enhanced methane production from PPMS. Design, fabrication and operation of lab scale continuous anaerobic reactor was studied with different organic loading rate. Finally, kinetics studies for continuous anaerobic reactor and mathematical modelling were studied in the present study.

1.2 OBJECTIVES

The main objectives of the study was to treat the pulp and paper mill sludge using anaerobic digestion. The purpose was also to find best strategy for improved biogas production and its efficiency by performing different pretreatment techniques. The scope of this thesis was limited to:

- Biochemical methane potential (BMP) (1 L capacity) test with different F/M ratio using cow dung as inoculum followed by batch studies of best F/M ratio attained (20 L capacity).
- Pretreatment of PPMS using thermal (hot air oven, autoclave, hot water bath, and microwave), electrohydrolysis, and biological (isolated bacteria) for enhanced hydrolysis/liquefaction step in anaerobic digestion.
- BMP (1 L capacity) test for best treatment techniques with different F/M ratio followed by batch studies (20 L capacity) of best F/M ratio.
- Design, fabrication and operation of a lab scale continuous anaerobic reactor (65 L capacity).
- Kinetics studies for a lab scale continuous anaerobic reactor and the development of a mathematical modelling on anaerobic digestion using research data.

1.3 NEED OF THE STUDY

The pulp and paper industry is one of the most polluting, energy and water intensive industries in the world. Due to the huge production of PPMS and improper management practices, the industry is facing many environmental effects as well as spending huge amount of fund in the management of produced PPMS. The traditional sludge management methods including landfilling and open

(illegal) dumping produces leachate that contaminates ground and surface water, and incineration emits greenhouse gases. This has forced the industry to look for alternative disposal options. With the increase in energy demand worldwide, concerns of energy security and climate change, anaerobic digestion a versatile technology that provides a potential alternative for managing the produced PPMS. Anaerobic digestion is a biological method to treat the organic matter that involves the transformation and decomposition of organic content, leading to a production of renewable methane rich energetic biogas and the stabilized digestate for agronomical purpose. Anaerobic digestion could reduce the greenhouse gases and leachate production in landfill and open dumping.

Although anaerobic digestion of sludge was widely studied, a very limited research have been studied with respect to PPMS, and mostly it was limited to batch study and very few continuous studies. Unlike anaerobic digestion of wastewater, anaerobic digestion of PPMS (both primary and biosludge) is still in its infancy (Meyer and Edward, 2014). This is primarily due to inherent recalcitrant characteristics of the lignocellulose content extant in the PPMS, which makes the hydrolysis stage as a rate-limiting step in anaerobic digestion. Pretreatment aims at improving the hydrolysis step, enhances the biogas yield, and demonstrats their ability to substantially reduce digestion time and thereby the required reactor size. Hence, there is also a need to study the reactor design and operation of the designed reactor in a continuous mode to enhance the biomethanization process for the lignocellulose waste materials.

1.4 SCOPE OF THE THESIS

To achieve the above mentioned objectives, different laboratory experiments were performed. In the first attempt, the experimental setup for the biochemical, physico-chemical analysis, and learning the protocols and operation of instruments were performed. Secondly, the performance of the BMP setup on basis of volatile solids with different F/M ratio was studied. Based on the previous study, a batch reactor with the best F/M ratio was studied to confirm the trend followed in BMP study. Then different pretreatment techniques were carried out for PPMS to enhance the hydrolysis step in anaerobic digestion. This was followed by a BMP test with different F/M ratio on basis of volatile solids and batch study with best F/M ratio was studied to confirm the improvement/ effect of pretreatment. Thirdly, design and operation of lab scale auger-plug flow anaerobic reactor with different volumetric organic loading rate was studied. Finally, kinetics and mathematical modelling was performed using the research data.

1.5 THESIS ORGANIZATION

This thesis has been organized into the following chapters:

- Chapter 1 gives a brief introduction of pulp and paper mill sludge problems, its treatment techniques and management methods, objectives, need of the study and scope of the thesis.
- Chapter 2 gives detail literature review of anaerobic digestion process, the processing of pulp and paper mill sludge for anaerobic digestion, optimum conditions required for anaerobic metabolic process, and different pretreatment techniques used in PPMS anaerobic digestion.

- Chapter 3 deals with collection and initial characterization of raw materials such as PPMS, cow dung. Experimental design of phase 1, 2, 3, 4 and 5 was given in flow chart. The detail procedure of biochemical and physico-chemical analysis and instrumental analysis are provided.
- Chapter 4 presents detail results and discussion during the BMP assay with varied F/M ratio and batch study with or without pretreatment of PPMS.
- Chapter 5 gives the detail results and discussion during lab scale continuous auger-plug flow reactor operation (AAPFR) with varied volumetric organic loading rate.
- Chapter 6 deals with the development and validation of mass diffusion model on the effect of moisture content for solid-state anaerobic digestion.
- Chapter 7 list the conclusions and future recommendation of the thesis.

"Unlike in wastewater treatment, anaerobic digestion of mill biosludge (waste activated sludge) and primary sludge is still in its infancy."

Elizabeth A. Edwards

Chapter 2

LITERATURE REVIEW

This chapter covers the detailed review of literature available related to the anaerobic digestion of pulp and paper mill sludge with or without pretreatment. Then it discuss the optimal condition and concentration required for anaerobic metabolic activity. Most related aspects of this study are presented here in summarized form.

2.1 BIOCHEMISTRY OF ANAEROBIC DIGESTION (AD)

Anaerobic digestion is the biological method used to treat organic matter that involves the transformation and decomposition of organic matter, leading to a production of renewable methane (CH_4) rich energetic biogas and the stabilized digestate through a number of metabolic stages mediated by several groups of microorganisms. Fig. 2.1 illustrates the schematics of various steps, microbes and metabolic products involved in the anaerobic digestion process.

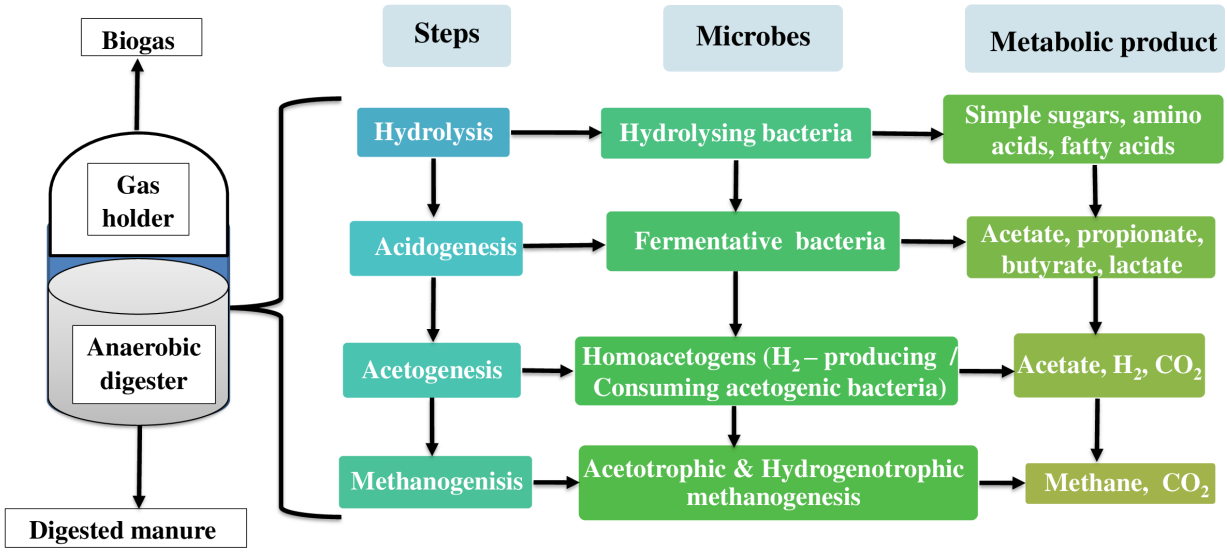

Fig. 2.1. Biochemistry and conversion steps in the anaerobic digestion process

Complex organic compounds such as carbohydrates, proteins and lipids are transformed into simple soluble products such as simple sugars, amino acids, and fatty acids, by the action of extracellular enzymes excreted by the hydrolysing bacteria. This step is known as hydrolysis or liquefaction. Hydrolysis could be a rate-limiting step in the overall anaerobic treatment processes of

waste containing lignocellulose, lipids and/or a significant amount of particulate matter (Van Haandel et al., 1994; Khanal, 2011). Polymers are transformed into soluble monomers by enzymatic hydrolysing bacteria. The reaction (2.1) is catalyzed by extracellular microbial enzymes known as hydrolyses or lyses.

$$nC_6H_{10}O_5 + nH_2 \xrightarrow{\text{Hydrolysis}} nC_6H_{12}O_6 \tag{2.1}$$

The second step is acidogenesis or acidification as given in reaction (2.2). The fermentative bacteria ferment the soluble products of the first step into simple molecules with lower molecular weight such as volatile fatty acids (VFA), alcohols, aldehydes, and gases like hydrogen (H_2) and carbon dioxide (CO_2).

$$nC_6H_{12}O_6 \xrightarrow{\text{Acid forming bacteria}} 3nCH_3COOH \tag{2.2}$$

Acidogenesis is the production of VFA (C>2) such as propionic, butyric, isobutyric, and valeric etc., Acidification is affected by a diverse group of bacteria, the majority of which are facultative anaerobes. The acidogenic bacteria are able to metabolize organic material down to a very low pH of around 4. Produced VFA along with ethanol are converted to acetic acid, H_2 and CO_2 by another group of bacteria known as hydrogen producing acetogenic bacteria. The acetic acid producing step is known as acetogenesis. The first three steps are often grouped together as acid fermentation. It is vital to note that in acid fermentation, no organic materials are removed from the liquid phase; it is just transformed into a form suitable for a subsequent process, which is methanogenesis. In the final step, acetate, H_2 and CO_2 are the primary substrates for the methanogenesis and converted into CH_4 and CO_2 as denoted in reaction (2.3). In this stage, organic material is removed by means of the production of biogas.

$$CH_3COOH \xrightarrow{\text{Methane forming bacteria}} CH_4 + CO_2 \tag{2.3}$$

CH_4 could be generated from two routes. The group of microorganisms involved in the generation of methane from acetate are known as acetotrophic or aceticlastic methanogens (reaction 2.3). The remaining methane is generated from H_2 and CO_2 by the hydrogenotrophic methanogens (reaction 2.4 - 2.6) (Lim et al., 2009; Izumi et al., 2010). Similarly, CO_2 can be hydrolysed to carbonic acid and to CH_4.

$$CO_2 + 4H_2 \xrightarrow{\text{Reduction}} CH_4 + 2H_2O \tag{2.4}$$

$$CO + H_2 \xrightarrow{\text{Hydrolysis}} H_3CO_3 \tag{2.5}$$

$$4H_2 + H_3CO_3 \xrightarrow{\text{Hydrolysis}} CH_4 + 3H_2O \tag{2.6}$$

In each of the sequential steps, the catabolic reactions described above develop together with anabolic activity. Free energy released in the above reactions are partially used for the synthesis of the anaerobic bacterial populations. Hence, a large fraction of digested organic matter is converted into biogas (85 to 95 %).

2.2 PULP AND PAPER MILL SLUDGE (PPMS)

Paper and paperboard has become an essential requirement of life. The present global paper and paperboard demand is 402 Mt per annum. There are more than 7745 mills producing 192 Mt of pulp. Over the last two decades (1991 to 2011), the paper demand has doubled from 243 to 402 Mt (RISI, 2015). Kulkarni (2013) reports that Indian pulp and paper mills have been operating at a capacity of 12.7 Mt of paper production and consumption of 11 Mt with 9.3 kg of consumption of paper per capita. The water use in pulp and paper mills is 10-100 m^3 per ton of produced paper and the sludge generation ranges from 0.2 to 0.6 wet tonnes per tonne pulp produced. Meyer and Edwards (2014) reported that energy conversion could be possible in the pulp and paper mill industry ranges from 4 to 400 Mt annually. If only 25 % of that produced sludge can be transformed into biogas, approximatively 100 TWh of electricity could be generated annually. At a glance in 2011, all biogas plants worldwide have generated approximately 40 TWh of electricity.

A diverse form of wastewater is produced during the pulp and paper production processes. Treatment of produced wastewater is done by the different techniques and their combination such as primary, secondary and tertiary treatment. Fig. 2.2 illustrates the schematic representation of the pulp and paper mill sludge production sites. Normally in most of the paper industry, primary treatment is followed by the biological/secondary treatment with sometimes-sequential anaerobic and aerobic systems. During the course of paper production processes, the varied form of solid waste is produced from industry. Wastewater that is generated during processes such as pulping and paper making process is the main source for the WWT. Hence, a huge amount of PPMS is produced in WWT. There was very limited data available for the total solid waste generation in the paper industry because of varied composition of feed material. Effluent from the pulp and paper industry is treated mostly by the primary and secondary treatment level. Due to the higher lignin content, most of the time pulping is inefficient and thus produce large amounts of primary sludge, varying from 0.3 to 1 m^3 of sludge per ton of paper produced (Priadi et al., 2014). The produced sludge is about 0.04 to 0.5 m^3 dry weight of sludge per ton of paper production in the North American paper mills (Bajpai, 2015). Mahmood and Elliott (2006) have reported that ratio between primary to bioslugde is about 70/30 in most of the Canadian pulp and paper industry. Conversely, this ratio can fluctuate among individual mills depending on the raw material usage (Stoica et al., 2009; Bajpai, 2015). Some industry produces only primary sludge and no biosludge, and vice versa.

2.2.1 Primary sludge

Primary sludge is originated from the clarification of processed water at the pulp and paper mill wastewater treatment plant. The sludge captured at the gravity-settling tank in the primary clarifier is referred as primary sludge. The primary sludge contains lignocellulose components (cellulose, hemicellulose and lignin), paper making fillers (kaolin and calcium carbonate), pitch, by-products of lignin and ash (de Alda, 2008). During the various paper production processes steps, there was an incomplete solid/liquid separation that contains about 80 % of the total suspended solid in the produced wastewater which was transferred as the primary sludge in the primary treatment.

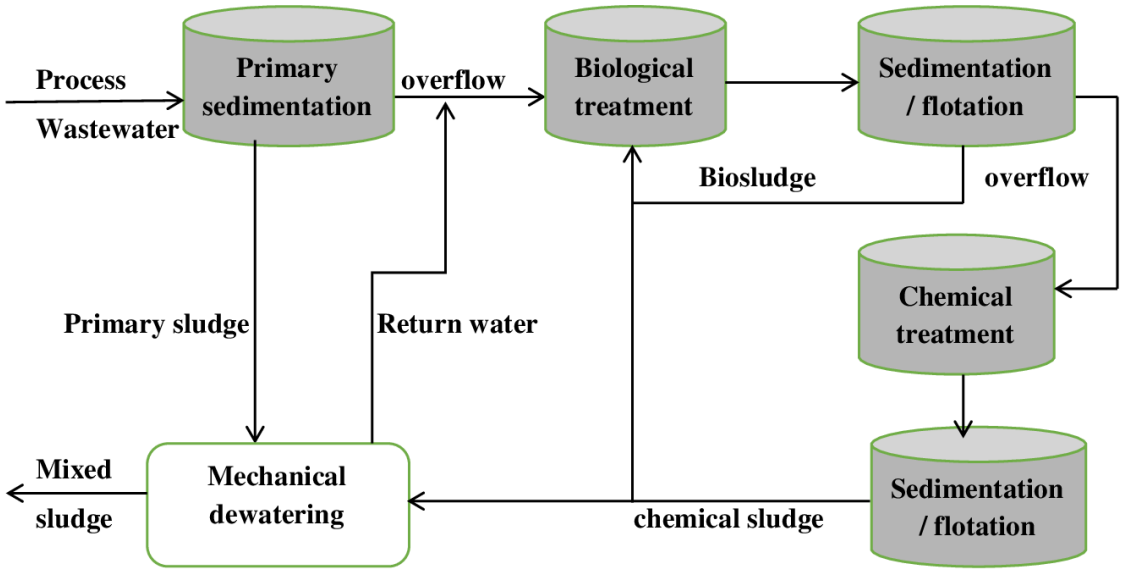

Fig. 2.2. Schematic representation of pulp and paper mill sludge production sites

The lost materials (fibre) from pulp mills are about 3 to 4 % and from waste paper mills vary about 15 to 30 % (Bajpai, 2015). Depending on the efficiency of the fibre recovery system, the primary sludge generation rate is varied. The produced primary sludge is relatively easy to dewater. Bajpai (2000) reported that produced primary sludge comprises of both inorganic and organic material, while the secondary sludge contains majorly organic materials. However, the anaerobic digestion of primary sludge is to date virtually unexplored (Meyer and Edwards, 2014). Some researchers have described the energy potential of the primary sludge through anaerobic digestion (Bayr and Rintala, 2012; Meyer and Edwards, 2014; Priadi et al., 2014). This primary sludge could be reincorporated for the biogas generation.

2.2.2 Secondary sludge or "Biosludge "

Secondary sludge is originated from the clarifier of the biological/secondary unit during wastewater treatment. The produced secondary sludge is primarily mixed with primary sludge to dewater and then disposed of through incineration or dumping in a landfill. Comparing this with the primary sludge, secondary sludge as the by-product of the secondary/biological treatment unit is far-off difficult to handle and dewater and even to dispose of. This sludge consists predominantly of excess microbial cell mass (produced from the secondary treatment/biological process), and unused chemical residuals from the pulping process (Kyllönen et al., 1988). More than half of the incoming organic pollution load is transformed into secondary sludge that comprises of about 0.5 to 2 % solids (KyllÃűnen et al., 1988). The volume of the secondary sludge is lesser than the primary sludge, because most of the heavy fibrous material, inorganic solids are clarified at the primary clarifier as primary sludge. Moreover, the country with strict environmental regulation will follow the tertiary treatment in addition to the primary and secondary treatment stages (Bajpai, 2000).

Bajpai (2015) reported that sludge produced from the Kraft pulping was around 0.1 m^3/adt but in mechanical and semi-chemical processes yield nearly 0.6 m^3/adt. Conspicuously, the quantity

of sludge produced from a pulp and paper mill using virgin material differs from a mill that uses secondary fibre. This is because the composition of virgin material, such as wood, agricultural residue, etc. is different from the recycled fibre. The amount of sludge produced from a recycled paper mill/secondary fibre mill has 2-4 times as much sludge as those from virgin mills (Bajpai, 2015). The raw material for recycled paper mill has a higher proportion of unrecyclable filler material. This problem is obvious in a mill consuming high filler material for the production of recycled paper products from office waste/ newsprints. On the other hand, sludge from the deinking mill has higher ash content. Generally, medium density fibreboard is manufactured by the mixing of sludge from the deinking mill and virgin mill sludge that contains both the primary sludge and secondary sludge at about 20 %. This proportion was characterized as suitable for the manufacture of medium density fibreboard. On comparing the ash content, primary sludge produced from the virgin mills has lower ash content than deinking mill sludge. The predominant characteristics such as a high holocellulose content, short with more fibres, low pH near to neutral, and high buffering capacity make PPMS the best resource for anaerobic digestion than making fibreboard. Obviously, great variation occurs within the different types of plants, based on the raw material and the paper manufacturing processes. The quantity of sludge generated from the aerobic treatment in pulp and paper mills is varied from 0.4 and 1.0 t of biosludge per t COD reduced (Hagelqvist, 2013), while at an anaerobic treatment of mill waste, it is only about 0.02 t of digesate per t COD removed.

2.3 ANAEROBIC DIGESTION OF PPMS

Historically, the anaerobic digestion process has been used for the treatment of sludge from the aerobic WWT plant and the waste from agriculture residue and animal manure. At the present time, anaerobic digestion has been used for different organic waste varied from the industrial, agricultural and municipal sectors (Mshandete et al., 2005). In recent times, a need has risen for the industrial sector such as pulp and paper mills for their treatment and disposal of produced excess sludge from the aerobic WWT plant. There are some disadvantages to the anaerobic digestion process such as a slow metabolic process, hence it has a long residence times, cannot able to accept shock loading, excessive foaming/formation of a thick layer in the top of the reactor. Moreover the presence of proper seed sludge is important during the initial start-up period because of the lower growth yield of anaerobic microbes. As if date, primary and secondary sludge has been mixed, thickened and then normally incinerated. This is not often energetically favorable or landfilled that have become restricted due to the emissions of greenhouse gases and a loss of resource (energy) (Bayr and Rintala, 2012).

Anaerobic digestion has become an alternative and reliable energy source (Ragauskas et al., 2006). The energy acquired from anaerobic digestion is renewable that can augment and eventually replace fossil transportation fuel (Himmel et al., 2007). Anaerobic digestion is attractive because it can reduce about 50 % of the sludge disposal/management costs. The disposal of sludge and its management cost account for about 60 % of the total operating cost of WWT plant (Monte et al., 2009; Xu and Lancaster, 2009). Rintala and Puhakka (1994) stated in a review paper that both primary and secondary sludge produced from WWT plants could be amenable for the anaerobic digestion. There was considerable scientific interest in the late 1990s for the anaerobic digestion of this substrate

(Jokela et al., 1997; Ratnieks and Gaylarde, 1997). During that time, experimental result using PPMS shows lower methane yield with longer solid retention time. Table 2.1 illustrates that anaerobic digestion of PPMS is acquiescent for biogas generation by comparing it with the varied substrate. Therefore, this review supports that anaerobic digestion of PPMS is technically feasible to use for biomethanation at an industrial level. Some studies on PPMS has also proven that the increased rate of solubilization and higher reduction in volatile solid content after digestion. Application of anaerobic digestion for mill-derived sludge has been limited by the requirement of a very long retention time of about 30-60 d and low overall degradation efficiency of the organic matter (30 to 50 %) (Meyer and Edwards, 2014).

Table 2.1. Biogas yield from different feedstock

Feedstock	Total solids (TS) (%)	Volatile Solids (VS) (% TS)	Biogas yield (m^3/kg VS)	Reference
Pig slurry	3-8	70-80	0.25-0.35	Holm-Nielsen et al. (2009)
Chicken slurry	10-30	70-80	0.35-0.60	Salminen and Rintala (2002)
Food waste	10-20	80-85	0.50-0.60	Dhamodharan et al. (2015)
Municipal biosludge	0.8-1.2	59-68	0.20-0.52	Le Hyaric et al. (2011)
Garden waste	60-70	90	0.20-0.50	Bouallagui et al. (2005)
Pulp and paper sludge	30-38	45-67	0.20-0.35	Bayr et al. (2013)

Table 2.2 exemplifies the previous experimental studies on anaerobic digestion of PPMS without pretreatment. It is clear that very limited experiments have been carried out and those were mostly batch assay and semi continuous without pretreatment. This appraisal shows that anaerobic digestion of PPMS without pretreatment shows that the specific methane yield ranged in between 30 and 200 mL/g VS or COD fed with volatile solid reduction rates varying from 21 to 57 %. Although the specific methane yield was at a minimum, the total amount of methane produced in the reactor was at a maximum. Because the methane production depends on the hydraulic retention time and the volatile solid reduction.

2.3.1 Pretreatment of PPMS

Considerable effort has been made to explore strategies and technologies that reduce the production of sludge in the line of wastewater (alternating oxic and anoxic environments), or in the line of sludge (sludge pretreatment prior to anaerobic digestion) and/or in the line of final disposal (incineration and pyrolysis) at the wastewater treatment plant of the pulp and paper industry. Among these strategies, the emerging pretreatment technologies prior to anaerobic digestion has become more prominent, to enhance digestibility of sludge material with the increased rate of production of biogas/methane (Meyer and Edwards, 2014).

Table 2.2. Results from previous experimental studies on AD of PPMS

Type of sludge	COD concen. (g/L)	VS/TS ratio	Retention time (d)	VS (or COD) removal η (%)	CH_4 yield (mL/g VS, COD fed)	Experimental setup	Reference
Kraft pulp mill WAS	13-100	0.57	NA	40	123	Pilot scale	Puhakka et al. (1988)
Mixture PS/WAS from chemi thermo mechanical pulp mill (70-90 % WAS)	18-53	0.75-0.93	15	41 (VSS)	90	Bench scale	Puhakka et al. (1992)
Mixture municipal sludge, PS and WAS from thermo mechanical pulp and paper mill	45	0.59	30	27	185	Bench Scale	Jokela et al. (1997)
WAS from bleaching chemi thermo mechanical pulp and paper mill + 10 % MGWL	NA	0.65	NA	57	270	Pilot scale	Lin et al. (2011b)
Kraft pulp and paper mill PS	1.4-1.8 (sCOD)	0.81-0.84	16-32	25-40	190-240	Bench scale (thermophilic)	Bayr and Rintala (2012)
Mixture Kraft pulp and paper mill PS + WAS (VS ratio 3:2)	NA	NA	25-31	25-40	150-170	Bench scale (thermophilic)	Bayr and Rintala (2012)
Pig waste and paper sludge (2:1)	35-50	NA	35	48	250	BMP assay	Parameswaran and Rittmann (2012)
Pulp and paper sludge and food waste	NA	0.57	30	71-87	432.3	Bench scale	Lin et al. (2013)
Codigetion of PPMS and food waste	NA	0.40	55	73-94	123-256	Batch reactor	Lin et al. (2013)
Mixture of WAS from thermo mechanical pulping / chemi thermo mechanical pulp mill + municipal WAS	NA	0.69	19	50 (VSS)	84	Batch	Hagelqvist (2013)

NA-Information not available

Due to the resistant and recalcitrant nature of lignocellulose content, biodegradation of PPMS is hindered and forms a major barrier in bioconversion of PPMS into biogas. The structural and compositional properties of lignocellulose holds the cellulose and hemicellulose together due to their sturdy and adhesive properties to form a rigid three-dimensional structure. Making the hydrolysis step a bottleneck for anaerobic digestion, forming a rate-limiting step in anaerobic digestion PPMS (Tiehm et al., 2001; Wood et al., 2009; Yunqin et al., 2010; Elliott and Mahmood, 2012).

Hence, the pretreatment is necessary to brittle the bottleneck present in anaerobic digestion of PPMS. During pretreatment, the cell wall get ruptured and then extracellular polymeric substances including organic debris and extracellular polymers get destructure and/or degraded. As a result, there is a release of freely available intracellular organic material for easy consumption by the acidogenic and methanogenic microorganisms. This mechanism of the disintegration of sludge can be enhanced by employing pretreatment technologies. This is important in sludge digestion because the major constituents of cells are the organic fractions, which are favorable substrate for microbial degradation. As mentioned above, although cellulose and hemicellulose present in the PPMS are hydrolysable, they are often covered by the lignin in PPMS and thus prevented from the enzymatic attack of anaerobic microorganisms. Therefore, pretreatment for deconstructing lignin incrustation prior to anaerobic digestion becomes essential. Over the last decade, different pretreatment methods have been investigated to improve the anaerobic digestion technology. They may be categorized as physical, chemical and biological pretreatment as shown in Fig. 2.3. Effects of different pretreatments on the physical/ chemical composition and structural alteration of lignocellulose material are displayed in Table 2.4. These methods could improve the accessibility of lignocellulosic materilas through various machanisms, such as breaking the covalent associations between the lignocellulosic components, depolymerizing lignin, decreasing cellulose crystallinity and dissolving hemicellulose (Taherzadeh and Karimi, 2008; Hendriks and Zeeman, 2009).

2.3.1.1 Physical pretreatment

Physical pretreatment is further grouped into mechanical and thermal pretreatment.

Mechanical pretreatment

In mechanical pretreatment, the PPMS could be comminuted into smaller pieces by numerous mechanisms such as chipping, grinding and milling. Mechanical pretreatment is done primarily to disrupt the crystallinity of cellulose (not applicable for biosludge) by breaking into smaller components resulting in higher specific surface area of the PPMS and reduced degree of polymerization, thus rendering the PPMS more amenable to succeeding enzymatic hydrolysis. On the other hand, mechanical pretreatment is energy-intensive and expensive, and moreover time-consuming.

The mechanical pretreatment is less effective than chemical pretreatment because it does not remove the lignin content (Zheng et al., 2014). Moreover, it has been observed that significant restriction in the cellulose accessibility also inhibits the cellulose enzyme. Therefore, the mechanical

pretreatment method is only used occasionally. Two experimental studies have examined the effect of high-pressure homogenization on PPMS (biosludge) digestability. Saha et al. (2011) conducted a batch experiment and observed an improvement in specific methane yield by 80 % after the 21 d of digestion period. Whereas, Elliott and Mahmood (2012) observed that the methane yield at an HRT of only 3 d was equivalent at an HRT of 20 d without pretreatment in a continuously fed reactor.

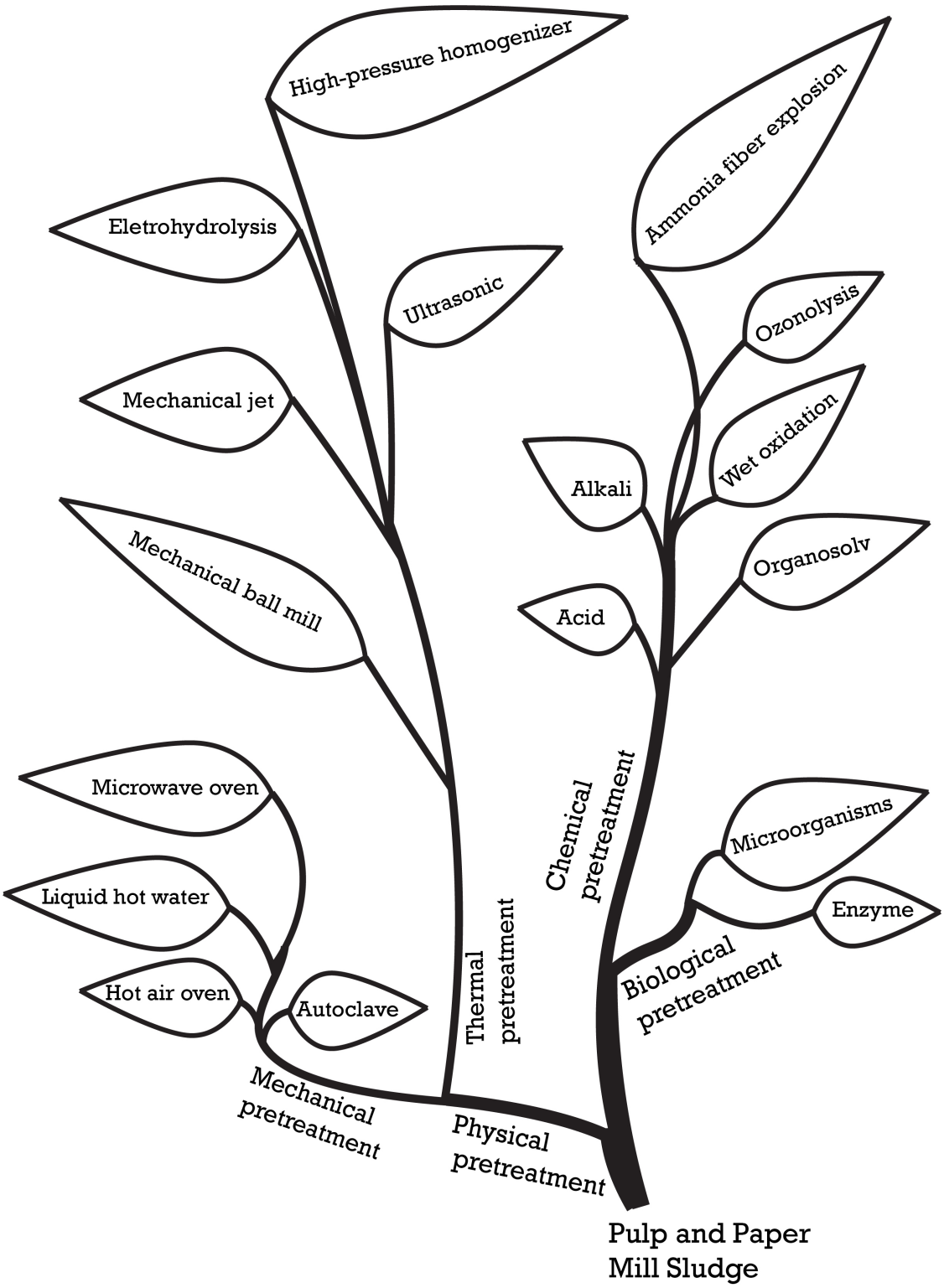

Fig. 2.3. Different pretreatment adopted in pulp and paper mill sludge

Thermal pretreatment

In thermal pretreatment, PPMS is rapidly heated by a different mode of heating such as hot air, liquid hot water, and steam over a period of stipulated time to encourage the holocellulose hydrolysis with rapid decompression. The sludge is subjected to temperature in the range of 150-200^{o}C, although lower temperature have also been reported. The pressure adjoining these temperatures are in the range of 600-2,500 kPa. The major key factors for thermal pretreatment are the treatment time, exposing temperature, moisture content and size of the particle. Thermal pretrearment is highly effective in increasing the accessible and susceptible surface area of cellulose and enhancing the degradability of cellulose to microbes and enzymes. All studies report a positive impact of thermal pretreatment on anaerobic digestion. Wood et al. (2009) conducted the three different pretreatment methods (thermal, caustic, and sonication) in a BMP assay with the PPMS (biosludge) from the Kraft and sulphite pulp mill. It was reported that thermal pretreatment at 170^{o}C appeared to be the most effective with a 55 % and 280 % increase in specific methane production, respectively. In another study, where PPMS was treated thermally at a lower temperature ($< 150^o$C) and then followed by a themophilic anaerobic digestion, which shows an increase in specific methane yield of 45 % (Bayr et al., 2013). The optimum conditions and magnitude of the improvement however vary considerably.

Ultrasound

Ultrasonic treatment works similar to mechanical pretreatment but utilise the acoustic waves, whereas microwave pretreatment applies the electromagnetic waves that disturbs the cell structure and flocs. Sonication is the powerful method to distract the sludge cells. The principles of this treatment relies on the induced cavitation process. Through the subsequent compression and expansion of the fluid under the influences of the ultrasonic waves, implosions are generated which gives rise to local extreme conditions (temperature of several 1000^{o}C and pressure of up to 500 bar (Saha et al., 2011)). Some studies have been investigating the effect of ultrasound on PPMS with the increased specific methane yield of up to 80 % (Wood et al., 2009; Saha et al., 2011; Elliott and Mahmood, 2012; Park et al., 2012). One study utilize the microwave pretreatment that showed increment of about 90 % (Saha et al., 2011).

Eletrohydrolysis

Electrohydrolysis pretreatment is the process of passing direct current through an ionic substance to solubilize the organic matter by breaking the bonds between polymers induced by the application of current through electrodes. The principle of electrohydrolysis pretreatment relies on electrophoresis, ohmic heating and electroosmosis resulting in the disintegration of particles and microbial cell lysis (Zhen et al., 2014). The higher liquefaction and delignification of lignocellulose matter can be possible in electrohydrolysis pretreatment, with the enhanced biogas production in anaerobic digestion.

Table 2.3. Results from previous pretreatment experimental studies on AD of PPMS

Type of sludge	COD (g/L)	VS/TS ratio	Retention time (d)	Type of pretreatment	VS/COD removal η (%)	CH_4 yield (mL/g VS)	Setup	Reference
Secondary / biosludge	14.1	0.77	NA	Alkaline (NaOH)	32	NA	Bench scale	Navia et al. (2002)
WAS from mechanical and chemical pulp and paper mill	NA	NA	20	1) Ultrasound (2-30 Wh/L)	NA	96-148	BMP assay	Gavala et al. (2003)
				2) Enymatic (mixture of Hydrolase, 40 mg/g)	NA	178		
				3) Ultrasound + Enymatic	NA	196		
				4) Untreated control	NA	43-155		
Sulfite pulp mill WAS	12	0.78	34	1) Hydrothermal (170^{o}C, 1 h)	65 (VSS)	185	BMP assay	Wood et al. (2009)
				2) Caustic (NaOH, pH 12, 140^{o}C, 1 h)	62 (VSS)	145		
				3) Ultrasound (20 kHz, 30 min)	28 (VSS)	120		
				4) Untreated control	NA	120		
Kraft pulp mill WAS	27	0.78	34	1) Hydrothermal (170^{o}C, 1 h)	31 (VSS)	115	BMP assay	Wood et al. (2009)
				2) Caustic (NaOH, pH 12, 140^{o}C, 1 h)	28 (VSS)	110		
				3) Ultrasound (20 kHz, 30 min)	$<$2 (VSS)	40		
				4) Untreated control	NA	20		
Bleaching chemi thermo mechanical pulp mill WAS	34-40	0.76	21	1) Microwave (50-175^{o}C, 2450 MHz)	23-24	50-95	BMP assay	Saha et al. (2011)
				2) Ultrasound (20 kHz, 15-90 min)	26-30	70-90		

				3) High pressure homogenization (83 MPa)	26	90		
				4) Untreated control	26	50		
Bleaching chemi thermo mechanical pulp mill WAS + PS (40:60 % v/v)	34-40	0.76	21	1) Microwave (50-175^{o}C, 2450 MHz)	16-24	55-75	BMP assay	Saha et al. (2011)
				2) Ultrasound (20 kHz, 15-90 min)	15-23	60-70		
				3) Untreated control	10	50		
WAS from bleaching chemi thermo mechanical pulp / thermo mechanical pulping mill (raw)	30	0.76	28	1) Combined caustic and Ultrasound	30	67	BMP assay	Park et al. (2012)
				2) Untreated control	21	85		
WAS from bleaching chemi thermo mechanical pulp / thermo mechanical pulping mill (thickened)	88	0.85	28	1) Combined caustic and Ultrasound	27	96	BMP assay	Park et al. (2012)
				2) Untreated control	23	88		
WAS from mechanical pulp mill	47	0.9	20	1) Mechanical shear (1500 rpm)	32 (VSS)	73	Bench scale reactor	Elliott and Mahmood (2012)
				2) Ultrasound (20 kHz)	39	90		
				3) High pressure homogenization (83 MPa)	58 (VSS)	91		
				4) Untreated control	29	77		

Pulp and Paper mill WAS	1	0.83	30	1) Hydrothermal (150^{o}C, 10 min)	NA	134	BMP assay	Bayr et al. (2013)
				2) Enzymes (mixture of Accelerases, 70 mg/g VS)	NA	114		
				3) Ultrasound (45 kHz, 30 min)	NA	114		
				4) Caustic (NaOH, pH 12)	NA	86		
				5) Acid (HNO_3, pH 3)	NA	61		
				6) Untreated control	NA	108		
Mixture of PS sludge and WAS + 3 % MGWL	NA	0.65	40	1) Alkalinen (NaOH, 80 g/k)	21-24	320	Bench scale	Tyagi et al. (2014)
				2) Untreated control	21-24	175		
Mixture of PS, rice straw and MGWL	NA	0.54	50	Microbial consortia (OEM1)	NA	429	Bench scale	Lin et al. (2017)

PS - Primary sludge, WAS - Waste activated sludge, MGWL - Monosodium glutamate waste liquor, NA - Information not available

2.3.1.2 Chemical pretreatment

Among the various pretreatment methods, chemical pretreatment is studied mostly. The paper industry uses the chemical pretreatment majorly for the delignification of cellulosic materials to manufacture the high-quality paper products. In recent times, by employing the modification in the pulping processes, the possibility of evolving effective and inexpensive pretreatment techniques has been considered. The chemical pretreatment has been studied with the primary goal of increasing the cellulose biodegradability by removing the lignin and/or hemicellulose, reducing the degree of polymerization, and destructure the crystallinity of cellulose component. Various chemical methods have been developed (Fig. 2.3), based on different operating principles. The major groups are (i) acid and alkaline (thermal) hydrolysis, (ii) ozonation, and (iii) advanced oxidation methods. These methods are described hereafter.

Acid and alkaline (thermal) hydrolysis

In chemical treatments, an acid or base is added to solubilize the sludge. The addition of acid or base avoids the necessity of temperatures and so the treatment methods are carried out in an open environment or moderate temperature. An overview of this pretreatment are presented in the Table 2.4. These methods are shown to be an effective albeit cumbersome methods for sludge solubilization since it is required for pH levels that are extreme, and its treated sludge needs subsequently to be re-neutralished. Hence, these pretreatments are limited in an industrial application. Lin et al. (2009) studied the effect of alkali pretreatment on PPMS solubilization and subsequent anaerobic digestion. It was reported that at an optimal sodium hydroxide (NaOH) dose of 8 g NaOH per 100 g total solid sludge, the solubilization and methane yield was increased by 83 and 183.5 % respectively. Alkali addition has been shown to increase solubilization of PPMS and improve biogas yield (Park et al., 2012).

Oxidative pretreatment

The oxidative pretreatment uses the oxygen or air at high temperature (260^{o}C) and pressure (10 MPa). Effective sludge solubilisation is achieved in a high percentage. Problems such as odour, corrosion and high-energy cost however restrict the practical application of this process. The most frequent studies of oxidative methods are ozonation (O_3) and peroxidation (H_2O_2), belonging to the advanced oxidation processes and based on the generation of hydroxyl (OH*) radicals which are extremely powerful oxidants. Elliott and Mahmood (2007) made an attempt to solubilise the COD of municipal biosludge using highly oxidative conditions using ozone. Reported that the ozone application is likely to cost intensive in relation to the accomplished enhancement in anaerobic digestibility of PPMS (Elliott and Mahmood, 2007).

2.3.1.3 Biological pretreatment

Generally, biological treatment is done with the wood degrading microorganisms such as the white rod, brown rod, soft rod fungi, and also with the bacteria and enzyme to alter the chemical

composition, and/or to destructure the PPMS material. The altered PPMS can easily provide the extracellular polymeric substance for enzyme digestion. Especially, fungi which has unique degradation characteristics for the lignocellulosic material. There is some common perception that the brown and soft rod fungi attack mainly on cellulose material and minor impact on lignin content while white rod fungi degrade the lignin component more actively.

The biological pretreatment seems to be a promising technique and has an evident advantage such as no chemical requirement, milder environment condition, lower energy input, moreover the working manner is environment-friendly. Conversely, biological pretreatment disadvantages are as apparent as its advantages because the metabolic activities involved in biological pretreatment are very slow and requires vast space to perform the treatment. Moreover, it requires careful control of environmental growth conditions. Karlsson et al. (2011) studied in batch experiments with a mixture of various hydrolases at a concentration of 40 mg/g total solids (TS) to PPMS (biosludge), and reported that specific methane yield increased by 35 %. Whereas in another study with a semi continuous fed bench scale reactor with the enzyme concentrations of 80 mg/g TS, the methane yield did not improved at all, and it was reported that this could be due to the unfavourable sludge viscosity.

