EXPERIMENTAL INVESTIGATION OF LIGNOCELLULOSIC BIOMASS FOR BIOGAS PRODUCTION

A Thesis

DOCTOR OF PHILOSOPHY

By

DIPTI YADAV

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GUWAHATI – 781039, INDIA
September 2019

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Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of

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This thesis is dedicated to

My Parents

&

Mentors

Whose endless faith, support and blessings always inspired me to move forward

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INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

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Declaration

I hereby certify that the information presented in this thesis is entirely my own account of my research and contains as its main content work expect where otherwise stated, which has not previously been submitted for a degree or diploma at this institute or any tertiary educational institution.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

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CERTIFICATE

It is certified that the work contained in the thesis entitled **Experimental Investigation of Lignocellulosic Biomass for Biogas Production** by **Dipti Yadav**, a student in the Centre for Energy, Indian Institute of Technology Guwahati, India, for the award of the degree of **Doctor of Philosophy** has been carried out under our supervision and this work has not been submitted elsewhere for the degree.

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ABSTRACT

Exacerbating environmental issues, the rising demand for energy, political apprehensions and the medium-way depletion of fossil fuels has generated the prerequisite for development of sustainable technologies based on renewable economic resources. Biogas is one of the potential alternatives biofuels identified so far and is economically feasible, which might benefit the future energy supply demands as well as contributing to a reduction of greenhouse gas emissions. Traditionally, livestock dung has been utilized as feedstock for biogas production but due to unavailability, imbalances and failure occurs in large scale biogas plants, lignocellulosic biomass has attained huge attention. Lignocellulosic biomass acknowledged as the most abundant low cost resources for renewable energy generation across the globe. The socio-economic connotation is also very prodigious. Energy crops, saw dust, marine weeds, agricultural residues or waste vegetables and woody plants fall under this category. The benefit of using this class of biomass does not compete with the arable land. In the Organisation for Economic Co-operation and Development (OECD) countries like India, China and Africa etc. lignocellulosic biomass disposed of openly, which origins environmental pollutions as well as root cause for many diseases. Although the complex structure of lignocellulosic biomass is a challenge for its utilization as feedstock in anaerobic digestion system. This work is intended to give an overview about certain aspects of the complex lignocellulosic biomass, providing the characterization, co-digestion, pretreatment and its potentiality for biogas production.

To evaluate the prospective of a variety of lignocellulosic biomass as biogas feedstocks, the characterization of different types of lignocellulosic biomass (bamboo dust, areca nut shell, rice husk, duckweed, saw dust and maize) abundantly found was investigated. The lignocellulosic biomass was characterized for volatile matter, moisture content, ash content and carbon, hydrogen, and nitrogen (CHN) content. Property analysis of lignocellulosic biomass was also done by Fourier transform spectroscopy, X-ray diffraction and thermogravimetric analysis. The volatile matter content in saw dust, bamboo dust, areca nut shell, rice husk, duckweed, and maize was found to be 82.79±1.5 %, 84.24±1.0 %, 79.3±1.4 %, 74.1±1.6, 84.4±1.0 and 92.24±1.0 respectively, indicating their potential for biogas production by anaerobic digestion. To identify the assessment of potential lignocellulosic biomass for biogas production, lignocellulosic biomass has been co-digested with cattle dung in different ratio to optimize the quantity as well as biogas production. The impact of co-digestion of lignocellulosic biomass with cattle dung in varying ratios (LB: CD= 90:10, 75:25, and 50:50 respectively) in batch type anaerobic digesters was performed at 37±2 °C temperature for 55 days. Results shows cumulative biogas or methane yields for a set of biomass materials digested separately in different ratio LB and CD. The LB: CD; 50:50 exhibited highest biogas production compares to other combinations. To enhance or exploit lignocellulosic biomass fully a thorough study on pretreatment has been conducted further.

Various physical, chemical and physicochemical methods have been employed for the pretreatment with aim of exposure of cellulose moieties in biomass (rice husk, arecanut shell and saw dust) for breakdown of lignocellulose. Banana peel ash solution (BPA solution) is a natural base and a potential source of potassium of 78.10mg/g. It has a very high pH. A study was conducted to explore and compare its effects against a conventional pretreatment method using sodium hydroxide alone and autoclave with sodium hydroxide, which are well known conventional alkali pretreatment methods. The effects of the pretreatment were investigated by biomass fibre analysis (Van Soest’s Method), Fourier transform infrared spectroscopy and thermogravimetric analysis. The fibre analysis results revealed that the BPA solution was able to degrade lignin up to 26.57% in saw dust, 12.59% in rice husk and 20.4% in areca nut shell.

Additionally, a novel microbubble-enhanced DBD plasma reactor has been tested to pretreat whole maize plants with the view of improving its digestibility; hence increasing methane-rich biogas production. Daily feeding of pretreated maize did not show any noticeable change in the biogas output over raw maize. However, using standard Biomethane Potential (BMP) tests, plasma-microbubble pretreated, and washed maize produced ~17% higher cumulative biogas volume compared to that of raw maize after 35 days. Unwashed, plasma-microbubble pretreated maize produced ~28% lower biogas likely due to the presence of inhibitors and residual reactive species. For anaerobic digesters operated with a relatively long residence time and fed with material that has high lignocellulose content, plasma-microbubble pretreatment could be beneficial in improving the biogas output.