The majority of the literature reports that the thermal pretreatment or the combination of different pretreatment (alkali-thermo or thermo-chemical) have been proposed and tested majorly for municipal wastewater treatment plant sludge. The combinations of different pretreatment techniques by employing physical and/or chemical principles has an ability to considerably increase the surface area, decrease the digestion time and thereby reduce the size of the reactor with the enhanced biogas production, and also with decreased excess sludge generation after digestion. However, most literature reported in the pulp and paper industry, reiterate the pretreatment potential of secondary sludge to enhanced biogas through the anaerobic bioconversion technology. Some literature has compiled the different pretreatments using PPMS (Mahmood and Elliott, 2006; Elliott and Mahmood, 2012). The experimental studies on pretreatment technologies in PPMS can be classified as the mechanical (Saha et al., 2011; Elliott and Mahmood, 2012), chemical (Navia et al., 2002; Lin et al., 2009; Tyagi et al., 2014), ultrasonic (Tiehm et al., 2001; Saha et al., 2011; Tyagi et al., 2014), enzymatic (Yunqin et al., 2010; Bayr et al., 2013; Lin et al., 2017) and thermal pretreatment methods (Wood et al., 2009; Bayr and Rintala, 2012) and combinations of different pretreatment (Elliott and Mahmood, 2012; Park et al., 2012; Bayr et al., 2013). Thus, pretreatment is done to upsurge the available soluble organic material and to the accelerate sludge digestion. Some literature report that sludge pretreatment has increased the methane production for both mesophilic (43-145 %) (Saha et al., 2011) and thermophilic (4-58 %) anaerobic conditions (Gavala et al., 2003; Bayr and Rintala, 2012).

Thermal processing, sonication, and mechanical integration have seemed to be prominent technologies for PPMS pretreatment. Results from the previous studies were conflicting. Either alone or a combination of ultrasonic, microwave oven and/or enzymatic pretreatment which did not illustrate the substantial effect on the methane production from PPMS (Karlsson et al., 2011). Bayr et al. (2013) studied the biological pretreatment in a batch experiment by adding a mixture of accelerases (70 mg/g of VS) to the biosludge from the paper industry and reported that succeeding anaerobic digestion does not have any improvement. Nevertheless, enzymatic pretreatment might have the potential to

escalate the sludge digestibility (Yunqin et al., 2010). Alkaline treatment is the most commonly investigated chemical method, alone or in combination with other pretreatment methods. However, the enhanced digestibility of sludge through acidification has been previously suggested, Elliott and Mahmood (2007) reported that acidification seems to be impossible due to the necessity of the high range of acid consumption. On the antagonistic, Elliott and Mahmood (2007) recommend that PPMS could be more amenable to pretreatment because of the higher volatile fraction and reported that the residence time of sludge in a biogas reactor have been substantially reduced from 15-25 d to 7 d. From today's standpoint, it is difficult to suggest which pretreatment techniques claims the utmost potential for PPMS. This is because of the proportion and composition of sludge might vary based on raw material, pulping, and the paper production process. Moreover, there was very limited literature that inspects the different pretreatment effects on PPMS.

From this review, it can be seen that previous experiment studies on anaerobic digestion of PPMS have been carried out majorly by BMP assay, with very hardly any being to a bench scale. On analyzing the pretreatment literature, it was perceived that the pretreatment studies have gained interest only in the last decade. Table 2.3 exemplifies the different pretreatment methods used for PPMS in the literature. The studies display that the specific methane yield from the PPMS varies extensively between 50 to 429 mL/g VS fed/degraded. The removal rates of volatile solids were about 20 to 65 % after pretreatment in the batch bioreactor. From the Table 2.3, it was observed that digestibility of sludge has clear recognizable trends for the production of methane from the sludge.

The effectiveness of pretreatments are in enhancing the digestibility of lignocellulose materials involves a range of mechanisms, which include the deconstruction the pretention by lignin, increasing the accessible surface area, decreasing the cellulose crystallinity and the degrees of polymerisation as mentioned in the Table 2.4 (Hu et al., 2007; Hendriks and Zeeman, 2009; Hu et al., 2016). These effects vary with different methods, such as thermal pretreatment promotes the hemicellulose and lignin solubilization (Yang et al., 2010; Hu et al., 2016). Acid pretreatment solubilise the hemicellulose more efficiently, making the cellulose better accessible for the acidogenic microorganisms in anaerobic digestion. Alkaline pretretment makes a swollen state of the lignocellulose material and creates a more accessible surface area and pore size (Hendriks and Zeeman, 2009). The ultrasonic and electrohydrolysis pretreatment could potentially decrease the cellulose crystallinity and the degrees of polymerization, and also increases the accessible surface area and pore size (Taherzadeh and Karimi, 2008). Despite all the different mechanisms, the essence of all these methods is to make the lignocellulose available to the acidogenic and methanogenic microorganisms to produce more biogas. Therefore, it is wise to integrate these pretreatments prior to the anaerobic digestion to enhance the digestibility of lignocellulose material.

2.4 CO-DIGESTION STRATEGY FOR PPMS

Mono-digestion of recalcitrant feedstocks such as PPMS, highly protein rich substrate or feedstocks with harmful compounds can often resulted in a slow digestion process and a low biogas yield. These limitations could be overcome by codigestion of different feedstocks with appropriate carbon/nitrogen (C/N) mixing ratio, inhibitors, feedstock biodegradability, and TS content.

Table 2.4. Effect of different pretreatments on the physical/ chemical composition and structural alteration of lignocellulose biowaste

Pretreatment	Increased accessible surface area	Cellulose decrystallization	Hemicellulose solubilization	Lignin solubilization	Formation of furfural/ HMF	Alteration in lignin structure
Mechanical ball mill/jet	∗	⋆	○	○	○	○
High pressure homogenizer	∗	●	●	●	●	●
Ultrasonic	∗	⋆	⋆	∗	○	∗
Electrohydrolysis	∗	∗	⋆	⋆	∗	∗
Microwave irradiation	∗	⋆	⋆	○	⋆	⋆
Hot air oven	∗	⋆	⋆	∗/⋆	○	∗
Autoclave	∗	○	∗	∗/⋆	∗	∗/⋆
Acid	∗	○	∗	⋆	∗	∗
Alkaline	∗	○	⋆	∗/⋆	⋆	∗
Oxidative	∗	●	○	∗/⋆	⋆	∗
Ionic liquid/ Organosolv	∗	∗	⋆	○	○	○
Ammonia fiber explosion	∗	∗	⋆	∗	⋆	∗
Microorganisms	∗	●	∗	∗/⋆	○	∗
Enzyme	∗	●	∗	∗/⋆	○	∗

∗ - Major effect, ⋆ - Minor effect, ● - Not determined, ○ - No effect, HMF - Hydroxymethylfurfural

Removal efficiency might be enhanced by codigestion methods of different inoculum/substrate (Dhamodharan et al., 2015). As PPMS contains less in nitrogen content in order to maintain proper C/N ratio and also to increase the removal efficiency, codigestion could be more feasible if the substrate/inoculum have high nitrogen content. There are some literature reports that the substantial differences in the methane yield from anaerobic digestion depends on the type of substrate, source, and composition of inoculum used. Ge et al. (2016) and another study (Xu et al., 2016) report that the significant influence of methane yield, start-up time and its stability of the solid/ liquid state anaerobic digestion based on the source and its composition of the inoculum. Yang et al. (2015) reports that the suitable inoculum can upsurge the degradation rate, increase the methane production, shorten the starting time, and have a more stable digestion process.

Teghammar et al. (2012) reported that the PPMS was added to a nitrogen-rich substrate mixture used in an industrial digester, which has the stabilizing effects on the digestion process. PPMS addition could prevent the accumulation of volatile fatty acids (VFAs), increase the methane yield by 15-34 %, and decrease the hydraulic retention time by five days. Considering the fact that PPMS or easily degraded feedstocks are not highly available and the problems associated with the pretreatments, codigestion could be a good strategy to increase the amount of produced biogas, by avoiding the costs and challenges associated with the pretreatments. However, more research is needed in this area for a better understanding of appropriate mixing ratios, effects on interaction, and the impact of codigestion.

2.5 CONTINUOUS SCALE ANAEROBIC DIGESTION

Anaerobic digestion has been investigated as a treatment method for wastewater streams in the pulp and paper industry (Rintala and Puhakka, 1994). Several waste streams are aamenable to anaeorobic digestion, but there are still few anaerobic digesters in operation in the industry. The most common configuration include anaerobic digeastion followed by an aerobic treatement. In Canada, there are four anaerobic bioreactors were installed to treat pulp and paper mill effluents, with only two in operation (Elliot and Mahmood, 2007). Worldwide, there were around 100 anaerobic digester at pulp and paper mills with 75 being Upflow anaerobic sludge blanket (UASB) (Kleerebezem and Macarie, 2003; Wood et al., 2009). In some cases, UASB have been used to replace anaerobic lagoons leading to cost savings associated with the use of biogas in lime kilns and other pulp and paper mill processes (Chinnaraj and Rao, 2006).

Puhakka et al. (1992) conducted a pilot-scale experiment with biosludge from a kraft pulp mill for the period of 21 months. After a start-up and acclimation/adaptation period of 50 d, HRT was varied between 24 to 8 d while mostly maintaining steady operation. Along with a progressive increment in loading rate varied from 2.2 to 5.2 g VS/L d. Initially specific methane yield increased from 130 to 200 mL/g VS fed, and then decreased to 100 mL/g VS fed. During that time VS removal rates ranged between 37 and 55 %. At a remarkably low HRT of 8 d, although the specific methane yield was at a minimum, the total amount of methane produced in the reactor was at a maximum. The latter exceeded the total amount of methane generated at a HRT of 20 d by the 250 times.

A potential strategy for large scale digestion might therefore be to anaerobically digest as much organic matter as possible while maintaining low HRTs. The remaining solids fraction of the digestate may subsequently be pretreated with different pretreatment section explained in earlier section, followed by re-injuction into the digester, or alternatively, dewatered and incinerated or used as land application. In another long-term study (9 months) Karlsson et al. (2011) digested biosludge from a kraft pulp mill and TMP pulp mill in two bench-scale reactor (CSTR) with the varied loading rate from 2 to 4 g VS/L d. They achieved 40 % of mean VS reduction rate in both reactors. The specific methane yield from the digestion of kraft mill biosludge remained relatively constant and on average 120 mL/g VS fed, whereas that of the TMP mill biosludge was approximately 180 mL/g VS fed. In both studies (Puhakka et al., 1992; Karlsson et al., 2011) the VS loadingrate could steadily be increased over the course of several months with a relatively small impact on specific methane yield. Numerous laboratory and pilot-scale studies have shown that the contrary to common perception, most other mill effluents are also to some extent anaerobically.

2.6 OPTIMUM CONDITION REQUIRED FOR AD

To maintain anaerobic microbes with a high metabolic activity, it is necessary to have a controlled environmental condition. Because methanogens are highly susceptible to changes in environmental conditions, so it is important to maintain the optimal conditions. The rate and extent of biogas production depends on: the nature of the substrate, temperature, total solid, volatile solid, pH, alkalinity, mixing, toxicity, nutrients, retention time, C/N ratio, and the organic loading rate etc. Table 2.5 portrays the optimal condition and concentration of organic and inorganic compounds.

2.6.1 Temperature

Like most other biological systems, the anaerobic process is strongly temperature dependent. The temperature has a vital effect on the physicochemical properties of the components found in the digestion substrate. It influences the growth rate and metabolism of microorganisms and the population dynamics in the anaerobic bioreactor. In an anaerobic system, there exists three optimal temperature ranges for the methanogensis such as psyshrophilic, mesophilic and thermophilic as mentioned in the Table 2.5. In general, anaerobic conversion rates increase with temperature up to 60^oC. Anaerobic process can still operate in a temperature range of 10-45^oC without major changes in the microbial ecosystem. Acetotrophic methanogens are one of the most sensitive groups to increasing temperature. The degradation of propionate and butyrate are also sensitive at higher temperature above 70^oC. It has also a significant effect on the partial pressure of hydrogen in digester, because it influences the kinetics of the syntrophic metabolism. An increasing temperature has several beneficial effects such as an increasing solubility of the organic compounds, the biological activity doubles for every 10^oC increase in temperature within the optimal temperature range, improved biological and chemical reaction rates, and an increasing death rate of pathogens. Whereas, at high temperatures (thermophilic) has counteracting effects such as there will be an increased fraction of free ammonia, and the increasing pKa of the volatile fatty acid will make the more susceptible to inhibition. Hence, it is important to maintain the stable operating temperature in the digester.

Table 2.5. Optimum condition and concentration of organic and inorganic compounds required for AD

Parameters	Optimum conditions (mg/L)	Reference
Temperature	Psychrophilic (5-15^{o}C)	Van Haandel et al. (1994)
	Mesophilic (35-40^{o}C)	
	Thermophilic (50-65^{o}C)	
pH	6.8-7.4	
Oxidation reduction potential	-200 to -350 mV	Le Hyaric et al. (2011)
Volatile fatty acids	>2,000 (acetic acid)	Chen et al. (2008)
	>6,000-8,000 (total volatile acids)	
Alkalinity	1,000-5,000 (as $CaCO_3$)	Metcalf et al. (2003)
Hydrogen partial pressure	10^{-3} atm (hydrogenetropic methanogens)	Khanal (2011)
	10^{-4} atm (propionic acid degradation)	
	1 atm (ethanol oxidation)	
C/N ratio	20-30	Wang et al. (2012)
Organic loading rate	Varies according to the substrate and inoculum	
Sulfide	Up to 250 (as H_2S at pH 6.4-7.2)	Chen et al. (2008)
	90 (as H_2S at pH 7.8-8.0)	
	>100 (as soluble)	
Heavy metals		
Copper (Cu)	150^{a}	Hayes and Theis (1978)
Cobalt (Co)	75^{a}	
Cadmium (Cd)	150	
Iron (Fe)	1800^{a}	
Chromium (Cr)	200-250	
Nickel (Ni)	100^{a}	

Zinc (Zn)	60[a]			
Total ammonia nitrogen	1,500-3,000 (at pH > 7.6)			
	600-800 (free ammonia)			
Nutrients	Stimulating	Moderately inhibitor	Strongly inhibitor	
Calcium	200	2500-4500	8000	Appels et al. (2008)
Magnesium	75-150	1000-1500	3000	
Potassium	200-400	2500-4500	12000	
Sodium	230-350	3000-5500	8000	

[a]Milligram of metal per kg of dry solids

2.6.2 Volatile fatty acids (VFAs) and oxidation reduction potential (ORP)

Volatile fatty acids are important intermediate compounds in the metabolic pathways of methane fermentation. At very high concentration, it causes microbial stress. Acetic, propionic, butyric, and valeric acids are the major constituents present during anaerobic biodegradation. The monitoring of their concentrations provides useful information regarding the digester performance and system toxicity. For instance, the yield of acetate increases slightly with the increasing of pH, whereas for the butyrate yield increased with decreasing pH. The yield of propionate does not relate to pH. The VFAs accumulation is very common in the initial days and it needs neutralisation during the start-up phase of any bioreactor. The accumulation of acetic acid over 2,000 mg/L and total VFAs concentration over 8,000 mg/L will affect the overall methanogenisis process. The total VFAs are related to the various factors: mainly production and consumption rate by microorganisms within bioreactor, solid retention time and leachate/digestate recirculation practises, pretreatment, and initial feed characteristics. To understand the toxicity effect, VFAs to alkalinity ratio is a good indicator. For the good working reactor it needs, the VFAs to alkalinity ratio to be $\leq$0.4; for failure of a reactor $\geq$0.8 was reported by Khanal (2011). For the anaerobic system, the ORP value should want to be maintained around -200 to -350 mV at pH 7.0, to obtain the growth of an obligate anaerobe in any medium. It is well established that the methanogens require a highly reducing environment with redox potential as low as -400 mV. A limited number of studies have been carried out to evaluate the effect of ORP in a mixed culture using methanogenesis in an anaerobic treatment Khanal (2011).

2.6.3 pH and alkalinity

An anaerobic process performance is adversely affected by slight pH changes away from optimum. Anaerobic can be grouped into two category: acidogens and methanogens. Methanogens are more susceptible to pH variation. Each group of microorganisms has a different optimum pH range. The optimum pH range for acidogens and methanogens are 5.5-6.5 and 7.8-8.2 respectively. Hence, the optimum pH for the combined cultures ranges from 6.8-7.4. Acidogens are significantly less sensitive to low or high pH, and acid fermentation will succeed over methanogensis, which may result in the souring of the reactor (Van Haandel et al., 1994). As PPMS is almost equivalent to netrual pH, there would not be a problem during the initial stage/ rector start-up. There is some literature reported that, due to degradation of organic material after the start-up stage in a batch reactor, drop in pH is due to the VFA accumulation (Raposo et al., 2006; Lim et al., 2009). Thus, methanogens are vulnerable to abrupt pH changes ahead of the optimum value, the anaerobic treatment system needs satisfactory buffering capacity (alkalinity) to mitigate the pH change. An anaerobic system operating within an acceptable range of pH, is predominantly controlled by natural alkalinity or self-produced alkalinity. In General, the alkalinity varies from 1,000 to 5,000 mg/L as $CaCO_3$ in anaerobic system.

2.6.4 Hydrogen partial pressure

Anaerobic microorganisms, especially methanogens are highly susceptible to changes in the hydrogen partial pressure. The anaerobic oxidation by hydrogen producing bacteria of higher

organic acids (propionate, butyrate, valeric, benzoate) is not thermodynamically favourable in a pure culture. However, in a coculture, hydrogen producing acetogenic bacteria and hydrogen consuming methanogenic bacteria exist as a symbiotic relationship between these groups. The hydrogen consuming methanogenic bacteria hastily scavenge the hydrogen and keep the hydrogen partial pressure level low in an anaerobic environment. This offers a thermodynamically favourable condition for the hydrogen producing acetogenic bactreia to breakdown the organic compounds into H_2, acetate and carbon dioxide as manifested by a negative Gibb's free energy change. This phenomena is commonly known as interspecies hydrogen transfer. The propionic acid, butyrate, and ethanol oxidation becomes thermodynamically favourable only at hydrogen partial pressure below 10^{-4}, 10^{-3} and 1 atm respectively. It is important to monitor the oxidation of propionate because it is more critical than the oxidation of other organic acids and solvents as many as 30 % of the electrons are associated with propionate oxidation (Khanal, 2011).

2.6.5 Carbon/Nitrogen ratio

Nitrogen is a major nutrient for the growth of microorganisms. The nitrogen uptake rate is varied for the aerobic and anaerobic microbes. Anaerobic bacteria use carbon 25-35 times higher than nitrogen. For better digestion, the carbon- nitrogen ratio should be about 25-30:1 in the substrate. Carbon acts as the energy source for the microorganisms, whereas nitrogen play a major role in increasing the microbial population. Nitrogen is most commonly supplemented by urea, aqueous ammonia, or ammonium chloride. If the nitrogen concentration is low, microbial populations remain less and the process will have a longer duration for the digestion of available carbon. Excess nitrogen, may cause ammonia formation, which affect the anaerobic microbes. In anaerobic digestion, nitrogen could be utilised in two ways by the microbes such as assimilatory (nitrate to nitrogen gas called as denitrification) and dissimilatory (nitrate to ammonia called as ammonification). More nitrate addition leads to ammonification whereas less nitrogen leads to a deficiency. As PPMS has less in nitrogen, high in carbon, proper mixing of substrate/inoculum having high nitrogen is needed for higher digestibility and methane yield.

2.6.6 Toxic materials

The toxicity of the anaerobic digestion processes is mediated by the substances present in the influent waste stream or through by-products of the metabolic activities. Toxic materials such as ammonia, heavy metals, halogenated compounds, and sulfide can be present in significant concentrations in industrial and municipal sewage sludge.

Ammonia is produced by the biological degradation of nitrogenous materials mostly in the form of protein and urea. There are two predominant forms of ammonia: ammonium (NH_4^+) and free ammonia (NH_3). The free ammonia is the more toxic of both; its concentration mainly depends on the three factor such as total free ammonia concentration, pH and temperature. The ammonia concentration 50-100 mg/L is a more beneficial anaerobic microorganisms (Khanal, 2011) whereas ammonia nitrogen exceeding 3,000 mg/L, itself becomes toxic and will be independent of pH.

Due to industrial activities, heavy metals find their way from wastewater and sludge. Some values of inhibitory concentration are listed in Table 2.5. Many enzymes and co-enzymes depend on the minimal trace amount of metals for their activation and activity; whereas at high concentration causes an inhibitory or toxic effect to microorganisms. Nickel and cobalt are particularly important because they are a structural constituent of factor F430 and vitamin B12 respectively, which is found only in methanogenic bacteria. Heavy metal toxicity follows the order Ni>Cu>Pb>Cr>Zn Hayes and Theis (1978), with iron considered more constructive than detrimental because it mediates sulfide toxicity. PPMS contain much less concentrations of these heavy metals. Hence, inhibition in anaerobic digestion due to heavy metals could be less contribution. This is not true in all cases because the concentration of heavy metals vary from industry to industry, since every industry using the different raw materials.

Sulphate is found in wastewater and hence in sludge. Under anaerobic condition, sulphate is used as an electron acceptor and hence reduced to sulphide by sulphate reducing bacteria. Sulphide is also produced during the degradation of sulphur-containing organic matter (protein) found in waste streams. The unionized sulfide (H_2S) is considered to be more toxic to methanogens than in its ionized form (HS^-).

2.6.7 Nutrients

Various nutrients including Na, K, Ca, Mg, and others are found in the influent of the digester, and can be released due to degradation of organic material or with compounds added for the pH adjustment. Even though nutrients are required for microbial growth, they can be toxic or inhibitory to the microorganisms and interfere with their metabolism when present in high concentrations (Chen et al., 2008). The presence of low concentration of nutrients are essential for the methanogenic bacteria. The level of inhibition depends on the concentration found in the sludge. The moderate and strong inhibitory concentration for Na, K, Ca and Mg are enlisted in the Table 2.5. Sodium is an important methanogenic bacteria because it works for the formation of ATP or the oxidation of NADH. The simultaneous addition of Ca and K in suitable concentrations is found to be beneficial in improving the efficiency of the anaerobic treatment processes. When the concentration of Na, K, Ca, and Mg is below 500 mg/L, both mesophilic and themophilic digester functioning will be improved. As PPMS contain very less concentration of nutrients, it is necessary to add essential quantities of nutrients to stimulate the better anaerobic digestibility for enhanced methane production.

2.7 APPLICATION OF ANAEROBIC TECHNOLOGY IN PULP AND PAPER INDUSTRY

The need for renewable energy has increased due to depletion of oil reserves and strict environmental regulations such as the Kyoto Protocol. Biogas as a potential renewable energy source can be generated by the anaerobic digestion of organic wastes. Biogas as a main by-product of anaerobic digestion contains about 65 % methane by volume. The high methane content makes the biogas a useful fuel that can displace natural gas in pipelines and in the industrial processes or can be converted to electricity and used for heating (Deublein and Steinhauser, 2011). Although the characteristics of produced sludge are quite different because of different production processes and

stages, the methane content generated from the anaerobic digestion process is similar that has been collected and used for heating and lighting in industry. Similar technology to but different substrate had been used in the year 1927, Ruhurverband utilized the biogas from sludge digestion in Iserlohn and Essen-Rellinghausen to generate power in order to operate the biological treatment plant, and also cooling water from the motorised engine was used for the digestion tank (Imhoff, 1938). Such use of biogas (digester gas) is common practice nowadays throughout the world at wastewater treatment plants.

Anaerobic digestion is hailed as an archetypal appropriate technology as it may help to meet the basic need of providing steam (heat) for digestion of woodchips in the pulping process and electricity for the industrial process. Owing to the production of biogas from the PPMS, the industry gains an earning that gives an additional source of income from the sale of electricity, heat and digestate (humus). There is some possible way to calculate the biogas production from an anaerobic digestion in relation to the feedstock composition. Moreover, this calculation does not take a process parameter (the type of digesters, the temperature of digestion etc.) into account but only based on average values.

For instance, an anaerobic digestion plant installed in a paper industry can treat 100 tonnes of PPMS per day, with the total solids at 34 %, volatile solids at 46 % of TS, can yield biogas up to 0.25 m^3/kg of VS feed. In a year the anaerobic digestion plant can produce about 3,889.3 m^3/yr of biogas that is 10.66 m^3 of biogas per hour. The biogas utilization in the form of combined heat and power (CHP) unit can produce 65.14 kW (the calorific value of biogas is 22 MJ/m^3). It is assumed that the conversion efficiency of a CHP unit for electricity and for heat to be 30 and 55 % respectively. In this case, the production of electricity and heat will be 171.20 and 313.83 MW per year respectively. This could be efficiently used for the industrial processes. Moreover, produced surplus heat can be used in the digester house for heating the digester and many other applications in an industrial level. Also the anaerobic process creates high-quality digestate which can be used as a soil conditioner (fertilizer) as it recycles nutrients and organic matter back into the local environment. As of now, there is no literature available on biosolids generated from the anaerobic digestion that can also be used as a soil conditioner (fertilizer). Therefore, the review suggest that further study is required on the digestate on a land application as a soil amendment as a post treatment of anaerobic digestion. Communities could therefore live in ecological balance with nature.

2.8 MODELLING ON ANAEROBIC DIGESTION

Several anaerobic process models are available ranging from simple kinetic models (first order kinetics) to highly complicated structured models such as ADM1 (Batstone et al., 2002). Most of the models have been developed for specific applications. Depending on the nature of biological reaction, inhibition mechanism has been included for the model development. Based on moisture content (MC) present in bioreactor in anaerobic technology, the AD could be classified into liquid state AD (LS-AD) operates at MC greater than 85 % and SS-AD functions at MC less than 85 % (Karthikeyan and Visvanathan, 2013). SS-AD has gained more consideration for the treatment of solid waste and its economical bioenergy production in recent years. SS-AD has an advantage than LS-AD in its solid waste handling (Xu et al., 2014). The SS-AD allows high loading capacity with

the smaller reactor volume, low energy and water consumption (Xu et al., 2014), fewer moving parts, lower energy input for heating and mixing, easier to handle end product, and a greater acceptance of inputs containing plastics, glass, and grit (Brown et al., 2012). The final end-product, compost like digestate left after the digestion is easy to transport with low cost (Li and Wang, 2011). A serious problem associated with the fibrous biomass causing floating and stratification in LS-AD could be easily resolved in SS-AD (Xu et al., 2013). Owing to this advantage and its simple design over LS-AD, the SS-AD has been broadly used throughout the world, almost 60 % of digestion in Europe in 2010 was based on SS-AD (Baere et al., 2010).

If the problem associated with the SS-AD such as accumulation of hydrolytic product, and the higher volatile fatty acid are not controlled timely, the adverse effect of MC could offset the anticipated advantages from the SS-AD. The understanding of fundamental mechanism based on the effect of MC on CH_4 production rate is necessary to optimize the SS-AD system. General observation is that the hydrolysis or methanogenesis be the rate limiting step for the LS-AD system by mean of CH_4 production rate. The studies on SS-AD by Abbassi-Guendouz et al. (2012) reported that by providing the enough quantity of inoculum, volatile fatty acid accumulation or pH drops can be rectified in a reactor feed with enough recalcitrant cellulosic biomass as a substrate. Li and Wang (2011) depicted that hydrolysis was the major constrained for the SS-AD. Batstone et al. (2002) developed structured model ADM1 that describes the multiple steps of biochemical and physic-chemical process in AD. Biochemical reaction are normally catalyzed by intra or extracellular enzymes that act on the biologically available organic material. Physicochemical reactions are not biologically mediated but encompass in ion association or dissociation, and gas liquid transfer. Abbassi-Guendouz et al. (2012) tried to fit the experimental data from the SS-AD into the LS-AD of ADM1 and noted that the value of the first order hydrolysis rate coefficient lower in the model. To explain mathematically, the lowered hydrolysis rate coefficient was attributed to the reduced CH_4 production rate from the cardboard waste under a low MC i.e. less than 80 %. The mechanism beneath the reduced CH_4 production rate was not revealed.

There was limited information regarding the compromised hydrolysis rate in AD under a lower MC (Vavilin et al., 2008) while it was generally perceived in studies of enzymatic hydrolysis for ethanol production from cellulosic biomass (Cara et al., 2007). Kristensen et al. (2009) depicted that excessive accumulated sugar could cause the diminished hydrolysis rate during the enzymatic hydrolysis of lignocellulose material under a low MC. The hydrolysis inhibition by the accumulated sugar under a low MC was a plausible elucidation for the SS-AD system (Ge et al., 2016). Some vital component was still missing. The prime difference between SS-AD and enzymatic hydrolysis is that SS-AD contain huge quantity of sugar consumer than their production of hydrolytic product, when hydrolysis is a rate-limiting step. This hurdles can be overwhelmed, only when microbes are able to access the sugars or else sugar accumulation phenomenon can occur due to increased mass diffusion resistance in SS-AD. Bollon et al. (2011) reported that mass diffusion coefficient was increased by two order of magnitude in SS-AD than LS-AD. The mathematical model is able to predict the correlation between different variables without understanding in-depth mechanism. The mathematical model developed could be compared against other statistical models for model validation.

2.9 OPERATION, MAINTENANCE AND TROUPE SHOOTING

Careful attention is needed for the successful operation of an anaerobic treatment system. Meticulous attention is needed especially of volatile fatty acids accumulation, shock loading, air exposure, sludge washout, on the availability of trace metals, nutrients and alkalinity and the maintenance of proper environmental conditions such as pH, temperature, and ORP. These attentions are quite often crucial during the start-up phase. Poor attention of this may lead to complete failure of the anaerobic reactor. Anaerobic digesters operate in a stable way if the solid levels and the alkalinity to acid ratio are controlled. In anaerobic waste stabilization, there exists a symbiotic relationship between acetogens and methanogens. This keeps the anaerobic system well balanced. By determining the individual VFA in the effluent, one can judge if the system is in balance or not. When the symbiotic relationship is disturbed due to either overloading, toxicity, nutrient deficiency, or biomass washout, there is an accumulation of VFA and their levels continue to increase. This may cause an abrupt drop in pH and subsequent souring of the anaerobic reactor. If corrective measures are not taken in a timely manner, the reactor may eventually fail.

The routine operation of the anaerobic reactor needs adequate maintenance and repairs, cleaning and start-up/shutdown procedures; (i) digester start-up, involving the startup sequence and actions needed to achieve the stable digestion condition, (ii) common operational problems and troubleshooting event occur when instabilities such as an increase in CO_2 or pH, poor supernatant quality and foam, faulty mixing or temperature, the consistency of the digester sludge, occurrences of a scum layer/blanket etc.

2.10 CONCLUDING REMARKS

Due to the huge production of PPMS and improper management practices, the industry is facing many environmental effects as well as spending a huge amount of funds in the management of produced PPMS. Since open dumping and incineration is major practice being followed, it is having a huge impact on the environment by leachate production, greenhouse gas emission and other air borne diseases. The primary reason is only due to the 40- 55 % composition of organic matter in the PPMS. From the above literature, anaerobic digestion is the best alternatives in sludge handling and management. Anaerobic digestion is superior in treating the organic waste into biogas due to its high-energy recovery and maintaining an industrial environment balance.

Although anaerobic digestion of sludge has been widely studied, very limited experimental studies has been done with respect to PPMS. Among those, most of the studies are related to biosludge, but in average a large fraction of the sludge generated in mills consists of primary sludge. Some of the pretreatment technologies, using physical or chemical principles, or often a combination of both have demonstrated that their ability to substantially reduce the digestion time and thereby the reactor size. In addition to that increased gas production and reduced excess sludge generation has been reported to have extra benefits associated with them. Some of the pretreatment technologies reviewed have started to make their way into full-scale implementation worldwide at several municipal facilities but not in the pulp and paper industry (Elliott and Mahmood, 2007). The pretreatment technologies

that appear to be at the forefront of preconditioning sludge include thermal processing, mechanical disintegration, electrohydrolysis, and sonication.

Therefore, research is needed in different pretreatments to destruct the lignocellulose content present in PPMS. Thermal-acid or thermal-alkali combinations could be more prominent for the lignocellulose material because thermal pretreatment promotes the hemicellulose and lignin solubilization (Yang et al., 2010; Hu et al., 2016), whereas acid pretreatment solubilise the hemicellulose more efficiently, making the cellulose more accessible. Alkaline pretretment makes the lignocellulose material to a swollen and creates a more accessible surface area and pore size (Hendriks and Zeeman, 2009). In addition, research is needed to link between the microbial community dynamics and the reactor operation performance. Research should consider the adaptation of anaerobic microorganisms to the lignocellulosic material. Therefore, large scale and long-term experiments with the PPMS as a substrate can be conducted to exploit the microbial resource and its ability of adaptation by detailed analyses of the microbial community and physiology.

In future, pulp and paper mills will be composed of integrated biorefineries where paper production is only one part of product line. Anaerobic technology plays a vital role in the transformation of lignocellulosic material into valuable marketable products beyond methane, such as ethanol, volatile fatty acids. In the next decades, even carbon dioxide may undergo a transition from waste to resource, because it could be used in the future to substitute the carbon sources from oil (Meyer and Edward, 2014).

"If we intend to provide a better life, and a better world, for future generations, we can't ignore the quality of the environment we leave them."

John Kasich

Chapter 3

MATERIALS AND METHODS

Different experimental approaches were used to accomplish the stipulated objectives. The research was carried out in different phases using pulp and paper mill sludge as substrate and cow dung as inoculum. The detailed methodology is summarized below.

3.1 EXPERIMENTAL FLOW CHART

In order to accomplish the objectives, the proposed research was carried out in five different phases (Fig. 3.1).

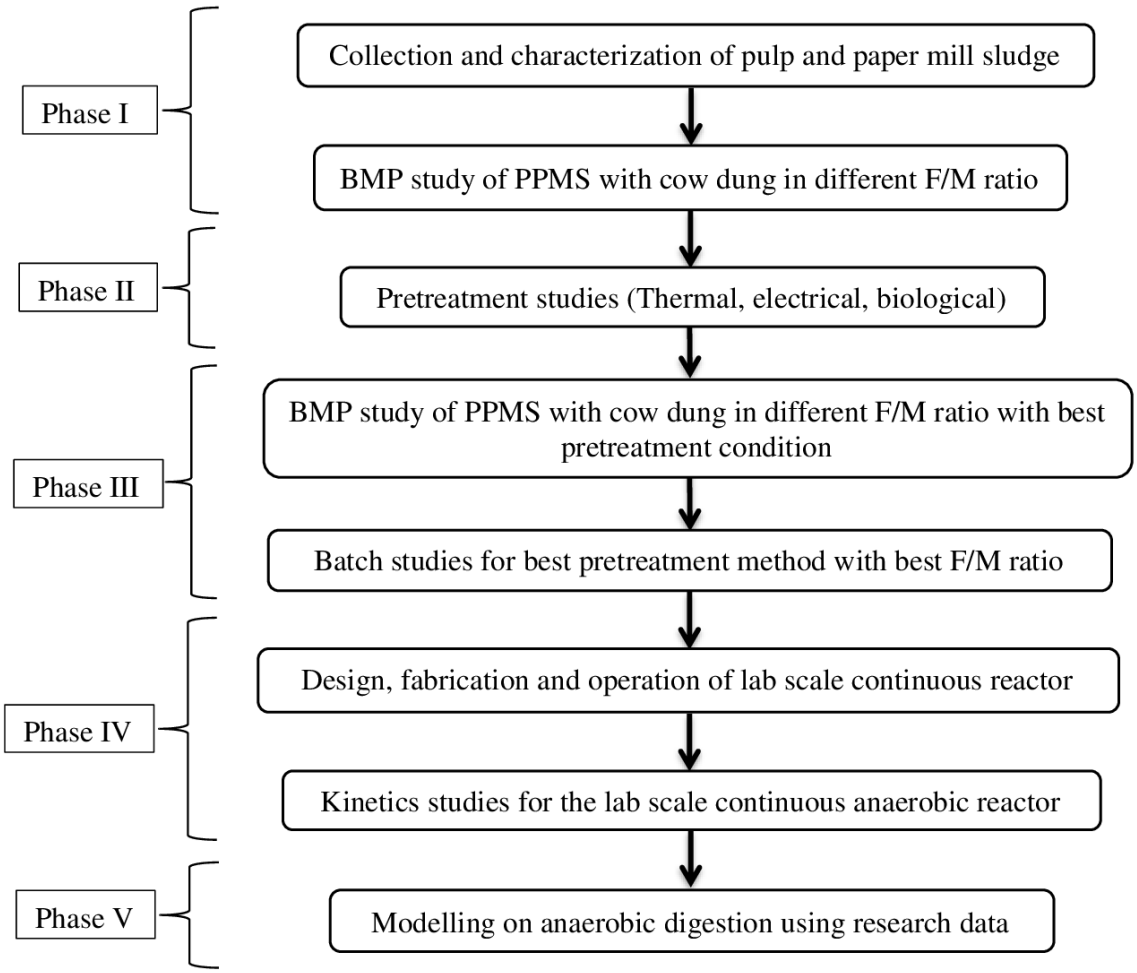

Fig. 3.1. Experimental flow chart of the research work

In phase I, the biochemical methane potential (BMP) test was conducted on the effects of different food/ microorganisms (F/M) ratio of the PPMS using cow dung as inoculum. In phase II, the effects of different pretreatment techniques (thermal (autoclave, hot air oven, hot water bath, microwave oven), electrohydrolysis (application of current), and biological (isolated bacterial strain)) on hydrolysis step was studied. In phase III, BMP test with different F/M ratio was studied for the screened pretreatment followed by batch experiments with optimum F/M were conducted for both control and the best pretreatment. In Phase IV, a lab scale continuous reactor study was conducted with different organic loading rate (OLR) and then its kinetics were studied. In phase V, mathematical modelling was done with research data obtained from the current study.

3.2 SUBSTRATE AND INOCULUM

The PPMS was collected from a Nagaon paper mill, at the Hindustan Paper Corporation Limited located at Jagiroad, Assam, India. Fig. 3.2 shows the Nagaon paper mill industry. Sludge samples were collected from the filter house (belt press) at the primary treatment of pulp and paper mill effluent treatment plant (ETP). The pulp and paper mill ETP has preliminary and primary treatment followed by a conventional aerobic activated sludge unit. After sampling at ETP, it was transported to the laboratory and kept at 4^oC prior to use. Cow dung was obtained from a nearby farm in Amingaon, Indian Institute of Technology Guwahati, North Guwahati, India and used as inoculum for the BMP and batch study.

Fig. 3.2. Source of substrate (PPMS) and filter press unit at the Nagaon paper mill (PPMS)

3.3 ANAEROBIC BMP SETUP OF PPMS WITH DIFFERENT F/M RATIO

The rate and extent of anaerobic digestion of PPMS was tested using the BMP assay as described in Dhamodharan et al. (2015). The BMP assay is nothing but a batch digestion to test anaerobic degradability in a sealed bottle with a mixture of substrate and inoculum with the defined nutrients. The anaerobic reactor (1,000 mL) were loaded with the mixture of PPMS (refers to food (F)) and cow dung (refers to a microorganisms (M)) for different F/M ratio (g/g) based on volatile solids (VS) content (Table 3.1), hereafter F/M ratio (g/g) is simply denoted as F/M ratio. After adding an essential macro (phosphorus as phosphate buffer (pH 7)) and micro nutrients (trace metals such as iron (ferric

chloride, 0.25 mg/L), calcium (calcium chloride, 27.5 g/L), magnesium (magnesium sulphate, 22.5 g/L), nickel (nickel chloride, 0.05 mg/L), and cobalt (cobalt nitrate, 0.05 mg/L)) as reported by the Speece and McCarty (1964) were added to the reactor. Then entire reactor was purged with nitrogen in order to make anaerobic condition. Anaerobic reactor was coupled to another aspirator bottle having 1.5 N sodium hydroxide (NaOH) solution that absorbs the CO_2 and H_2S and displaces NaOH solution that represent CH_4 production (Fig. 3.3). Thymol blue indicator was added to the aspirator bottle, to conform the displacement was only because of NaOH solution. Initially the aspirator was in blue colour due to addition of indicator, once the capacity of NaOH solution was exhausted, it turns into white colour. All the reactor were maintained at room temperature varies from 30 to 38^{o}C. Manual mixing was done for less than one minute (10 times of forward and backward) at three to four time in a day. During the entire experiment period, most of the parameters were monitored properly such as temperature, pH, stirring intensity, physical and chemical characteristics of substrates that affects BMP test (Browne and Murphy, 2013). Fig. 3.3 shows the schematic diagram and experimental anaerobic BMP setup used for the current study.

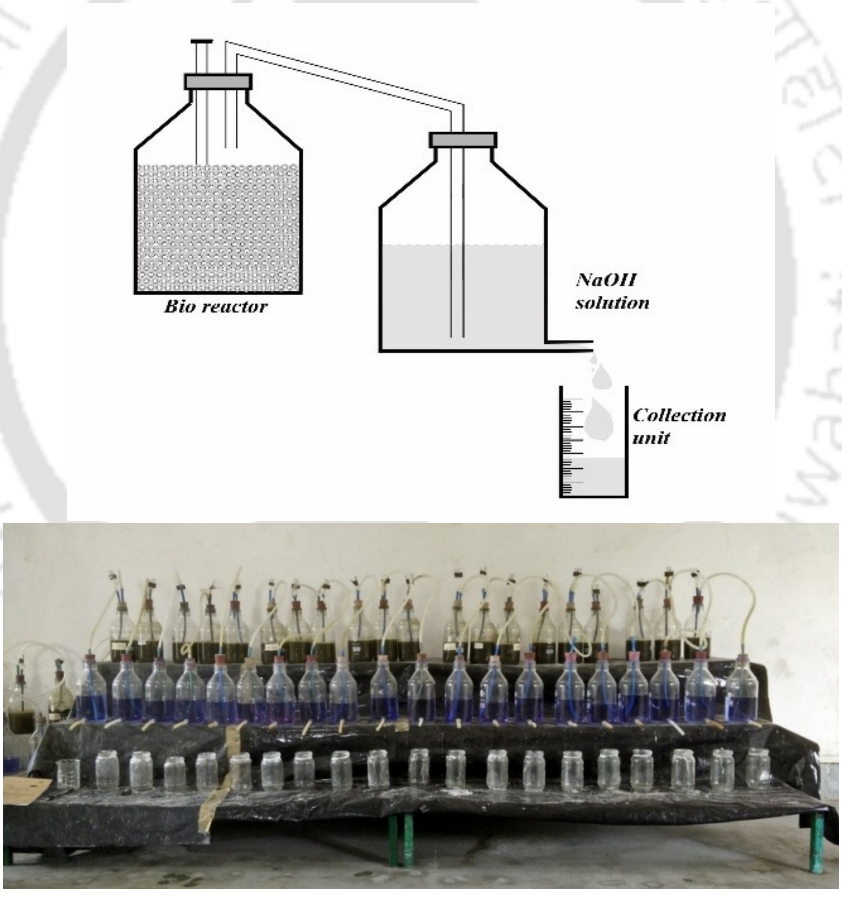

Fig. 3.3. Schematic and experimental anaerobic setup used for the current study

3.4 PRETREATMENT METHODS

3.4.1 Thermal pretreatment

For each thermal pretreatment study, 50 g of PPMS was added to 150 mL of distilled water and mixed properly to get a slurry form. The pretreatment study was split into two approach. The

first approach based on temperature study, by which the prepared samples were kept at different temperature at a particular time. After obtaining the suitable temperature, the second approach based on time study, prepared samples were kept at varied times with the selected best temperature inside the pretreatment vessel (heating source). Control sample without any kind of pretreatment was kept outside for each and every pretreatment. Fig. 3.4 shows the instruments used for the pretreatment study.

Table 3.1. Composition of feedstock used for BMP assay

F/M ratio	Dosage of each material (g)			
	PPMS	Cow dung	Distilled water	Total weight
Control		100	600	700
0.5	55	100	545	700
1.0	110	100	490	700
1.5	165	100	435	700
2.0	220	100	380	700
2.5	275	100	325	700
3.0	330	100	270	700

3.4.1.1 Hot air oven

Samples were placed in conical flasks into a hot air oven (SONNU-220/230 volt AC; temperature 0 to 200^{o}C (Fig. 3.4(a))). According to conditions proposed by Wood et al. (2009), temperature was set at 70, 80, 90, 100 and 110^{o}C for 30 min. After evaluating the better temperature, a trial to evaluate the effect of exposure time was set with 30, 60, 90, 120 and 150 min.

3.4.1.2 Autoclave

Samples were placed in conical flasks into an autoclave (EQUTRON-7421SLEFA; temperature 0-135^{o}C with 30 psi (Fig.3.4(c))). Temperature was set at 80, 90, 100, 110 and 120^{o}C for 20 min according to the procedure proposed by Gabhane et al. (2011) and Menardo et al. (2012). After evaluating the best temperature, a study to evaluate the effect of time was carried for 20, 40, 60 and 80 min exposure time.

3.4.1.3 Hot water bath

Sealed conical flasks containing PPMS were placed in a hot water bath (IKONTM; temperature 0 to 110^{o}C (Fig. 3.4(d))) at different temperatures: 60, 70, 80, 90 and 110^{o}C for 30 min according to the literature (Bayr et al., 2013; Cho et al., 2013). Once the best temperature was chosen, experiments were replicated at different exposure times: 30, 60, 90 and 120 min with the selected best temperature. The pretreatment temperature and time was selected based on preliminary study and literature.

(a) Hot air oven (b) Microwave oven (c) Autoclave

(d) Hot water bath (e) Electrohydrolysis

Fig. 3.4. Instrumentation used for the pretreatment study

3.4.1.4 Microwave oven

Sealed conical flasks containing PPMS were irradiated into a microwave oven (Samsung CE118KF; frequency 2450 MHz; temperature 40-250^{o}C (Fig. 3.4(b))) at 100, 120, 140, 180, 200, 220 and 250^{o}C for 2 min reaction time and 2 min stand time. The irradiation conditions were chosen according to the previous studies by Saha et al. (2011) and Tyagi et al. (2014). When cooking time is set above 2 min effervescence may be caused.

3.4.2 Electrohydrolysis pretreatment

For electrohydrolysis pretreatment study, 200 g of PPMS was added to 600 mL of distilled water and mixed properly to get a slurry form. Based on the preliminary literature (Yuan et al., 2011; Zhen et al., 2014) pretreatment condition such as applied voltage and time were selected. The pretreatment study was split into two approach. The first approach based on applied voltage (V) study, the samples was kept at a different applied voltage at a particular time. After getting the suitable voltage, the second approach based on a time study, the samples were kept at varied time with the best voltage in the plastic tank. The samples kept outside without any treatment was used as the control.

Electrohydrolysis pretreatment consist of cylindrical plastic feed tank (10 cm diameter and 30 cm height), DC power generator, graphite electrode, flash mixer, RPM regulator, multimeter, ammeter and tachometer. The feed tank was half filled with PPMS in the ratio of 1:3 (PPMS: distilled water) to get a slurry form. As PPMS contain high total solid content, three parts of water was added to the PPMS in order to make a slurry condition to avoid clogging and also to provide enough passage way for DC. With the help of graphite electrode, DC was supplied to the sample kept in the plastic tank. Two graphite electrodes as cathode and anode are half immersed into the sample, and placed

at a distance without touching flash mixture and wall of the plastic reaction tank (Fig. 3.4(e)). With the help of an insulated the flash mixture that was rotating at 300 rpm, the sample kept in a plastic feed tank were in suspension. The rpm of the flash mixture was maintained by the rpm regulator and with the tachometer. Ammeter and multimeter were used to measure the current and applied voltage respectively. Fig. 3.5 gives the schematic representation of the electrohydrolysis pretreatment setup.

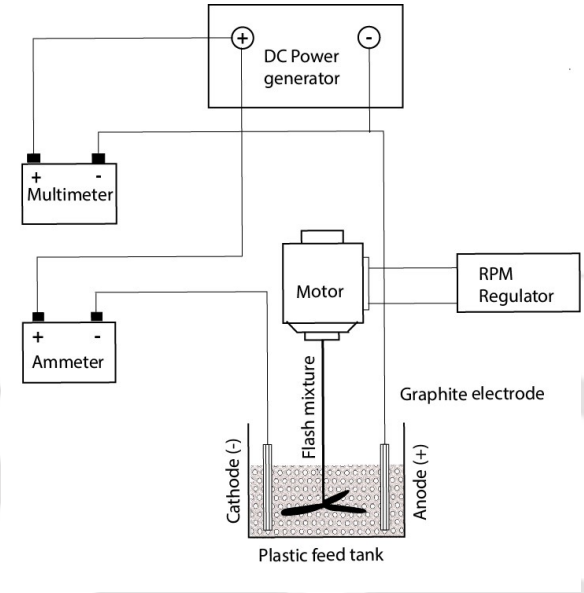

Fig. 3.5. A schematic diagram of the experimental setup of electrohydrolysis pretreatment

Table 3.2. Bacterial strain with its accession number used for biological pretreatment

Sample Name	Strain ID	Gene Bank Accession No.	Nearest Relative
Brown Millipede (Diploda)	BRb2	MF187530	*Paenibacillus sp.* VKVVG1
Cow dung	CDb1	MF136787	*Bacillus mojavensis* VKAK1
Silver fish	SFa2	MF099900	*Citrobacter werkmanii* VKVVG4
Soil	UN3d2	MF098756	*Bordetella muralis* VKVVG5

3.4.3 Biological pretreatment

The bacterial strain isolated from the millipede (BRb2), Cow dung (CDb1), silver fish (SFa2) and soil (UN3d2) was used to pretreat the PPMS for enhancing the hydrolysis prior to anaerobic digestion. Table 3.2 shows the bacterial strain with its gene bank accession number. This bacterial strain was cultivated in autoclaved carboxymethyl cellulose (CMC) media for 2 d at 37^{o}C, 120 rpm. After two days of cultivation, the bacterial culture was ready for inoculation. The PPMS was inoculated with different dosages of bacterial culture (10^7 and 10^8 CFU/mL). The inoculated PPMS samples were kept inside a shaking incubator at 37^{o}C, 120 rpm. A sample kept aside without inoculation of any bacterial strain was used as control.

3.5 ANAEROBIC BMP SETUP WITH DIFFERENT F/M RATIO FOR SCREENED PRETREATMENT

The procedure explained in section 3.3 had been followed to setup the anaerobic BMP with different F/M ratio for screened pretreatment.

3.6 BATCH REACTOR SETUP FOR BEST F/M RATIO

Batch setup (large scale of BMP assay) was an extended study of BMP assay to find the maximum methane production time and retention time. Batch study was conducted in a 20 L plastic bottle with the working volume of 15 L capacity (20 times of BMP). Batch experiment was studied for best F/M ratio of both control (without pretreatment) and pretreatment BMP assay. Fig. 3.6 shows the batch reactor setup with water displacement method studied in this research work. As explained in BMP setup, essential macro and micronutrients were added to the reactor. Nitrogen was purged to the reactor to make anaerobic condition then the reactor was connected to gas collection system. The experiment was conducted for 70 d. The samples were collected periodically (once in a week) for analysis. The biogas produced was measured daily using a water displacement method.

Fig. 3.6. Batch reactor setup with water displacement system

3.7 DESIGN OF CONTINUOUS ANAEROBIC REACTOR

Conventionally, a design of the biogas digester depends on the scale of application, the nature of the feedstock and the microorganisms involved Patinvoh et al. (2017). The design usually aims to provide a good environment that enables efficient contact between the microorganisms and substrate. To make the substrate/feedstocks more beneficial for methane production, it is important to develop a new digester that could over compete older digester drawbacks such as longer hydraulic retention time, lower degradation efficiency, and in a way allow for a higher biogas yield to be achieved. To minimize the costs associated with a large digester volume, a digester design which can allow for a shorter hydraulic retention time is necessary. As PPMS is a lignocellulose fibrous feedstock, with a high TS material, and is easily settable in the anaerobic digester, continuous stirring is needed. Hence, a continuous stirred tank reactor or plug flow reactor would be more applicable with some

upgradation in the reactor, such as a conveying mechanisms inside reactor could be more appropriate. Such a design with conveying devices might offer the stirring mechanisms, avoids the settable solids in digester, and give more contact surface area for anaerobic microorganism and the feedstock.

The lab scale anaerobic reactor was designed based on data obtained from previous batch studies and from the review of literature. In general, the anaerobic reactor design based on empirical approach such solids retention time (SRT), volatile solids (VS) loading rate, and volumetric organic loading rate (VOLR), etc.

Solids retention time (SRT): The optimum retention time needed for effective digestion can be assessed from laboratory study or by evaluation of existing operating plants based on the maximum bioenergy production as a function of SRT. In general, the SRT may vary from 15 to 30 d and 5 to 15 d for mesophilic thermophilic digestion, respectively. This approach does not take the waste characteristics. Digester size can be estimated by knowing the volume of waste and substrate produced.

Volatile solids loading rate: This approach also does not take into account the feedstock characteristics. This was a commonly used approach for sizing an anaerobic reactor. In general, the VS loading rate for a thermophilic digester could be twice that of mesophilic conditions.

Volumetric organic loading rate (VOLR): Simply VOLR called as an organic loading rate (OLR). This approach is most commonly used for designing an anaerobic digester. OLR is given by Eq.3.1.

$$OLR = \frac{C_i \cdot Q}{V} \tag{3.1}$$

where, OLR is the volumetric organic loading rate (kg VS/m^3 day), is influent concentration (kg VS/m^3), Q is a feed flow rate (m^3/day), and V is the bioreactor volume (m^3). Here C_i and Q are known parameters for a given feed stream.

Dimension of the AAPFR a) Volume of reactor = 65 L = 0.065 m^3. b) Volume of digester = 50 L = 0.050 m^3. c) Volume of gasholder = 15 L = 0.015 m^3. d) Length of auger = 60 cm = 0.6 m. e) Diameter of auger = 25 cm = 0.25 m. Detailed dimension for each components in the AAPFR had been given in Fig. 3.7.

3.7.1 Operation of a lab scale anaerobic auger plug flow reactor (AAPFR)

The continuous lab scale study was carried out in two place: one at the Department of Civil Engineering, IIT Guwahati, India (Fig. 3.9)and another one at the Centre for Agricultural Renewable Energy and Sustainability (CARES), University of Guelph (UoG) Ridgetown campus at Ridgetown, Ontario, Canada (Fig. 3.8). AAPFR reactor study at India was fed with the PPMS as a substrate and cow dung as an inoculum. Whereas at Canada, the reactor was fed with the corn silage as a substrate that was obtained from the dairy research centre at UoG Ridgetown campus. Digestate obtained from an industrial scale anaerobic reactor at CARES (1500 m^3) was used as inoculum. Both the reactor were installed in the open environment.

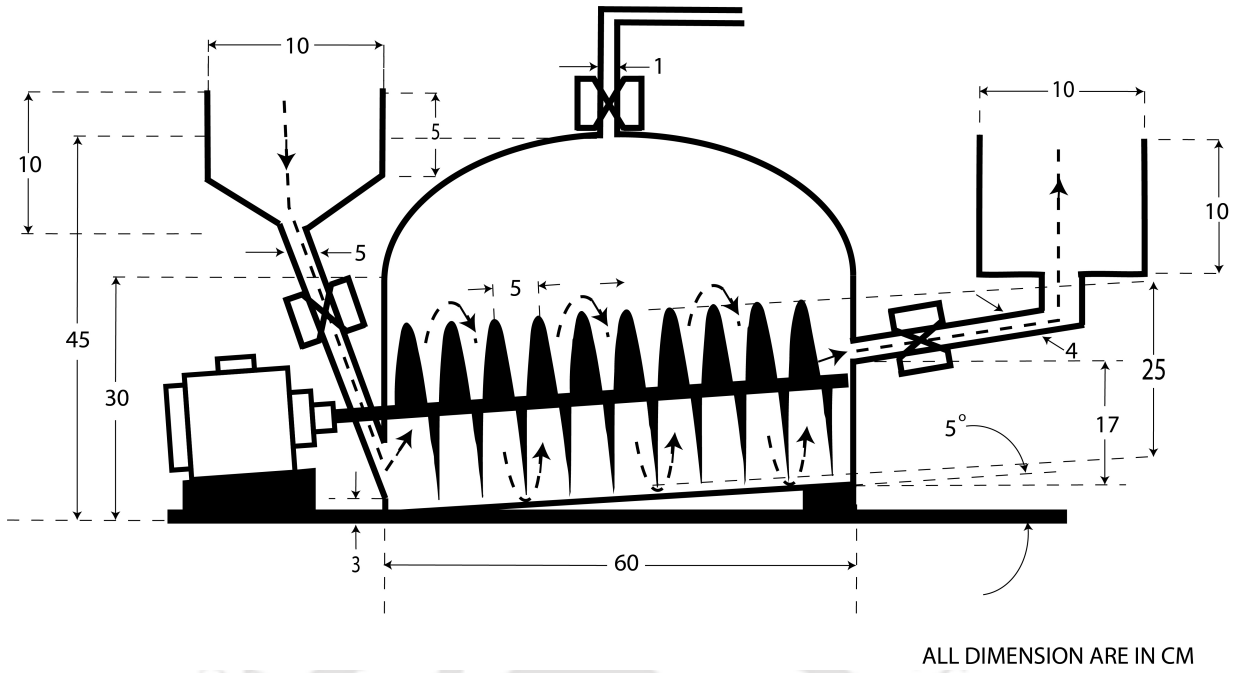

Fig. 3.7. Schematic view designed anaerobic auger plug flow reactor (AAPFR)

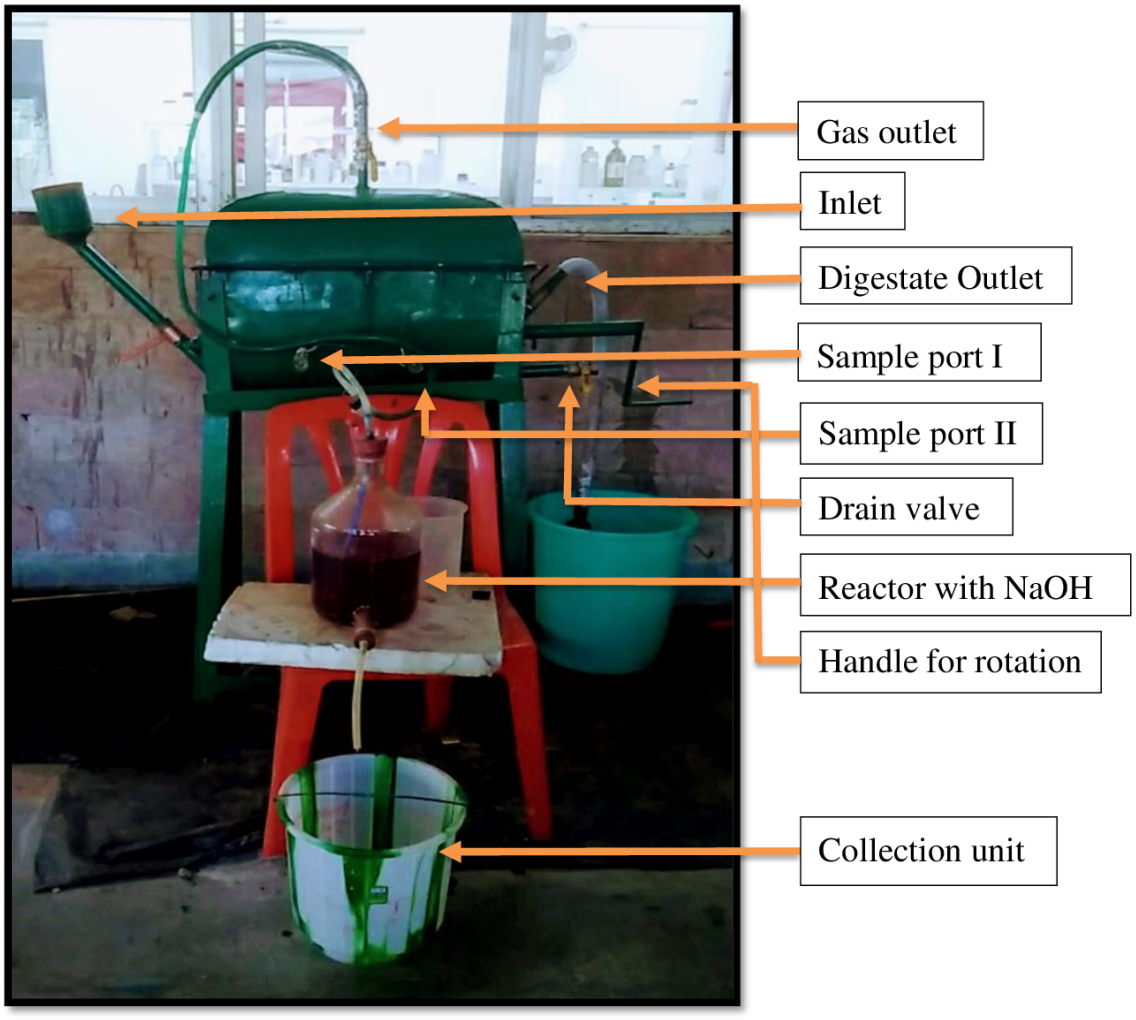

Fig. 3.8. Pictorial view of continuous AAPFR with the gas measured through water displacement method at IIT Guwahati, India

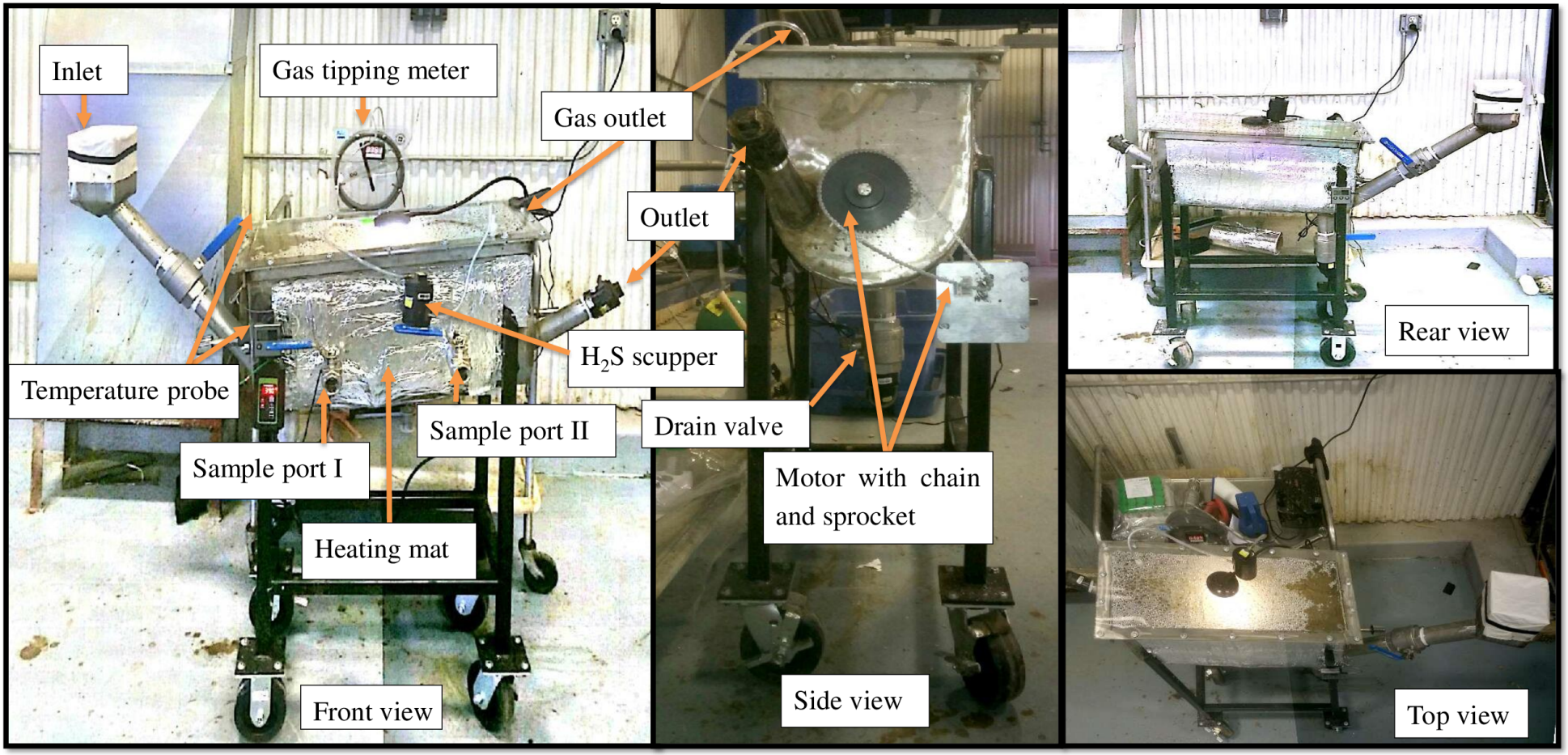

Fig. 3.9. Pictorial view of continuous anaerobic auger plug flow reactor (AAPFR) at UoG, Canada

A 65 L anaerobic auger plug flow reactor (AAPFR) with a 50 L working volume was used for the continuous study as shown in Fig. 3.8. The reactor was operated in a fill and draw mode with a daily feeding. The auger fitted inside the reactor acting as the conveying device with the plug flow operation. The auger fitted inside the reactor was equipped with a motor (with chain and sprocket) for rotation (5 rotation per day). For startup, the reactor was inoculated with the digestate obtained from the CARES. The reactor was covered with a heating mat to maintain the thermophilic temperature. Beginning with an OLR of 3.5 kg VS/m^3 day, the OLR was increased stepwise and maintained for 30 d for each OLR. The samples were taken once in a three-day period at different sampling port (Inlet, port I, port II and outlet) (Fig. 3.8 and 3.9). The biogas produced was measured daily using a multi-chamber rotor gas meter (RITTER). Biogas composition was analyzed thrice a week using the multitec 540 (SEWERIN).

3.8 KINETIC STUDY AND MATHEMATICAL MODELLING

In order to characterize each treatments and to evaluate the effect of F/M ratio on methane yield, three kinetic models were used as shown in Table 3.3. The modified Gompertz and logistic function model were selected because these models follow the sigmoidal function that correlate the growth of the methanogenic bacteria with the methane production in an anaerobic reactor (Donoso-Bravo et al., 2010; Li et al., 2012; Parameswaran and Rittmann, 2012). Another model transference function that

Table 3.3. Equation used for the kinetic study

Model	Equation
ModiiňĄed Gompertz model	$Y = M \cdot exp\left\{-exp\left[\frac{R_m \cdot e}{M}(\lambda - t) + 1\right]\right\}$
Logistic function model	$Y = \frac{M}{1+exp\left[\frac{4 \cdot R_m \cdot (\lambda - t)}{M} + 2\right]}$
Transference function model	$Y = M\left\{1 - exp[-\frac{R_m \cdot (t-\lambda)}{M}]\right\}$

follow the first order curve to relate the methane production with the microbial activity (Redzwan and Banks, 2004; Donoso-Bravo et al., 2010) was also used in this study. The bio-kinetic parameters such as potential methane production (M), the maximum methane production rate (R_m) and duration of lag phase (λ) were calculated by using the cumulative methane production data from the BMP assay. Where, Y represents the cumulative methane production volume (mL) with respect to time t (d), M is the potential CH_4 production (mL CH_4), R_m is the maximum rate of methane production (mL CH_4/d), λ is the lag phase time (d) and e is constant equivalent to 2.71. The bio-kinetics variables such as M, R_m and λ can be calculated using MATLAB R2015a by adjusting the pair of experimental data (Y,t) in a non-linear regression method.

Developed mathematical model to predict the maximum CH_4 production rate under a varied condition of MC with the mass diffusion limitation and hydrolysis inhibition is evaluated using the simultaneous ordinary differential equation in MATLAB R2015a.

3.9 PARAMETERS ANALYZED

Different experimental methods were used in this study to accomplish the stipulated objectives. Physico-chemical and biochemical analysis of samples for phase I-IV were carried out in an Environmental Engineering laboratory, Department of Civil Engineering, IIT Guwahati. Physico-chemical analysis for the continuous reactor was carried out at the CARES, University of Guelph Ridgetown campus at Ridgetown, Ontario, Canada. Experimental procedures for different parameters were explained below.

3.9.1 Physico-Chemical analysis

According to the standard methods, parameters such as total solids (TS), volatile solids (VS), ash content, moisture content (MC) and soluble Chemical oxygen demand (sCOD) were analyzed (APHA, 2005). To measure pH, 10 g of PPMS and cow dung were taken in a conical flask with 100 mL of deionized water and mixed for 2 h at 150 rpm in a horizontal shaker and measured using digital pH meter. For initial characterization, the PPMS samples were dried in a hot air oven at 105^{o}C until all moisture was removed, then powered and sieved in 0.2 mm to measure the total Kjeldhal nitrogen (TKN) and soluble and total chemical oxygen demand. The total organic carbon (TOC) was calculated from VS with a factor of 1.8 (Adams et al., 1951). Total nitrogen (TN) was analyzed using the Kjeldahl method and NH_4-N using KCl extraction (Tiquia and Tam, 2000). The TN was calculated as follow Eq.3.2:

$$TN = \frac{14 \cdot (S - B) \cdot N}{Wt.} \tag{3.2}$$

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

Volatile fatty acids (VFA) was analyzed using pH titration method of DiLallo and Albertson (1961). A sample of 50 mL was taken in a 100 mL beaker and titrate to pH 3.5 to 3.0 with 0.05 N H_2SO_4. The sample was continuously stirred with a magnetic stirrer during titration. The titrated sample was gently boiled for a minimum of 3 min. Then the boiled sample was kept outside without any disturbance to reach original room temperature. Then the sample was titrated with standard 0.05 N NaOH from pH 4.0 to 7.0. The volatile acid alkalinity and VFA was calculated as follow (Eq.3.3)

$$Volatileacidalkalinity = \frac{mL0.05NNaOH \cdot 2500 \cdot DF}{mLSample} \tag{3.3}$$

- Case 1. $>$ 180 mg/L volatile acid alkalinity,

  Volatile fatty acids = volatile acid alkalinity $\times$ 1.50,

- Case 2. $<$ 180 mg/L volatile acid alkalinity,

  Volatile fatty acids = volatile acid alkalinity $\times$ 1.00

The ratio of volatile organic acid to alkaline buffer capacity (FOS/TAC) was analyzed using the FOS/TAC analyzer according to the Nordmann method, which was developed by the Hach Lange

Laboratory in Germany (Lossie and Putz, 2008). A sample of 5 mL, diluted with 50 mL deionized water was used to titrated by 0.1 N of H_2SO_4 solution up to pH 5.0 to calculate the TAC value (3.4), expressed in mg/L of calcium carbonate ($CaCO_3$). Then the FOS value was obtained after a second titration step between pH 5.0 to 4.4 (Eq.3.5), expressed in mg/L of acetic acid (CH_3COOH).

$$TAC(mg/L)(CaCO_3) = \frac{A \cdot C_{tit} \cdot 50045}{V_{sam}} \tag{3.4}$$

$$FOS(mg/L)(CH_3COOH) = ((B \times 6.64) - 0.15) \times 500 \tag{3.5}$$

Where, A represents volume of titrant at pH 5.0 (mL), C_{tit} is concentration of titrant (ec/L), Vsam is the volume of sample (5 mL fixed by the FOS formula, for the Nordmann method), and B is the volume of titrant by difference between pH 5.0 to 4.4.

Biogas composition was analyzed using a multiple gas measuring device (Multitec 540-SEWERIN). The multitec device use the infrared measuring techniques for methane and CO_2.

3.9.2 Heavy metal analysis

The Flame photometer (Systronic 128) was used for analysis of Na, K and Ca concentration. The total concentration of Zn, Cu, Mn, Fe, Ni, Pb, Cd, Mg and Cr was measured using an atomic absorption spectrometer (AAS) (Varian Spectra 55B) after the digestion of 0.2 g sample with a 10 mL of H_2SO_4 and $HClO_4$ (5:1) mixture in the block digestion system (Pelican equipments Chennai, India) for 2 h at 300^{o}C.

3.9.3 Biochemical analysis

Lignin was analyzed using the National renewable energy laboratory (NREL) procedure (Templeton and Ehrman, 1995; Ehrman, 1996). Cellulose was obtained using the method adopted by Updegraff (1969). Hemicellulose was determined from the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF) using the method provided by Goering and Van (1975).

3.9.4 Instrumental characterization

The surface alteration was identified by Field Emission Scanning Electron Microscopy (FESEM (Zeiss, Sigma, EHT:2.00 kV, Mag:5.00 KX)). Double coated gold samples were used for the imaging, to put off degradation and for charge build up. Changes in lignocellulosic fraction were extensively studied by using Fourier transform infrared (FT-IR). The FT-IR spectrum (PerkinElmer Spectrum 2) was recorded from 4000 to 400 per cm at 16 scan with a resolution of 4 paer cm. 1 mg of dried PPMS was mixed with 300 mg of KBr in a mortar, then the mixture was compressed for 3 min at 10 MPa to set up the sample disc. Moreover, the cellulose crystallinity was characterized by X-ray diffractograms (XRD) with the Bruker, D-8 advance from 5 to 35^{o}C of diffracting angle (2θ) at the scanning speed of 5^o/min. The crystallinity index was measured from an empirical method (Gabhane et al., 2011) using the following Eq.3.6.

$$I_C = \frac{I_{cr} - I_{am}}{I_{cr}} \tag{3.6}$$

Where I_C is the crystallinity index, I_{cr} is the maximum diffraction intensity at peak position at 2θ = 31^o and I_{am} is the intensity at 2θ = 18^o.

3.10 INSTRUMENTS USED

Table 3.4 shows the different instruments that were used during the investigation and experimental analysis.

Table 3.4. Different instrument used for experimentation

Parameter analysis	Instrument Company	(Make/Model)
Sieving	Sieve	Unique drawing & survey emporium
Moisture content	Class II High Accuracy	Satwick scale industry
pH	μ pH system 361	Systronics
Volatile solids	Muffle furnace	International commercial traders
COD	COD digester	HACH
Centrifuge	Micro centrifuge	Thermisci
FOS/TAC	FOS/TAC analyzer	HACH
Biogas	Multiple gas measuring device	Multitec 540 -SEWERIN
Imaging	FESEM	Zeiss Sigma EHT
Spectra analysis	FTIR	PerkinElmer
Spectra analysis	XRD	Bruker D-8
Heavy metals	AAS	Varian Spectra 55B

"Environmental pollution is an incurable disease. It can only be prevented."

Barry Commoner

Chapter 4

ANAEROBIC DIGESTION OF PPMS, WITH AND WITHOUT PRETREATMENT IN BMP AND BATCH ASSAY

This chapter deals with the results and discussion of (phase I to IV) BMP test as control (without pretreatment) and pretreatment study followed by BMP test and batch studies of the PPMS.

4.1 Phase I: BMP ASSAY (without pretreatment) WITH DIFFERENT F/M RATIO USING COW DUNG AS INOCULUM

Objective of phase I: This phase was to evaluate the feasibility of PPMS as substrates for biogas production and to investigate the rate and extent of anaerobic digestion of different F/M ratio and its reaction kinetics. The study was evaluated at five different F/M ratios (i.e. 0.5, 1.0, 1.5, 2.0 and 2.5).

4.1.1 Characterization of the PPMS

The characterization of the PPMS was shown in Table 4.1. The PPMS showed 62 to 75 % and 59 to 68 % of MC and VS percentage, respectively. The high VS/TS ratio were desire for the biogas production. As PPMS was derived from the plant source that consisted of cellulose, hemicellulose, and lignin, its characteristics varied in a good ranges for the anaerobic digestion. The wide range of VS/TS percentage assures it utilizable organic content, As PPMS is almost equivalent to neutral pH and there would not be a problem during the initial stage/rector start-up. PPMS has less in nitrogen, high in carbon, proper mixing of substrate/inoculum having high nitrogen was needed for higher digestibility and methane yield. The percentage of VS/TS ratio along with sCOD concentration conform its appropriateness for greater methane production. The parameters that were characterized could compared with literature (Eskicioglu et al., 2011; Elliott and Mahmood, 2012) and perceived within the reported values. The observed values were found very much optimum for the anaerobic digestion process.

4.1.2 Anaerobic BMP assay results and discussions

Five different F/M ratio varied from 0.5 to 2.5 were tested over the period of 45 d at ambient temperature between 30-38^{o}C in order to evaluate the AD of PPMS. The F/M ratio was the most significant parameter for the anaerobic digestion of sludge material that conducted in batch study. During initial startup period in anaerobic system, inoculum play a vital role to balance the syntrophobacter and methanogens. Pandey et al. (2011) depicted that population balance helps in syntrophic metabolism that was thermodynamically feasible in AD process.

Table 4.1. Initial characterization of pulp and paper mill sludge

Parameters	Values		
Moisture content (%)	70.16±3.40		
pH	7.39±0.004		
Electrical conductivity (dS/m)	1.51±0.06		
VFA (mg/L)	468.75±35.0		
Total COD (mg/L)	64000±3000		
Soluable COD (mg/L)	1600±175		
Total Solids (TS) (%)	29.83±0.73		
Volatile Solids (VS) (%)	57.94±6.36		
Ash content (%)	16.06± 1.35		
Total organic carbon (TOC) (%)	25.52±0.75		
TKN (%)	1.03±0.01		
Ammonical-N (mg/kg)	30.15±0.07		
Nitrate- N (mg/kg)	61.71±0.14		
Total Phosphorous (mg/kg)	2265.5±0.39		
Available Phosphorous (mg/kg)	918.5±1.49		
Lignin (%)	5.68±0.23		
Hemicellulose (%)	6.53±0.25		
Cellulose (%)	32.49±0.370		
Carbohydrate (mg/L)	119.60±8.14		
Sulfate (mg/L)	721.44±45.13		
Heavy metals (g/kg dry matter)			
Sodium (Na)	1.34±0.05	Calcium (Ca)	39.31±0.27
Magnesium (Mg)	5.09±0.13	Potassium (K)	0.71± 0.02
Cadmium (Cd)	7.06±0.17	Lead (Pb)	8.61±0.25
Mercury (Hg)	3.43±0.03	Arsenic (Ar)	23.48±0.06
Copper (Cu)	0.15±0.01	Chromium (Cr)	0.88±0.43
Nickel (Ni)	0.22±0.003	Zinc (Zn)	0.18±0.003
Iron (Fe)	2.21±0.04	Manganse (Mn)	1.26 ±0.01

Note: (mean ± SD, n=3) SD - standard deviation

The conversion of organics material to methane involves a number of group of microorganisms carrying out rather specific reaction. Degradation of organic content present in the PPMS was

decomposed by the different kinds of bacteria such as hydrolysing bacteria, fermentative bacteria, Homoacetogens (H_2 - producing/Consuming acetogenic bacteria), and methanogens (acetotrophic and hydrogenotrophic methanogenesis). In order to interpret for the biogas production from the organic source, the following mechanism were used in this current study. The microbes used for the interpretation were taken from the anaerobic reactor and these microorganisms were not limited to (Gujer and Zehnder, 1983; Karthikeyan and Visvanathan, 2013).

The initial steps in anaerobic digestion was cell lysis that means breakdown of cell wall of microorganisms which leads to release of fluid (extracellular enzyme), that might be utilized by hydrolysing bacteria. The breakdown and/or decomposition of organic compound such as carbohydrate component made up of cellulose and hemicellulose leads to the increment of sCOD and VFA that was attributed by the two groups such as hydrolysing bactetria (*Clostidios, Bacillus sp., Bifidobacterium sp., staphylococcus sp.,*) and fermentative bacteria (*Acetovibrio cellulolytic, Butvrivibrio sp., Slelnomonas sp.,*). Later the produced higher VFA or the product of fermentative bacteria was consumed by the Homoacetogens (*Acetobacterium sp., Clostridium sp., Syntrophomonas sp.,*). Because the produced higher VFA were used as a carbon source for the homoacetogenic microorganisms. Later produced VFA along with ethanol were converted into acetic acid, H_2 and CO_2 by the hydrogen producing acetogenic bacteria. Finally produced acetate, hydrogen and carbon dioxide were used by the methanogenic microorganisms (*Methanobacterium sp., methanobacillus sp., Methanosarcina sp., Methanothrix sp.,*) for the production of biogas from the organic source.

Table 4.2. The 7 d average methane production rate on PPMS

Time (d)	Methane production rate (mL/d)					
	Control	F/M 0.5	F/M 1.0	F/M 1.5	F/M 2.0	F/M 2.5
1-7	39	50	61	62	63	61
8-14	76	82	95	99	110	98
15-21	48	102	78	97	97	87
22-28	37	64	61	77	82	65
29-35	13	44	55	63	67	51
36-45	9	34	42	53	55	47

Methane production

Since the quantity of methanogens present in the AD is unrealistic to determine experimentally, the VS reduction from the system was used for the proximity. In general perception, the rate of methane production is a measure of activity of biomass (methanogenic microorganisms) in the anaerobic condition. The production of methane was varies with many factor such as inoculum, VS, VFA, and temperature. This study observed that AD of PPMS with F/M ratio 2.0 has maximum methane production of 2.47 L, followed by F/M ratio 1.5 has 3.30 L. Next to that, F/M ratio of 2.5 and 1.0 were observed with moreover same cumulative methane production with 2.95 and 2.87 L respectively.

Methane production was quantified on the basis of per day methane production rate (Fig. 4.1(a)) and cumulative methane production (Fig. 4.1(b)). It was experienced that the initial methane production rate was similar for all F/M ratios except 0.5 (7-d average). After the 7^{th} d, F/M ratio 2.0 and 1.5 has higher methane production and consistent until 21 d around 82-110 and 77-99 mL/d respectively. As substrate was lignocellulose material (plant source), it was perceived that initially methane production was due to easily available volatile fraction of the substrate. Nevertheless, its solubilisation takes place very late after the 14 d but it was consistent up to 35 d with similar changes in all F/M ratios. The best wise order of F/M ratios for AD of PPMS based on the production of methane gas is 2.0>1.5>2.5>1.0>0.5. The variation in the rate of methane production in BMP of PPMS on 7 d average with different F/M ratio during the study period was shown in Table 4.2.

Volatile solids (VS)

Mass loss from the anaerobic digestion system was indicated by the decrement in VS that was correlated directly to the biogas production. High degradation designates higher VS reduction that yield more biogas. In general, reduction of VS mostly depends on the activity and adaptability of the inoculum towards substrate in the anaerobic system. In the current study maximum VS reduction was perceived in F/M ratio 2.0 with 15.73 % followed by F/M ratio 1.5 with 13.54 % that yield the cumulative methane production of 3.47 L and 3.30 L respectively. VS reduction from the AD of PPMS with different F/M ratio was shown in Fig. 4.1(c). In the current study VS reduction order follows the 2.0>1.5>1.0>0.5>2.5. The loss of VS was analogy with the mass loss from the system in the form of biogas. Even though VS reduction was less, due to active inoculum, the gas production was higher than Parameswaran and Rittmann (2012) with PPMS material.

Soluble COD

The transformation of complex organic molecules to simple soluble product by the action of extracellular enzymes emitted by the hydrolysing bacteria which leads to the increase in sCOD in the batch anaerobic reactor. Later, due to the decomposition of organic acids by the group of microorganisms such as homoacetogens and methanogens could leads to decrease in sCOD. The BMP with different F/M ratio present varied substrate removal rate in form of sCOD. The concentration of the sCOD of each F/M ratio was initially spiked and then decreased, after that each F/M ratio reached their peak values on the 28^{th} d. Because of digestion of initial 14 d, the insoluble organic fraction such as polymeric organic substance present in PPMS was majorly hydrolysed and solubilized by acidogenic bacteria, causing the great increase of sCOD. A similar founding had been already reported by others researcher (Wang et al., 2005). Fig. 4.1(d) shows the sCOD profile during AD with different F/M ratios. The removal rate of sCOD was higher in this study with same industrial sludge (Karlsson et al., 2011), but VS removal was a little lower. As substrate was lignocellulose material, it was observed that initially solubilisation was due to easily available volatile solid. Nevertheless lignocellulose content gets solubilized after 21 d.