The purifying process of methane enrichment from biogas generates many new prospects for its utilization but up gradation may increases the process cost. Therefore, study has been conducted to assess the effect of activated carbon (AC) addition on anaerobic digestion (AD) process for the augmentation of biogas as well as methane production. For this study activated carbon has been used in 3 different concentrations 1%, 5% and 10% respectively in batch type 1 L laboratory scale anaerobic digester at 37 °C for 25days HRT. Furthermore, continuous stirring tank reactor (CSTR) AD experiment has been conducted with activated carbon 1% and activated carbon 1% with raw maize and observed that addition of AC 1% with maize enhanced the biogas as well as methane content. This study proves that addition of AC in AD system not only improved biogas and methane production alone but it also supports the most recalcitrant LB for faster hydrolysis with higher efficiency.

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Place: IIT Guwahati

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NOMENCLATURE

GTOE	:	Gigatons of Oil Equivalent
GW	:	Gigawatt
TW	:	TeraWatt
MW	:	Megawatt
kJ/litre	:	1 kJ into Litre-atmosphere
kg/m^3	:	Kilogram per cubic metre
kg/sec/m	:	1 (kilogram / second) / meter
m/sec	:	Meter per second
mm	:	Milimeter
M	:	Molar
MJ/m^3	:	Mega Joule per cubic meter
L CH_4/kg VS	:	Specifuc methane yield
MPa	:	Mega Pascal
MJ/Kg	:	Mega Joule per kilogram
t	:	Time (s)
T	:	Temperature (°C)
R_2		Coefficient of determination of a linear regression

ABBREVIATIONS

IEA	:	International Energy Agency	AD	:	Anaerobic digestion
REN 21	:	Renewable Energy Policy Network for the 21st Century	pH	:	Potential hydrogen
NBMMP	:	National Biogas and Manure Management Programme	DM	:	Dry matter
DAI	:	Day after incubation	TAN	:	Total ammonia nitrogen
LB	:	Lignocellulosic biomass	VFAs	:	Volatile fatty acids
CH_4	:	Methane	CO_2	:	Carbon dioxide
H_2S	:	Hydrogen sulphide	O_2	:	Oxygen
IARI	:	Indian Agricultural Research Institute	PRAD	:	Planning Research and Action Division
AFPRO	:	Action for Food Production	NGO	:	Nongovernmental organization
HRT		Hydraulic retention time	EDX	:	Energy dispersive x-ray
CSTR	:	Continuous stirred tank reactors	SEM	:	Scanning electron microscope
ASTRA	:	Application of Science and Technology to Rural Areas	IISc	:	Indian Institute of Sciences
C/N	:	Carbon to nitrogen ratio	MSW	:	Municipal solid waste
VS	:	Volatile solid	H_2SO_4	:	Suphuric acid
SSAD	:	Solid state anaerobic digester	IIT	:	Indian Institute of Technology
RPM	:	Revolutions per minute	MC	:	Moisture content
DP	:	Degree of polymerization	NaOH	:	Sodium hydroxide
FTIR	:	Fourier Transform Infrared spectroscopy	XRD	:	X-diffractometer
TGA	:	Thermogravimetric Analysis	ASTM	:	American Society for Testing and Materials
HHV	:	Higher heating values	LHV	:	Lower heating value
NDF	:	Neutral detergent fibre	ADF	:	Acid detergent fibre
ADL	:	Acid detergent lignin	CI	:	Crystalline index
N	:	Nitrogen atom	N_2	:	Nitrogen gas
NO_x	:	Nitrogen oxide		:	
GC	:	Gas chromatograph	O	:	Oxygen
CNG	:	Compressed natural gas	H	:	Hydrogen

VM	Volatile matter	Si	Silicon
FC	Fixed carbon	CV	Calorific value
COD	Chemical oxygen demand	IS	Indian standard
C	Carbon	BD	Bamboo dust
TA	Total alkalinity	DW	Duckweed
M	Maize	AN	Arecanut Shell
LR	Loading rate	RH	Rice husk
TS	Total solid	SD	Saw dust
TERI	Tata Energy Research Institute	LR	Loading rate in kg VS day-1
OLR	Organic loading rate in kg VS day-1 m-3	CD	Cattle dung
CHP	Combined heat and power	HCl	Hydrogen chloride
APHA	American Public Health Association	ASTM	American Society for Testing and Materials
KVIC	Khadi andVillage Industries Commission	KOH	Potassium hydroxide
AC	Activated carbon	BPA	Banana peel ash

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CHAPTER – 1

INTRODUCTION

1.1 MOTIVATION

Fossil fuel plays a dominating role in energy sector for both the heat and power production. Amongst the fossil fuel, coal contributes significantly in power production. It is reported that 81% of the global primary energy consumption originated from fossil fuel with consumption of oil, coal and natural gas accounting for 4.4 GTOE, 3.7 GTOE and 3.0 GTOE respectively in 2016 (“IEEJ Outlook” 2019). Monotonic increase in price and depletion of reserve of fossil fuel attracted researchers towards alternative fuel. Moreover, the dependence on fossil fuels is associated with remarkably adverse impacts on the environment such as increased levels of air pollution, depletion of natural landscapes and climate change (IPCC, 2013), etc. The challenge is how to meet these growing energy needs in a more sustainable and environmentally stable way (Edenhofer et al. 2013). One of the present energy policies is therefore focused on the use of biomass for the production of biofuels, such as biogas, bioethanol, syngas, etc. so as to decrease the greenhouse gas productions and improve the energy security. According to IEA 2018 report biomass contributes 8% of the world's power generation and if it is utilized efficiently and appropriately will increase the overall energy generation (IEA, World

Energy Outlook 2018).