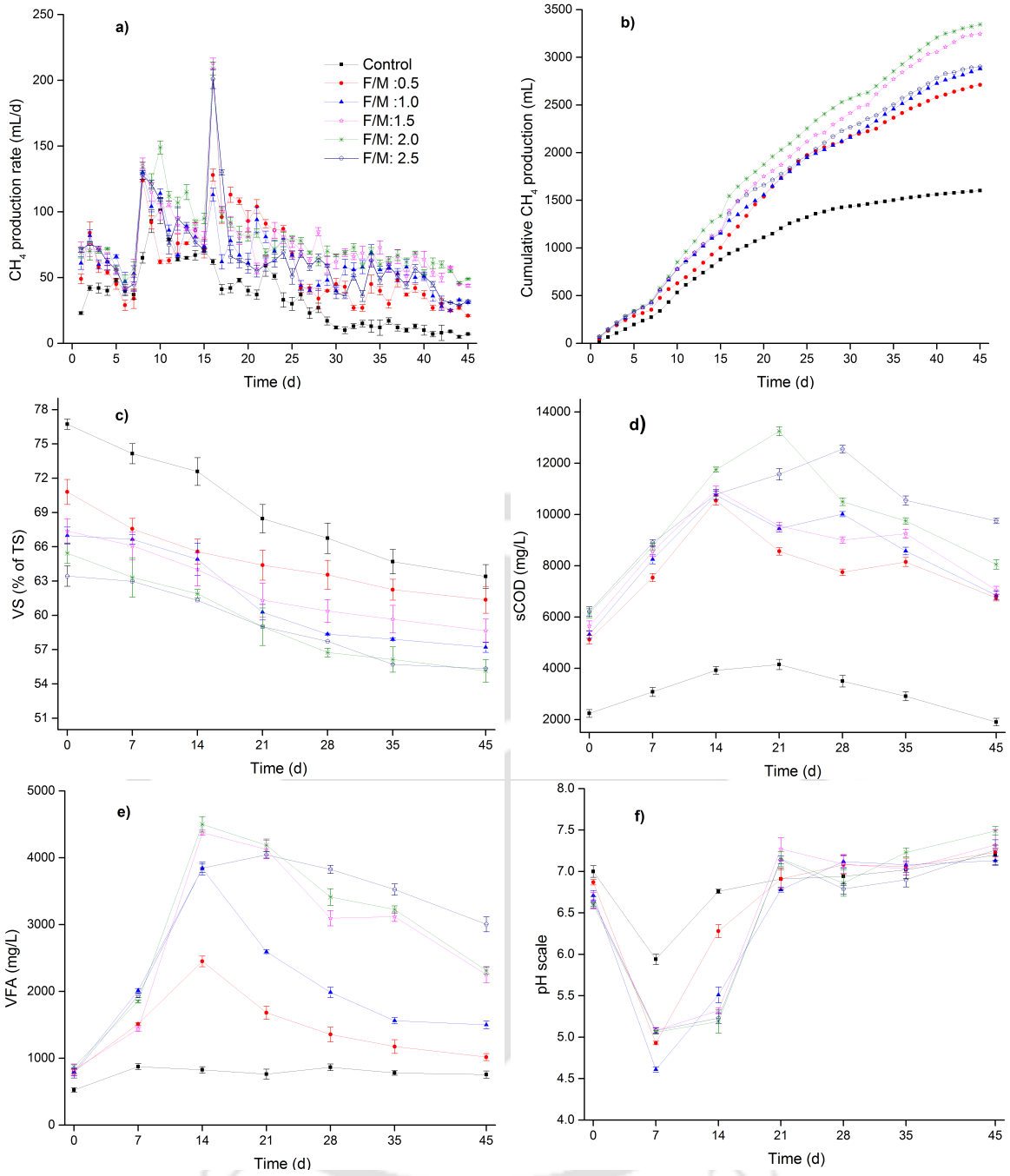

Fig. 4.1. Variation of VS, VFA, sCOD and pH in relation with methane production. **a)** Effect of daily CH_4 production at different F/M ratio **b)** Cumulative methane production at different F/M ratio **c)** Effect of VS at different F/M ratio **d)** Effect of sCOD at different F/M ratio **e)** Effect of VFA at different F/M ratio **f)** Effect of pH at different F/M ratio

Volatile fatty acids (VFA)

By varying the F/M ratio from 0.5 to 2.5, different pattern of VFA profile was observed during the methane producing period. The VFA value augmented initially was attributed by the soluble product from the hydrolysing bacteria could be fermented by the fermentative bacteria to form a mixture of organic acids, CO_2 and H_2. Then produced organic acids were started to decrease because of methanogens, as the result increased biogas production starts (Jeris and McCarty, 1965). The

decomposition and/or solubilisation of substrate (lignocellulose material) after 28 d, increase in VFA concentration was observed in F/M ratio 2.5 then it was decrease at the final stage, but in case of F/M ratio 0.5 and 1.0, it started to decreased after 14 d. This was attributed due to the lower substrate concentration in a reactor that induce the enzyme poorly in the biomass. In addition to that substrate level phospholylation occurs in methanogenesis, to outcompete this level a high concentration of substrate was required. On the other hand F/M ratio 2.5, has a higher VFA production resulted in a lowering the pH that inhibit methanogenesis due to the accumulated VFA in the reactor. Several investigators showed the detrimental effects of low pH that inhibits methanogens (Sallis and Uyanik, 2003; Raposo et al., 2006; Lim et al., 2009). Fig. 4.1(e) shows the VFA profile during AD of PPMS with different F/M ratios. This result suggests that methane yield was significantly inhibited at a higher F/M ratio and shows the optimal F/M ratio is indispensable to proper AD. Hence, F/M ratio 2.0 was considered as optimum F/M ratio for PPMS.

pH

In AD technology, both acetogens and methanogens experienced a syntrophic relationship to keep anaerobic reactor in well balanced condition. pH is also one of the simple test to experience this symbiotic relation (Buswell and Mueller, 1952). For increased yield of gas production, an optimum pH should range from 6.8-7.2, although the gas production was satisfactory when pH varied from 6.6-7.6 as well. In AD, pH from the bioreactor is a function of digestion and/or solubilisation of organic matter. That may be perceived from of increment or decrement of sCOD, concentration of volatile fatty acids produced, bicarbonate alkalinity of the system and the amount of carbon dioxide produced. From this experiment it was perceived pH values varied in the range 4.8-7.5 during AD (Fig. 4.1(f)), as PPMS was identified to be slightly acidic substrates (Table 4.1). The pH value was drop on 0-7 d and rising on day to end of the digestion. During the initial 7 d volatile acid production was more that was due to more solubilisation of easily available organic fraction, hence in the F/M ratio 1.0 and 0.5 has pH 4.61 and 4.93 respectively. During the course of time gas production was significantly affected due to the consumption of produced VFA was lower than the production of acid.

4.1.3 Influence of F/M ratio on Specific Methanogenic Activity

The specific methanogenic activity (SMA) of anaerobically digested pulp and paper mill sludge was spiked with the increasing in their F/M ratio ranging from 0.5 to 2.0 as shown in Fig. 4.2. Due to the solid liquid separation process happens at the optimum F/M ratio, the aspect of phase changes considerably and so medium would not be paste for loner time. The experimental result revealed that SMA at low F/M ratio was significantly lower than that of higher F/M ratio. Thus, at the higher and optimum F/M ratio provides the sufficient and maximal SMA for the enhanced methane production. The SMA looks to be experimentally liner relation with the F/M ratio as shown in Fig. 4.2. The SMA was spiked by 1.5 time when the F/M ratio increased from 0.5 to 2.0. This type of similar linear relation was experienced by the Pommier et al. (2007) and Le Hyaric et al. (2011) for samples from paper/cardboard and municipal solid waste respectively. Le Hyaric et al. (2011) highlighted that the SMA from the MSW as a source and propionate as a substrate. But in this study, SMA of the pulp

and paper mill sludge was used as the substrate. Anaerobic microorganisms makes the lignocellulosic material to destabilize, extracellular polymeric substances gets started to degrade as an upshot easily available intra cellular polymeric substance for the methanogenic organisms.

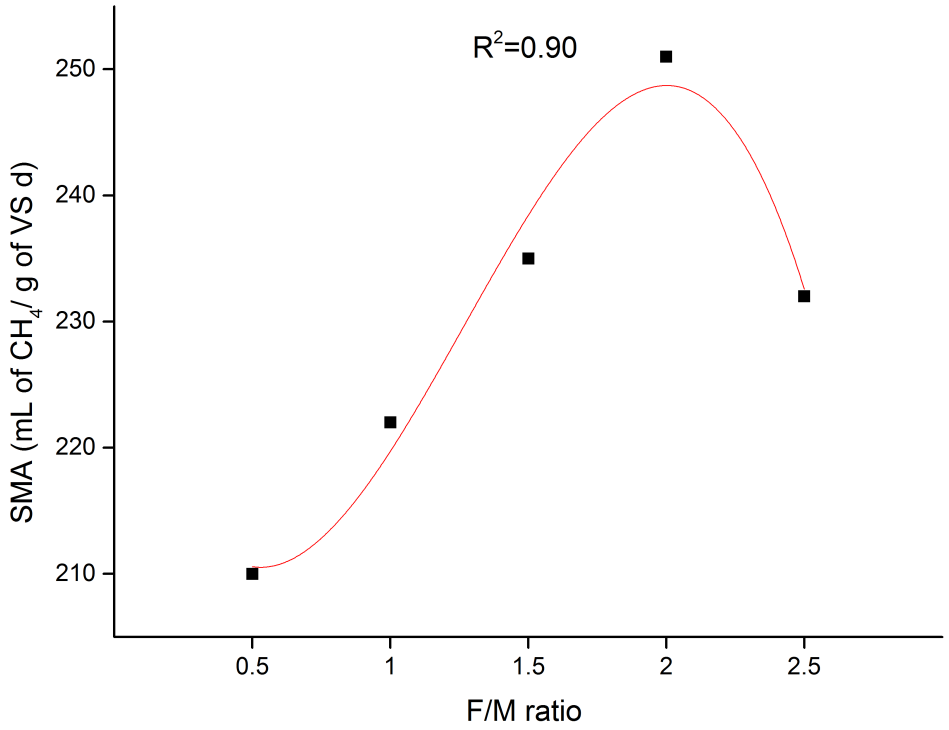

Fig. 4.2. Effect of F/M ratio on the specific methanogenic activity of the PPMS

4.1.4 Kinetic study

By using modified Gompertz, the accumulative methane production were fitted to find out the enhancement in efficiency of different F/M ratio. The rate of methane production values could afford significant information regarding inoculation for the digester system. The methane production rate could be the function of digestion time, that would be recalculated for each F/M ratio and evaluated parameter shows regression value (R^2) higher than 0.77 for each run. The value for the bio-kinetics parameters are presented in the Table 4.3. From the Table 4.3, the parameter M and R_m escalate in all the cases in that F/M ratio has increased. The higher quantity of M (potential methane production) is because of higher VS removal and the grander generation of methane is attributed due to the adequate amount of microorganisms present in the F/M ratio 2.0. On the contrary, duration of lag phase has been increased when F/M ratio greater than 2.0 because of higher solid content, creating the hydrolysis process hard to proceed increasing the period of acclimation for the microorganisms. According to other researcher (Raposo et al., 2012; Xu et al., 2014), the diminishing of maximum methane production rate is correlated with the mass transfer problems because of increasing in higher TS content.

Based on the parameter evaluated for the proposed model as displayed in the Table 4.3, the highest methane production (higher value of M) would be for the F/M ratio 2.0. The smaller differences for the maximum methane production rate (R_m) with regard to the F/M ratio of other than 2.0.

Various researcher (Donoso-Bravo et al., 2010; Huiliñir et al., 2014) were used secondary sludge, and determined the bio-kinetics parameters for the modified Gompertz equation. Donoso-Bravo et al. (2010) used the secondary sludge of paper industry and reported form value that the 243.5 mL/g of VS while in this study of primary sludge showed 264.5 mL/g of $VS_{degraded}$. The applicability of modified Gompertz model was well proven in earlier itself in the area methane production. Hence, the current study displays that F/M ratio 2 has higher regression value (R^2=.98) for PPMS which could provide useful kinetic data for the future installation of anaerobic reactor in this industrial sector.

Table 4.3. Kinetics value of AD of PPMS with different F/M ratio in control treatment

F/M ratio	M (L CH_4)	R_m(L CH_4/d)	λ (d)	R^2	Y (L CH_4)
0.5	2.8525	0.0710	0.0029	0.789	2.5031
1.0	3.1440	0.0933	0.0025	0.772	2.9229
1.5	3.5471	0.0863	0.0026	0.961	3.0840
2.0	3.6664	0.0965	0.0028	0.979	3.2853
2.5	3.2347	0.0856	0.0035	0.813	2.9040

4.1.5 Conclusion

The present study revealed that the PPMS generated from pulp and paper industry has a high potential for energy recovery in the form of biogas. Five F/M ratios varied from 0.5 to 2.5 based on VS was studied. According to the experimental result on gas production, VS reduction and SMA, F/M 2.0 was perceived as best. When F/M ratio greater than 2.0, methane yield was decreased, due to increased VFA production reduces the reactor pH and inhibits methanogens activity. Bearing in mind of sCOD, VFA, pH and rate of methane production, each reactor go through different pathway to attain maximum methane production. From the sCOD and VFA profile shows that pretreatment was necessary to fasten the biodegradation. Therefore, it can be concluded that PPMS has a high energy potential and in the further phase different pretreatment technique has been carried out to fasten the biogas production.

4.2 PHASE II: SCREENING PRETREATMENT FOR ENHANCED HYDROLYSIS STAGE

Objective of phase II: This phase was focused on different pretreatment techniques because of inherent recalcitrant characteristics of PPMS (lignocellulose content), turns hydrolysis step into a rate-limiting stage in the anaerobic digestion process. To accelerate the hydrolysis step, different pretreatment was investigated or screened for higher methane production. Even though there was extensive research on the effect of different pretreatment was available, the correlation between the structural and compositional properties remains unclear and contradictory. Hence, this study was carried out to predict the optimal pretreatment conditions and to elucidate the changes produced in the structure and composition of the PPMS after pretreatment.

4.2.1 Thermal pretreatment

4.2.1.1 The effect of sCOD and VFA in different heating processes

- ***Hot air oven pretreatment***

The effect of temperature and time on PPMS heated by the hot air in hot air oven was shown in Fig. 4.3(a) and (b). It was observed from the Fig. 4.3(a) that the increase in temperature has shown increase in solubilization rate which was measured in the form of sCOD and VFA. The sCOD and VFA increased up to 80^oC and then it got reduced. The reduction was attributed by the higher vaporisation of volatile compounds produced from the pretreatment effect than the solubilization rate at higher temperature (Appels et al., 2010). The pretreated sample at 80^oC has high sCOD and VFA. Hence, in the time study samples were kept at the temperature of 80^oC for different time duration. From the time study Fig. 4.3(b), the sample kept at the 90 min showed the highest sCOD with the increment of 1.61 times and VFA of about 1.95 times increment than the control PPMS sample. Ariunbaatar et al. (2014) also reported that the pretreatment at lower temperature needed a high pretreatment exposure time (at least 1 h) for a better solubilization rate. Wood et al. (2009) depicted that the hydrothermal pretreatment of sulfite and Kraft pulp mill waste activated sludge at 170^oC for 60 min showed a better solubilization.

- ***Hot water bath pretreatment***

The hot water bath pretreatment temperature study as shown in Fig. 4.3(c), there was not much increment in sCOD and VFA when compared to hot air oven pretreatment. As the temperature got increased, there was a slight ascend in sCOD and VFA. The decline in sCOD at 90^oC may be due to the loss of VFA through vaporization (Appels et al., 2010). Therefore, 80^oC temperature was selected for the time study. During the time study as shown in Fig. 4.3(d), the samples were kept for varied time period at the selected best temperature of 80^oC. As the exposure time (reaction time) was increased, there was a steep spike in the solubilization rate that was measured in the form of sCOD and VFA. From the Fig. 4.3(c-d), it was perceived that the sample kept at 80^oC for 90 min showed highest sCOD of about 1.23 times and VFA of about 1.88 times when compared to the untreated control sample in hot water bath pretreatment.

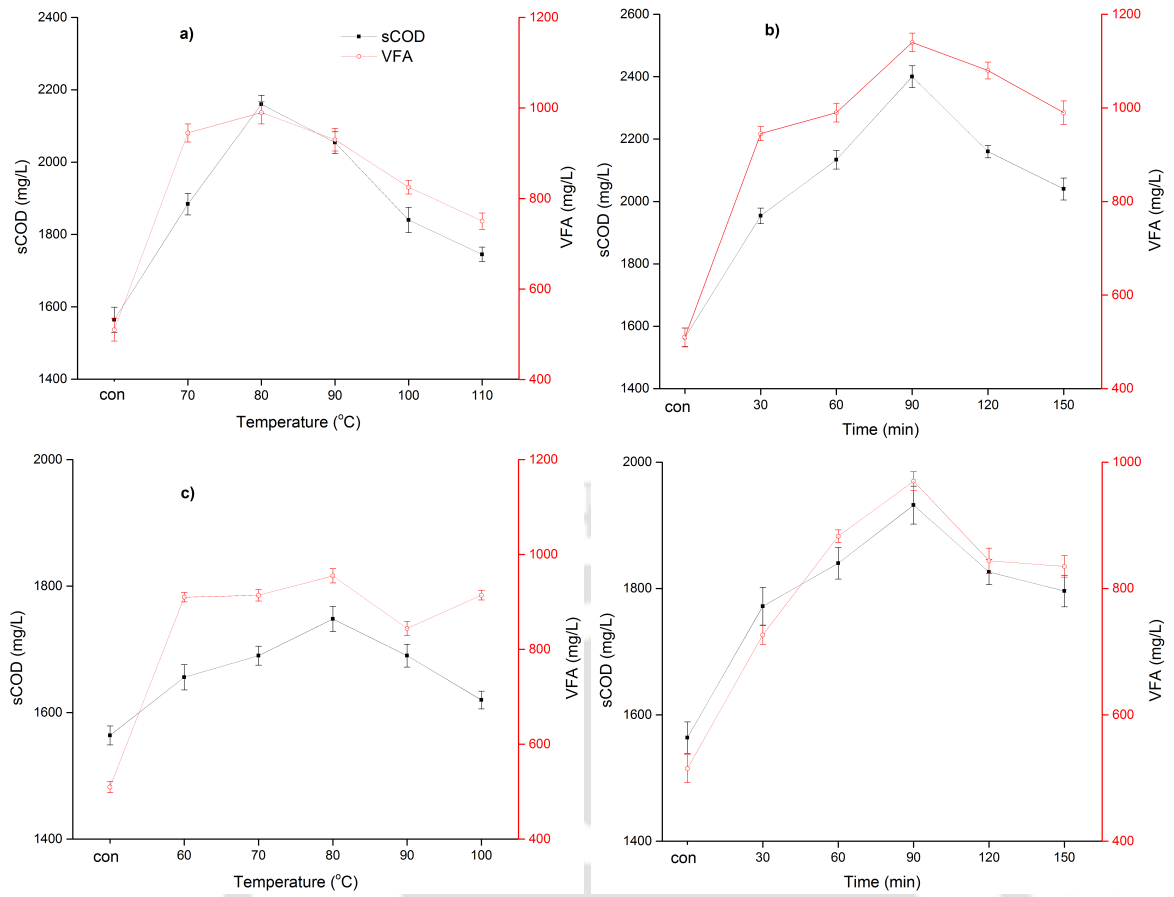

Fig. 4.3. The effect of temperature and time on COD and VFA different samples kept inside hot air oven and hot water bath. **a)** The effect of temperature on the COD and VFA in hot air oven for 30 min **b)** The effect of time on the COD and VFA in hot air oven for 80^{o}C **c)** The effect of temperature on the COD and VFA in hot water bath for 30 min **d)** The effect of time on the COD and VFA in hot water bath for 80^{o}C

- ***Autoclave pretreatment***

The effect of temperature and time on autoclave pretreated PPMS was shown in Fig. 4.4(a) and (b). As the temperature was being increased, the soluble compounds in PPMS got increased liquefaction/solubilization, thus sCOD increased by rises in VFA. From the Fig. 4.4(a), it was apparent that temperature at 110^{o}C has high sCOD and VFA. For time study, the samples were kept at the temperature of 110^{o}C with varied time in the interval of 20 min as shown in Fig. 4.4(b). As the exposure time was extending, there was a rise and fall of sCOD profile that was evident by ascend and descend of VFA profile. The sample kept at 40 min inside the autoclave showed highest sCOD of about 1.51 times and VFA of about 1.71 times than the untreated control sample. These results are in accordance with previous pretreatment studies on wheat and barley for ethanol production (Menardo et al., 2012).

- ***Microwave oven pretreatment***

From the Fig. 4.4(c), the temperature effect due to the microwave irradiation kept at 2 min reaction and 2 min standby time on microwave oven pretreatment was visible. It was perceived that the sCOD

increases with rises in temperature, whereas there is a reduction in VFA. The decline in VFA was attributed by a minor solubilization rate than the vaporisation of volatile compounds formed from the pretreatment. The sCOD spiked with the hike in temperature up to 140^{o}C and then it was reduced. Pretreatment at higher temperature promote the condensation and precipitation of soluble compounds lead to low water activity, formation of melanoidins refractory compounds due to the coupling of chemical reaction between the reducing sugars and amino acids (Hendriks and Zeeman, 2009). Saha et al. (2011) also studied the effects of temperature on microwave oven at varied temperature of 50, 75, 100, 125, 150 and 175^{o}C and optimised the temperature at 175^{o}C for 10 min, showed higher solubilization rate on paper mill sludge. Thus, PPMS pretreated at 200^{o}C for 2 min showed an increment of 39.4 % sCOD (1.39 times) when compared to untreated PPMS sample in this study.

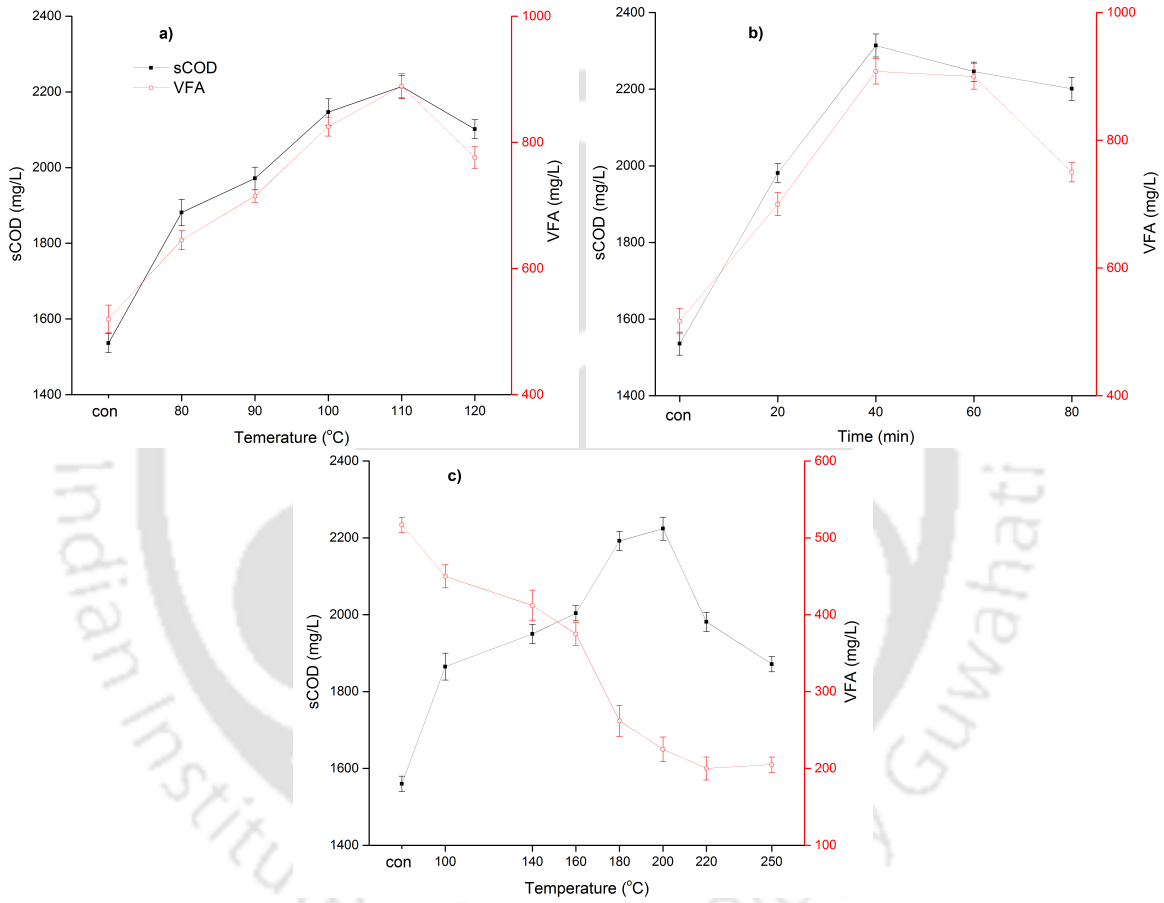

Fig. 4.4. The effect of temperature and time on COD and VFA for different samples kept in an autoclave and microwave. **a)** The effect of temperature on the COD and VFA in autoclave for 20 min **b)** The effect of time on the COD and VFA in autoclave for 110^{o}C **c)** The effect of temperature on the COD and VFA in microwave oven for 2 min

4.2.2 Electrohydrolysis pretreatment

4.2.2.1 Effect of sCOD and VFA with applied voltage

The effect of applied voltage with constant time on PPMS treated by DC current in electrohydrolysis pretreatment was shown in Fig. 4.5(a). It was observed from the Fig. 4.5(a) that the increase in applied voltage has shown increase in solubilization rate which was measured in the

form of sCOD and VFA. The sCOD and VFA increased up to 15 V and then it got reduced. Further increasing the applied voltage shows decrease in sCOD and VFA. At higher applied voltage, there is a release of higher quantity of electron by the DC current but there was not sufficient protons from the substrate. During the reaction time there is a higher rate of vaporization of volatile compound produced from the pretreatment effect than the solubilization rate (Zhen et al., 2014). This may be the reason for reduction in sCOD and VFA at higher voltage. The increase in sCOD and VFA at 15 V was due to the release of sufficient electron by the DC current that was enough to neutralize the protons released from the PPMS. Thus, 15 V is found to be best for the time study.

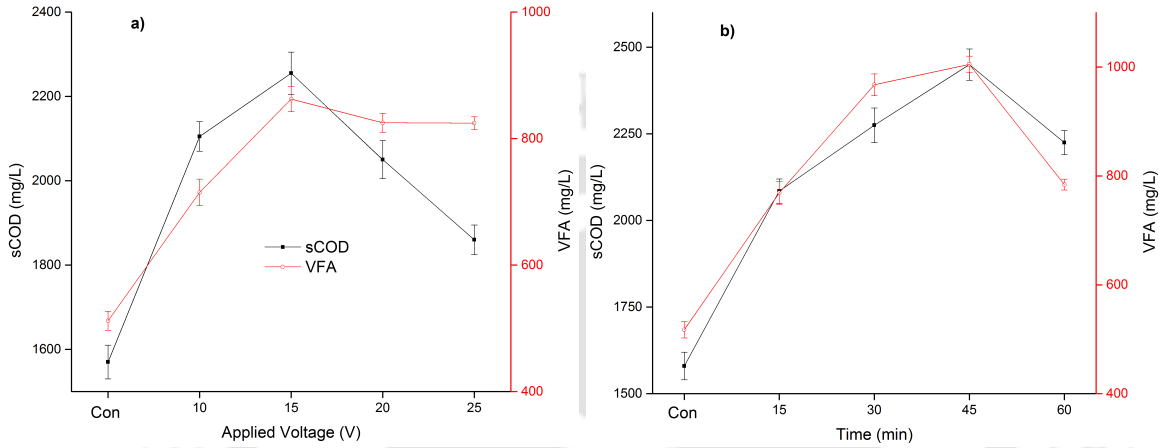

Fig. 4.5. The effect of applied voltage and time on sCOD and VFA in electrohydrolysis pretreatment. **a)** The effect of applied voltage on the sCOD and VFA for 20 min **b)** The effect of time on the sCOD and VFA at 15 V with different time intervals

4.2.2.2 Effect of sCOD and VFA with time

Hence for the time study, the samples were kept at 15 V for the different time duration. It was observed from the Fig. 4.5(b) that the increase in exposure time has shown increase in solubilization rate which was measured in the form of sCOD and VFA. From the time study Fig. 4.5(b), the sample kept at the 45 min showed the highest sCOD with the increment of 1.91 times and VFA of about 1.85 times increment in electrohydrolysis pretreatment than control sample of PPMS. Zhen et al. (2014) obtained an increase of 1.5 times sCOD in waste activated sludge for the sample pretreated at 20 V for 40 min. Thus, 15 V for 45 min is found to be best pretreatment conditions for the elctrohydrolysis pretreatment of PPMS.

4.2.2.3 Effect of current and time for applied voltage and time

It was observed that at the same applied voltage, the current is gradually increasing with the time of exposure (Table 4.4 and 4.5). Increase in conductivity caused by solubilization of particulate organic matter by the breaking of bonds between polymers induced by application of DC current. It was found that increase in applied voltage, then the slope of current versus time also increased. When the sample pretreated at 10 V, there was no change in current whereas at 15 V showed an increase of current 0.005 amp. At higher voltages, there was more energy available to cause rapid solubilization.

Table 4.4. Variation of current and resistance with different applied voltage (V) at particular time (20 min)

Time (min)	Current (amp)	Resistance (ohm)	Current (amp)	Resistance (ohm)	Current (amp)	Resistance (ohm)	Current (amp)	Resistance (ohm)
	at 10 V		at 15 V		at 20 V		at 25 V	
0	0.029	344.828	0.050	300.000	0.075	266.667	0.10	250.000
5	0.029	344.828	0.055	272.727	0.075	266.667	0.10	250.000
10	0.029	344.828	0.055	272.727	0.075	266.667	0.11	227.273
15	0.029	344.828	0.055	272.727	0.075	266.667	0.11	227.273
20	0.029	344.828	0.055	272.727	0.076	263.158	0.11	227.273

Values are the mean of two pretreatment experiment

Table 4.5. Variation of current and resistance with the different time duration at 15 V

Time (min)	Current (amp)	Resistance (ohm)	Current (amp)	Resistance (ohm)	Current (amp)	Resistance (ohm)	Current (amp)	Resistance (ohm)
	at 15 (min)		at 30 (min)		at 45 (min)		at 60 (min)	
0	0.040	375.000	0.051	294.118	0.049	306.122	0.047	319.149
5	0.040	375.000	0.051	294.118	0.049	306.122	0.047	319.149
10	0.041	365.853	0.052	288.462	0.051	294.118	0.047	319.149
15	0.041	365.853	0.052	288.462	0.051	294.118	0.049	306.122
20			0.052	288.462	0.051	294.118	0.049	306.122
25			0.052	286.260	0.052	288.462	0.049	306.122
30			0.024	625.000	0.053	283.019	0.050	300.000
35					0.054	277.778	0.050	300.000
40					0.056	267.857	0.051	294.118
45					0.056	267.857	0.051	294.118
50							0.051	294.118
55							0.051	294.118
60							0.051	294.118

Values are the mean of two pretreatment experiment

By analyzing the resistance versus time, it was observed that at the same applied voltage, the resistance is gradually decreasing as the time of exposure increases (Table 4.4 and 4.5). This is because of hydrolysis of polymer into simple soluble monomers. As the time of exposure increased, more and more particulate matter is getting solubilized. The sample pretreated at 15 V for 15 min shows 2.6 % reduction of resistance whereas at 45 min shows a 12.5 % reduction of resistance. This huge difference in reduction of resistance at 15 V for longer exposure time, there is more energy available to break the bonds between polymers. Very few literature is available, Zhen et al., (2014) investigated the effect of combined electrical-alkali pretreatment to enhance the hydrolysis of waste activated sludge for anaerobic digestion process but does not revealed the effect of current or resistance with time.

4.2.3 Biological pretreatment

4.2.3.1 The effect of sCOD and VFA in different isolated bacterial strain

The Fig. 4.6 shows effect of different microbial pretreatment on solubilization of PPMS which was measured as sCOD and VFA. To enhance the solubilization of PPMS, different isolated microorganisms were employed to pretreat the PPMS. To increase the solubilization or liquefaction process an appropriate assortment of bacterial strain/culture is necessary. As the day was extending,

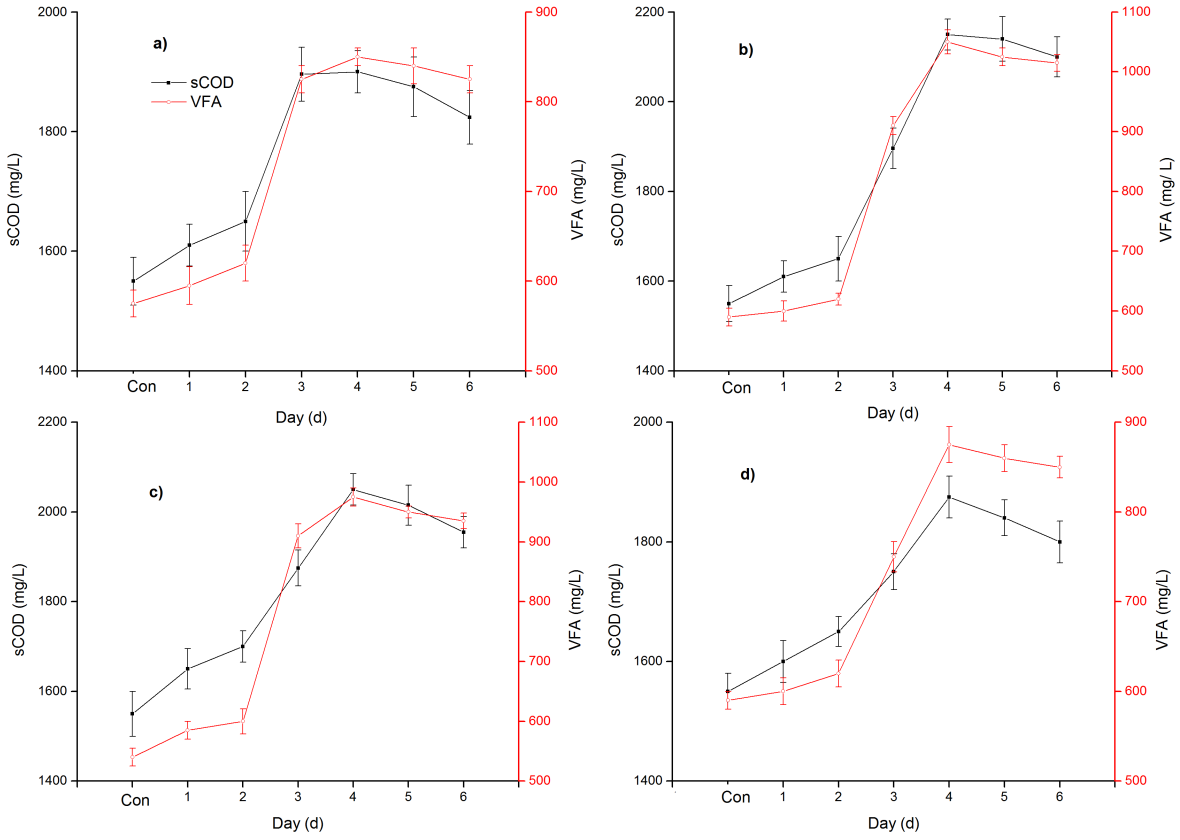

Fig. 4.6. The variation of sCOD and VFA with respect to isolated bacterial stain. **a)** *Paenibacillus sp.* (BRb2) (at 10^8 CFU/mL) **b)** *Bacillus mojavensis* (CDb1) (at 10^8 CFU/mL), **c)** *Citrobacter werkmanii* (SFa2) (at 10^7 CFU/mL), **d)** *Bordetella muralis* (UN3d2) (at 10^7 CFU/mL)

the soluble compounds presents in PPMS got increased solubilization, thus sCOD increased with rise in VFA in all four microbial culture pretreatment. This is due to the activity of microbial culture on the soluble compounds which causes increment in sCOD and VFA. During bacterial pretreatment, initially the bacteria starts to consume the easily available soluble organic matter, once it got over, the bacteria secrete the exoenzymes to solubilize the particulate organic matter thereby increasing the quantity of soluble organic matter and the bacterial population, leading to increase in sCOD. The sCOD and VFA increased hand in hand as the number of days increased and after reaching the utmost peak, it starts decreasing. From the Fig. 4.6, it was observed that on day 4 has high sCOD and VFA for all the microbial strain with different degree of solubilization rate. The microbial pretreatment with *Bacillus mojavensis* showed the highest solubilization on 4 d followed by *Citrobacter werkmanii*, *Paenibacillus sp.*, and *Bordetella muralis*. Yuan et al. (2014) also observed increment in the concentrations of sCOD and VFA on 4 d of microbial pretreatment of corn stalk. The different degree of solubilization rate follows as: *Bacillus mojavensis* (CDb1) (1.38 times or 38.80 %), *Citrobacter werkmanii* (SFa2) (1.32 times or 32.25 %), *Paenibacillus sp.* (BRb2) (1.22 times or 22.5 %), and *Bordetella muralis* (UN3d2) (1.20 times or 20.97 %). In bacterial pretreatment, time required for solubilization was the key administering feature because the release of soluble organic matter augment with an increment in time (Kavitha et al., 2017). After few days, as the availability of food decreases, the bacteria starts to undergo endogenous decay due to food limitation that leads to the decrement in sCOD (Barua and Kalamdhad, 2018).

4.2.4 Holocellulose and lignin content effect on pretreatment methods

The compositional analysis was performed to identify the changes in composition such as cellulose, hemicellulose, and lignin before and after different pretreatments of PPMS. Table 4.6 presents the pretreatment conditions and lignocellulose contents of untreated and pretreated PPMS. The efficacy of different pretreatments has been identified by comparing the increase in solubilization/ liquefaction of the pretreated PPMS with the untreated (control) sample. It was observed that hot air oven pretreated PPMS showed the highest increase in acid soluble lignin (1.78), insoluble lignin (1.30 times), and cellulose (1.20 times) and reduced in the hemicellulose content (1.08 times) than the untreated sample (Table 4.6). The increment in acid soluble and cellulose portrays that pretreatment has the higher liquefaction and delignification characteristic. Thus, these results suggest that the presence of higher acid soluble lignin could be easily fermentable by fermentative microorganisms and also the cellulose could be easily accessed by the microbes when compared to the untreated PPMS. Due to the dissolution and depolymerization/repolymerization reaction at the thermal pretreatment (Li et al., 2007), there was a slight increment of acid insoluble lignin because of the redistribution of lignin on the fiber surface again than untreated PPMS. As hemicellulose are short and branched chain, helps in built a network between the cellulose and lignin. Due to its nature, there is a slight reduction in hemicellulose percentage after the pretreatment in all samples. This reduction may be attributed to the deconstruction of lignocellulose content and its solubilization of acid soluble lignin and cellulose after the pretreatment. The increment in acid soluble and cellulose portrays that the pretreatment has the different degree of liquefaction and delignification characteristic.

Table 4.6. Pretreatment conditions and lignocellulose contents of untreated and pretreated PPMS

Pretreatment	Temperature (^{o}C)	Time (min)	Acid insloluble lignin (%)	Acid soluble lignin (%)	Hemicellulose (%)	Cellulose (%)
Control			2.30±0.12	3.53±0.12	6.27±0.75	32.75±1.26
Thermal						
Hot air oven	80	90	3.01±0.11	6.27±0.08	5.89±0.59	38.75±1.37
Hot water bath	80	90	2.54±0.08	6.41±0.18	5.75±0.46	36.14±1.46
Autoclave	110	40	2.56±0.17	6.11±0.05	5.88±0.62	35.46±1.85
Microwave oven	200	2	2.89±0.13	6.05±0.21	5.76±0.76	36.49±1.05
Electrical						
Electrohydrolysis	15 (V)	45	2.91±0.11	6.17±0.08	5.88±0.59	37.75±1.37
Biological						
Paenibacillus sp.	35	4^{th} d	2.55±0.17	5.67±0.09	6.07±0.37	35.64±1.04
Bacillus mojavensis	35	4^{th} d	2.67±0.09	6.01±0.13	5.98±0.42	36.55±0.99
Citrobacter werkmanii	35	4^{th} d	2.70±0.16	4.86±0.16	6.08±0.28	35.57±1.26
Bordetella muralis	35	4^{th} d	2.40±0.12	5.14±0.25	5.97±0.33	34.46±0.89

Similar results also obtained in the previous literature (Li et al., 2007). Suggesting that the presence of higher acid soluble lignin could be easily fermentable by the fermentative microorganisms (Patel et al., 2015) and the cellulose present in the pretreated PPMS has been easily accessed by the microbes when compared to the untreated PPMS.

The reduction in the hemicellulose content may leads to the formation fermentation inhibitor such as furfural from xylose, hydroxymethylfurfural (HMF) from glucose, and acetic acid from acetate groups attached to the hemicellulose (Sharma et al., 2015; Gaur et al., 2016). During pretreatment, hemicellulose is breakdown mainly into xylose and small amount of glucose. The hemicellulose reduction may be attributed by the deconstruction of lignocellulose content and its solubilization of acid soluble lignin and cellulose after the pretreatment. The order of better pretreatment follows as: thermal (hot air oven) followed by electorhydrolysis (15 V for 45 min) and biological *Bacillus mojavensis* (CDb1). The experimental data of the lignocellulose content has quite a similar trend after different pretreatment. On analysing the results by ANOVA, lignocellulose content varied significantly between the different thermal pretreatment at the 5 % level. It was observed that there was an increase in cellulose (P=0.0096), acid soluble (P=0.0053) and insoluble lignin (P=0.0012), but decrease in hemicellulose (P=0.0009) content after different pretreatment processes.

4.2.5 Instrumental characterization

4.2.5.1 FESEM

The physical structure of the untreated and pretreated PPMS were studied by FESEM. The FESEM images of untreated (control) and thermally pretreated PPMS (Fig. 4.7). The control sample has rigid compacted smooth surface characterizing the existence of tightly bound lignin in the cell wall, whereas in pretreated sample shows, its morphological changes (deteriorated) in their surface layer. The response of different pretreatment on PPMS was varied greatly. Based on the different pretreatment, the surface destructured was happened after pretreatment. Hot air oven pretreated PPMS has totally destructed surface matrix, surface with hollows and cracks for hot water bath pretreated PPMS, rough scaly deconstructed outer layer for autoclave pretreated, loosely concaved textured surface for microwave pretreated, surface with fissures and large hallow for electrohydrolysis pretreated PPMS, whereas in biological pretreatment showed its morphological changes with more concaved texture for *Paenibacillus sp.* (BRb2), larger hallow matrix for *Bacillus mojavensis* (CDb1), cracked meta surface for *Bordetella muralis* (UN3d2) and *Citrobacter werkmanii* (SFa2) were observed. Thereby, different degree of morphological changes had observed after different pretreatment be an idea of surface destructured leads to an increased bioaccessibility of cellulose for the acidogenic microorganisms.

4.2.5.2 FT-IR

The structural and components change in cellulose, hemicellulose, and lignin can be extensively identified by using the characteristic absorbance band at Fourier Transform Infrared (FT-IR) spectrum. Morán et al. (2008) have been well documented the characteristic FT-IR bands of cellulose,

hemicellulose, and lignin. The characteristic bands changes in relative absorbance were also applied to determine the structural and components change after pretreatment (Hu et al., 2010). The FT-IR spectra of pretreated and unpretreated pulp and paper mill sludge are illustrated in Fig. 4.8. The efficacy of different pretreatment effect on structural changes has been compared with MorÃąn et al. (2008) as denoted by wavenumber reported and the corresponding assignment (wavenumber picked in this pretreatment study) are enlisted in Table 4.7. After pretreatment the relative absorbance of the lignin characteristic bands are slightly decreased, whereas the relative absorbance of the cellulose are slightly increased, indicating a changes in the relative structural and components of lignocellulose content.

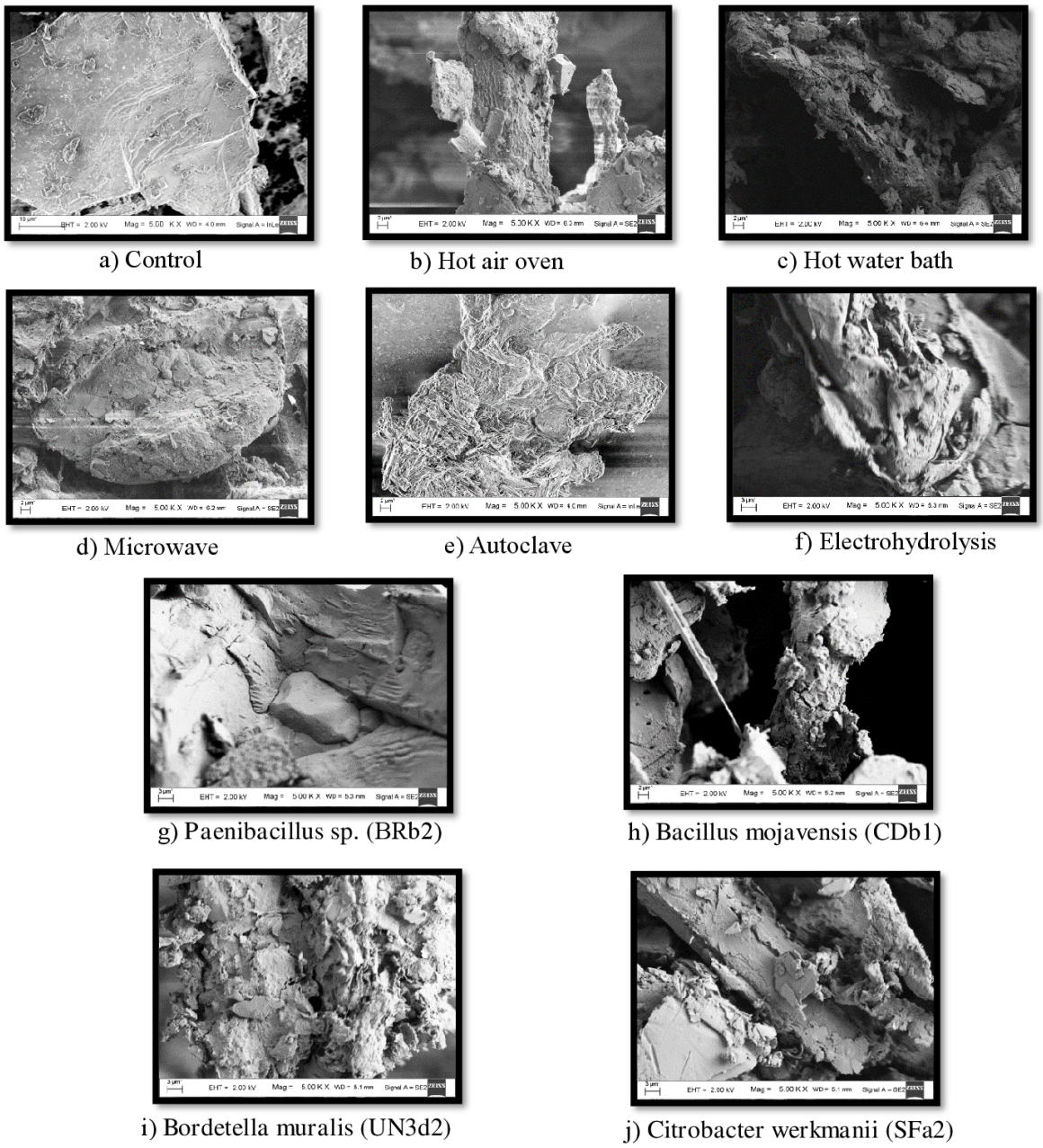

Fig. 4.7. FESEM images of unpretreated and pretreated PPMS **a)** Control, **b)** Hot air oven, **c)** Hot water bath, **d)** Microwave, **e)** Autoclave, **f)** Electrohydrolysis, **g)** *Paenibacillus sp.* (BRb2), **h)** *Bacillus mojavensis* (CDb1), **i)** *Bordetella muralis* (UN3d2), and **j)** *Citrobacter werkmanii* (SFa2)

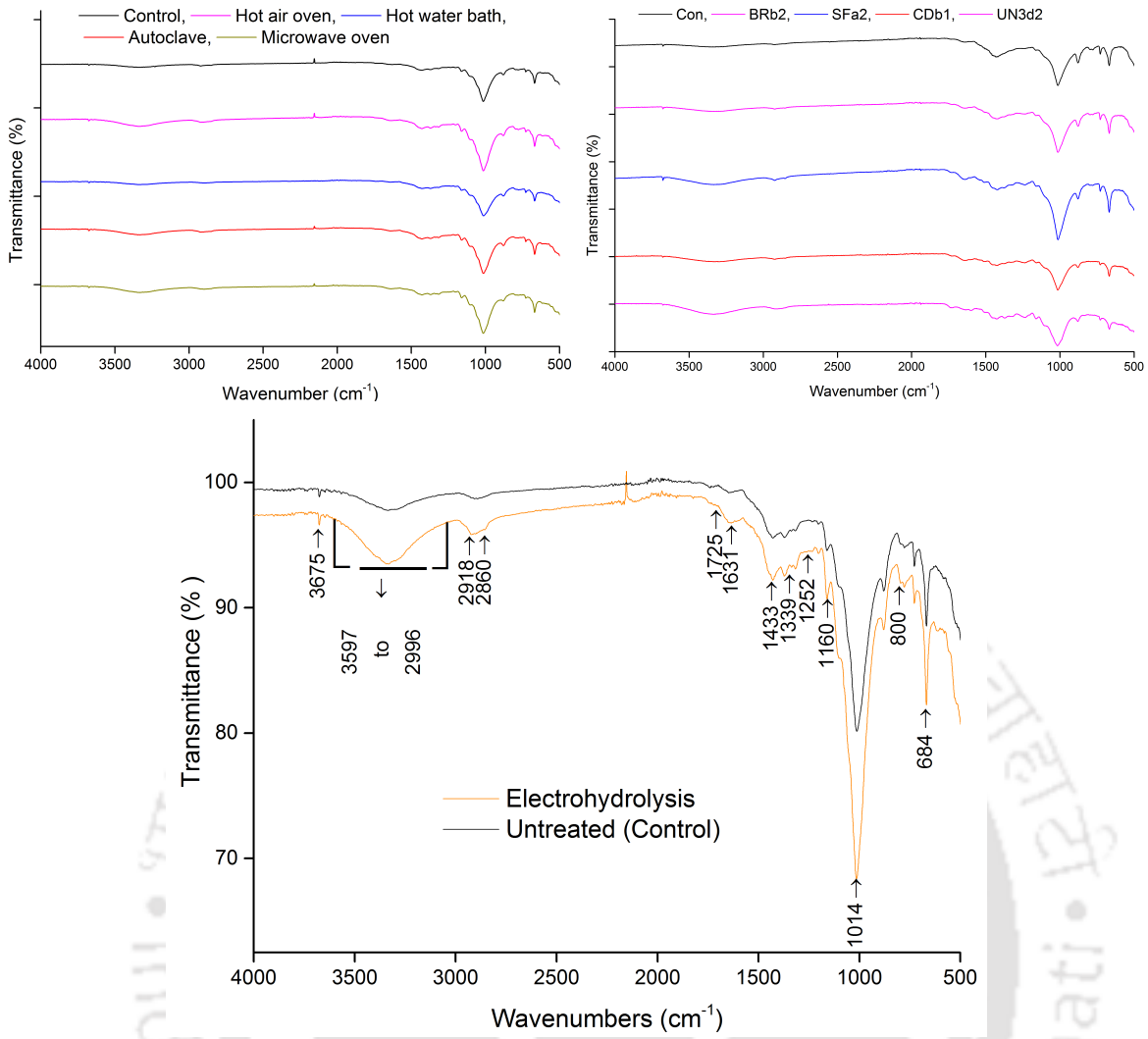

Fig. 4.8. FTIR spectra of untreated and pretreated pulp and paper mill sludge

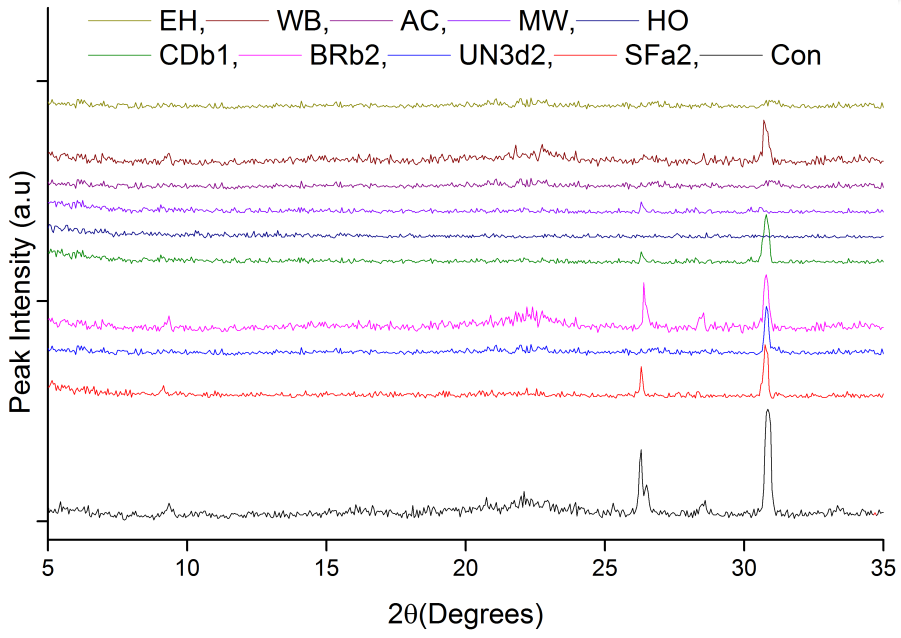

Fig. 4.9. XRD of the untreated and pretreated pulp and paper mill sludge

Table 4.7. Characteristics FT-IR bands of pretreated pulp and paper mill sludge

Fiber component	Wavenumber reported (cm^{-1})	Wavenumber picked (cm^{-1})	Functional group	Assignment
Cellulose	1048	1014	C-OH	O-H bond bending
	1270-1280	1252	C-O-C	Aryl-alkyl ether vibration
	1640	1631	Fiber-OH	Absorbed water bending
	2890	2875	H-C-H	Alkyl, aliphatic deformation
	4000-2995	3346	OH	Acid, methanol stretching
	4000-2995	3675	OH	Acid, methanol stretching
Hemicellulose	1048	1014	C-OH	O-H bond bending
	2890	2860	H-C-H	Alkyl, aliphatic deformation
	4000-2995	3337	OH	Acid, methanol stretching
	4000-2995	3675	OH	Acid, methanol stretching
Lignin	700-900	684	C-H	Aromatic hydrogen deformation
	700-900	800	C-H	Aromatic hydrogen deformation
	1048	1014	C-OH	O-H bond bending
	1158	1160	C-O	Phenyl bending
	1430	1433	O-CH_3	Methoxyl-O-CH_3 deformation
	2890	2860	H-C-H	Alkyl, aliphatic deformation
	4000-2995	3337	OH	Acid, methanol stretching
	4000-2995	3675	OH	Acid, methanol stretching

Pretreated PPMS showed reduced intensity than the untreated (control) PPMS, this was due to chemical modification undergone by the substrate during the pretreatment. The reduced intensity depicts the rupture of OH stretch, aromatic rings of lignin, C-O, C=O, C-C-O bond of cellulose, hemicellulose and lignin, commending trouble free bioaccessibility of the treated PPMS than the untreated PPMS. To the best literature known so far, there was no study used the FT-IR analysis for the structural changes at pretreatment stage, whereas the reduction in organic content and aliphatic chain during anaerobic digestion (Marcato et al., 2009; Yang et al., 2009).

4.2.5.3 XRD

By studying the changes in cellulose crystallinity, the effectiveness of different pretreatment on PPMS was evaluated. Fig. 4.9 shows the XRD spectra of untreated (control) and pretreated PPMS. The sharp peaks in control sample (Fig. 4.9) indicates the higher crystalline in nature, while amorphous substance do not possess the sharp peaks. Reduced peaks after pretreatment such as hot air oven (HO), hot water bath (HB), microwave oven (MW), autoclave (AC) and electrohydrolysis (EH)

portrays the reduction in their crystallinity of cellulose. Reduction in biological pretreatment was not that much when compared to the untreated PPMS. Table 4.8 display the changes in crystallinity of cellulose for control and pretreated of PPMS. After pretreatment, there was a serious reduction in cellulose crystallinity. Yang et al. (2009) depicted that any reduction in crystallinity of cellulose would contribute to an increased fragility and susceptibility of cell wall for the cellulolytic microorganisms. These results are in accordance with previous pretreatment studies for ethanol production (Yang and Wyman, 2008; Gabhane et al., 2011). The order of reduction in crystallinity after pretreatment was EH>HO>HB>MW>CDb1>SFa2>AC>UN3d2>BRb2.

Table 4.8. Changes in the cellulose crystallinity after different pretreatment

Types	Pretreatment	Crystallinity index (I_C)
Control		0.9137
Thermal	Hot air oven (HO)	0.6833
	Hot water bath (HB)	0.7666
	Autoclave (AC)	0.8285
	Microwave oven (MW)	0.7965
Electrical	Electrohydrolysis (EH)	0.6283
Biological	*Bacillus mojavensis* (CDb1)	0.8123
	Paenibacillus sp. (BRb2)	0.8469
	Bordetella muralis (UN3d2)	0.8346
	Citrobacter werkmanii (SFa2)	0.8127

4.2.6 Conclusions

It was observed that the pretreatment has affect the PMSS lignocellulose contents at different proportions. It was perceived that the organic and inorganic compounds are efficiently solubilized after the different pretreatment. Compositional and instrumental analysis such as FESEM, XRD, and FT-IR spectra showed that the different pretreatment has different proportional effect on lignocellulose content. However, the hot air oven and electohydrolysis pretreatment offered better results in solubilisation rates measure in the form of sCOD and VFA. The XRD and FT-IR spectroscopic characterization shows the development of aliphatic, unsaturated and carbonyl carbon functionalities in the pretreated samples at higher severities. FESEM picture also qualities the change in structure after the pretreatment. Thus, pretreatments serve to disrupt the lignocellulosic structure, making the cellulose easily accessible to acidogenic microorganisms. From this phase II, it was inferred that the hot air oven pretreatment at 80^oC for 90 min exposure time, electrohydrolysis pretreatment at 15 V for 45 min, and biological pretreatment *Bacillus mojavensis* (CDb1) on 4^{th} d with 10^8 CFU/mL showed a better solubilization rate in the hydrolysis stage. Thus, pretreatment showed a significant effect on lignocellulose content to speed up the hydrolysis step in anaerobic digestion process.

4.3 PHASE III: EVALUATING SCREENED PRETREATMENT FOR ENHANCED METHANE PRODUCTION IN BMP AND BATCH TEST

Objective of phase III: This phase was to evaluate the efficacy of the best pretreatment screened out in the previous phase. To delineate the anaerobic biochemistry and to investigate the rate and extent of reaction kinetics after pretreatment, this study was carried out in BMP and Batch test.

4.3.1 Thermal pretreatment (Hot air oven)

4.3.1.1 Effect of methane production after pretreatment

In order to rupture the lignocellulose cell wall of PPMS, and to improve the methane production potential, thermal pretreatment was carried out. In anaerobic digestion of PPMS (lignocellulosic waste material), first process step named hydrolysis was a rate-limiting step because of the recalcitrant inherent arrangement. By doing the thermal pretreatment, the recalcitrant structure get destructure. Hence the extracellular polymeric substances such as lignin and hemicellulose gets restructure. Because of pretreatment, acetogenic microorganisms acquire readily available intracellular substance like cellulose in the PPMS. Due to the restructure, gladly accessible cellulose gets degraded as early as in order to spike the SMA to produce enhanced methane gas.

Fig. 4.10 shows that variation of sCOD, VFA, VS and pH in relation to methane gas production. Due to the degradation of organic matter present in the PPMS, the sCOD concentration (Fig. 4.10(a)) starts to increase up to 14 d. This is because of the hydrolysis (major or minor effect by pretreatment) and acidogensis stage by the fermentative bacteria present in the anaerobic reactor. After 14 d sCOD concentration get decreased because of the higher activity of the acetogenic and methanogenic bacteria which was resulted in the increased daily gas production (Fig. 4.10(e)). During the first 14 d, the activity of the fermentative bacteria was higher than acetogenic and methanogenic bacteria. As a result, there was a higher VFA production (Fig. 4.10(b)). Then the produced VFA was actively taken up by the acetogenic and methanogenic microorganisms, hence VFA production is lesser in the latter half of the anaerobic process. The activity of the acetogenic and methanogenic bacteria is lesser than fermentative bacteria in the initial seven days and so there was a drop in pH (Fig. 4.10(c)) during the starting period. In the later period, due to the symbiotic relation with each other bacteria such as fermentative, acetogenic and methanogenic microorganisms, there was no drop in the pH. After pretreatment, it had been observed that, there was a clear trend such as increasing initially then diminishing in sCOD and VFA concentration, while in unpretreated paper mill sludge shows zig-zag manner in the sCOD and VFA concentration profile. That zig-zag manner is attributed to the degradation of lignin and/or hemicellulose in the later segment which was very hard for digest by the anaerobic microorganisms.

4.3.1.2 Theoretical methane yield and biodegradability

The theoretical methane yield (TMY) from the PPMS was calculated based on elemental compositions of organic substrates using the Buswell and Mueller (1952).

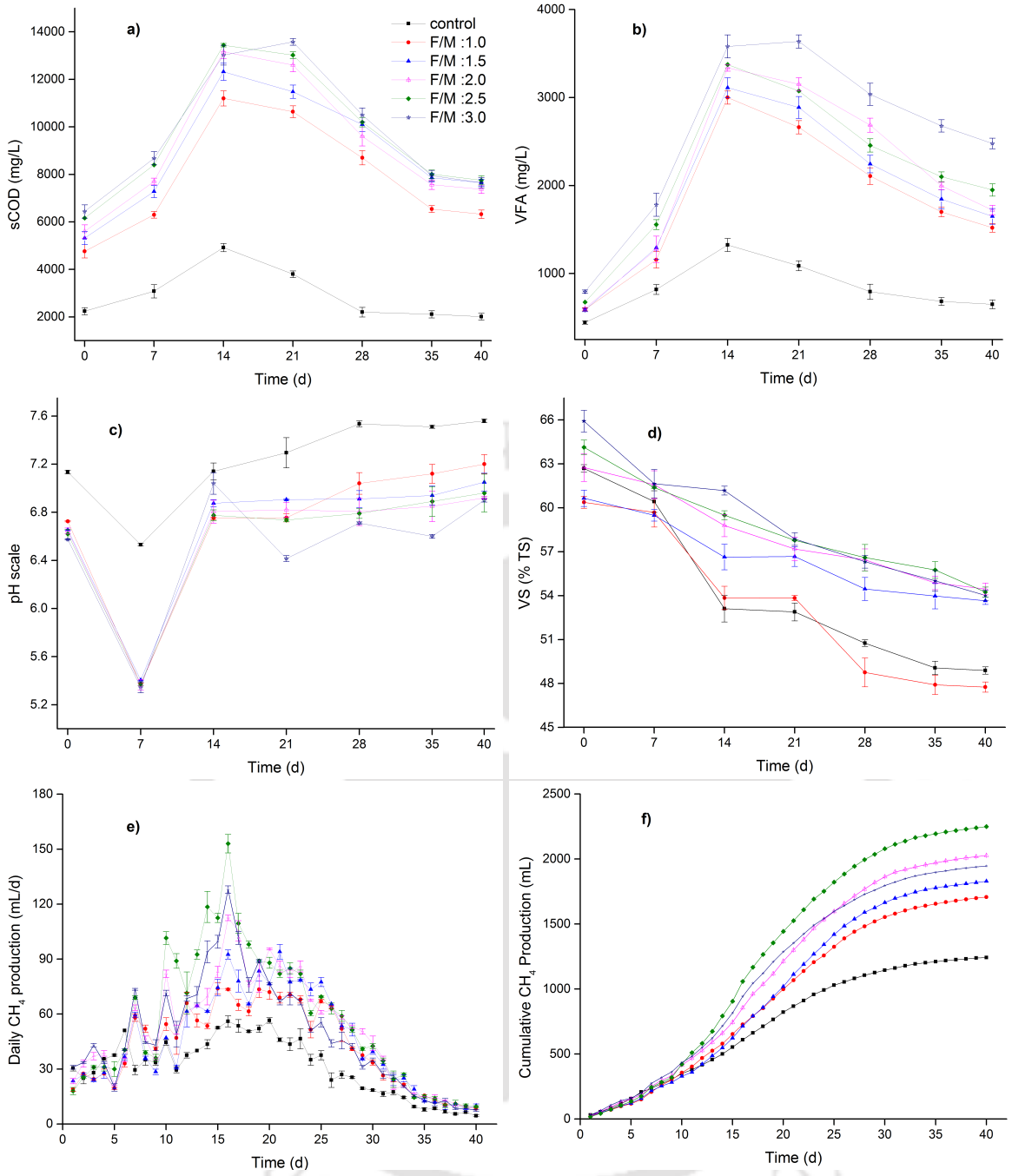

Fig. 4.10. Variation of pH, VFA, sCOD, and VS in relation to CH_4 production. **a)** Effect of sCOD at different F/M ratio **b)** Effect of VFA at different F/M ratio **c)** Effect of pH at different F/M ratio **d)** Effect VS at different F/M ratio **e)** Effect of daily CH_4 production at different F/M ratio **f)** Cumulative CH_4 production at different F/M ratio

Based on the following Eq. (4.1) and Eq. (4.2), the theoretical methane yield and biodegradability of the PPMS has been calculated.

$$C_nH_aO_bN_c + (n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4})H_2O \rightarrow (\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8})CH_4 + (\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8})CO_2 + cNH_3 \quad (4.1)$$

$$TMY\frac{mLCH_4}{gVS} = \frac{22.4 \cdot 10,000 \cdot \frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}}{12n + a + 16b + 14c} \quad (4.2)$$

Anaerobic biodegradability of the substrate could be calculated by dividing the experimental methane yield to theoretical methane yield.

4.3.1.3 Influence of F/M ratio on specific methanogenic activity and biodegradability

The specific methanogenic activity (SMA) of thermally pretreated PPMS was spiked with the increasing in their F/M ratio ranging from 1.0 to 2.5 as shown in Fig. 4.11. Due to the solid-liquid separation process happens at the optimum F/M ratio, the aspect of phase changes considerably and so medium would not be paste for longer time. The experimental result revealed that SMA at low F/M ratio was significantly lower than that of higher F/M ratio. Thus, at the higher and optimum F/M ratio provides the sufficient and maximal SMA for the enhanced methane production. The SMA appears to be experimentally liner relation with the F/M ratio (Fig. 4.11). The SMA was spiked by double the time when the F/M ratio increased from 1.0 to 2.5. The SMA of thermally pretreated PPMS was increased by 1.5 times than untreated PPMS in previous study. This type of similar linear relation was experienced by the Pommier et al. (2007) and Le Hyaric et al. (2011) for samples from paper/cardboard and municipal solid waste respectively. Le Hyaric et al. (2011) highlighted that the SMA from the MSW as a source and propionate as a substrate. But in this study, SMA of the thermally pretreated PPMS was used as the substrate. Thermal pretreatment makes the lignocellulosic material to destructure, extracellular polymeric substances gets started to degrade as an upshot easily available intra cellular polymeric substance for the methanogenic organisms.

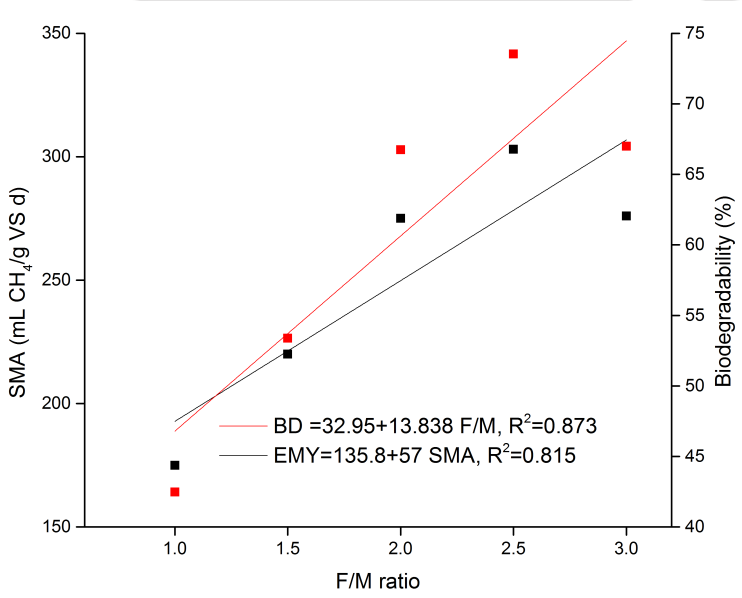

Fig. 4.11. Effect of F/M ratio on the specific methanogenic activity (SMA) and biodegradability of thermally pretreated PPMS

Due to the complex structure of lignin makes the PPMS (lignocellulose wastes) hard to digestion and shows low methane production and biodegradability (Li et al., 2013). This is because of higher lignocellulose material at high substrate concentration was difficult to biodegradable in anaerobic environment. Therefore, investigating the methane production potential with the influence of substrate concentration on biodegradability is necessary. Fig. 4.11 shows the correlation between the substrate

concentration (F/M ratio), specific methane yield and biodegradability. Positive correlations were found between the substrate concentration and experimental methane yield with a R^2=0.815, between biodegradability and substrate concentration with a R^2=0.873. Due to high values of correlation coefficient (R^2) indicate the derived linear models could be used to evaluate the methane production potential and biodegradability. By increasing the F/M ratio, there is a spike in the methane yield. After reaching the maximum and optimum F/M level, the SMA shows diminishing level of methane yield. Thus, the methane yield was significantly increased as SMA with the F/M ratio between 1.0 and 2.5. It was found that the substrate concentration with F/M ratio higher than 2.5 has low yield of methane yield and biodegradability as shown in Fig. 4.11. Thus, the F/M ratio 2.5 be a critical point in anaerobic digestion of thermally pretreated PPMS.

4.3.1.4 Energy assessment

An energy assessment of anaerobic digestion of thermally pretreated PPMS was carried out for evaluating its scalability based on the experimental results obtained in this study. The following assumption were made in this work, (1) PPMS used was homogeneous in mixture, (2) the density (ρ) (kg/m^3) and specific heat capacity C (kJ/kg·^{o}C) of PPMS were assumed to be the same as that of water, (3) no mass was lost during the thermal pretreatment, (4) the ambient temperature was constant at 27^{o}C. The input energy needed for the thermal pretreatment was calculated as the energy required to heat the PPMS from ambient temperature (T_a) to pretreatment temperature (T_p), i.e. 80^{o}C, subtracted by the heat recovered (by mean of heat exchanger with an efficiency of 85 %) when cooling down PPMS from T_p to T_a. The input energy was calculated by the Eq. (4.3) which was modified from the Passos and Ferrer (2015).

$$Q_{in} = \rho VC(T_p - T_a) + \omega(t_{rise} + t_{hold}) - \phi\rho VC(T_p - T_a) \tag{4.3}$$

where Q_{in}: input energy for thermal pretreatment (kJ); ρ: density of the PPMS (kg/m^3); V: useful volume of the reactor (m^3); C: specific heat capacity (kJ/kg·^{o}C); T_p: pretreatment temperature (80^{o}C); T_a: ambient temperature (27^{o}C); ω: power required to maintain pretreatment temperature by the heating device (kJ/s); t_{rise}: time elapsed for rise from ambient to pretreatment temperature (^{o}C); t_{hold}: holding time for pretreatment (^{o}C); ϕ: heat recovery from pretreated PPMS; T_r: mesophilic anaerobic digestion temperature (^{o}C). The energy output from the anaerobic digestion of PPMS was calculated from the highest methane yield based on Eq. (4.4)

$$Q_o = P_{CH_4}\xi V\rho\eta \tag{4.4}$$

where Q_o: output energy from the anaerobic digestion of PPMS (kJ); P_{CH_4}: methane yield (m^3 CH_4/kg VS); ξ: lower heating value of methane (kJ/m^3 CH_4); V: useful volume of the reactor (m^3); ρ: density of the PPMS (kg VS/m^3); η: energy conversion efficiency (%) which was assumed to be 90 % according to Passos and Ferrer (2015).

$$\Delta Q = Q_o - Q_{in} \tag{4.5}$$

Results was expressed as energy balance ΔQ (kJ) for anaerobic digestion of thermally pretreated PPMS. The energy balance was estimated as the difference between the energy output to energy input as denoted in Eq. (4.5). Table 4.9 shows the parameters used for the energy assessment were summarized as supplementary information.

Table 4.9. Different parameters used for energy assessment

Parameters	Unit	Value
Ambient temperature (T_a)	C	27
Pretreatment temperature (T_p)	C	80
Digestion temperature (T_r)	C	35
Density of PPMS/water (ρ)	kg/m^3	1000
Specific heat capacity of PPMS/water (C)	kJ/kg/C	4.18
Volume of PPMS for a single experiment (V)	m^3	9.89×10^{-3}
Lower heating value of CH_4 (ξ) (q)	kJ/m^3	35800
Heat recovery by heat exchanger (ϕ)	%	85
Energy conversion efficiency of CH_4 (η)	%	90
Power consumption by hot air oven (ω)	kJ/s	1.2
Time elapsed for temperature rise from ambient to pretreatment temperatures (t_{rise})	s	1200
Holding time at pretreatment temperature (t_{hold})	s	5400

The energy assessment of thermally pretreated PPMS was carried out from the experimental results in BMP assay. Since the global energy balances were estimated by subtracting the energy input to energy output, positive value indicates the net energy production in the system. As it can be observed in this study, input energy needed for thermal pretreatment (220 g of PPMS) at 80^{o}C for 90 min was calculated to be 8530 kJ, while the output energy was calculated based on lower heating value of methane as 17,266 kJ. Thus, the net energy of 8735 kJ was gained after thermal pretreatment at 80^{o}C. This study evaluated the feasibility of low temperature thermal pretreatment for improving the anaerobic digestion of PPMS. This study also evident to minimize the drawbacks of conventional thermal pretreatment process including the high energy consumption, inhibitory compounds formation and unfavorable pH/alkalinity decrease. According to the experimental results, low temperature thermal pretreatment followed by anaerobic digestion process is a simple and sustainable strategy for waste stabilization and utilization in the pulp and paper mill industry.

4.3.1.5 Application of kinetic model

The accumulated methane production curve attained for the each treatment were consequently simulated using the three proposed kinetic models in order to examine the SMA of PPMS in function with the F/M ratio. The simulated value i.e the bio-kinetics parameters is presented in Table 4.10.

Fig. 4.12 shows the suggested models and experimental data fitting. The modified Gompertz is one with the best fit in all treatment, the logistic function was also prophesying the behavior of the data from the experiment but little lower than the modified Gompertz model (Table 4.10). The modified Gompertz and logistic function model shows that there was no significant variance between the model and experimental data. In disparity, transference function could not replicate the experimental data for all F/M ratio used, the parameter M has enormously higher values (Table 4.10). Thus, modified Gompertz and logistic function pronounces the adequate methane production from the PPMS. The similar trend of a fitting order was attained by Zwietering et al. (1990) and Huiliñir et al. (2014).

Table 4.10. Kinetic parameters and goodness of fit attained on thermally pretreated PPMS

F/M ratio	M (L CH_4)	R_m (L CH_4/d)	λ (d)	Experiment CH_4 (L)	Y (L-CH_4)	Model
1.0	2.1377	0.0535	0.0010	1.708	1.7189	Gompertz model
	1.8371	0.0537	0.0009	1.7849		Logistic model
	3.8512	0.0545	0.0010	1.9232		Transference model
1.5	2.3707	0.0558	0.0011	1.829	1.8429	Gompertz model
	1.9921	0.0562	0.0019	1.9179		Logistic model
	4.2251	0.0054	0.0010	2.0910		Transference model
2.0	2.5375	0.0641	0.0010	2.027	2.0478	Gompertz model
	2.1825	0.0644	0.0012	2.1284		Logistic model
	4.9314	0.0653	0.0097	2.2967		Transference model
2.5	2.7156	0.0740	0.0099	2.250	2.2660	Gompertz model
	2.3802	0.0742	0.0010	2.3567		Logistic model
	5.36415	0.0774	0.0010	2.5455		Transference model
3.0	2.3030	0.0657	0.0012	1.946	1.9622	Gompertz model
	2.0488	0.0656	0.0010	2.0356		Logistic model
	4.9026	0.0719	0.0013	2.1757		Transference model

Under the batch condition, the modified Gompertz and logistic model assume that the specific growth rate of methanogenic organisms (SMA) is directly proportional to the methane produced from the anaerobic rector, with the growth curve seems to be sigmoidal production trend (Altaş, 2009). These two models were modified in order to take the biological parameter as proposed by Zwietering et al. (1990). By meaning the Monod equation, these Gompertz and logistic function does not consider the substrate consumption rate, but only the number of microorganisms is accountable. In all the F/M ratio, value for the M in transfer function is varied from the experimental value and also its value quite higher than the modified Gompertz and logistic function model. This higher value for M may be attributed to the biogas production value and not the methane production alone. From the Table 4.10, the value for the parameter M (potential methane production) has high that do not

match with the range of experimental and other models data. This behavior is replicated in the shape of the curve. Very few researcher uses this model, when lag phase is nearly equal to zero and consider only the exponential and stationary phase in the gas production (Donoso-Bravo et al., 2010; Li et al., 2012).

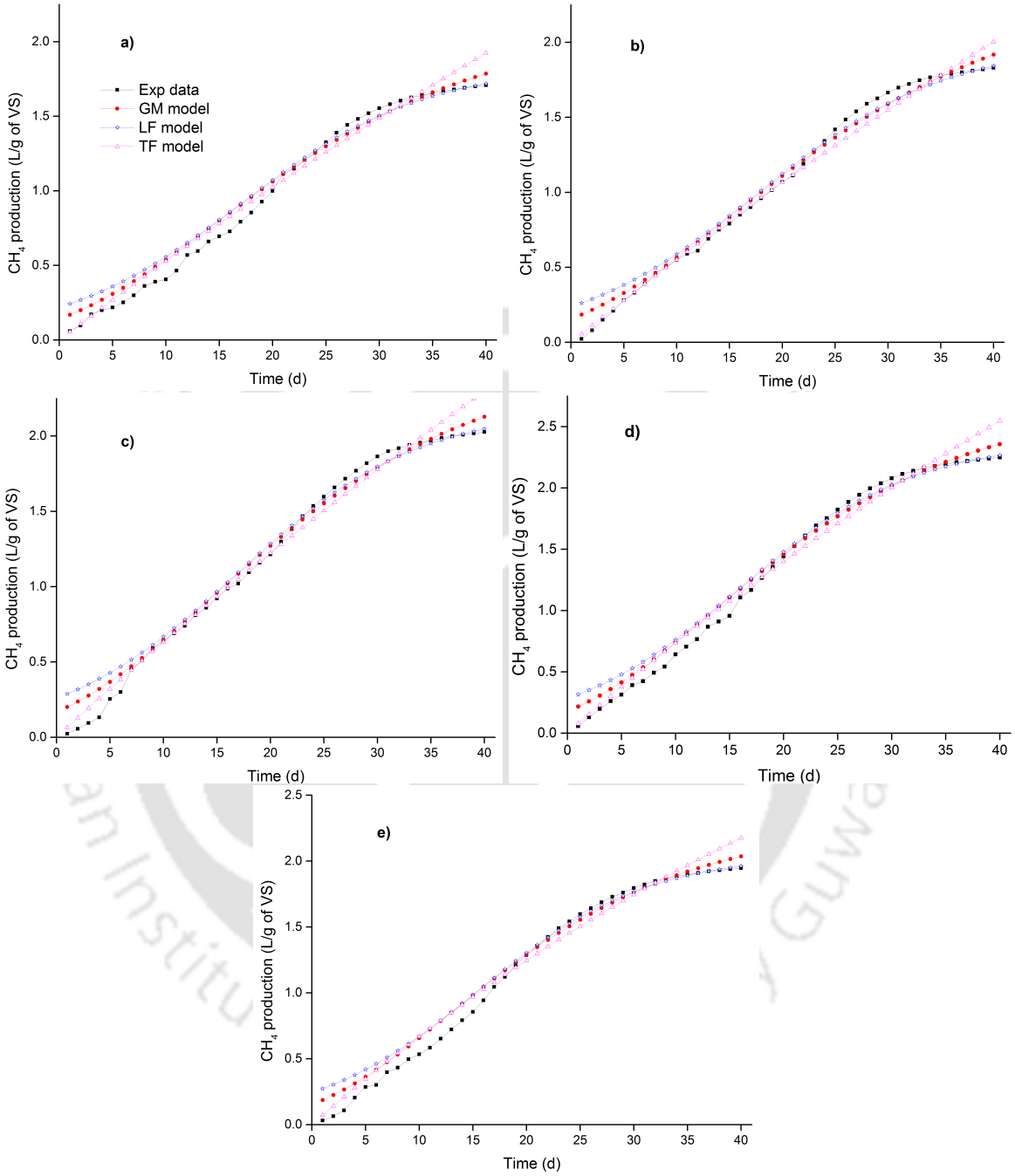

Fig. 4.12. Models fitted with cumulative CH_4 production from BMP assay of thermally pretreated PPMS with different F/M ratio. **a)** with F/M :1.0 **b)** with F/M :1.5 **c)** with F/M :2.0 **d)** with F/M :2.5 **e)** with F/M :3.0

Considering the parameter M and R_m (Table 4.10), its value increased by increase in F/M ratio in all the models, the higher value of M shows the higher removal of volatile solid and greater production of methane. Increasing of R_m (maximum rate of methane production) may be attributed to the pretreatment makes the cell wall destructure and readily available intracellular substance from

the sludge material for the acetogenic and methanogenic organisms. On the contrary, duration of lag phase has been increased for the F/M ratio greater than 2.5. This is because of higher substrate concentration (solid content), creating the hydrolysis process hard to proceed and increase the period of acclimation for the microorganisms.

Based on the fit shown in Table 4.10, the highest methane production (M) is for F/M ratio 2.5, slighted variation of maximum rate of methane production (R_m) with each F/M ratio, but lag phase time (λ) become constant for all the treatment. The cumulative methane production (Y) from the experiment and from simulated model values would be almost equivalent to modified Gompertz and the very slight variation in the logistic function, while for transference function has board variation. Parameswaran and Rittmann (2012) also used modified Gompertz equation for paper sludge and attained the value for M is 35.49 mL CH_4/g VS. This is 8.5 times low than obtained value from this work (303 mL CH_4/g of VSremoved). Concerning on parameter R_m, Parameswaran and Rittmann (2012) got the value 0.64 mL/h that was 2.8 times lower than this work (1.78 mL/h).

4.3.2 Electrohydrolysis pretreatment (15 V for 45 min)

4.3.2.1 Effect of methane production after pretreatment

Fig. 4.13 shows the variation of sCOD, VFA, VS and pH in relation to methane gas production after electrohydrolysis pretreatment. In this study, methane production was quantified on the basis of daily methane production rate (Fig. 4.13(a)) and cumulative methane production (Fig. 4.13(b)). Maximum substrate utilization rate was observed in the first 14 d of digestion, because the electrohydrolysis pretreatment made PPMS more readily degradable. Methane yield increased with the F/M ratio up to the optimum level of 2.5, then diminished at F/M ratio 3.0. This observation was likely because higher total solid content leads to the inhibition of hydrolysis and mass transfer problems (Xu et al., 2014). It was observed PPMS with the F/M ratio 2.5 has maximum methane production of 2.49 L, followed by F/M ratio 3.0 has 2.30 L. The methane yield was calculated to be 301$\pm$3 mL CH_4/g VS degraded, which was an increase of 13.8 % when compare to an earlier study assessing the biogas potential of PPMS (264$\pm$4 mL CH_4/g VS degraded).

Due to the degradation of organic matter present in the PPMS, the sCOD concentration (Fig. 4.13(c)) increased within 7 d after electrohydrolysis pretreatment. This observation can be explained by increased access to substrate for hydrolytic microbes, which had a higher initial activity than acetogenic and methanogenic groups. After 7 d, sCOD concentration decreased as acetogenic and methanogenic increased, converting sCOD into biogas (Fig. 4.13(a)).

By varying the F/M ratio from 1.5 to 3.0, different patterns of VFA accumulation were observed during the methane-producing period (Fig. 4.13(e)). The VFA concentration increased in the first 7 d due to fermentation of soluble products, forming a mixture of organic acids, CO_2 and H_2. Then the produced organic acids (VFA) were started to decompose by the acetogenic and methanogenic microorganisms, resulted in increased biogas production. Rate of VFA uptake and conversion by acetogens and methanogens increased as their activity level increased. But in the latter half of the anaerobic process, rate of VFA consumption decreased, as available substrate was used up by the

microorganisms, hence VFA concentration was low in the last phase of digestion.

Mass loss from the anaerobic digestion system was indicated by VS reduction, which was directly correlated to biogas production (Fig. 4.13(d)). In this study, VS reduction order followed: 2.5>3.0>2.0>1.5. VS reduction reflects biodegradation, and is analogous to mass from the system as biogas.