The abundantly available biomass resources are potential candidate for generation of biogas through anaerobic digestion (AD). Biogas is one of the potential alternatives identified so far and is economically feasible. It has the lowest financial input to output energy and unlimited in potential and eco-friendly in nature due to which same is attracted wide attention. According to REN 21 (2016) report global biogas electricity has a share of about 20% in total bio-power production and 4% in global heat generation. The global installed biogas capacity reached to 15 GW in 2015. Table 1.1 shows global data for installed biogas capacity and biogas generated electricity.

Table 1.1 Biogas installed capacity and power productions (Scarlat et al. 2018)

Country/Continent	**Biogas Installed Capacity (MW)**	**Country/Continent**	**Biogas Generated Electricity Production(MW)**
Europe	10400	Europe Union	58000000
North America	2400	United States	14000
South America	147	China	350
Asia	711	Brazil	450
Africa	33	India	187

In India, the National Biogas and Manure Management Programme (NBMMP) promotes the manufacture of family size biogas plants for generating fuel for cooking and organic compost. In 2014, there were about 4.75 million farm size biogas plants as compared to a potential to build about 12 million biogas plants, which could produce more than 10 billion m^3 biogas/year (MNRE 2012). India aims at setting up 110,000 biogas plants from 2014 to 2019. So far, the installed electricity capacity of biogas plants has reached 179 MW in 2015 and 187 MW in 2016 (Scarlat et al. 2018).

Out of all the biomasses, the second generation biomass containing lignocellulose is a prospective feedstock for the biofuels production. Woody crops, saw dust, rice husk, agricultural residues or waste vegetables and plants fall under the category. There is a vast reserve of the same in the worlds. This class of biomass does not compete with the first generation biomass (food crop). The present obtainability of lignocellulosic biomass in India is expected about 500 million metric tons per year (Kumar et al. 2015). It is estimated the data for the biomass availability to be about 120-150 million metric tons per annum corresponding to a potential of about 18000 MW (https://mnre.gov.in/biomass-powercogen).

In India post-harvest generates huge amount of agricultural crop residues/waste which has been disposed of openly in the nearby society without any treatment which is a significant source of greenhouse gas emissions and propagation locations for disease vectors such as flies, mosquitoes, cockroaches, rats, and other pests (India: Urbanisation, sustainable development and poverty alleviation, INTL 442). Consequently, later it causes disease like chikungunya, plague, dengue, malaria, lung diseases etc. In the year 2007 in Kerala, the mosquito-borne viral disease chikungunya, erupted, claiming 40 lives while 7,000 people were admitted in hospitals in the South and Central districts of Kollam, Pathanamthitta, Kottayam and parts of the capital city (http://iictenvis.nic.in/Database/Chikungunya_outbreak_in_Kerala_2007_1057.aspx).

According to World Resources (1996-97), another calamity reported the epidemic of pneumonic plague in September 1994 in Surat, Gujrat, which are also consequences of the large quantities of garbage that remained uncollected offering a perfect home for rats (Mavalankar). In addition to this, digestate (outlet slurry) from biogas production can be utilized as fertiliser, just like compost, having the additional nutrients concentration as compost which reduce the use of chemical fertilizers in farmstead, and reduces nutrient discharge and escapes methane emissions.

Traditionally, animal dung has been recognized as the major raw material for biogas plants but due to unavailability and unequal distribution, globally a lot of interest has been generated to explore alternative feed materials like lignocellulosic waste.

Inopportunely, the nature of lignocellulose biomass limits their application in anaerobic digestion. The probable reasons are (1) the indissolubility and recalcitrant structure prevent lignocellulose from being efficiently hydrolyze; (2) the lower amount of nutrient content makes lignocellulosic materials a poor nutrient source for degrading microorganisms (Sawatdeenarunat et al. 2015; Monlau et al. 2013). Therefore, surface disruption is essential in order to efficiently utilize the lignocellulosic biomass based biogas plant. Many researchers studied the application of different pretreatments in order to boost substrates biodegradability (Monlau et al. 2013; Taherzadeh and Karimi 2008; Zheng et al. 2014). However, there is still a need of finding or optimizing pretreatment methods, as the existed approaches can be cost-demanding, time consuming, have difficulties in full-scale applications or are associated with the production of inhibitors to the anaerobic digestion microbiome (Hendriks and Zeeman 2009; Kratky and Jirout 2011). Henceforth, the problem with lignocellulose degradation should be addressed without deteriorating the feasibility of biogas system. Additionally, deeper insights of the biomethanation enhancement methods like co-digestion of substrates and addition of activated carbon or biochar can provide important knowledge in order to augment the overall process efficacy of lignocellulose-based anaerobic digestion.

1.2 BIOGAS COMPOSITION AND PROPERTIES

The biogas generated is deliberated a clean and environmentally friendly renewable fuel and mainly composed of CH_4 (55–75%) and CO_2 (25–45%), small amounts of water vapour, traces of hydrogen sulphide, ammonia and hydrogen, and possibly other contaminants like siloxanes (Nijaguna 2001; Chandel and Sukumaran 2017). The properties of biogas are presented in Table-1.2.

Table 1.2 Properties and characteristics of biogas (Nijaguna 2001)

Particulars	**Usual Value**	**Range**
Calorific value (kJ/litre of raw biogas)	21.5	20.1 – 25.9
Effective molecular weight	27.35	24 – 29
Density (kg/m^3)	1.0994	0.96 – 1.17
Specific gravity	0.94	0.82 – 1.00
Viscosity (kg/sec/m)	1.297 x 10^{-5}	
Optimum air to fuel ratio (15% biogas)	5.5: 1	
Flammability(biogas in air)	9% - 17%	
Burning velocity (m/sec in air)	0.25	

1.3 ANAEROBIC DIGESTION AND BIOGAS PRODUCTION

AD process involves the degradation and balance of organic materials under anaerobic conditions by a microbial consortium of microorganisms leading to the production of an energy rich biogas. The AD process can be categorized into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis and in each individual category, different groups of facultative or obligatory anaerobic microorganisms are involved as shown in Fig. 1.1 (Nijaguna 2001) and the details are mentioned below.

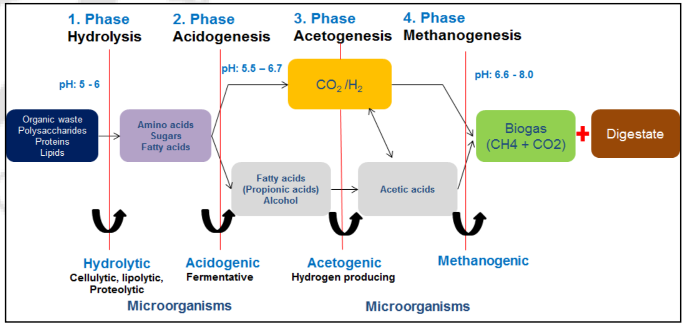


Fig. 1.1 Anaerobic Digestion process

The degradation process takes place in digesters that are aimed to provide ideal environmental conditions for microorganism like mixing, temperature, pH. Digesters are categorized according to the feeding arrangement i.e. batch, continuous, semi-continuous, the number of stages i.e single stage or two stages. The temperature of the AD process psychrophilic 0-15° C, mesophilic 30–40 °C or thermophilic 50–60 °C conditions (Mittal 1996), and the fluid-dynamic i.e. plug flow, completely mixed, hybrid, which in turn depends on the feedstock concentration (AD process can operate under wet, <15% dry matter, or dry, >15% dry matter, conditions). The AD process comprises a sequences of bioprocesses in which different leading microbes hydrolysed organic matter and soluble materials into biogas in the absence of oxygen (Gerardi 2003). The details for all four phases in AD are mentioned below.

1.3.1 HYDROLYSIS

In the first phase of AD, complex biodegradable materials such as proteins, carbohydrates, and lipids are hydrolysed into simple soluble products by extracellular enzymes, such as cellulases, amylases, proteases, and lipases, emitted by fermentative bacteria (Kayhanian 1995). The main degradation reactions at this stage are as follows:

$$(C_6H_{10}O_5)n + nH2O \rightarrow nC_6H_{12}O_6 \qquad (1.1)$$

$$(R\text{-}CHNH_2COOH)n + nH2O \rightarrow nR\text{-}CHOH_2COOH + nNH_3 \qquad (1.2)$$

$$C_3H_5\,(OCOR)_3 + 3H2O \rightarrow C_3H_5\,(OH)_3 + 3RCOOH \qquad (1.3)$$

1.3.2 ACIDOGENESIS

This process involves biodegradation through which simple monomers produced by the first phase (hydrolysis) are further converted into volatile fatty acids, such as acetic acid, propionic acid, and butyric acid and trace alcohols, ketones, carbon dioxide, ammonia, hydrogen sulphide, and hydrogen(Madigan et al. 2008).

1.3.3 ACETOGENESIS

The hydrogen-producing acetogenic bacteria decompose and convert propionic acid, butyric acid, and alcohols into acetic acid, hydrogen, and carbon dioxide, while the homoacetogenic bacteria convert hydrogen and carbon dioxide into acetic acid (Kangle et al. 2012). The main reactions involved in this stage include:

$$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2 \qquad (1.4)$$

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2 \qquad (1.5)$$

$$CH_3\,(CH_2)_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2 \qquad (1.6)$$

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \qquad (1.7)$$

$$4CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2H_2O \qquad (1.8)$$

$$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O \qquad (1.9)$$

1.3.4 METHANOGENESIS

In this phase, the acetic acid, hydrogen, and formic acid, methanol, and methylamine are converted into methane (Madigan et al. 2008). It has been reported that about two-thirds of the methane produced comes from acetic acid, while the rest comes from hydrogen and C_1 compounds (formic acid, methanol, and methylamine). The major reactions at this stage include:

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (1.10)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (1.11)$$

$$\mathrm{HCOOH + 3H_2 \rightarrow CH_4 + 2H_2O} \quad (1.12)$$

$$\mathrm{CH_3OH + H_2 \rightarrow CH_4 + H_2O} \quad (1.13)$$

$$\mathrm{4CH_3NH_2 + 2H_2O + 4H^+ \rightarrow 3CH_4 + CO_2 + 4NH^+} \quad (1.14)$$

Anaerobic digestion process is achieved by diverse anaerobic species of bacteria that contribute in different phases of the process of transforming lignocellulosic biomass comprising of organic compounds, which are classified into carbohydrates, lipids, and proteins to biogas. Consequently, particular optimum parameters are also requisite for appropriate performance of each species of microorganism at different phases (Yadvika et al. 2004). It is must that in operating parameters is controlled so that it enhances the microbial activity and thus increases the degradation ability of the process. The generation of biogas is determined by certain operative factors *viz.*, temperature, pH, pre-treatment, particle size, agitation, rate of organic load and retention time. Any instant variation in these parameters can negatively affect the digestion process, hence the production of biogas (Yadvika et al. 2004; Gerardi 2003). Biogas technology has prospective to deliver the demand of energy in an economical and environmentally beneficial way (IPCC 2013 report). It discovers its applicability ranging from cooking fuel to generating electricity and fuel vehicles (Kapdi 2004). Additionally, the main product (biogas) and the digestate (outlet) slurry can be regarded as a by-product which is used in preparing briquettes for fuel in stoves or as fertiliser for agricultural applications (Chandel and Sukumaran 2017). Therefore, there are unique advantages of biogas technology over other bioenergy technologies (producer gas, pyrolysis, etc.) from consumers' perception.