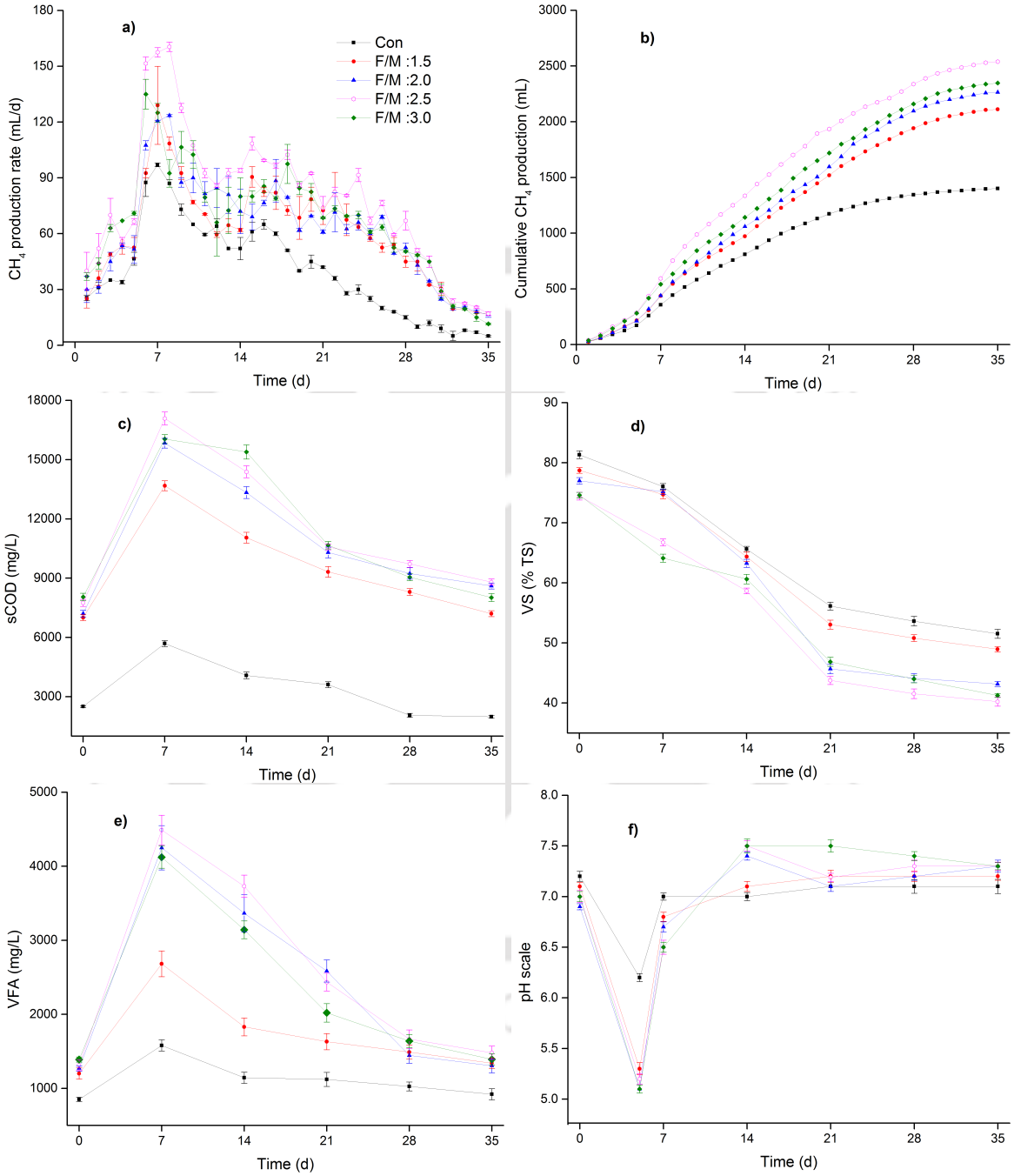

Fig. 4.13. Variation of pH, VFA, sCOD, and VS in relation with CH_4 production. **a)** Effect of daily CH_4 production at different F/M ratio **b)** Cumulative CH_4 production at different F/M ratio **c)** Effect of sCOD at different F/M ratio **d)** Effect of VS at different F/M ratio **e)** Effect of VFA at different F/M ratio **f)** Effect of pH at different F/M ratio

There was a drop in pH (Fig. 4.13(f)) during the initial 7 d period, that coincided with peak VFA concentration, as the activity of fermentative microbes were greater than acetogens and methanogens in the anaerobic process. However, as the anaerobic microbial community came into balance, a further decrease in pH was not observed in earlier BMP study. It was perceived in the study that the pH varied in the range of 5.1-7.5 in the BMP assay (Fig. 4.13(f)). During the initial 7 d volatile acid production was more that was attributed by high solubilisation of easily available organic matter, so the pH value was drop in initial period 0-7 d and later it was rising up to the end of the digestion. This is because of acidogens, acetogens and methanogens experienced a syntrophic relationship to keep anaerobic reactor in well balanced condition. The experimental data of the daily gas production has a quite similar trend; hence, it was exposed to an analysis of variance (ANOVA). The methane yield has seen to be significantly varied at the 5 % level at the different F/M ratio varies from 1.5 to 3.0.

4.3.2.2 Influence of F/M ratio on specific methane yield (SMY) and biodegradability (BD)

The specific methane yield of pretreated PPMS was increased with the increasing in their F/M ratio as shown in Table 4.11. After reaching the maximum and optimum F/M level, the SMY shows diminishing level of methane yield. Thus, the methane yield was significantly increased as SMY with the F/M ratio between 1.0 and 2.5. The experimental result revealed that SMY at low F/M ratio was significantly lower than that of higher F/M ratio. Thus, at the higher and optimum F/M ratio provides the sufficient and maximal SMY for the enhanced methane production. The SMY appeared to increase in linear manner. The SMY was spiked by double the time when the F/M ratio increased from 1.5 to 2.5. The SMA of pretreated PPMS was increased by 1.3 times (30 %) than untreated PPMS in previous study. This type of similar linear relation was experienced by the Pommier et al. (2007) and Le Hyaric et al. (2011) for samples from paper/cardboard and municipal solid waste respectively.

Table 4.11. Methane production and biodegradability of PPMS after eletrohydrolysis pretreatment

F/M ratio	Specific methane yield (mL/g VS)	Theoretical methane yield (mL/g VS)	BD (%)
1.5	230	412	53.39
2.0	287	412	65.75
2.5	301	412	70.54
3.0	274	412	67.99

Due to the complex structure of lignin makes the lignocellulose wastes hard to digestion and shows low methane production and biodegradability (Li et al., 2013). This is because of higher lignocellulose material at high substrate concentration was difficult to biodegradable in anaerobic environment. Specific methane yield and biodegradability were summarized in Table 4.11 with different F/M ratio. It was found that the SMY and BD was significantly reduced when F/M ratio higher than 2.5 (Table 4.11). Thus, the F/M ratio 2.5 be a critical point in anaerobic digestion of pretreated PPMS.

4.3.2.3 Energy assessment

An energy assessment of electrohydrolysis pretreated PPMS with subsequent anaerobic digestion was carried out to assess the scalability of the process, based on the experimental results obtained in this study. The input energy needed for the electrohydrolysis pretreatment was calculated by summing the energy required to pretreat the PPMS (consumption of DC supply) and energy consumption for the mixing devise. Thus, input energy was calculated by the Eq. (4.6).

$$Q_{in} = [(V_V \cdot I_A) \cdot t] + (\omega \cdot t) \tag{4.6}$$

where Q_{in}: input energy for pretreatment (kJ); [Power (J/s) equal to voltage (V_V) multiply by current (I_A)] V_V: voltage used for pretreatment (V); I_A: current used for pretreatment (A); t: time elapsed for pretreatment (s); ω: energy consumption for mixing (J/s).

The energy output from the electrohydrolysis pretreated PPMS was calculated from the highest methane yield based on Eq. (4.4). The energy balance was estimated as the difference between the energy output to energy input as denoted in Eq. (4.5).

Based on experimental results from the BMP assay, the energy balance was estimated for the electrohydrolysis pretreated PPMS and subsequent anaerobic digestion. Consideration energy is a key issue for assessing the viability of pretreatment techniques at large scale (Passos and Ferrer, 2015). Since global energy balances are estimated by subtracting energy input from energy output, a positive value indicates net energy production in the system. In the current study, energy input needed for an electrohydrolysis pretreatment at 15 V for 45 min was calculated to be 4,040 kJ, while the output energy was calculated based on lower heating value of methane as 17,264 kJ. Thus, the net energy gain for the electrohydrolysis process was 13,224 kJ, which was 1.51 times higher than previous thermal pretreatment. This study evaluated the feasibility of electrohydrolysis pretreatment for improving the anaerobic digestion of PPMS. Thus, the current study also evident to reduce the drawbacks in conventional pretreatment such as high-energy consumption reduced inhibitory compounds formation, and unfavourable pH/alkalinity decrease. According to the experimental results, electrohydrolysis pretreatment followed by anaerobic digestion process is a simple and sustainable strategy for waste stabilisation and utilisation in the pulp and paper mill industry. This study suggests that electrohydrolysis pretreatment succeeds in enhancing the methane yield, even though the energy consumed for the pretreatment seems higher. In light of the results, it is worth mentioning that the methane yield of PPMS may have been underestimated, because BMP test were not carried out using inoculum acclimated to PPMS digestion. While the results of BMP tests are useful to compare pretreatment conditions, continuous reactors with a microbial community acclimated to PPMS should better be used to verify the energy balance of the process.

4.3.2.4 Kinetic study

Two kinetic models, the modified Gompertz and logistic function, were used to simulate the accumulated methane production curve for each treatment which is the function of digestion time.

The simulated bio-kinetics values are presented in Table 4.12. Fig. 4.14 shows the experimental and suggested fit from each model. The modified Gompertz model provided the best fit for all treatments, but the logistic function was also able to predict biogas production kinetics (Table 4.12). Huilinir et al. (2014) attained a similar trend of a fitting order using the same models. The modified Gompertz and logistic function models follow that the specific growth rate of methanogenic organisms is directly proportional to the methane produced from the batch anaerobic reactor, with the growth curve following a sigmoidal production trend (Altas, 2009). These two models were modified in order to take the biological parameter into account, with a slow growth rate at the beginning and end of the digestion period, with an exponential increase in the time between. As the substrate concentration (varied F/M ratio) was studied in this study, with a degradation of volatile solids (mass loss in a reactor) which was directly related to the biogas production from the selected substrate that was experienced in the modified Gompertz and logistic model.

Table 4.12. Kinetic parameters and goodness of fit attained from electrohydrolysis pretreatment

F/M ratio	M (L-CH_4)	R_m (L CH_4/d)	λ (d)	R^2	Experiment CH_4 (L)	Y (L CH_4)	Model
1.5	2.103	0.0867	0.0013	0.9868	2.001	2.064	Gompertz model
	2.154	0.0767	0.0015	0.9829		2.050	Logistic model
2.0	2.446	0.0887	0.0014	0.9948	2.104	2.249	Gompertz model
	2.324	0.0857	0.0012	0.9880		2.229	Logistic model
2.5	2.789	0.0859	0.0012	0.9915	2.495	2.514	Gompertz model
	2.654	0.0824	0.0011	0.9884		2.498	Logistic model
3.0	2.689	0.0960	0.0013	0.9808	2.227	2.338	Gompertz model
	2.434	0.0974	0.0014	0.9779		2.321	Logistic model

In all the F/M ratio treatments, the experimental value for M was lower than predicted by both the modified Gompertz and logistic function models. Considering the parameter M and R_m (Table 4.12), its value increased along with increases in F/M ratio in all the models, the higher value of M reflects the greater removal of VS and greater production of methane. Increases in R_m (maximum rate of methane production) may be due to the electrohydrolysis pretreatment, which the PPMS structure to destructure and readily available intracellular substance for the acetogenic and methanogenic microorganisms. However, it was observed that when F/M was greater than 2.5, the lag phase was longer. This phenomenon was likely due to reduced hydrolytic efficiency caused by mass diffusion limitations and increased acclimation period for microorganisms.

Based on the fit shown in Table 4.12, the highest methane production (M) is for F/M ratio 2.5, with slight variation in maximum methane production rate (R_m) for each F/M ratio, and lag phase time (λ) being almost constant for all treatments. The cumulative methane production (Y) from the experiment was almost equivalent to predictions from the modified Gompertz model, and with slight deviation from the logistic function model. Parameswaran and Rittmann (2012) used modified

Gompertz equation for paper sludge and reported a value for M of 35.49 mL CH_4/g VS, which is 8.5 times lower than the value found in this work (301 mL CH_4/g VS).

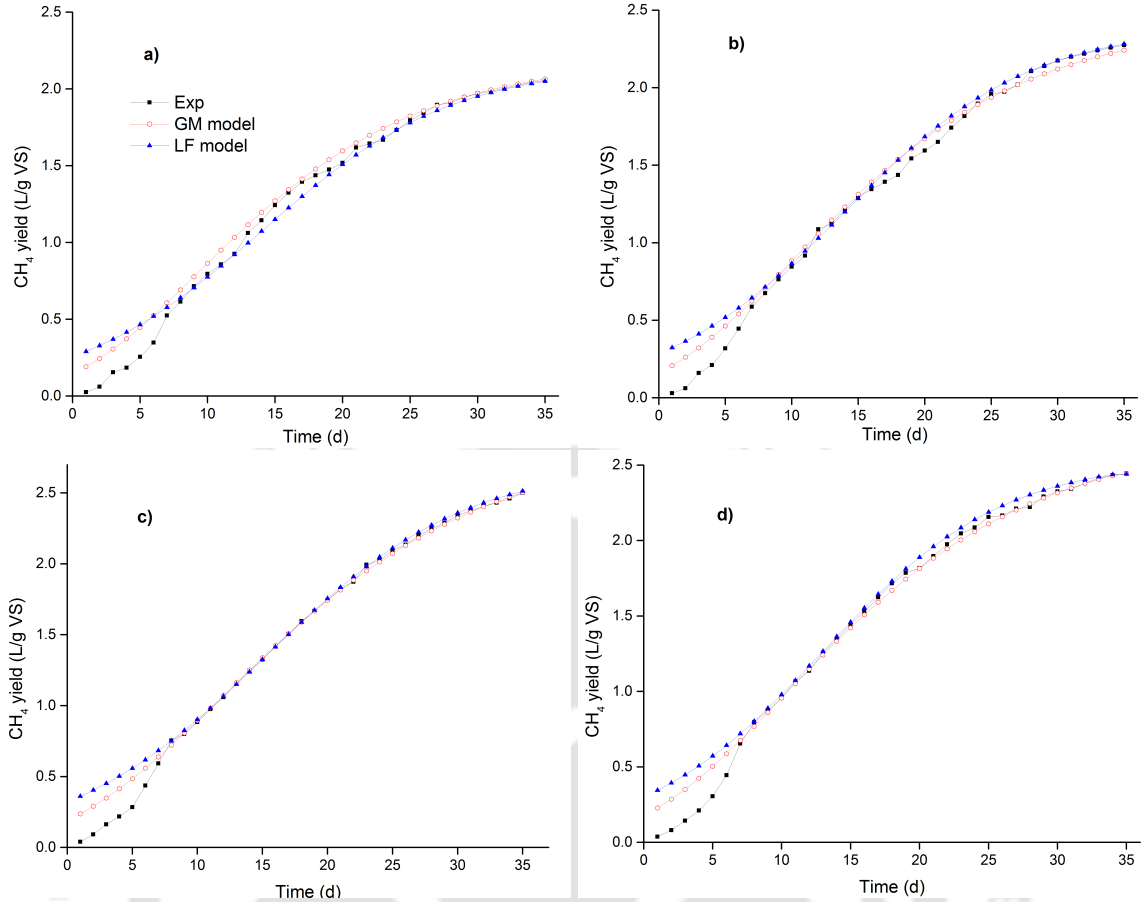

Fig. 4.14. Models fitted with cumulative methane production from BMP assay by using PPMS with different F/M ratio. **a)** with F/M: 1.5 **b)** with F/M :2.0 **c)** with F/M :2.5 **d)** with F/M :3.0

4.3.3 Biological Pretreatment (*Bacillus mojavensis* (CDb1))

4.3.3.1 Effect of methane production after microbial pretreatment

To study the efficacy of microbial pretreated PPMS, BMP test was carried out with CDb1 microbial culture because CDb1 only illustrates 1.37 times increased solubilization than other microbial culture. Hence, PPMS was inoculated with 10^8 CFU/mL of CDb1 and inoculated PPMS was incubated at 37^{o}C for 4 d. After 4 d of pretreatment, cow dung was added to the pretreated PPMS and employed for BMP study with varied F/M ratio. Fig. 4.15 shows the degradation pattern of different biochemical parameters such as pH, VFA, sCOD and VS in relation with the daily and cumulative methane production. Methane generation rate was a significant factor for evaluating the performance of anaerobic digester loaded with varied F/M ratio that were examined as the daily methane generation rate (Fig. 4.15(a)) and cumulative methane production (Fig. 4.15(b)) of microbial pretreated PPMS. No lag phase was observed during the AD of microbial (CDb1) pretreated PPMS. Methane generation started from the very first day itself for pretreated PPMS.

A sharp increase of in daily methane production rate was observed with entire F/M were observed,

whereas maximum methane production was experienced in F/M ratio 2.5. For microbial pretreated PPMS, the F/M ratio 2.5 demonstrated the maximum daily and cumulative methane production when compared to other F/M ratios. By the end of 30 d, methane generation stabilized and attain steady phase for each and every F/M ratio. Yunqin et al. (2010) reported that the reduction in hydrolysis period after biological pretreated PPMS with compost derived enzymes. Initial day (time required for inoculation period) of microbial pretreatment was observed to be time consuming, when compared to the thermal and thermos-chemical pretreatment studies but microbial pretreatment is ecofriendly and dose not required additional energy or costly chemicals. Van Kuijk et al. (2015) reported that an effective strain and its ideal culture conditions only makes the pretreatment process efficient by reducing the pretreatment time and organic matter loss.

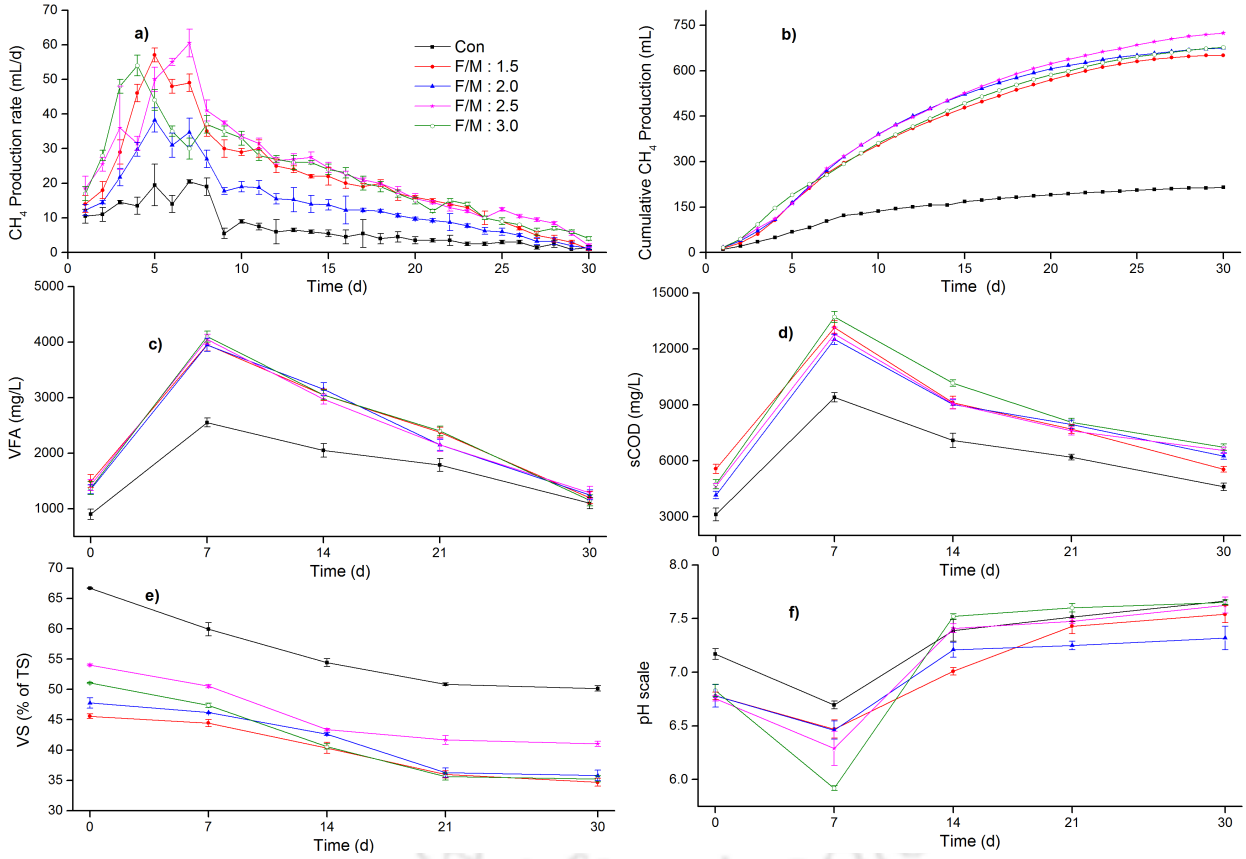

Fig. 4.15. The variation of pH, VFA, sCOD, and VS in relation with CH_4 production in biological pretreatment. **a)** Effect of daily CH_4 production at different F/M ratio **b)** Cumulative CH_4 production at different F/M ratio **c)** Effect of VFA at different F/M ratio **d)** Effect of VFAS at different F/M ratio **e)** Effect of VS at different F/M ratio **f)** Effect of pH at different F/M ratio

Weekly analysis of samples illustrates the variation of biochemical reaction undergone during BMP test of microbial pretreated PPMS and the importance of F/M ratio. As the digestion time was extending, the sCOD (Fig. 4.15(d)) and VFA (Fig. 4.15 (c)) concentrations of microbial pretreated PPMS got increased initially then started to decrease as experienced in the previous thermal and electrohydrolysis BMP test. Both VFA and sCOD increased hand in hand up to utmost peak and then it started to decrease. The VFA and sCOD concentration was highest on 7^{th} d for each and every

F/M ratio of microbial pretreated PPMS. An increase in the VFA concentration in starting period was observed due to the higher activity of the acidogens and the steady fall in the concentration of VFA illustrates the higher acitivity of methanogens than the acidogenic bacteria (Lin et al., 2017). After 7 d of digestion time, both sCOD and VFA started to decreasing as the soluble organics started to exhausting. sCOD increased with the passage of time, this is because microbial pretreatment made the soluble organics of PPMS easily bioaccessible for the forthcoming microorganisms precursor.

Mass loss from the anaerobic digestion system was indicated by VS reduction, which was directly correlated to biogas production (Fig. 4.15(e)). In this microbial pretreated PPMS study, VS reduction order followed: 2.5>3.0>2.0>1.5. The VS reduction reflects biodegradation, and it was analogous to mass loss in the form of biogas in the system.

Maintaining neutral pH inside the reactor is necessary, as subtle biochemical equilibrium between the acidogens and the methanogens should be prevalent (Khanal, 2011). There was a drop in pH (Fig. 4.15(f)) during the initial 7 d digestion period, that coincided with sharp peak of VFA concentration, this was attributed due to the high activity of fermentative microbes than acetogens and methanogens. However, as the anaerobic microbial community came into picture in the later period, all the reactor attains around neutral pH that could be more favorable conditions for the methanogens to produce more methane. It was perceived in the microbial pretreated BMP study that the pH scale was varied very short range (6.0-7.5) when compared to earlier thermally pretreated (5.1-7.5) PPMS. Therefore, it can be reported that microbial pretreatment enhanced the solubility of PPMS, there by increased methane production from 264 mL/g VS degraded (without pretreatment) when to 295 mL/g VS degraded.

4.3.4 Batch studies for best pretreatment method with best F/M ratio

Batch reactor (20 L) study was carried out with best F/M ratio attained from the BMP assay of control, hot air oven (HO) and electrohydrolysis (EH) pretreatment over the period of 75 d at ambient temperature between 30-38^{o}C in order to calculate the optimum hydraulic retention time (HRT) for PPMS. Therefore, batch anaerobic studies were conducted for pretreated and unpretreated PPMS to evaluate the rate and efficacy of hot air oven and electrohydrolysis pretreatment.

4.3.4.1 Effect of methane production

This study observed that methane production from control has lower than the pretreated PPMS. Methane production was quantified on the basis of per day methane production rate (Fig. 4.16(a)) and cumulative methane production (Fig. 4.16(b)). The steep curve of cumulative CH4 production of Hot air oven and electrohydrolysis pretreated PPMS provides the evident for superior degradation than the untreated PPMS (Fig. 4.16(b)). From the Fig. 4.16(a-b), the results lay emphasis on the easily bio-accessible soluble organic matter of PPMS that was amenable to acetogenic and methanogenic bacteria after thermal and electrical pretreatment and the existence of highly resistant lignocellulose cell wall of untreated PPMS was hard to degrade by the acetogenic and methonogenic bacteria.

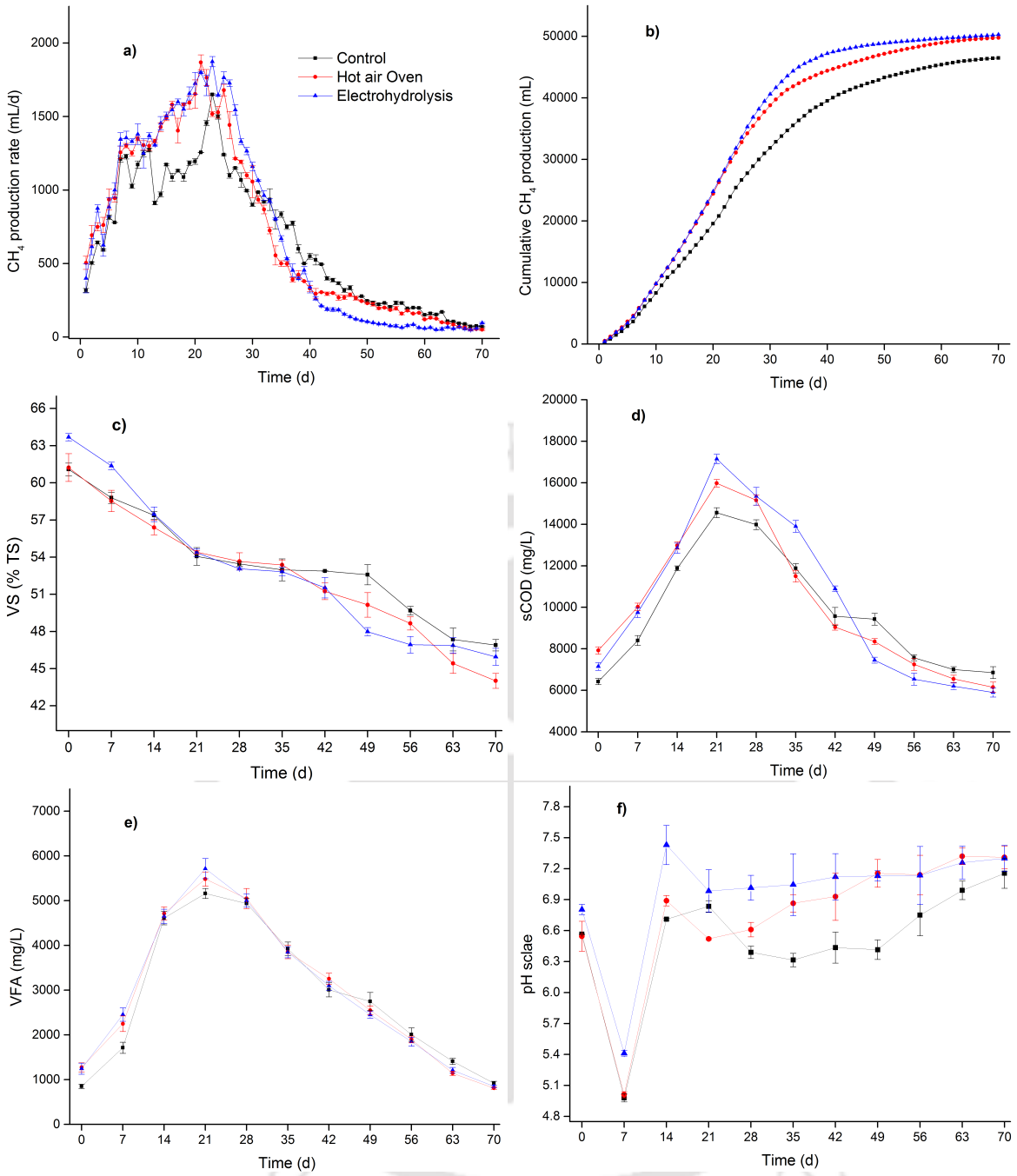

Fig. 4.16. The variation of pH, VFA, sCOD, and VS in relation with CH_4 production in batch reactor. **a)** Effect of daily CH_4 production at best F/M ratio **b)** Cumulative CH_4 production at best F/M ratio **c)** Effect of sCOD at best F/M ratio **d)** Effect of VS best F/M ratio **e)** Effect of VFA at best F/M ratio **f)** Effect of pH at best F/M ratio

It was experienced that the initial methane production rate was similar for all batch reactor (10 d average). After the 10^{th} d, there was an increment in methane production from pretreated reactor (Fig. 4.16(b)). As substrate was lignocellulose material (plant source), it was perceived that initially methane production was due to easily available volatile fraction of the substrate. Thereby, thermal and electrohydrolysis pretreatment aid in discharging the extracellular and intracellular biopolymers of the PPMS into the soluble aqueous phase. The discharge of soluble organic matter during pretreatment assists in accelerating the biodegradability of the substrate or enhancing the methane generation.

Nevertheless, its solubilisation takes place very late after the 14 days but it was consistent up to 40^{th} d with some changes in control and pretreated reactor. The best wise order of batch reactor for AD of PPMS based on the methane production was EH=HO>con. The cumulative CH_4 production on the 70^{th} d of hot air oven and electrohydrolysis pretreated PPMS was 49,952±150 mL of CH_4 and 50105 mL of CH_4 respectively, whereas for untreated PPMS was 42,068±12 mL of CH_4. Therefore, it can be started that thermal (hot air oven) and electrohydrolysis pretreatment has a significant effect on the methane production rate.

Weekly analysis of the samples illustrates the variation undergone in biochemical reaction during batch reactor study of PPMS with or without pretreatment. The Mass loss from the anaerobic digestion system was indicated by the decrement in VS that was correlated directly to the biogas production. High degradation designates higher VS reduction that yield more biogas. In the current batch study, maximum VS reduction was perceived in hot air oven pretreated with 28.04 % followed by electrohydrolysis pretreated with 27.84 %, where as in control 23.23 %. VS reduction from the AD of PPMS in batch was shown in Fig. 4.15(c).

Short chain fatty acids are key intermediate products formed during the anaerobic digestion process and they can indicate the process stability, stable, overloaded or inhibition. The total VFA concentrations in relation to methane production pH was shown in Fig. 4.15(e). The sCOD and VFA concentrations of pretreated PPMS increased with passage of time for each pretreatment. The results of the VFA analysis showed an exponential increase in VFA concentration in batch reactors running on pretreated PPMS when compared to untreated (Fig. 4.16(e)). Yu et al. (2004) started that the increase in VFA concentrations signifies the increased quantity of soluble organics that can be easily converted to methane. Within the first seven days itself VFA became 2,550 mg/L and 2,450 mg/L from 1,125 mg/L in the batch reactor running on electrohydrolysis and hot air oven pretreated PPMS while the VFA increased to just 1,205 mg/L from 865 mg/L for the batch reactor running on untreated PPMS. This is in line with the VS reduction in batch reactors of pretreated and untreated PPMS. An increase in the VFA concentration in starting period was observed due to the higher activity of the acidogens and the steady fall in the concentration of VFA illustrates the higher acitivity of methanogens than the acidogenic bacteria (Lin et al., 2017).

The production of VFA was increased until 21 d in all batch reactor, Due to the degradation of organic matter present in the PPMS, the sCOD concentration (Fig. 4.16(d)) starts to increase up to 21 d. This is because of the hydrolysis (major or minor effect by pretreatment) and acidogensis stage by the fermentative bacteria present in the anaerobic reactor. After 21 d sCOD concentration get decreased because of the higher activity of the acetogenic and methanogenic bacteria which was resulted in the increased daily gas production (Fig. 4.16(a)). During the first 21 d, the activity of the fermentative bacteria was higher than acetogenic and methanogenic bacteria. As a result, there was a higher VFA production as seen in Fig. 4.16(e). Then the produced VFA was actively taken up by the acetogenic and methanogenic microorganisms, hence VFA production is lesser in the latter half of the anaerobic process. The activity of the acetogenic and methanogenic bacteria was lesser than fermentative bacteria in the initial seven days and so there was a drop in pH (Fig. 4.16(f)) during the starting period. As the methanogens are sensitive to acidic conditions, so they come into action at the

end of the acidogenic phase. In the later period, due to the symbiotic relation with each other bacteria such as fermentative, acetogenic and methanogenic microorganisms, there was no drop in the pH.

4.3.4.2 Effect of lignocellulose content in AD of PPMS

Organisms use diverse mechanisms involving multiple complementary enzymes, particularly glycoside hydrolases to deconstruct lignocellulose (Cragg et al., 2015). Lignocellulose deconstruction is achieved under a wide range of environmental conditions, such as pH, redox potential, temperature, and pressure. Rumen microorganisms are a complex anaerobic microbial consortium which are mainly found in a specific stomach of ruminant animals (Barnes and Keller, 2003). Due to the higher cellulolytic activities of ruminant microorganisms promote higher rate of lignocellulose degradation that conventional inoculums such as anaerobic sewage sludge (Yue et al., 2013). The higher VFA yield during digestion is the key factor to assert an advantage of using ruminant microorganisms against the other inoculum for lignocellulose digestion. During anaerobic digestion of lignocellulose, cellulose and hemicellulose are the main component reduced and converted into biogas. The cellulose, hemicellulose and lignin degradation pattern during the digestion of PPMS were shown in Fig. 4.17.

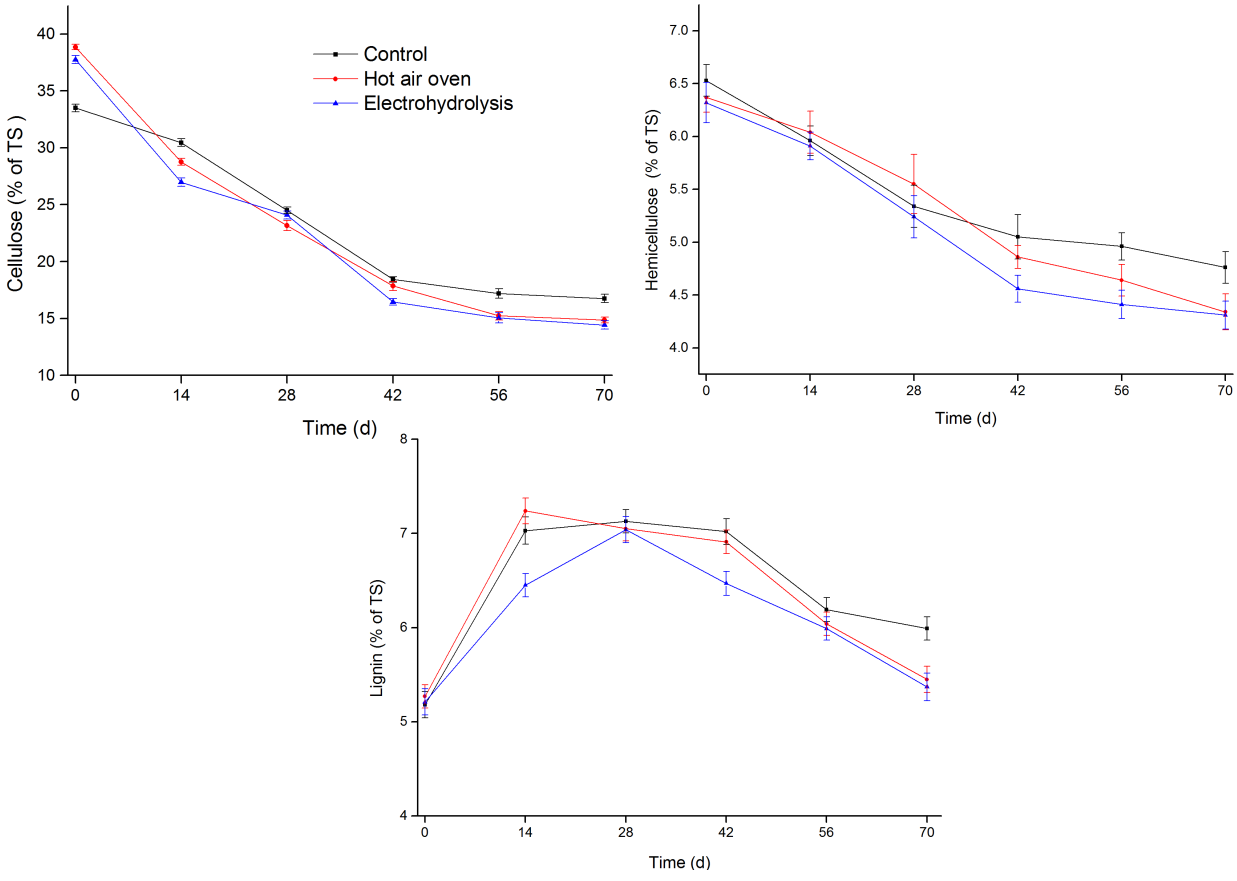

Fig. 4.17. The lignocellulose degradation pattern in anaerobic digestion of PPMS

The lignocellulose is degraded by only a small group of microorganism that produce specific enzymes. These secreted enzymes breaks the bonds that link the carbon atoms together within these molecules lead to degradation of lignocellulose content. From the Fig. 4.17, it was observed that lignocellulose degradation got improved after thermal and electrohydrolysis pretreatment with

improved biogas/methane production as seen in previous section. The cellulose and hemicellulose content got reduced, right from starting of digestion of PPMS in a batch reactor, whereas lignin content increased initially up to day 14, later it start to decrease until end of the digestion period. The cellulose and hemicellulose degradation rate was in agreement with the biogas production. Hemicellulose showed lower degradability than cellulose. During the anaerobic digestion, due to activity of acetogenic and methanogenic microoraisms, the cellulose content present in the PPMS had reduced gradually. The degradation competences of cellulose was spiked after thermal and electrohdrolysis pretreatment. The cellulose degradation rate in the electrically treated reactor was 61.80 % after 70 d of digestion followed by thermally pretreated (61.72 %) and then control reactor (49.95 %), which showed a good adaptability of PPMS on lignocellulose degradation. The result was in accordance with the Gu et al. (2014). Due to pretreatment, cellulose removal percentage was augmented from 49.95 to 61.80 %. Lin et al. (2009) also experienced that same percent of cellulose removal rate. On the other hand, the lignin content got increased initial digestion period, later it started to degrade by the anaerobic microbes but experienced no obvious lignin degradation change was not observed. The increment in initial days was due to the production of smaller molecules such as D-glucose, glucomannose etc., from the rate limiting step called hydrolysis Similar result was in accordance with the Xu et al., 2013 and Gu et al., 2014. In this study, hemicellulose showed lower degradability than cellulose that was due to difficult to detach the hemicellulose content from lignin present in PPMS. The highest hemicellulose degradation rate was observed in the thermally pretreated reactor (32.86 %) followed by electrohydrolysis pretreated reactor (31.85 %) and then control reactor (25.10 %). This result suggests that pretreated PPMS had better adaptability in lignocellulose degradation than untreated PPMS. Higher cellulose and hemicellulose degradation was experienced with increased biogas production. It could be concluded that the biogas production was related to cellulose and hemicellulose degradation.

4.3.5 Conclusions

This phase presented the effect of pretreatment with varied F/M ratio in thermal (hot air oven), electrohydrolysis, and biological (isolated bacterial strain: CDb1) in a BMP study. It was observed that methane/biogas production was improved after pretreatment at varied degree of benefit. The specific methane yield and biodegradability of PPMS were improved after pretreatment that was confirmed in the batch reactor too. The result revealed that the specific methane production potential was increased from 264±5 to 303±4 mL of CH_4/g VS degraded (for thermal pretreatment), 301±3 mL of CH_4/g VS degraded (for electrohydrolysis pretreatment), and 295±3 mL of CH_4/g VS degraded (for biological pretreatment). A net energy of 8,735 kJ was gained after thermal pretreatment and 13,224 kJ was gained after electohydrolysis pretreatment. The results from batch reactor study revealed that the cellulose degradation rate was increased in electrically treated reactor (61.80 %) followed by thermally pretreated (61.72 %) than control reactor (49.95 %), which showed a good adaptability of PPMS on lignocellulose degradation. Hemicellulose showed lower degradability than cellulose. In addition to that, kinetic models such as modified Gompertz, logistic function, and transference function model were studied. Among that modified Gompertz and logistic function

models showed a satisfactory methane production curve for untreated and pretreated PPMS and forms an exact sigmoidal methane production curve similar to experimental methane production curve. These sigmoidal trends confirms the methane production and its curve is proportional to the specific growth rate of methanogens that was usually seen in microbial growth curve. These models can be used to present the methane production and lag phase with the correlation coefficient range from 0.972 to 0.999. Predicted methane production rate (R_m) of pretreated PPMS was higher from untreated PPMS for modified Gompertz when compared to other two models. In both raw and pretreated PPMS, the transference function model showed values that was different from those perceived experimental. Regression values were always lower, when compared to the other two models. The transference model does not fit the points of curve such as lag phase during digestion. So this model would describing the biogas production instead of production of methane only. Parameter M (methane production potential) shows high values that do not fall in the range of methane produced from raw and pretreated PPMS. This behaviour could be connected to the shape of the curve, considering only the exponential and stationary stage in the gas production during digestion. Therefore, this model could be used only when lag phase is zero and/or close to zero. Based on three models, the highest methane production could be for eletrohydrolysis pretreated followed by thermal pretreatment. By considering the bio-kinetics parameter M (methane production potential) and R_m (maximum methane production rate) increases after pretreatment, while parameter λ (lag phase) decreases. This increase/decrease conforms the effectiveness of pretreatment. These increment in M and R_m attributed to the greater removal of volatile solids after pretreatment and the better methane production. Whereas decreased lag phase (λ) confirms the reduction in the digestion time and most of the biogas produced in initial half of the digestion period. It was observed that the modified Gompertz and logistic function model reproduce exact methane production curve which was found experimentally. It could be concluded that the pretreatment had greater impact in the biogas production.

"Water and air, the two essential fluids on which all life depends, have become global garbage cans."

Jacques-Yves Cousteau

Chapter 5

SEMI-CONTINUOUS LAB SCALE ANAEROBIC AUGER PLUG PLOW REACTOR (AAPFR)

This chapter dealt with the results and discussion of fed batch continuous anaerobic auger plug flow reactor (AAPFR), kinetic model development and its application.

5.1 PHASE IV: LAB SCALE AAPFR STUDIES FED WITH PPMS AND CORN SILAGE

Objective of phase IV: This study was focused on optimization and operation of AAPFR study at mesophilic condition. The study was majorly emphasized on the effect of increasing organic loading rate (OLR) on the methane production in long-term experiments. Based on the long-term experimental data, further study was carried out on development of kinetic model for AAPFR.

5.1.1 Acclimatization of AAPFR

Lab scale AAPFR studies have been performed with two different substrate with similar characteristics (Table 5.1) such as studies with PPMS (with cow dung as inoculum) and corn silage (with digestate as inoculum). The operating conditions for AAPFR, such as hydraulic retention time (HRT) and OLR for anaerobic digestion of PPMS as a substrate have been chosen/taken from the previous batch and BMP study, whereas the AAPFR fed with the corn silage had been operated more than 130 d with increasing organic loading rates (OLR).

The AAPFR was setup at open environment in both study place with the operating temperature 25-33^{o}C. For start-up the AAPFR at India, the initial fermentation condition of anaerobic digestion experiment was determined with the inoculum ratio 165 g/kg of TS according to the earlier feasibility study of batch and BMP tests. Based on the former condition, the feedstock for anaerobic digestion composed of 450 g PPMS, cow dung 200 g and distilled water 2000 mL. For start-up the AAPFR at Canada, the reactor was begin with an OLR of 3.5 kg VS/m^3/d. after start-up, the reactor was loaded with OLR of 3.5 kg VS/m^3/d and gradually increased to 8.8 kg VS/m^3/d. For each OLR step, the reactor was maintained at 30 d, different dosage of corn silage and digestate were fed into the reactor (Table 5.2).

It is well known that AD are sensitive to environmental conditions and easily influenced by operational parameters. To improve the efficiency, the influence of temperature, pH, C/N ratio, mixing intensity and other parameters on AD of different substrate alone has been studied intensively. However, AD of agricultural residue such as corn silage (lignocellulose material) have not studied

that much in depth. Therefore, studies on effect of increase in OLR, the operational parameters have been focused to improve the digestibility. However, the different operational conditions could result in differences in substrate characteristics that affect anaerobic performance.

Table 5.1. The physical and lignocellulose characterization of PPMS and corn silage

Parameters	Substrate (PPMS)	Inoculum (Cow dung)	Substrate (Corn silage)	Inoculum (Digestate)
Moisture content (%)	70.16±3.40	80±4	63.90±1.50	95.03±0.17
Total Solids (TS) (%)	29.83±0.73	20±2	36.11±0.25	4.96±0.74
pH	6.39±0.004	7.5±0.3	4.37±0.76	7.92±0.05
VFA (mg/L)	468.75±35.0	ND	2075±150	1235±125
Volatile Solids (VS) (%)	67.94 ±6.36	82.05±3	92.61±3.45	81.61±0.96
Ash content (%)	16.06 ±1.35	2.01±0.45	4.06±0.35	1.07±0.01
Total organic carbon (%)	25 ±5	46±5	36±6	42±4
C/N ratio	30-35	ND	35-45	ND
TKN (%)	1.03 ±0.01	ND	2.48±0.24	ND
Lignin (%)	5.68 ±0.23	ND	8.4±0.25	ND
Hemicellulose (%)	6.53 ±0.25	ND	16.8±0.15	ND
Cellulose (%)	32.49 ±0.37	ND	34.45±0.47	ND

Note: (mean ± SD, n=3) SD: standard deviation; ND: not determined

Table 5.2. The organic loading rate and composition of the feedstock for AAPFR operation

OLR (kg VS/m^3 d)	SRT (d)	Dosage of each material (g)		Total dosage (kg/d)
		Corn silage	Digestate	
3.5	25	330	1250	1.58
5.0	21	450	1450	1.90
6.5	17	610	1900	2.51
8.8	13	810	2500	3.31

5.1.2 Operation of AAPFR at IITG, India

5.1.2.1 Methane production profile from AAPFR

The AAPFR had been operated for 75 d with (thermal pretreatment) or without pretreatment at specified OLR (6.3 kg VS/m^3/d). Fig. 5.1 shows the daily methane production profile of AAPFR fed with PPMS (with or without pretreatment) at 21 d HRT and 6.3 kg VS/m^3/d. For the first stage

of semi-continuous experiment after starting up on 0-10 d, a sharp increase of methane production was observed as seen Fig. 5.1. This effect resulted from the fact that the methanogens presents in the digestate (inoculum) was highly active for biogas production. Thermal pretreatment allowed to improve the removal yield, thus biogas (methane) production also improved too as seen from the Fig. 5.1. The methane content of biogas produced by PPMS is of same order of methane content during the production stage in both studies, whereas after thermal pretreatment biogas yield was improved to 23 %, thereby increased the rate of methane production after thermal treatment. Thus, thermal pretreatment allowed an improvement in anaerobic digestion performance around 23 % (for biogas production) for a treatment at 90^{o}C. The methane yield obtained from the continuous study was not significantly different from the BMP and batch study of PPMS, and experimental methane yield was an equal to 310 mL CH_4/g of VS. Thus is accordance with literature results (Bougrier et al., 2007; Lin et al., 2011a).

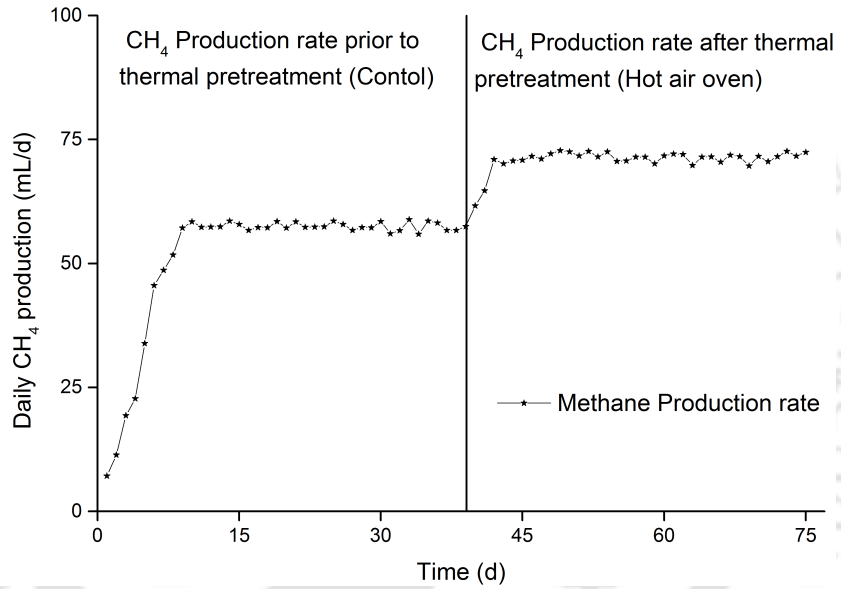

Fig. 5.1. Methane production profile of AAPFR fed with PPMS (with and without pretreatment) at 21 d HRT and 6.3 kg VS/m^3/d

5.1.2.2 Biogas content (%) (CH_4 and CO_2), pH, sCOD and VFA concentrations profile in the semi-continuous AAPFR

The Fig. 5.2 shows the biogas content, pH, sCOD, and VFA concentrations in semi-continuous fed, laboratory-scale AAPFR at mesophilic anaerobic condition. The biogas composition was stabilized after day 10 with CH_4 and CO_2 content maintained at approximately 56-60 % and 39-42 %, respectively for both control and thermal pretreatment (Fig. 5.2). However, an obvious increase in methane production were observed on the first 10 d of semi-continuous experiment, and then a smooth increase of methane production and a stable value of pH were observed until 75 d, when a fill and draw mode with one feeding per day with an OLR of 6.3 kg /m^3 d based on VS fed. The sCOD and VFAs concentrations were always less than 7 mg/L and 3 mg/L, respectively during this period, which were suitable for the acetogens and methanogenic microorganisms. Lin et al. (2011a)

achieved similar VFA concentration range with mesophilic anaerobic digestion of paper mill sludge. The sCOD concentrations maintained in the range of 3 to 8 kg/m^3 which was consistent to the sCOD trend obtained by Salminen and Rintala (2002) and Lin et al. (2011a).

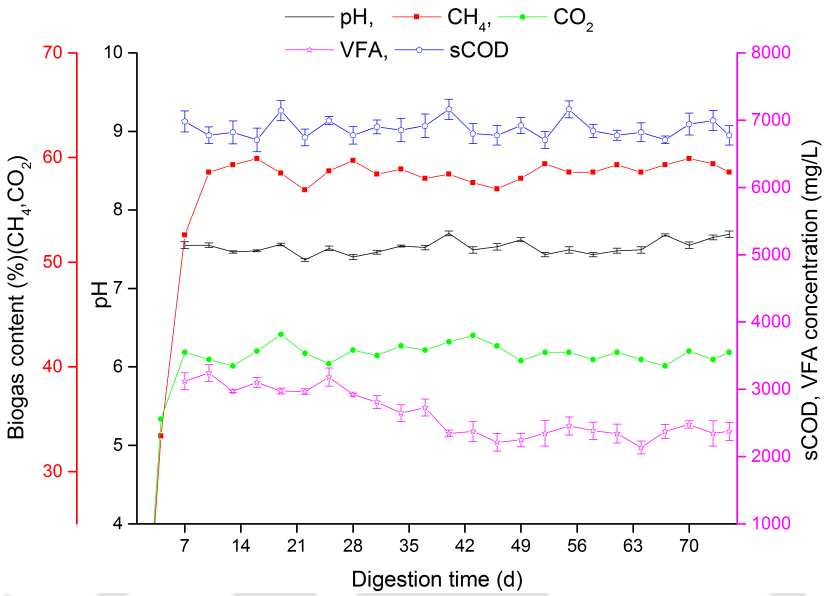

Fig. 5.2. The variation of Biogas content (%) (CH_4 and CO_2), pH, sCOD and VFA concentrations in the semi continuous AAPFR

Due to thermal pretreatment at (80^{o}C for 90 min) lower temperature, the refractory compounds like melanoidines were not formed during and after pretreatment of PPMS. Hence, pretreated PPMS becomes more solubilized, which could be used efficiently for biogas production. Thermal pretreatment allowed an improvement in methane production from 264±4 mL CH_4/g VS for control to 310±5 CH_4/g VS for pretreated PPMS. During this experimental period, change in pH was not observed, throughout the digestion time pH was in optimum range. In addition, biodegradable organic substance can be removed through conversion into methane and CO_2 during anaerobic process, resulting in sCOD decrease in the digester. Total VFAs concentration followed the same general trend as the vales of sCOD (Fig. 5.2), accounting for most of the sCOD.

5.1.3 Operation of AAPFR at UoG, Canada

5.1.3.1 Methane production profile from AAPFR with varied OLR

The reactor performance data in the course of digestion time with special emphasis on biogas production, pH value , VFA (FOS) concentration, buffer capacity (TAC), and FOS/TAC ratio in the influent, port-I, port-II, and effluent (Fig. 5.3). It was clearly seen from the Fig. 5.3, which demonstrate the effect increasing OLR upon other parameters. For the first stage of the AAPFR experiment, after start-up on 0-10 d with the OLR 3.5 kg VS/m^3/d, a sharp increase of biogas production was observed. Unfortunately, during this period different other parameters has not been recorded. This sharp peak in biogas production was resulted from the fact that the OLR in AAPFR was lower than that in the full-scale reactor from which the inoculum was usually obtained and there

were some VFA remained in the reactor when the start-up stage ended. This causes the greater consumption of VFA than generation in the first stage (Lin et al., 2011a; Wan et al., 2011). However, there was further increase in biogas production for each step increase in OLR (Fig. 5.3(a)). The start-up phase of AAPFR lasted for a shorter time (10 d) and also for Lin et al. (2011a) than that (14 d) reported by Zupančič and Jemec (2010) when the process got fully adapted to the substrate. Throughout experimental period, the pH of the AAPFR was within the optimum range (Fig. 5.3(b)) for both acidogenic and methanogenic microorganisms.

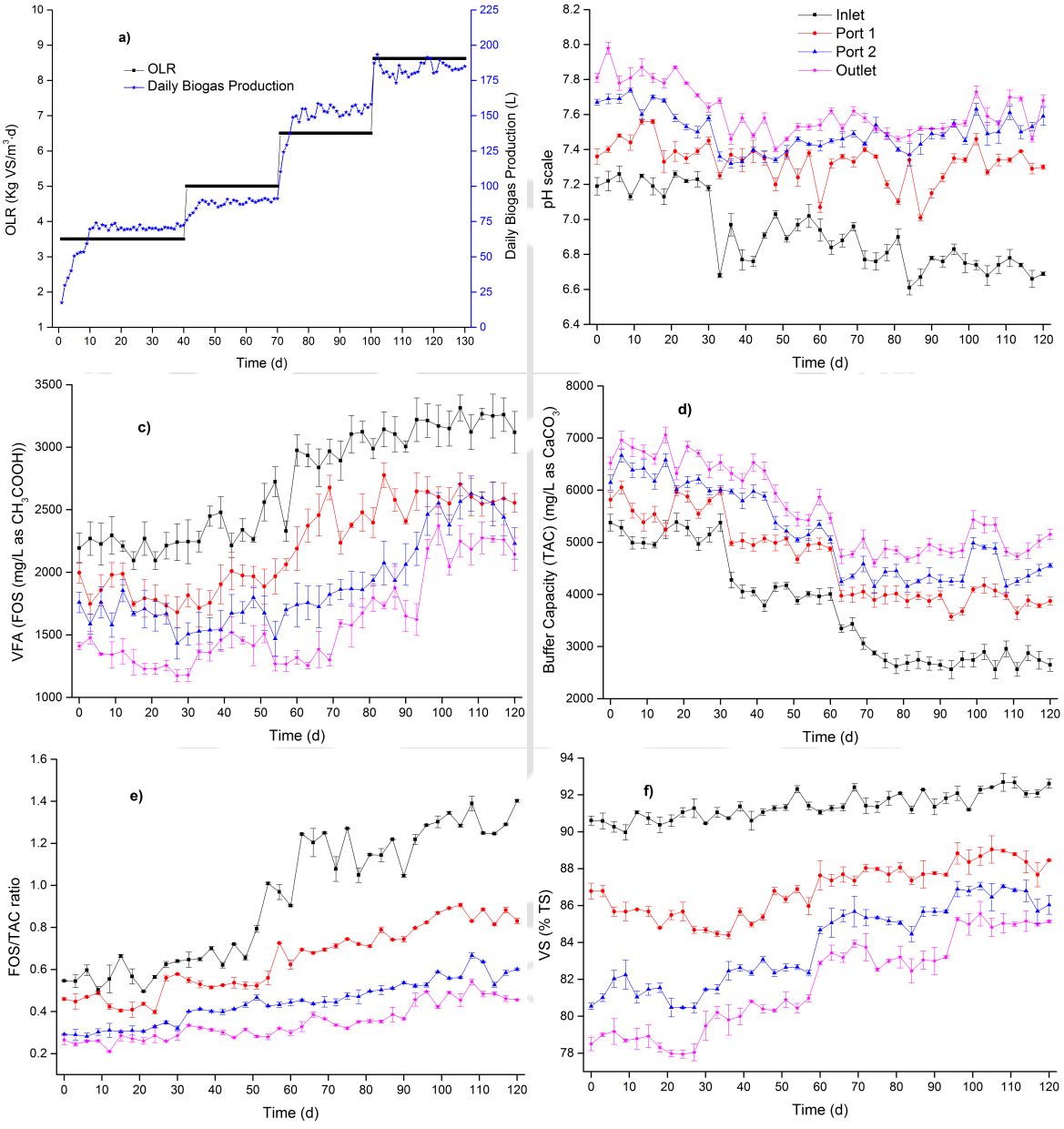

Fig. 5.3. The variation of biogas production rate (a), pH scale (b), VFA (as FOS) concentrations (c), buffer capacity (as TAC)(d), FOS/TAC ratio (e) and VS reduction (f) with OLR increased in the semi-continuous AAPFR experiment

After start-up phase, every 30 d interval (i.e on 40th d, 70th d, and 100th d) the OLR was increased, resulting in a further increase of biogas production and an optimum pH range throughout the experimental period. But VFA concentrations (FOS value) at the outlet was increase in increasing

OLR and buffer capacity (TAC value) at the outlet was decrease with increase in OLR such as behave as vice versa. The AAPFR performance was better also stable and attain a maximum CH_4 yield of 0.410 m^3/kg based on VS degraded at OLR loaded at 6.5 kg VS/m^3/d (Table 5.4). During OLR of 8.8 kg VS/m^3/d, the reactor attain maximum CH_4 production rate 0.787 m^3/m^3/d but decrease in CH_4 yield of 0.360 m^3/kg based on VS degraded when compared to OLR 6.5 kg VS/m^3/d (0.410 m^3/kg). The maximum CH_4 yield obtained in this work (0.410 m^3/kg based on VS) was lower than that (0.598 m^3/kg) attain from the co-digestion of thickened waste activated sludge and fat, oil, and grease (FOG) because of the more optimal mixing substrate for methane production by Wan et al. (2011).

5.1.3.2 pH profile from AAPFR

The pH profile (Inlet, port-I, port-II, outlet) during the long-term semi-continuous experiment was shown in Fig. 5.3(b). Fig. 5.4 shows the daily pH profile of inlet and outlet of AAPFR. The pH is the crucial factor in maintaining good performance of an anaerobic system. The pH value were stable in the range of 6.5-7.8, which was an optimum pH value for the methanogens. This was mainly due to the greater consumption of the organic acids generating from the substrates (corn silage) and the further more formation of ammonia in the reactor. Although corn silage was acidic in nature (Table 5.1), it have higher buffer capacity and hence no base material was used to adjust the pH value in the reactor during the long-term semi-continuous experiment. At the beginning, pH value was little higher due to low OLR and hence it has lower buffer capacity. The increasing in OLR resulted in a decrease in pH value. This could be attributed due to increasing rate of acidogensis and non-proportional growth of methanogens (which consumes CO_2 as a substrate to produce methane). A slight difference in pH value (Fig 5.4) was noticed during the digestion period, probably attributable to their slight difference in alkalinity and buffering capacity during the digestion process.

5.1.3.3 Volatile fatty acids (VFAs as FOS) profile from AAPFR

The VFAs play an important role in maintaining an efficient anaerobic digestion performance due to its strong effects on pH and alkalinity in the digester (Buyukkamaci and Filibeli, 2004). In addition, VFAs can also be used as a good parameter to signal the process imbalance in anaerobic digestion. Fig. 5.3(c) displays the VFAs (FOS) profile (Inlet, port-I, port-II, outlet) during the long-term semi-continuous experiment. Fig. 5.4 shows the daily VFAs profile of inlet and outlet of AAPFR operation. The VFAs concentrations at outlet were always less than 2.0 kg/m^3 up to OLR 6.5 kg/m^3/d, which were suitable for the acetogenic and methanogenic microorganisms. Riau et al. (2010) and Wan et al. (2011) attain similar VFAs concentration range in mesophilic anaerobic digestion from sewage sludge. In general, biodegradable organic substances can be removed through conversion into CH_4 and CO_2 during the anaerobic process, resulting in VFAs reduction in the digester. As shown in Fig. 5.3(c) and Fig. 5.4, the initial and final VFAs concentration was increased with increase in OLR and reduction in SRT. However, the OLR at 8.8 kg/m^3/d shows VFA accumulation thus an obvious increase of VFA concentration (1,500 to 2,300 mg/L as acetic acid) (Fig. 5.4) in the effluent was observed and methane yield decreased significantly from 0.410 to 0.360 m^3/kg.

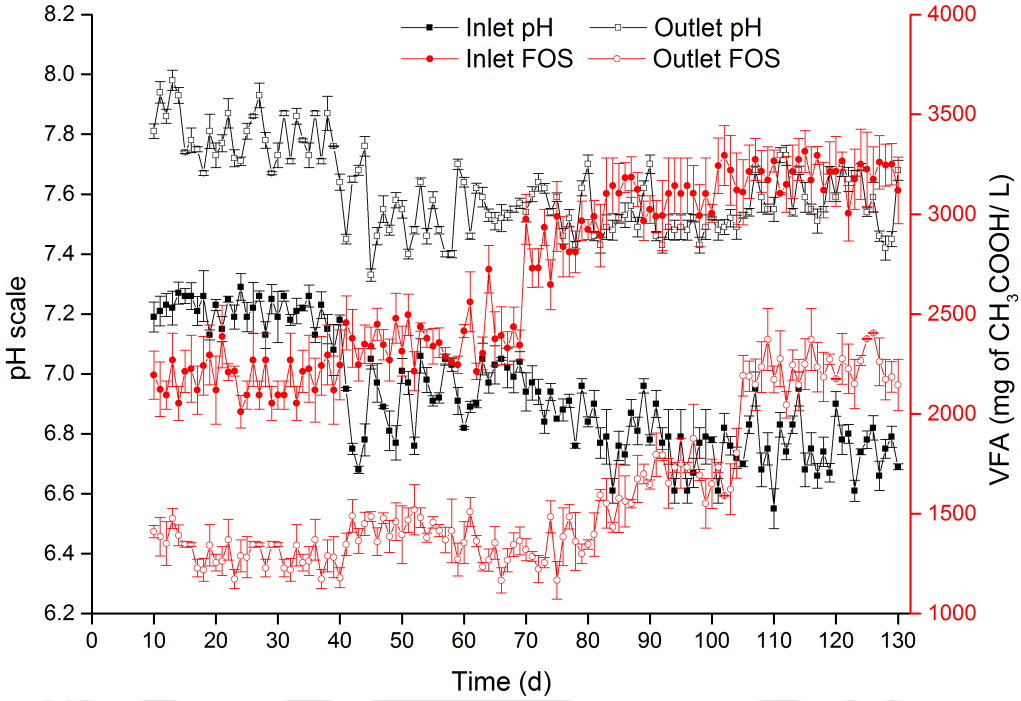

Fig. 5.4. The daily variation of pH and VFA (acetic acid) (FOS) concentrations during the course of AAPFR operation

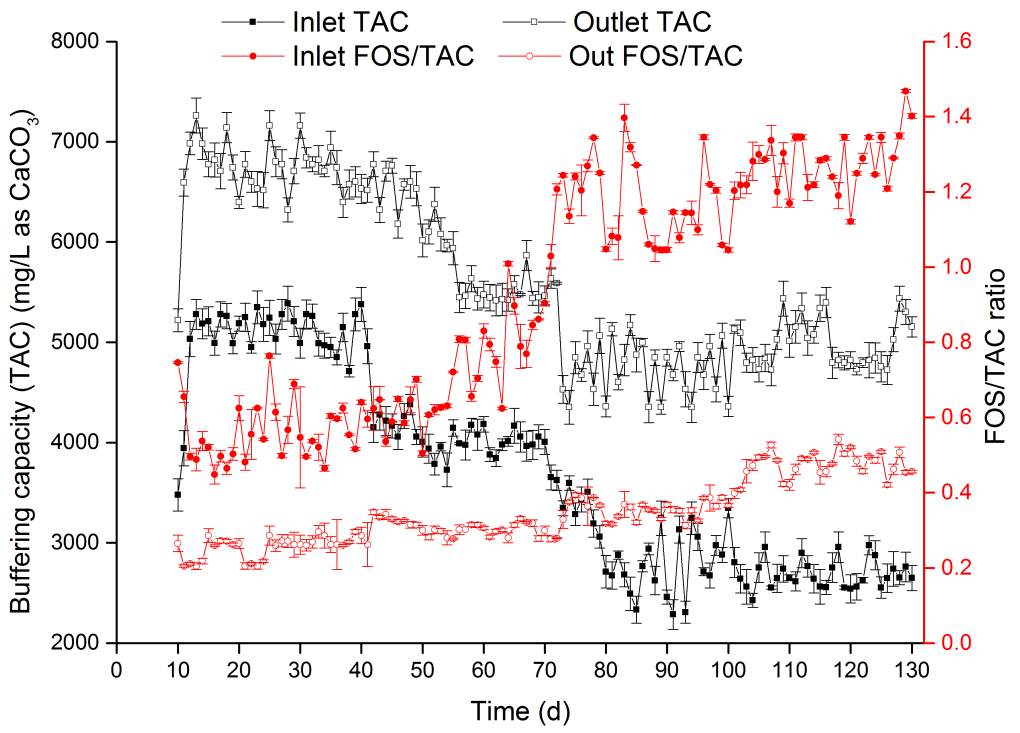

Fig. 5.5. The daily variation of buffering capacity (TAC) and FOS/TAC ratio during the course of AAPFR operation

5.1.3.4 Buffering capacity (TAC) and FOS/TAC ratio profile from AAPFR

During the digestion process, alkalinity is a better indicator of process performance and directly shows the digester buffering capacity. This could be managed by adjusting the pH value, thus

pH adjustment could provide way to improve the self-buffering capacity in the system to meet the requirements of the microbial populations. Buffering capacity could affects the activities of the specific acidogenic and methanogenic microbial population, and consequently influence the process stability. In general, the buffering capacity (alkalinity) varies from 1,000 to 5,000 mg/L as $CaCO_3$ in anaerobic system Khanal (2011). Fig. 5.3(d) shows the buffering capacity (TAC) profile (Inlet, port-I, port-II, outlet) during the long-term semi-continuous experiment. Fig. 5.5 shows the daily buffering capacity profile of inlet and outlet of AAPFR. It was observed in Fig. 5.3(d) and Fig. 5.5; the initial and final buffering capacity was decreased with increase in OLR. As corn silage (substrate) and digestate (inoculum) have higher buffering capacity observed when the reactor was loaded with low OLR. During this time of operation, biogas production and methane yield were also in increasing trend as increasing in OLR. When the AAPFR loaded with 6.5 kg/m^3/d OLR, the reactor attains the optimum buffering capacity and hence there was high methane yield 0.410 m^3/Kg VS based.

Table 5.3. Thumb rule for assessment of FOS/TAC ratio during anaerobic digestion

FOS/TAC ratio	Background	Measure to be taken for remediation
>0.6	Highly excessive biomass input	Stop adding biomass
0.5-0.6	Excessive biomass input	Add less biomass
0.4-0.5	Reactor is heavily loaded	Monitor reactor more closely
0.3-0.4	Biogas production at a maximum	Keep biomass input more constant
0.2-0.3	Biomass input is too low	Slowly increase the biomass input
<0.2	Biomass input is far too low	Rapidly increase the biomass input

The FOS/TAC ratio is the indicator for accessing anaerobic digestion process. The application of FOS/TAC value was very high in industrial scale operation in most of the European and North American. The FOS/TAC ratio can be used as a good parameter to signal biomass content in anaerobic digester. TAC value is the estimation of the buffering capacity of the sample and the FOS value corresponds to the VFAs content. Table 5.3 shows the general thump rule followed in anaerobic digestion system to assess the FOS/TAC ratio. Fig. 5.3(c) displays the FOS/TAC ratio (Inlet, port-I, port-II, outlet) during the long-term semi-continuous experiment of AAPFR. It was observed from Fig. 5.3(e) and 5.5, the initial (huge increment) and final FOS/TAC ratio was increased with increase in OLR. There was high FOS/TAC ratio (up to 1.5) in the inlet, the AAPFR can able to handle without any trouble in its operation. However, at OLR 8.8 kg/m^3/d the AAPFR experience the high excessive biomass content in the digester. This was attributed to the lower removal efficiency (Table 5.4) at high loading rate. In this study, it was perceived that the OLR at 6.5 kg/m^3/d experiences the higher biogas production with optimum FOS/TAC ratio range (0.3-0.4).

5.1.3.5 Volatile solids (VS) reduction profile from AAPFR

The mass loss from the anaerobic digestion system was indicated by the decrement in VS that was correlated directly to biogas production. Higher degradation designates higher VS and TS

reduction that yield significantly more biogas. The reduction was mainly depends on the activity and adaptability of the inoculum towards substrate in the anaerobic digestion system. The AAPFR presented the various rate of removal in term of VS and TS. The reactor fed with corn silage showed a faster substrate removal rate. Fig. 5.3(f) portrays the VS reduction profile (Inlet, port-I, port-II, outlet) during the long-term semi-continuous experiment of AAPFR. An increase in biogas production was observed with an increase in OLR, despite of decrease in VS and TS removal percent as shown in Table 5.4. This may be attributed to the fact that although there was a decrease in VS removal rate at higher loading rates; even than the VS removal rate was not that much low at higher OLR than that at lower OLR as seen in Fig. 5.3(f). As corn silage is lignocellulose material, difficult to degrade at lower retention time and hence low VS reduction was obtained in the present study.

Table 5.4. The percent reduction of VS and TS, average production of biogas content and methane yield at varied OLR during AAPFR operation

OLR (kg VS/m^3d)	Percent reduction (%)		Average daily production rate (L/d)			CH_4 yield (m^3/kg)
	VS	TS	Biogas	CH_4	CO_2	
3.5	62.04	57.16	69.62	45.32	24.31	327.31
5.0	57.36	52.35	86.61	55.98	30.63	333.48
6.5	49.53	44.34	145.37	92.12	53.26	410.00
8.8	36.67	31.09	181.02	106.85	74.17	360.21

5.1.3.6 The composition of biogas at varied OLR in AAPFR operation

Table 5.5 presents the composition of average biogas content at varied OLR during AAPFR operation. Fig. 5.6 portrays the volumetric concentrations of CH_4, % and CO_2,%), hydrogen sulfide ($H2_S$, ppm), and temperature profile during the course of AAPFR operation. The increase in biogas production was observed with an increase in OLR (Table 5.5 and Fig. 5.6) in AAPFR operation. This may be attributed to the fact that the increased availability of organic matter from the substrate. In addition to this, increase in OLR resulted in a decrease in methane content and increase in H_2S concentration (Table 5.5 and Fig. 5.6). This can be attributed to increasing rate of acidogenisis and non-proportional growth of methanogens (which consumes CO_2 as substrate to produce CH_4). However, the reactor showed a stable operation at an OLR 6.5 kg/m^3/d. The reactor lost its stability at an OLR 8.8 kg/m^3/d, which was apparent by decrease in biogas production and its methane content. The result obtained were in agreement with the observation made by other workers (Lin et al., 2011a; Linke, 2006) with respect to varied OLR.

The biofilter filled with activated charcoal (20 g) were used to scrub the H_2S concentrations from the biogas produced from the AAPFR. The H_2S concentrations profile from the Table 5.5 and Fig. 5.6, it was observed that activated charcoal has high adsorption capacity to scrub the H_2S concentrations. As biogas production was in huge quantity, activated charcoal attain equilibrium in very short duration. It could be suggested that the somewhat bigger biofilter has been used to scrub the H_2S concentrations.

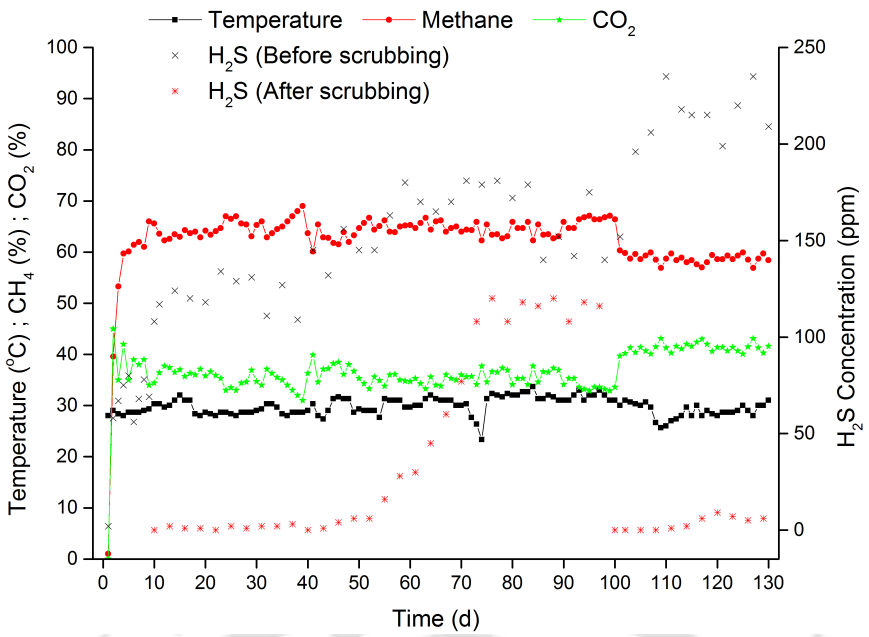

Fig. 5.6. The concentrations of CH_4, (%), CO_2, (%), H_2S, (ppm), and temperature (^oC) profile during the course of AAPFR operation

In this study, it was observed that the critical OLR for AAPFR fed with corn silage and digestate was 6.5 kg/m^3/d with a 17 d SRT gives higher biogas production. According to Lin et al. (2011a), the loading rate and SRT for PPMS and digestate were typically in the range of (1.5 to 6.0) kg/m^3/d with 15 d SRT. From this long-term experiment, it was perceived that anaerobic digestion of corn silage and digestate was feasible in plug flow reactor up to 12 % TS content. It indicated that this AAPFR was more efficient to treat various other agricultural residue for methane production.

Table 5.5. The composition of biogas (average) at varied OLR during AAPFR operation

OLR (kg VS/m^3 d)	SRT (d)	Volume		H_2S concentrations (ppm)	
		CH_4	CO_2	Before scrubbing	After scrubbing
3.5	25	65.09	34.91	108.0	1.4
5.0	21	64.63	35.37	157.1	19.6
6.5	17	63.37	36.63	164.1	110.9
8.8	13	59.03	40.97	215.5	3.3

5.1.4 Development of kinetic model for AAPFR

The simple model developed here defines the biogas production process for an AAPFR. To develop the kinetic model mass balance equation with equal mass flow of input and output (m_o) can be written as (mass of the biogas is neglected).

$$dv(\frac{dC_o}{dt}) = m_o C_o - m_o(C_o + dx) + dvr_c \quad (5.1)$$

The substrate removal rate (r_c) as a function C_o is expressed as first order kinetic can be written as

$$\frac{dC_o}{dt} = r_c = -kc \tag{5.2}$$

At steady state $\frac{dC_o}{dt} = 0$ and combining Eq. (5.1) and (5.2). Equation for the HRT has been obtain

$$HRT = \frac{1}{k} log \frac{C_o}{C} \tag{5.3}$$

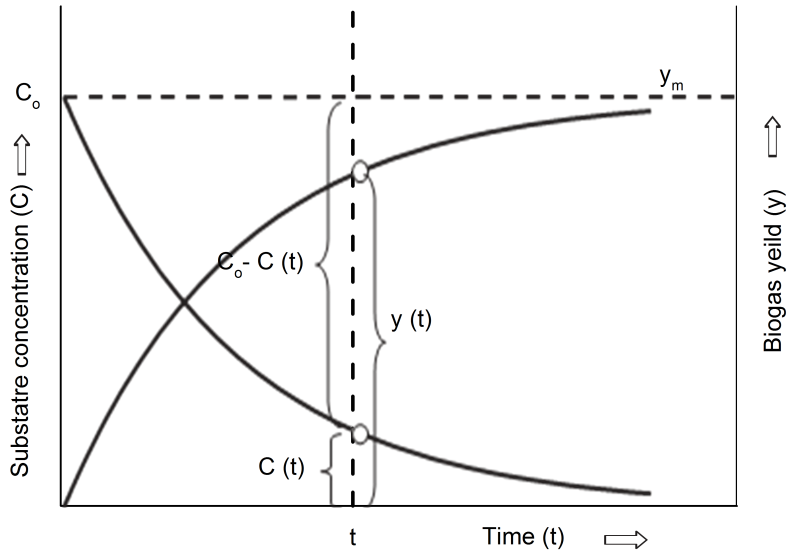

Fig. 5.7. Correlation between the substrate degradation and biogas (CH_4) production in the anaerobic digestion process

The overall correlation between substrate concentration C and biogas yield (y) at time t is shown in Fig. 5.7. The biodegradable fraction present in the complex organic substrate is disintegrated into biogas yield according to Eq. (5.4) and (5.5), respectively.

$$\frac{C_o - C(t)}{C_o} = \frac{y(t)}{y_m} \tag{5.4}$$

$$\frac{C_o}{C} = \frac{y_m}{y_m - y} \tag{5.5}$$

The hydraulic retention time of a plug flow reactor can be written as Eq. (5.6) by using Eq. (5.5).

$$HRT = \frac{1}{k} log \frac{y_m}{y_m - y} \tag{5.6}$$

and the biogas yield from the volatile solids fraction can be written as:

$$y = y_m(1 - e^{-(k.HRT)}) \tag{5.7}$$

For dimensioning the reactor size of the AAPFR, both the OLR and HRT are the most applied parameters in practice. The Eq. (5.6) and (5.7) can be rewritten as Eq. (5.8) and (5.9), respectively by using the OLR=C_o/HRT.

$$OLR = \frac{kC_o}{log\frac{y_m}{y_m - y}} \tag{5.8}$$

$$y = y_m[1 - e^{-\frac{kC_o}{OLR}}] \tag{5.9}$$

In order to dimension the reactor size by means of HRT or OLR, it is necessary to calculate the y_m, and k. Based on y_m, k and Co, both the reactor size and reactor performance data can be calculated. The maximum biogas yield (y_m) is equivalent to the ultimate anaerobic biodegradability and results when the OLR value is near zero. The maximum biogas yield (y_m) can be calculated from a single batch test, whereas the calculation of k could be obtained from the long-term experiments in a AAPFR.The value for k could be obtained by plotting log[y_m/(y_m-y)] against HRTor 1/OLR. The slope of the straight line yields k or kC_o , respectively.

Therefore, biogas yield y can be expressed as an absolute proportion p (p=y/y_m), and the correlation between HRT and p results from Eq. (5.11). Fig. 5.8 shows the absolute proportion of p for y_m for different value of HRT and k. The Eq. 5.10 indicates that k increases with decrease of HRT for a constant p. For example, in order to obtain 90 % of y_m, and for k = 0.1, the HRT requires 23 d.

$$HRT = \frac{1}{k} log(\frac{1}{1-p}) \tag{5.10}$$

$$p = 1 - e^{(k.HRT)} \tag{5.11}$$

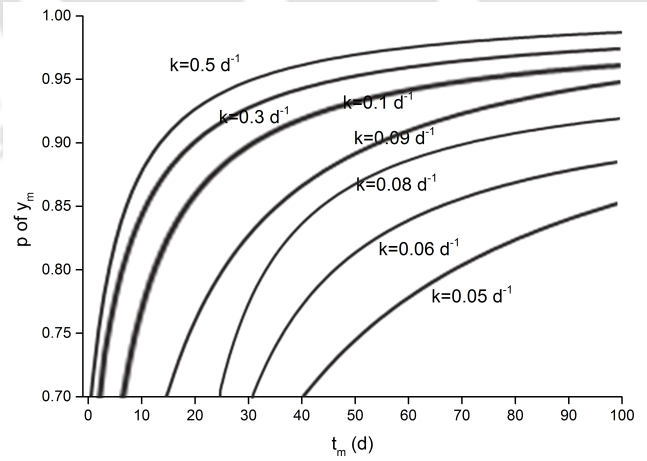

Fig. 5.8. Absolute proportion p of y_m for different value of HRT and k

5.1.4.1 Application of kinetic model

Results from the long-term mesophilic anaerobic digestion with corn silage (C_o=110 kg of VS/m^3) as described above were used to apply model. However plotting of all observed biogas yield and CH_4 in the biogas against the corresponding value of OLR resulted in both decrease of y and CH_4 with

increase of OLR (Fig. 5.9). The maximum biogas yield can be obtained from curve fitting according to Eq. (5.9) and resulted to 0.869 m^3/kg of biogas production (Fig. 5.9).

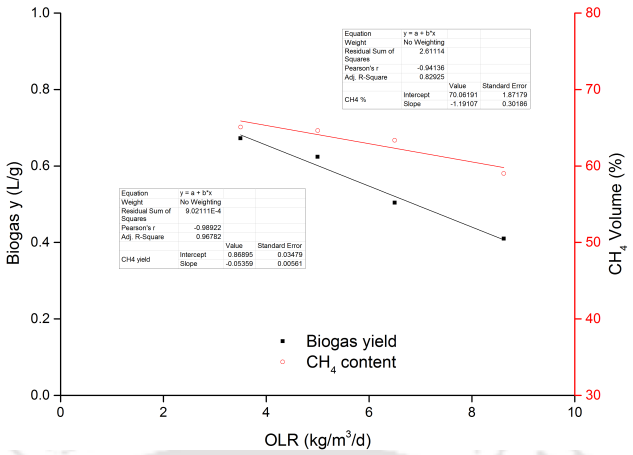

Fig. 5.9. Effect of OLR on biogas yield (y) and CH_4 composition from AAPFR experiment

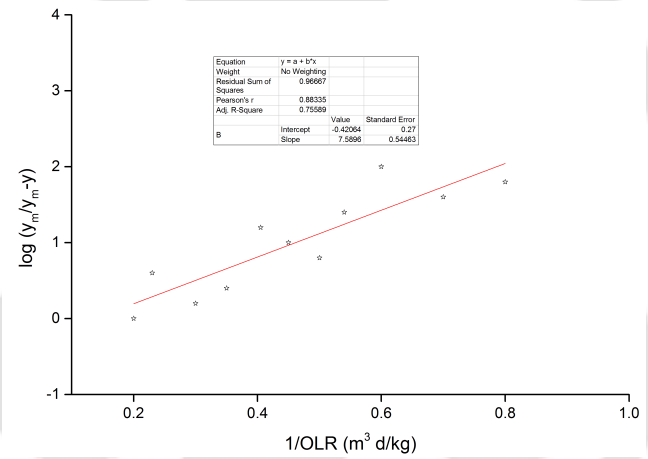

Fig. 5.10. The graph of $log\frac{y_m}{y_m-y}$ and 1/OLR from semi-continuous AAPFR experiment

The reaction rate constant k resulted from the plot of log[y_m/(y_m-y)] against 1/OLR and the slope of k.Co= 7.589 kg/m^3/d as well as k=0.0689 d^{-1} due to C_o=110 kg/m^3.The value of k (0.0689 d^{-1}) in this experiment was a little lower than 0.089 d^{-1} obtained by Linke (2006). This increased k value for Linke, (2006) was because the potato waste biodegradation was at higher degree. However, by means of this parameter, the reactor performance data can be calculated. For example, in order to obtain 80 % and 90 % y_m, the required HRT resulted from Eq. 5.10, was 26 d and 33 d, respectively (Fig. 5.8). However, longer HRT will certainly reduce the utilization efficiency of the reactor and decrese the biogas production per d. Therefore, it is necessary to choose an optimal HRT to obtain the highest p and the reactor utilization efficiency in practice.

5.1.5 Conclusions

In this phase, the lab scale AAPFR experiments had been studied with the special emphasis on effect of increasing OLR on the methane production in long-term experiments. AAPFR had been

designed for the treatment of organic solid waste with the special emphasis on more biogas recovery from the lignocellulose material such as PPMS, corn silage etc. The AAPFR experimental studies were carried out in two place: one at India, IITG (Environmental lab) campus and another one at Canada, University of Guelph Ridgetown Campus. At India the AAPFR was operated for 75 d with thermal pretreatment (30 d) and without pretreatment (30 d) at 21d HRT with specified OLR (6.5 kg VS/m^3/d). The methane yield obtained from the continuous study was equal to 310 mL CH_4/g of VS degraded. The biogas composition was stabilized after day 10 with CH_4 and CO_2 content maintained at approximately 56-60 % and 39-42 %, respectively for both control and thermal pretreatment. At Canada, study was majorly emphasized on the effect of increasing OLR on the methane production in long-term experiments (130 d) in corn silage (lignocellulose material). The increase in biogas production was observed with an increase in OLR. In addition to this, increase in OLR resulted in a decrease in methane content and increase in H_2S concentration. However, the reactor showed a stable operation at an OLR 6.5 kg/m^3/d. The reactor lost its stability at an OLR 8.8 kg/m^3/d, which was apparent by decrease in biogas yield and its methane content. Using the continuous lab scale experimental data, a new kinetic model had been developed. The biogas yield (y) as a function of maximum biogas yield (y_m), reaction rate constant (k), and HRT are described based on a mass balance and first order kinetic in a AAPFR. In this study, the values for ym and k were obtained as 0.789 L/g and 0.068 per d. respectively. This simple model can be apply for dimensioning plug flow reactor digesting different organic wastes such as agricultural residue, animal waste slurries, biogas crops etc.

"Earth provides enough to satisfy every man's needs, but not every man's greed."