1.4 LIGNOCELLULOSIC BIOMASS

In recent years, many attempts have been made to utilize lignocellulosic biomass, such as wood, agricultural waste residues, water hyacinth plants and rice husk, etc. for biogas production (Yadav et al. 2016). Resources rich in lignocellulose, such as plant and agricultural leftovers are the most promising renewable carbon-based feedstock for biomethanation without competing with the arable land (Sawatdeenarunat et al. 2015; Chandra et al. 2012). To overcome this challenge many researchers worldwide are working on characterization and pretreatment methods to exploit the potential of lignocellulosic biomass efficiently. Few studies reported on the characterization of lignocellulosic biomass still lack clear analysis for sustainable and systematic bioenergy conversion. Characterization studies of lignocellulosic biomass reveals the complex feedstock compositions and its biochemical and thermal behavior in conversion processes. Yield can be anticipated and mathematical models can be designed to understand the products and economic analysis. Most significantly, technology can be designed to make biomass conversion process more effectual, effective, and better to address the diminishing fossil fuel production. LB mainly comprises cellulose, hemicelluloses and lignin and has the potential to be used as feedstock for biogas production. Though, the compact crystalline structure and the fact that recalcitrant lignin physically guards the cellulose and hemicelluloses content, makes these materials more resilient to enzymatic hydrolysis and AD (Mckendry 2000). Hence, an appropriate pretreatment technique is required to increase the hydrolysis or digestibility of lignocellulosic material. However, pretreatment technology related to production of biogas from lignocellulosic biomass has many pros and cons and it's very necessary to identify appropriate pretreatment technology which enhances the biogas production. It should be cost effective, less detrimental to the environment and importantly it can be practiced in large scale AD plants. The utilization of lignocellulosic biomass for biogas feedstock has significant benefit over animal manure as socio-cultural barrier for household biogas plant (Rupf et al. 2016). Sometimes people don't wanted to handle cattle dung for family biogas plant due to socio-economic inhibition so in that case lignocellulosic biomass would be the ideal feedstock for popularizing the same in the society.

1.5 CO-DIGESTION AND ADDITION OF ACTIVATED CARBON FOR THE ENHANCEMENT OF BIOGAS PRODUCTION

Along with pretreatment prior to anaerobic digestion, co-digestion was also investigated for the utilization of lignocellulosic materials. It has been comprehended that co-digestion can be an intriguing alternative for increasing biogas production and biodegradation efficacy (Martinez et al. 2016). Co-digestion is the instantaneous digestion of a uniform mixture of two or more substrates. The use of co-substrates can increase the biogas yield due to the optimistic synergism developed in the digestion process, providing a better nutritional balance and suitable moisture content required in the digester feed (Mata-Alvarez et al. 2000). Cattle dung usually comprises high ammonia concentrations (TAN-6000mg/l), which can cause inhibition in the AD process (Yenigun and Demirel 2013). On the contrary, lignocellulosic biomasses are difficult to hydrolyze as they comprise a substantial portion of lignin. Conversely, co-digestion of animal dung and biomass has been shown to be a successful way to utilize these materials in an anaerobic digestion process; together the materials complement each other and the possibility of the inhibition decreases. Moreover, hardly any studies have been reported for the optimized combination of cattle dung and LB for the enhancement of biogas production. In recent years another interesting research with respect to biogas enhancement i.e. effect of additive in anaerobic digestion has taken the platform. Kumar et al. (1987) analyzed the influence of adding commercial charcoal to cattle dung slurry as feedstock on biogas yield. Addition of 5 % of commercial charcoal to cattle dung slurry on the dry weight basis raised the yield by 17 and 34.7 % in batch and semi-continuous processes, respectively. In addition, the remaining activated charcoal in outlet slurry (digestate) could serve as soil improver if the digestate is composted or used as a fertilizer (Monlau et al. 2015). Luo and Lu (2014) had reported application of eco-compatible biochar and its possible mechanisms to promote AD are the adsorption of inhibitive compounds, promoting direct interspecies electron transfer, and acting as an effective microbial immobilization method. The use of additive in biogas technology is a comparatively new thing and more insightful study is prerequisite to optimize the quantity of additive addition and its effect on biogas and methane enhancement.

1.6 AIM AND OBJECTIVES

The aim of the present work is to experimentally investigate the potentiality of lignocellulosic biomass as feed material for biogas production.

The following are the scope of the work

1. Characterization of locally available lignocellulosic biomass to identify its potential for biogas production.
2. Performance evaluation of biogas production and effect of various parameters for co-digestion of cattle dung with lignocellulosic biomass.

3. Experimental study on different kind of pretreatment methods to identify the efficacy in breakdown of lignocellulosic biomass structure.
4. Study on enhancement of biogas production with addition of activated carbon in feed material.

1.7 OUTLINE OF THE THESIS

The present thesis consists of 7(seven) chapters. **Chapter 2** describes the extensive review of literature focused on governing factors and strategies for biogas production from lignocellulosic biomass, characterization, pretreatment methods, addition of activated carbon and co-digestion. **Chapter 3** deals with the detail characterization of lignocellulosic biomass as potential feedstock for biogas production. Assessment of various co-digestion mixtures of cattle manure and biomass in order to enhancement the biogas methane production is presented in **Chapter 4**. In **Chapter 5**, the pretreatments of lignocellulosic biomass with physico, chemical, thermochemical and natural base method are discussed. Further, a novel lignocellulosic biomass pretreatment reactor has been designed and tested to investigate pretreatment efficacy with maize as lignocellulosic biomass. Furthermore, the study was conducted to analyze plasma pretreated maize as feedstock for the enhancement of biogas production in laboratory scale AD reactors. The effects of activated carbon addition on AD process to enhance of methane production is presented in **Chapter 6**.Finally, conclusions and scope for future work is presented in **Chapter 7**.

CHAPTER – 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter deals with the review of literature related to biogas production. Starting with the biogas technology, history and development of biogas in India, production of biogas from various feed materials are highlighted. Moreover, literature related to the effect of different parameters on biogas production is also discussed. Special emphasis is given to the state of the art related to biogas production from lignocellulosic biomass (LB) and pertaining problems are elaborated.

2.2 BIOGAS TECHNOLOGY

Biogas is a very versatile and attractive technology producing clean fuel from organic wastes. It can be utilized for cooking, heating, cooling, electricity production as well as for automotive applications (Prasad et al. 2017). Biogas produced through AD process yields mainly methane (55–65%) and carbon dioxide (25–45%) (Manyi-loh et al. 2013; Horváth et al. 2016; Miltner et al. 2017; Kougias and Angelidaki 2018) with typical calorific value of 21.5 MJ/m^3 (Sahota et al. 2018). Manyi-loh et al. (2013) had reported that the biogas can be produced from various organic feedstock such as landfill, municipal solid waste, agro-industrial, animal manure and energy crops, etc. Worldwide biogas digesters available in a wide range from simple household digesters and covered lagoons to highly mechanized continuous stirred tanks reactors with modern sensors for process monitoring and control. According to Horvath et al. (2016) production of biogas contributes to the production of renewable and sustainable energy since biogas works as a flexible and predictable alternative for fossil fuels.

2.3 HISTORY OF DEVELOPMENT OF BIOGAS PRODUCTION IN INDIA

Biogas technology has a long history in India. The technology found attention in 1950s but the actual development started in 1960s.

- The first use of biogas has been reported in 1887 for lighting at Matunga leper asylum, Bombay (Mumbai), utilizes human waste to generate gas to meet its lighting needs (Khanal 2011).
- In 1937, first successful biogas plant was commissioned by S.V. Desai – a microbiologist of the Indian Agricultural Research Institute (IARI).
- In 1960, the Structural Engineering Research Centre, Roorkee, has developed and introduced ferro-cement gas holders instead of steel drums. This type of gas holder is believed to be cheaper, and with a longer life. It is also claimed to have lesser maintenance costs.
- In 1961, Mr. Jashbhai Patel developed a floating drum biogas plant model named Gramalaxmi III which was more productive, requiring less maintenance with a longer life.
- Khadi and Village Industries commission (KVIC) promoted Gramalaxmi III model and the model became famous as KVIC model. The main advantage of this model was its constant pressure gas output because of its floating drum.

- A significant development was achieved during the year 1961 by the Planning Research and Action Division (PRAD) of Uttar Pradesh, India. PRAD reported successful implementation of a fixed dome digester named as JANATA model. This model is an improved version of Chinese biogas model. It is 30% cheaper than the conventional KVIC model of same capacity.
- Action for Food Production (AFPRO), a NGO developed a biogas plant model called Deenbandhu meaning ‘friend of poor’ in the year 1984. It was an improvement of Janata type model and soon became popular because of its low cost.
- The department of Application of Science and Technology to Rural Areas (ASTRA) under the Indian Institute of Sciences (IISc), Bangalore in the year 1982 has modified the KVIC design and showed that it is possible to get gas within only 35 days of feeding the plant.
- Ganesh model is modified version of Chinese model and made of angular steel and plastic foil (1999).
- Mahanta et al. (2003) had developed a new biogas model named “Samridhi”. It is an adapted Deenbandhu model to duplex digester, which has been successfully implemented in North Eastern region of India. This duplex digester is constructed in such a way that it can provide 5 m^3 of biogas production per day in comparison to 3 m^3 Deenbandhu biogas plant of same size.

2.4 AVAILABILITY OF DIFFERENT ORGANIC WASTE AND THEIR CHARACTERISTICS FOR BIOGAS PRODUCTION

Traditionally, animal dung and sewage sludge has been utilized as ideal feedstock for biogas production to generate energy as well as to reduce the solid waste pollution. reported that animal dung, which contains high moisture and recalcitrant portions, has lower methane yield per volatile solids (VS) or chemical oxygen demand (COD) than easily degradable feedstock such as food waste. Worldwide, enormous amounts of animal manure are generated each year due to increase in large scale mechanized poultry breeding industries (Food and Agriculture Organization of the United Nations, 2013). Chicken litter is an encouraging feedstock and found in plenty for biogas production via AD, which has not been completely exploited hitherto (Nie et al. 2014). Abouelenien et al. (2009) studied that chicken litter has a higher portion of biodegradable organic matter than other animal wastes but he had also reported the presence of uric acid and undigested proteins which follows to production of high amounts of unionized ammonia and ammonium ions. The chicken manure has higher potential for methane yield and shows even higher nitrogen content compared to other animal manure (Zhang et al. 2013). Xu et al. (2018) reported that pig litter has higher methane yield than cattle dung possibly due to a higher protein and lipid content, less lignin, and less slowly degradable carbohydrates. In the last decade, many researchers studied the potentiality of animal manure for biogas production. However, due to uneven availability, inappropriate C/N ratios, low pH, poor buffering capacity and high ammonia concentration (Jr et al. 2001; Zhang et al. 2013) of animal dung attracted interest towards utilization of LB for co-digestion and subsequently efficient biogas production (Wang et al. 2014).