Mahatma Gandhi

Chapter 6

MODELLING ON ANAEROBIC DIGESTION

This chapter deals with the development and validation of mathematical modelling on solid-state anaerobic digestion (SS-AD). The detailed methodology has given in this chapter.

6.1 PHASE V: A MASS DIFFUSION MODEL ON THE EFFECT OF MOISTURE CONTENT FOR SOLID-STATE ANAEROBIC DIGESTION

Objective of phase V:This phase was emphasized on development of mathematical model based on experimental data. The methane production rate increases with a decrease in moisture content (MC) up to the optimum threshold level and diminish in a feedstock fed with the PPMS (lignocellulosic material) in SS-AD. The interpretation on two-faceted effect of moisture content could not be elucidated by the conventional Anaerobic Digestion Model No 1 (ADM1). This study proposed that the decreased moisture content causes augmented mass diffusion resistance by the accumulation of hydrolytic product. This could lead to the reduced production of methane gas. Based on the hypothesis, a new solid state anaerobic digestion model was developed based on mass diffusion limitation and hydrolysis inhibition.

6.1.1 Overview of model development

The understanding of fundamental mechanism based on the effect of MC on CH_4 production rate is necessary to optimize the SS-AD system. General observation was that the hydrolysis or methanogenesis be the rate-limiting step for the Liquid state-anaerobic digestion (LS-AD) system. Li and Wang (2011) depicted that hydrolysis was the major constrained for the SS-AD. Abbassi-Guendouz et al. (2012) tried to fit the experimental data from the SS-AD into the LS-AD of ADM1 and noted that the value of the first order hydrolysis rate coefficient lower in the model. To explain mathematically, the lowered hydrolysis rate coefficient was attributed to the reduced CH_4 production rate from the cardboard waste under a low MC i.e. less than 80 %. The mechanism beneath the reduced CH_4 production rate was not revealed.

There was limited information regarding the compromised hydrolysis rate in AD under a lower MC (Vavilin et al., 2008) while it was generally perceived in studies of enzymatic hydrolysis for ethanol production from cellulosic biomass (Cara et al., 2007). Kristensen et al. (2009) depicted that excessive accumulated sugar could cause the diminished hydrolysis rate during the enzymatic hydrolysis of lignocellulose material under a low MC. The hydrolysis inhibition by the accumulated sugar under a low MC was a plausible elucidation for the SS-AD system (Ge et al., 2016). Some

vital component was still missing. The prime difference between SS-AD and enzymatic hydrolysis is that SS-AD contain huge quantity of sugar consumer than their production of hydrolytic product, when hydrolysis was a rate-limiting step. These hurdles can be overwhelmed, only when microbes are able to access the sugars or else sugar accumulation phenomenon can occur due to increased mass diffusion resistance in SS-AD. Bollon et al. (2013) reported that mass diffusion coefficient was increased by two order of magnitude in SS-AD than LS-AD. Current study aimed to develop the mathematical model based on the influence of MC on limited mass diffusion rate that leads to the hydrolysis inhibition caused by the aggravated hydrolytic product during hydrolysis steps in the SS-AD system. The mathematical model is able to predict the correlation between different variables without understanding in-depth mechanism. The developed mathematical model could be compared against other statistical models for model validation.

The magnitude of hydrolysis inhibition caused by the internal mass diffusion resistance is one of the prime difference in SS-AD and LS-AD. This prime difference was not considered in ADM1. The mathematical model developed for the SS-AD in this study could interpret the influence of MC on the rate of CH_4 production, which was based on limited mass diffusion that cause hydrolysis inhibition by the gathered hydrolytic product.

6.1.2 Basic assumption and its physical process

The model for the proposed study was developed for the SS-AD in a batch reactor fed with PPMS and cow dung. Prior to loading in a batch reactor, PPMS and cow dung were well mixed. From these mixing it was assumed that cow dung was completely dispersed into each and every point of the microflora and evenly scattered in sludge bed. To utilize mathematical simulation, the substrate layer was assumed to be enclosed with each microflora of small sphere in a three dimensional in the sludge bed. Each substrate layer was in contact with numerous thin substrate layer. The void between the spheres was neglected because of its microscale. During the period of digestion, each microflora excretes extracellular hydrolytic enzyme to the surrounding substrate layer that converts the holocellulose to sugars. It was assumed that the substrate layer has higher sugar concentration at the outer surface (S) and lower concentration at the inside microflora (S'), due to the consumption of sugars by the microbes. Because of the concentration gradient, there was a mass diffusion by sugar that diffuses into the inside of microflora. It forms the sugarshed in the substrate layer (S) by permitting the sugars to flow towards each microflora (S') (Fig. 6.1).

It was difficult to determine the microflora diameter. Thiele et al. (1988) reported in a well-mixed anaerobic digester that the diameter typically varies in the range of 10-100 μm to represent the syntrophic behavior of microflora. Based on the theory of mass diffusion, there was a diffusion boundary, that should persist at a certain distance from the surface of each microflora and beyond this boundary there will not be a diffusion to happen. Casey et al. (2000) depicted in a well-mixed reactor that depending on the hydrodynamic condition these diffusion boundary layers may range from 10-1,000 μm. Due to the digestion of organic material, there should be a release of cellulase from the microflora that was assumed to be distributed evenly in the substrate layer. There was a gradient of cellulase concentration persists in the substrate layer, very rare research was found in this

regard to cellulase diffusion in the solid state.

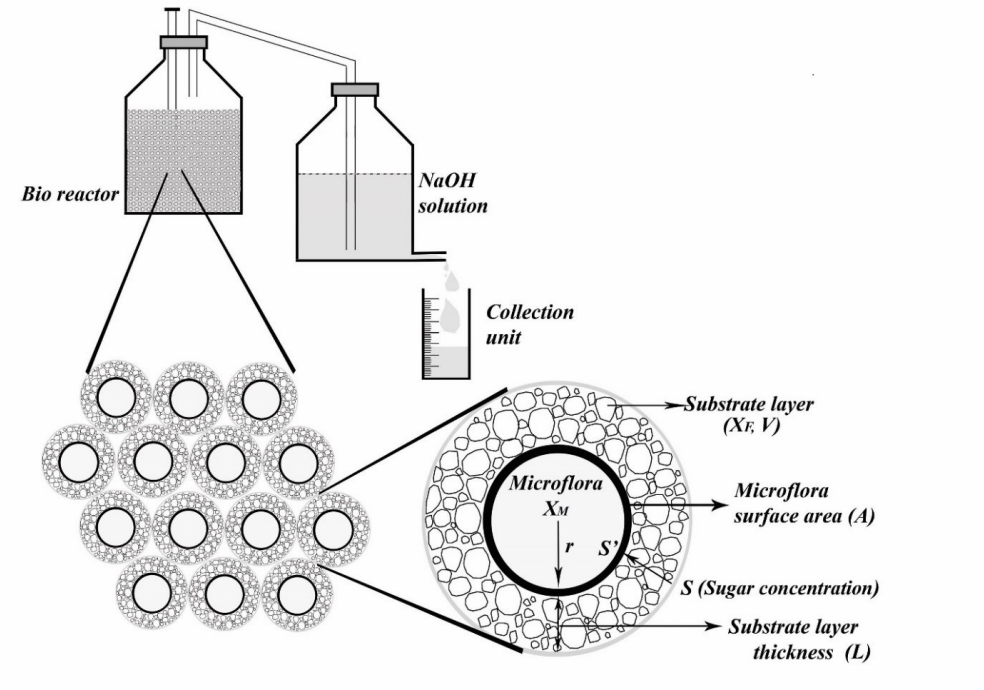

Fig. 6.1. Schematic sketch of solid-state anaerobic digestion for the model hypothesis

Based on the above premise, the proposed model was developed for the AD in which hydrolysis is the rate limiting step, problem like acidification in overload reactors were not inclusive in this model prediction. Other steps involved in AD process such as acidogenesis, acetogenesis and methanogenesis were pooled into a single step because these steps occurred fast enough, and only a few soluble monomers stayed in a microflora. Due to its synergic microbial community, microflora was assumed to be living in a close affinity and densely packed in order to provide a thermodynamically favorable condition. McCarty and Smith (1986) reported in AD that maximum allowable distance for a syntrophic community is 1-2 μm (De Bok et al., 2004). It was assumed that due to its densely packed, each microflora function like a discrete micro reactor that could be expanded up to merging with the adjacent micro reactor. According to the available literature, the decay rate of anaerobic microbes was around 0.01 per d, that was lesser than 5 % of maximum growth rate as reported by Batstone et al. (2002). In order to avoid complexity in this model development, biomass decay was not included but hydrolytic inhibition because of the accumulation of hydrolytic product was considered that was the major difference for this proposed model.

6.1.3 Development of mathematical model

This study aimed to develop the mathematical model to validate the hypothesis based on the influence of MC by utilizing / solving the following models such as biological reaction model, mass diffusion model, mass balance model, and pseudo-steady state model. Fig. 6.2 shows a procedural schematic flow of the various models interlinking for model development and its validation.

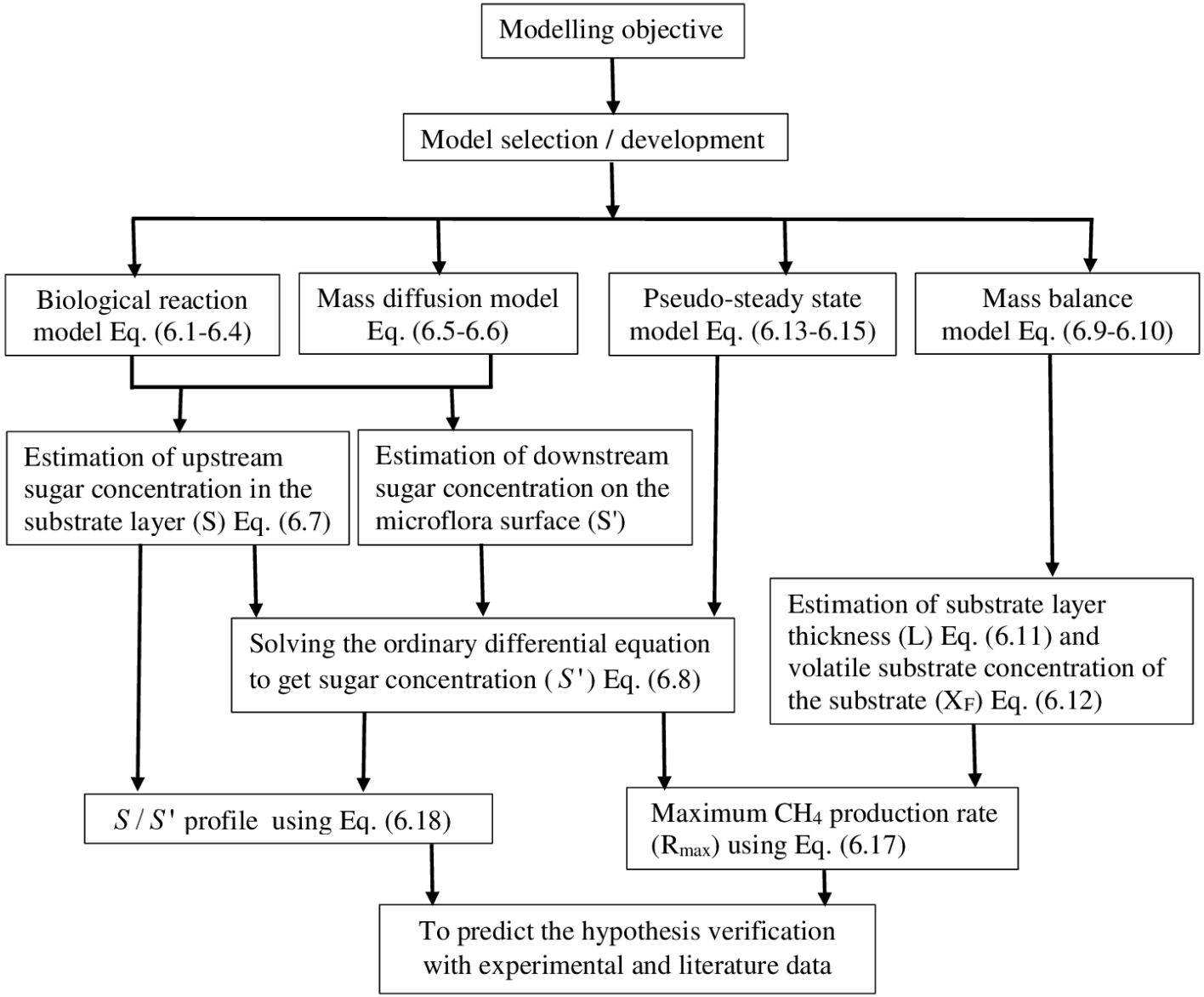

Fig. 6.2. Proposed hypothesis estimation procedure for mathematical modelling

6.1.3.1 Biological reaction model

The biological reaction model could be defined as the use of computational simulations of biological reaction in a system to analyze and visualize the complex connection between the biological reactions. Based on ADM1 (Batstone et al., 2002), hydrolysis rate was assumed to follow first order kinetics without any inhibition as shown in Eq. (6.1). The reduction in hydrolysis rate due to inhibition was described by the Eq. (6.2).

$$R_h = k_h X_F \tag{6.1}$$

$$R_i = k_i S \tag{6.2}$$

R_h and R_i are the hydrolysis rate and its reduction rate due to inhibition of sugar, k_h and k_i are first order hydrolysis rate coefficient and inhibition coefficient of hydrolysis. X_F and S are the volatile substrate concentration and sugar concentration in the substrate layer as shown in Fig. 6.1. Abbassi-Guendouz et al. (2012) supported this assumption by observation from the SS-AD that due to a reduction in the hydrolysis rate coefficient there was a decline in the CH_4 production rate under a low MC (i.e. less than 80 % MC). Modenbach and Nokes (2013) perceived enzyme hydrolysis for ethanol production under high sugar concentration where the activity of the hydrolytic enzyme was inhibited.

The main motive for using a low MC with lignocellulose material for CH_4 production in SS-AD is similar to ethanol production from that substrate, which reduces the water consumption rate, diminishes equipment and energy cost. Kristensen et al. (2009) reported for the ethanol production that they encountered the similar problem such as inhibition of cellulase by the end product causes rate limiting for the hydrolysis of lignocellulose material under a low MC. There was no exact kinetic equation defining this phenomenon, all the experimental result so far utilizes the inverted linear relation between the hydrolytic enzyme activity and the sugar concentration on the microflora surface (S') (Roberts et al., 2011).

Based on Monod equation, the consumption of diffused sugar into the microflora can be described by Eq. (6.3) in which utilization rate of sugar is R_u, the half saturation coefficient, the maximum specific growth rate, yield of growth and microflora surface sugar concentration are K_s, μ_{max}, $Y_{\Delta M/S'}$ and S'. X_M is the inoculum volatile solid (VS) concentration because it is impractical to determine experimentally the concentration of the methanogens in microflora.

$$R_u = \frac{\mu_{max} S' X_m}{(K_s + S') Y_{\Delta M/S'}} \tag{6.3}$$

The rate limiting step is the hydrolysis, there was an insufficient sugar for the microbial utilization and so $K_s \gg S'$ in the Eq. (6.3). Eq. (6.3) is revised as Eq. (6.4).

$$R_u = \frac{\mu_{max} S' X_m}{K_s Y_{\Delta M/S'}} \tag{6.4}$$

Biological reaction model only form the basis for the model development and to validate the model hypothesis. Eq. (6.4) is a pseudo 1^{st}-order kinetics that considers the inhibition mechanism for the reduction in volumetric rate of CH_4 production.

6.1.3.2 Mass diffusion model

The model used for mass diffusion could be described by Fick (1855) that the molar flux developed during the diffusion is directly proportional to the concentration gradient. Based on FickâĂŹs law, the diffusion of sugar from the substrate layer to the microflora can be defined by Eq. (6.5).

$$R_d = \frac{D_e A}{LV}(S - S') \tag{6.5}$$

R_d and D_e are the diffusion rate and the effective mass diffusion coefficient of sugars. A, L and V are the surface area of microflora, substrate layer thickness and volume. S and S' are the concentration of sugar in the upstream substrate layer and on the downstream microflora surface as shown in Fig. 6.1. Sugar inhibition is easy to find in saccharification process because cellulase is the single catalyst, the determination of sugar gradient in microscale from SS-AD would be challenging. Bollon et al. (2013) used mass diffusion limitation form foundation for the accumulation of substrate in SS-AD that leads to partial inhibition and reduced overall process efficiency.

There was a strong proof by theories for the effective diffusion coefficient for the fluid (gas-liquid) stream, the effective diffusion coefficient for the solid-like material such as SS-AD has mere literature. Masaro and Zhu (1999) reported that the effective diffusion coefficient of solid state has a wide range which may vary by the factor higher than 10^{10}. Xu et al. (2014) depicted that currently there is no kinetic model available to predict the D_e in an SS-AD. An empirical stretch in an exponential equation was used in this study, to predict the D_e as shown in Eq. (6.6), that was utilized by the Masaro and Zhu (1999) for the polymeric substances. The D_e was tentatively assumed for the lignocellulose waste material.

$$D_e = De^{-\alpha C^v} \tag{6.6}$$

α and v are the two scaling parameters. C is the concentration of diffusion obstacles and an equivalent ratio ($C=X_F/\alpha_F$) between volatile content (X_F) to the organic faction in the PPMS (α_F) and its units is g/L.

By combining Eqs. (6.1), (6.2) and (6.5) provides the change in sugar concentration in the substrate layer (S) as given by Eq. (6.7)

$$\frac{dS'}{dt} = R_h + R_i - R_d \tag{6.7}$$

The combination of Eqs. (6.4) and (6.5) provides the change in sugar concentration on the microflora surface (S') as given by Eq. (6.8)

$$\frac{dS'}{dt} = R_d - R_u \tag{6.8}$$

As shown in Fig. 6.2, the parameter needed to predict the change in sugar concentration in the substrate layer (S) could be estimated by combining and solving the biological reaction model (Eq. 6.1-6.4) and mass diffusion model (Eq. 6.5 and 6.6). Change in sugar concentration on the microflora surface (S') could be estimated by solving the biological reaction model (Eq. 6.1-6.4), mass diffusion model (Eq. 6.5 and 6.6) and pseudo-steady state model. Eq. (6.8) is a combination of mass diffusion and biological reaction model, which give the change in sugar concentration from substrate layer to microflora surface needed to the model development.

6.1.3.3 Mass balance model

A mass balance mostly called as a material balance is based on the conservation of mass to analyze physical systems. The mass flow can be identified by considering the material entering and leaving a system which might have some unknown. The F/M ratio (n) could be calculated from the Eq. (6.9) for the batch anaerobic reactor. The (n) was represented by the ratio of VS between the F/M and r is the microflora radius.

$$n = \frac{\frac{3}{4}\pi\left[(L+r)^3 - r^3\right]X_F}{\frac{4}{3}\pi r^3 X_M} \tag{6.9}$$

$$\frac{3}{4}\pi(L+r)^3 X_{MC} = \frac{3}{4}\pi\left[(L+r)^3 - r^3\right]\frac{X_F}{\alpha_F} + \frac{3}{4}\pi r^3\frac{X_M}{\alpha_M} \tag{6.10}$$

The MC (X_{MC}) can be estimated based on Eq. (6.10) by adding up the mixture of MC by PPMS and cow dung, in which α_F and α_M are the organic fraction present in the food and microorganisms.

$$L = \left[\frac{X_M(\alpha_M n + \alpha_F)}{X_{TS}\alpha_F\alpha_M}\right]^{1/3} r - r \tag{6.11}$$

$$X_F = \frac{X_M X_{MC}\alpha_M\alpha_F n}{X_M\alpha_M n - X_{MC}\alpha_F\alpha_M + X_F\alpha_F} \tag{6.12}$$

Eq. (6.11) is used to determine the substrate layer thickness from the Eq. (6.9). The Eq. (6.12) substrate layer contain MC could be determined from the Eq. (6.10). From the Eq. (6.11) and Eq. (6.12), the parameter such as L and X_F needed for the model development to predict the R_{max} as shown in Fig. 6.2.

6.1.3.4 Pseudo steady state model

The CH_4 production from the AD is a function of digestion time which describes the sigmoidal curve. In that low CH_4 production at initial and end of the digestion time while maximum CH_4 production at in between these digestion time. By employing the modified Gompertz model equation, maximum CH_4 production rate can be calculated from the Eq. (6.11) as described by Zwietering et al. (1990).

$$Y_t = M_{max}\cdot exp\left\{-exp\left[\frac{R_{max}\cdot e}{M_{max}}(\lambda - t) + 1\right]\right\} \tag{6.13}$$

Y_t, M_{max}, λ, and t are the cumulative CH_4 production during the time t, maximum CH_4 production

potential, maximum rate of CH_4 production, lag phase time and the digestion time.

By considering the hydrolysis is the rate limiting step and it was assumed to follow the pseudo steady state, in which the intermediate compounds concentration does not change with time as described below

$$\frac{dS}{dt} = 0 \tag{6.14}$$

$$\frac{dS'}{dt} = 0 \tag{6.15}$$

On solving the Eq. (6.7), Eq. (6.8), Eq. (6.14), and Eq. (6.15) in order to get S' as defined in Eq. (6.16)

$$S' = \frac{k_h X_F}{k_i + \left[\frac{k_i VL}{D_e A} + 1\right] \frac{\mu_{max} X_M}{K_S Y_{\Delta M/S'}}} \tag{6.16}$$

The maximum rate of CH_4 production (R_{max}) can be estimated by assuming that there was a negligible change in the initial concentration of substrate and inoculum as X_F^0 and X_M^0 by multiplying the CH_4 yield coefficient ($Y_{\Delta CH_4/\Delta S}$) as given in Eq. (6.17)

$$R_{max} = \frac{k_h X_F^0 Y_{\Delta CH_4/\Delta S}}{\frac{k_i K_S Y_{\Delta M/S'}}{\mu_{max} X_M^0} + \frac{k_i VL}{D_e A} + 1} \tag{6.17}$$

In the Eq. (6.17), the known parameter such as X_F^0 and L can be calculated from the Eq. (6.11) and Eq. (6.12). Some other parameters for evaluating the Eq. (6.17) can be used from the Table 6.1. Few unknown parameters such as k_i, α, and v can be computed by MATLAB R2015a using the experimental cumulative CH_4 production andthen it was used to verifying the proposed hypothesis. The Eq. 6.17 is the model developed to estimate the maximum rate of CH_4 production (R_{max}) under hydrolysis inhibition and mass diffusion limitation is used for statistical analysis and its validation as ascribed in Fig. 6.2.

Table 6.1. Parameters used for mathematical modelling

Description	Symbol	Value	Unit	Reference
Maximum specific growth rate	μ_{max}	8	1/d	Rosen and Jeppsson (2006)
Half saturation coefficient	K_S	0.14	g/L	
Hydrolysis rate coefficient	k_h	10	1/d	
Microbial growth yield	$Y_{\Delta M/S'}$	0.04	g/g	
Glucose diffusion coefficient at 37^oC	D	9.8×10^{-6}	cm^2/s	Hobbie and Roth (2007)
Methane yield	$Y_{\Delta CH_4/\Delta S}$	303	mL/g	This study
Volatile inoculum concentration	X_M	112.45	g/L	This study

Table 6.2. Parameters used for Simulation of experimental and literature data

α_F	α_M	$k_i(1/d)$	α	ν	Reference
0.63	0.80	2.77×10^{7}	1.59×10^{-4}	1.87	This study
0.92	0.50	1.22×10^{5}	1.23×10^{-2}	0.96	Xu et al. (2014)
0.77	0.50	9.25×10^{7}	2.45×10^{-5}	2.01	Abbassi- Guendouz et al. (2014)

6.1.4 Verification of model

The experimental data from current study was initially verified with eight data points obtained from the batch anaerobic reactor using PPMS as a lignocellulose substrate by varying the MC with F/M ratio equals to 2. Fig. 6.3 shows the experimental and its proposed model prediction of maximum rate of CH_4 production (R_{max}) by using the parameters listed in Table 6.2 (current study). From the Fig. 6.3, it was observed mathematically with R^2= 0.90 for the description of experimental results. The maximum rate of CH_4 production (R_{max}) was spiked with the decrease in MC up to 83 % and then R_{max} was dropped gradually when lowering the MC. This description gives a SS-AD performance with the satisfactory verification for the model fitness of proposed model.

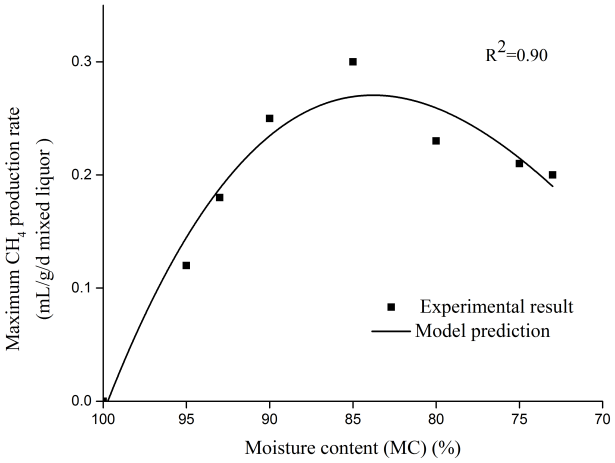

Fig. 6.3. Simulation based on influence of MC on maximum CH_4 production rate (R_{max}) for the data obtained from this study

To further test the hypothesis in this study, literature data from the experiment conducted by Xu et al. (2014), who studied the SS-AD in batch reactor with the corn stover as a feed stock and digestate obtained from the mesophilic anaerobic digester treating sewage sludge was used as inoculum. The 14 data points were acquired from the anaerobic batch reactor with the F/M ratio equal to 2 and MC varied between 99 to 72 %. Their result showed that maximum rate of CH_4 production increase with the decrease in MC up to optimum condition then R_{max} decreased with the decrease in MC which also showed bell shaped curve with R^2=0.85. For this simulated Fig. 6.4(a), parameters were listed in Table 6.2. The Fig. 6.4(a) depicts that R_{max} value is higher at the MC range 78 % to 85 %. This type of close correlation between the experimental as well as literature data provide the evident support

to hydrolysis inhibition because of mass diffusion limitation to validate the hypothesis proposed in the model development. Developed model to estimate the maximum rate of CH_4 production R_{max} was also satisfactorily fit data from the other literature data such as Le Hyaric et al. (2011). The data were not shown because few data points were found in their studies compared to Xu et al. (2014) and current study.

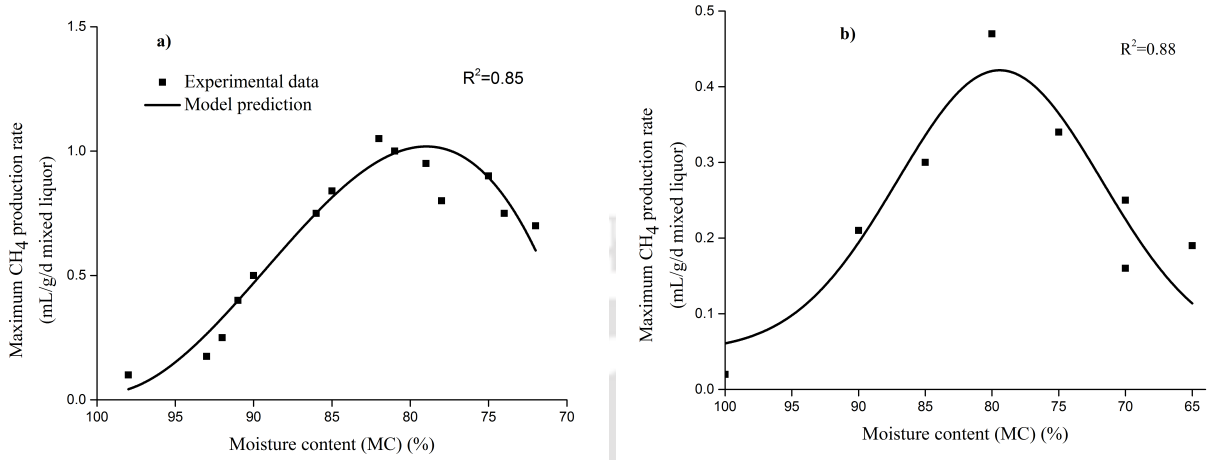

Fig. 6.4. Simulation based on the influence of MC on maximum methane production rate (R_{max}) a) For the data from Xu et al. (2014) b) For the data from Abbassi-Guendouz et al. (2012)

The statistically obtained data from the Abbassi-Guendouz et al. (2012) were also used to fit the maximum rate of CH_4 production (R_{max}) as Eq. (6.17) for model validation. Abbassi-Guendouz et al. (2012) conducted batch experiment with the effect on total solids in SS-AD, used the cardboard waste as a feedstock and the digestate from the anaerobic reactor treating the organic fraction of the municipal solid waste was used as inoculum at F/M=20. To validate statistically in current study, MC was taken into consideration. Abbassi-Guendouz et al. (2012) used the ADM1 to establish the statistical relation between the total solid and the accumulated CH_4 production from cardboard. Abbassi-Guendouz et al. (2012) reported that the reduced CH_4 production under a low MC was due to the increased gas/liquid mass transfer limitation, without going in-depth of hydrolysis inhibition and mass diffusion. Again bell shaped curve with the peak fall between 85 to 80 % MC as shown in Fig. 6.4(b). The parameter used for the simulation were listed in Table 6.2. The Eq. (6.17) was derived from a proposed theoretical hypothesis, used for the experimental data from current study and data by the Abbassi-Guendouz et al. (2012) and Xu et al. (2014) for the statistical prediction that were well agreed with each other.

6.1.5 Implication of model

CH_4 production rate had a two-faceted effect by varying the MC in AD which was seen in Fig. 6.3 and 6.4. The organic solid present in the LS-AD gives primary energy source for the microbial growth and these microbes contributes CH_4 production which was represented by the Monod equation in Eq. (6.4). CH_4 production was depicted in the bell shaped curve in left hand side. Right hand side in bell shaped curve is attributed to the inhibition effect due to its growing mass diffusion resistances as noted in Eq. (6.2) in SS-AD systems. In all AD systems, this two-faceted effect due to MC would exist,

but its deciding dominant facet may depends on the magnitude of MC. Fig. 6.3 and 6.4 depicts that within a range of MC between 85 to 80 %, there is a turning point where the rate of CH_4 production had changed from increasing as MC decreased, to diminishing. This turning point is the optimum MC for the effective CH_4 production in SS-AD.

This two-faceted effect of MC can be interpreted better by the hydrolysis inhibition due to its mass diffusion. The diminishment of CH_4 production rate owing to mass diffusion itself was not a direct constrains and other possibilities that was inhibition also considered. In AD process under hydrolysis limited condition, all the hydrolyzed product will convert into CH_4 and CO_2. As an outcome, strong mass diffusion resistance was persisted with the steeper gradient on mass diffusion to cause the transformation of hydrolytic product to microbes. For this mechanism to proceeds, hydrolysis rate should want to synchronize with methanogenesis rate regardless of magnitude on mass diffusion limitation at steady state. Since, it was not able to perceive in this experiment. To interpret the rate limiting hydrolysis process step, the effect on lower MC was considered for SS-AD. The hypothesis on inhibition mechanism in SS-AD due to mass diffusion resistances and its mathematical support was shown in Fig. 6.3 and 6.4. Vavilin et al. (2008) reported that hydrolysis inhibition was caused by many hydrolytic products under a low MC. Hydrolysis rate would be conceded if this inhibition was removed at timely by the downstream consumers.

6.1.6 Prediction of the model

As shown in Fig. 6.2, the range of S/S' can be calculated to estimate the extent of the accumulated hydrolytic product with the derived Eq. (6.18). S/S' is the ratio of the concentration of substrate layer to microflora. By expending the microflora radii (r) (10 and 100 μm) and diffusion resistance (L) (0 to 100 μm), the profile of S/S' could be calculated with the use of three varied effective diffusion coefficient (D_e) as displayed in Fig. 6.5. Bollon et al. (2013) and Xu et al. (2014) were the two available literature to our best knowledge used the D_e magnitude in SS-AD and predict that D_e order was lowered by two factor than water (D). The reduction of D_e in SS-AD was attributed by the low MC makes diffusion only through the capillary structure in the solid substrate than a bulk solution. The parameters such as internal porosity and tortuosity were affected by the capillary structure at less than MC range of bout 85-80 %, therefore diffusion coefficient was reduced.

$$\frac{S}{S'} = 1 + \frac{LVX_M\mu_{max}}{K_s Y_{\Delta M/S'} D_e A} \tag{6.18}$$

In the Eq. (6.18) three different D_e (10^{-5}, 10^{-6}, 10^{-7} cm^2/s) was used for the simulation to define the D_e in SS-AD. Fig. 6.5 shows three different trends. The first trend, the accumulation of hydrolytic product (S/S') was contributed by the decline in D_e, e.g. around 1,000 times less product accumulation in LS-AD (D_e= 10^{-5} cm^2/s) as compared to SS-AD (D_e = 10^{-7} cm^2/s) at the same diffusion distance (L). The second trend, by increasing the diffusion distance (L) the product accumulation was increased quadratically. The third trend, the microflora size also had a vital effect on S/S', particularly r varied from 10 to 100 μm such as abundant surface area was available in larger microflora that causes sugar influx in the substrate layer. Fig. 6.5 also signify that effective

hydrolysis only occurred within the nearer boundary of microflora surface due to the accumulated product inhibition. This provides the plausible elucidation for the observation that effective hydrolysis occurred at adjacent hydrolytic microflora as reported by Wang (2011). A hydrolytic inhibition zone is whispered in SS-AD that provide a lower rate of CH_4 production than LS-AD because it does not have this zone. By combining this three trend, it was concluding that under lower MC, there was austere hydrolytic product inhibition in SS-AD due to mass diffusion limitation.

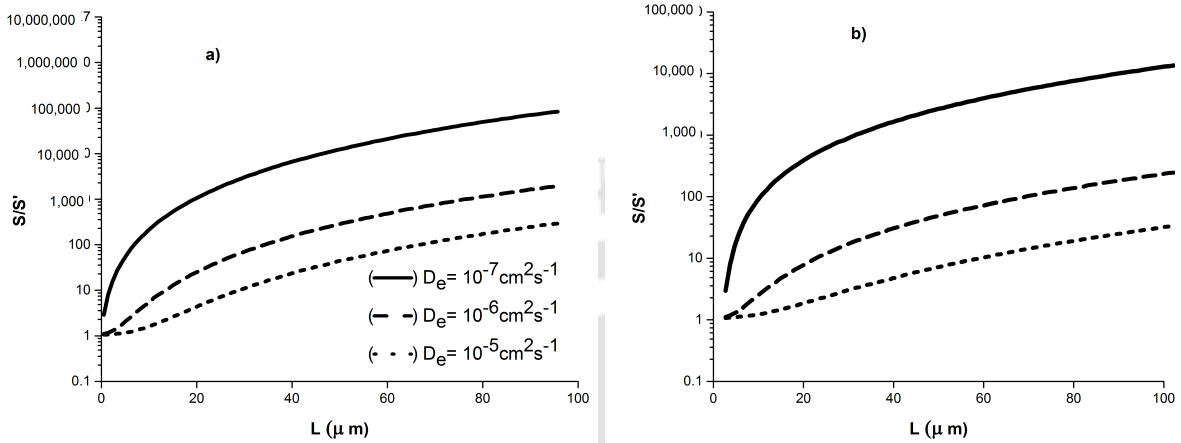

Fig. 6.5. Relation inbetween S/S´ratio and diffusion distance (L) at (a) r =10 μm (b) r =100 μm

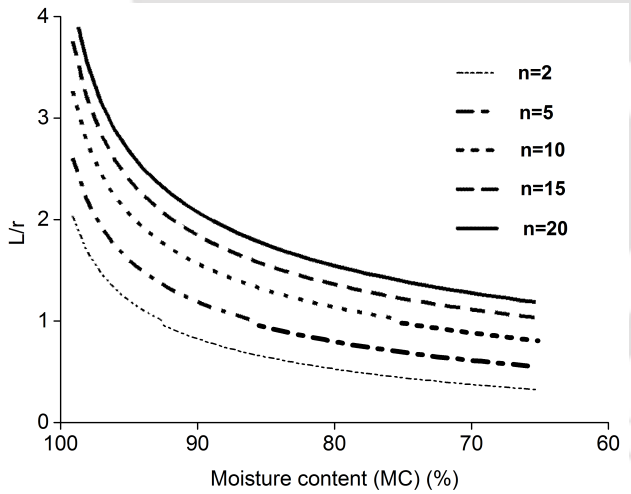

Fig. 6.6. Relation in-between L/r and MC (%) varying (n) from 2 to 20

The extent of product accumulation was determined by giving significance to the microflora radius and mass diffusion distances, but it is essential to find their possible range for the SS-AD system. There was a very few informative literature available regarding this aspect (Xu et al., 2014). By using the Eq. (6.9) the comparative ratio of L/r for the SS-AD could be calculated. Fig. 6.6 shows that two different trend. One trend shows that by increasing the L/r ratio, there was an increase in F/M ratio. While another trend shows that by decreasing the MC there was a diminution in L/r ratio. It could be realized (Fig. 6.6) that L/r ratio varied in the array from 1.3 to 2. That means (L) and (r) should vary from the range of 10-200 μm and 10-100 μm in an SS-AD. From this study, it was perceived quantitatively for the existence of mass diffusion would lead to hydrolysis inhibition region in SS-AD.

6.1.7 Conclusion

The effect of MC on mass diffusion in SS-AD of PPMS was studied. AD of lignocellulose organic content showed the threshold limit between 80 to 85 % of the MC. CH_4 production rate was diminished when it was lower than 80 % MC. Introducing the mass diffusion mechanism that causes hydrolysis inhibition in SS-AD was capable of delivering the interpretation on two-faceted effect of MC in SS-AD. The developed model is able to address the theoretical contextual for the experimental data and its statistical analysis. The proposed model is significant to realize the performance deterioration in SS-AD with the decreasing MC. The better degree of agreement between model simulation and the literature as well as experimental data verified that the deterioration in the volumetric rate of methane production. This could be attributed to hydrolysis inhibition as a result of limited mass diffusion in solid state anaerobic digestion. This proposed model could provide a useful information for the future installation of the SS-AD for enhanced CH_4 production.

"Pollution is nothing but the resource we are not harvesting. we allow them to disperse because we've been ignorant of their value."

R. Buckminster Fuller

Chapter 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

The anaerobic digestion of PPMS generated from pulp and paper industry has a high potential for energy recovery in the form of biogas. In the phase I, Experimental result on gas production, VS reduction and SMA, F/M 2.0 was perceived as best for control (untreated) PPMS. On seeing the trend sCOD, VFA, pH and rate of methane production, each reactor go through different pathway to attain maximum methane production. It was perceived from the sCOD and VFA profile shows that pretreatment was necessary to fasten the biodegradation.

From pretreatment study, it was observed that the pretreatment has affect the PMSS lignocellulose contents at different proportions. During the time of pretreatment, both the organic and inorganic compounds were efficiently solubilized after the different pretreatment. Compositional and instrumental analysis such as FESEM, XRD, and FT-IR spectra showed that the different pretreatment has different degree of effect on lignocellulose content. However, the thermal (hot air oven), electohydrolysis, and biological (*(Bacillus mojavensis)* pretreatment offered better results in solubilisation rates measure in the form of sCOD and VFA. The XRD and FT-IR spectroscopic characterization shows the development of aliphatic, unsaturated and carbonyl carbon functionalities in the pretreated samples at higher severities. FESEM picture also qualities the change in structure after the pretreatment. Thus, pretreatments serve to disrupt the lignocellulosic structure, making the cellulose easily accessible to acidogenic microorganisms. From this phase II, it was inferred that the hot air oven pretreatment at 80^{o}C for 90 min exposure time, electrohydrolysis pretreatment at 15 V for 45 min, and biological pretreatment (*(Bacillus mojavensis)* on 4 d with 10^8 CFU/mL showed a better solubilization rate in the hydrolysis stage.

Further study was carried out (Phase III) to find the efficacy of screened pretreatment in the previous phase. This phase presented the effect of pretreatment on improved methane production in BMP and batch test. The specific methane yield and biodegradability of PPMS were improved after pretreatment that was confirmed in the batch reactor too. The result revealed that the specific methane production potential was increased from 264±5 to 303±4 mL of CH_4/g VS degraded (for thermal pretreatment), 301±3 mL of CH_4/g VS degraded (for electrohydrolysis pretreatment), and 295±3 mL of CH_4/g VS degraded (for biological pretreatment). In addition to that, three kinetic models were studied. Among that modified Gompertz and logistic function models represents and reproduce the experimental data, while modified Gompertz model has better fit in all run. The results

from batch reactor revealed that the cellulose degradation rate was increased in electrically treated reactor (61.80 %) followed by thermally pretreated (61.72 %) then control reactor (49.95 %), which showed a good adaptability of PPMS on lignocellulose degradation.

In phase IV, Anaerobic auger plug flow reactor (AAPFR) experimental studies were carried out in two place: one at India, IITG (Environmental lab) and another one at UoG, Canada. At IITG, the AAPFR was operated for 75 d with thermal pretreatment for 30 d and without pretreatment (30 d) at 21 HRT with specified OLR (6.5 kg VS/m^3/d). The CH_4 yield obtained from the continuous study was not significantly different from the BMP and batch study, and experimental CH_4 yield was equal to 310 mL CH_4/ g of VS degraded. At UoG, study was majorly focused on effect of increasing OLR on the CH_4 production in long-term experiments (130 d) with corn silage (lignocellulose material) as substrate. The increase in biogas production was observed with an increase in OLR. In addition to this, increase in OLR resulted in a decrease in CH_4 content and increase in H_2S concentration. However, the reactor showed a stable operation at an OLR 6.5 kg/m^3/d. The reactor lost its stability at an OLR 8.8 kg/m^3/d, which was apparent by decrease in biogas yield and its methane content. The biogas composition was stabilized after 10 d with CH_4 and CO_2 content maintained at approximately 58-65 % and 39-42 %, respectively.

Further study in phase V, a development of mathematical modelling on a mass diffusion model on the effect of moisture content (MC) for the solid-state anaerobic digestion was studied. This model proposed that the decreased MC causes augmented mass diffusion resistance by the accumulation of hydrolytic product and lead to the reduced methane gas production. According to this hypothesis, a new solid-state anaerobic digestion model was developed based on mass diffusion limitation and hydrolysis inhibition. The better degree of agreement between model simulation and the literature as well as experimental data verified that the deterioration in the volumetric rate of methane production.

7.2 RECOMMENDATION FOR FUTURE WORK

- Combination of different pretreatment for the PPMS to enhance the biodegradability and methane production.
- Improvisation and optimization of AAPFR for other lignocellulose material and co-digestion of different organic waste streams.
- Improvisation of developed mass diffusion mathematical model with the limited models.
- A more through economic analysis should be performed involving sizing and capital costs of the equipment to better estimate the savings potential of pretreatment and anaerobic digestion of PPMS in AAPFR.

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