Dewil et al. (2008) had reported sewage sludge management as a foundation of public health and environmental protection because of concern about the large quantity, potentially environmental risk and the higher cost for disposal which accounts for up to 50% of the total waste water treatment expenses. Biogas is the most widely employed process for sludge utilization because it has the advantage of decreasing organic content as well as generating renewable energy (Dai et al. 2013). Generally, sewage treatment produces two wastes: primary or raw sludge and activated or secondary sludge (https://en.wikipedia.org/wiki/Sewage_sludge_treatment). Activated sludge that is easily decomposed compared to raw sludge because it contains more fat, protein and carbohydrates as opposed to activated sludge which consists of recalcitrant bacterial cells (https://nptel.ac.in/courses/105105048/M22L36.pdf). Davidsson (2007) reported the methane yields of sewage sludge in the range of 224-381 L CH4/kg VS in Sweden. Many researchers had reported biogas production from first generation biofuels and are shown in Table 2.1.

Table 2.1 Biogas yield from first generation biofuel feedstock (Scarlat et al. 2018)

Feedstock	**DM (%)**	**VS (% of DM)**	**Methane yield (L CH_4/kg VS)**	**Methane yield (L CH_4/kg fresh)**
Pig slurry	3-8	70-80	250–350	6–22
Cattle slurry	6-12	70-85	200–250	8–25
Poultry manure	10-30	70-80	300–350	21–84
Maize silage	30-40	90-95	250–450	68–170
Grass	20-30	90-95	300–450	55–128
Alfalfa	20-25	90-95	300–500	57–118
Potatoes	20-30	90-95	280–400	54–128
Sugar Beet	15-20	90-95	230–380	31–72
Straw	85-90	80-90	200–250	136–202
Vegetable Waste	85-90	80-90	200–251	136–203
Organic Waste	10-40	75-90	350–450	26–180
Slaughterhouse residues	35	90-95	550–650	173–216
Sewage sludge	5-10	75	300–400	11–30

- DM = Dry matter, VS= Volatile solids

Recently use LB as feed material is getting popularity because of its quality of non-edible, self-adaptable, self-reliant, quick growth, higher yield and low nutrient requirements (https://en.wikipedia.org/wiki/Lignocellulosic_biomass). Many researchers found that the failure in biogas plants are due to mono-digestion of animal dung and therefore have recommended possible solutions to overcome this problem called co-digestion (Wang et al. 2014). In current scenario biomass waste are available in aquaculture, forestry, agricultural, industrial and domestic sectors (Cherubini 2010); and among these material sources, LB is an abundantly available carbon-rich and land-based feedstock, which can improve the independency on gas and oil (Kumar and Saini 2015).

Prasad, Rathore, and Singh (2017) reported that agricultural sector provides plentiful LB residues which have been estimated around 30 % of worldwide agricultural productivity. Kumar and Saini (2015) had reported that a corn residue which comprises husk, stover and cob contains high content of cellulose and hemicelluloses which have been usually utilized as the feedstock in AD.

Chen (2016) in his studies reported that a large quantity of agricultural waste is produced every year in China and the main types of this waste are rice straw, wheat straw, and corn stover which accounts for approximately 47%, 25% and 28% respectively of total crop residue. The waste management of rice straw via incineration or returning to farmland can raise serious environmental problems such as greenhouse gas emissions (Menke, Garivait, and Gadde 2009). Zhou et al. (2017) investigated that rice straw is mainly composed of cellulose and hemicellulose (around 30% and 18%, respectively) and a silicified surface layer. In AD process, the main problem with the rice straw as with most LB residue is the recalcitrance to hydrolysis and low nitrogen content (Agrawal et al. 2018). Paepatung et al. (2009) experimented on untreated rice straw and found methane production up to 306.2 ml/g VS which is closed to the methane yield of rice straw (340 ml CH4/g VS) in another study. To fasten the slow degradation of LB residues, pretreatment methods such as size reduction, acid/alkaline, heat treatment, fungal treatment etc. are prerequisite.

Miscanthus lutarioriparius is another extremely encouraging herbaceous perennial grass that produces cane-like stems with high biomass production (https://en.wikipedia.org/wiki/Miscanthus). Li et al. (2017) reported average annual dry biomass yield of *M. lutarioriparius* that can reach as high as 32t ha^{-1} or 33.4t ha^{-1}. These values are comparable to those of maize but higher than those for hemp and *Pennisetum*, or elephant grass. Frigon and Guiot (2010) had utilized energy crop residues like maize and miscanthus and woody crops like willow, poplar and oak for biogas production. It was also reported that the biogas production with woody LB require pre-treatment before utilization in AD for efficient biogas production. Tsapekos et al. (2015), Sondergaard et al. (2015), Fotidis (2014), Li et al. (2013), Kougias (2015), D'Este et al. (2017) and other few researchers had worked on methane yield from different feedstock which is illustrated in Table 2.2.

Table 2.2 Methane yield from different feedstock (adopted from Kougias and Angelidaki 2018)

Category	**Feedstock**	**Methane yield (mL-CH4/g VS)**	**Reference**
Livestock manure	Cattle manure	242–399	Tsapekos et al. (2015) Zarkadas et al. (2016)
	Mink manure	239–428	Sondergaard et al. (2015) Tsapekos et al. (2017) Li et al. (2013)
	Pig manure	313–322	Tsapekos et al. (2015)
	Poultry manure	107–438	Li et al. (2013) Kougias et al. (2017) Fotidis (2014)
Agricultural wastes	Barley	322–335	Sondergaard et al. (2015)
	Corn silage	270-298	Labatut et al. (2011) Frigon and Guiot (2010)
	Fruit and Vegetable waste	153- 342	Tsapekos et al. (2015) Sondergaard et al. (2015) Tsapekos et al. (2017)
	Meadow grass	282–388	O-Thong et al. (2012)
	Palm Oil Mill Effluents	378–503	Li et al. (2013) Menardo et al. (2015)
	Rice straw	279–280	Labatut et al. (2011) Li et al. (2013)
	Switchgrass	122–246	Sondergaard et al. (2015)
	Wheat	245–319	Li et al. (2013)
Oil/LCFA	Rapeseed Oil	704	Menardo et al. (2011) Kougias (2015)
	Oleic acid	837-0.3	Kougias (2015)
Household	Kitchen waste	541–683	Li (2013) Li (2015)

Municipal/ Industrial Wastes	Organic fraction of municipal solid waste Solid cattle slaughterhouse wastes Sewage sludge	300–570 561–657 249–274	Pages-Diaz (2014) Davidsson (2007) Pages-Diaz (2014) Borowski et al. (2014) Cabbai et al. (2013)
Macroalgae	*Laminariadigitata* *Saccharinalatissima*	359±5 285±19	D'Este et al. (2017) D'Este et al. (2017)
Notes: Results are based on biochemical methane potential tests. Differences in values may be attributed to the specific chemical composition of the tested substrates			

2.5 LIGNOCELLULOSIC BIOMASS AND ITS STRUCTURE

LB is one of the most abundant organic matters on earth but its bioconversion to methane via AD is hampered by the cell wall as well as the shielding of cellulose and hemicellulose by impermeable, ingestible lignin (Weiland 2010). Yang et al. (2015) had studied methane yield of LB and found it to be low. It has been found that theoretical methane yield in various cases can be as little as 10%. Figure 2.3 illustrates the minutiae of LB structure drawn by Rubin 2008.

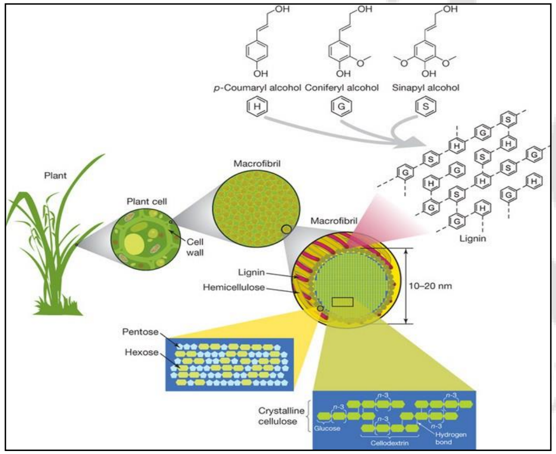


Fig. 2.1 Structure of lignocellulose (Rubin 2008)

2.5.1 CELLULOSE

Cellulose is the main component of higher plants, comprising 20-40% of the cell wall (https://en.wikipedia.org/wiki/Cell_wall). Cellulose is an unbranched polymer chain, organized by glucose units, which are linked by β-1, 4-glycosidic bonds (George 2015). George (2015) also reported that in cellulose the degree of polymerization can be up to 14000 glucose units, where each glucose unit is rotated 180°, relative to the adjoining unit. The cellulose chains form tight aggregates in the form of three dimensional microfibrils. These are stabilized with hydrogen and van der Waals linkages. Each glucose unit is linked via two intra-chain hydrogen bonds and two to three inter-chain bonds. This styles the structure compactly packed and stable. Each microfibril consists of 30-36 parallel cellulose chains. The half-life of crystalline cellulose at a neutral pH is 100 million years (Wilson 2011).

2.5.2 HEMICELLULOSE

Hemicellulose is the polysaccharides of the cell wall that are not part of the cellulosic microfibrillar which is found in the matrix phase (https://en.wikipedia.org/wiki/Hemicellulose). The matrix phase consists of different hemicellulose as well as pectines, and they are attached via hydrogen bonds to the cellulose fibers (Taherzadeh and Karimi 2008). Also, they have reported that hemicelluloses are a group of different branched polysaccharides, which are comprised of both pentoses e.g., xylose and arabinose and hexoses e.g., glucose, mannose, and galactose. The contribution and constitution of hemicellulose varies from different plants and different cells. They can be divided into (1-3, 1-4)-β-D-glucans, heteroxylans, heteroglucans, heteromannans (galactoglucomannans and glucomannans) and arabino-3, 6-galactans (O'Dwyer 1934). Sjostrom (1993) reported hardwood and agricultural plants such as grasses and straw, the dominant hemicelluloses is xylan, while for softwoods, it is glucomannan. Sjostrom 1993 also stated that lignocelluloses consist of approximately 20-35% hemicelluloses and the degree of polymerization is often around 200.

2.5.3 PECTINS

Pectins are one more type of polysaccharides that occur in the primary cell wall of grasses (Satari et al. 2019). These polysaccharides are hydrophilic and highly diverse in nature (Abiola 2017). Gama, Van Dyk and Pletschke (2015) reported that the major components in pectins to be: galacturonic acids, followed by rhamnose, arabinose, galactose, fucose, and apiose.

2.5.4 LIGNIN

Lignin is the third most abundant substance next to cellulose and hemicellulose in nature. Davin (2008) reported lignin as complex, amorphous, branched polymers constructed of different phenylpropane units. The monomeric composition of lignin varies; however, most monolignols are derived from p-coumaryl alcohol, coniferyl alcohol or sinapyl alcohol (Hendriks and Zeeman 2009). Lignin can also be designed as a mixture of p-hydroxyphenyl, guaiacyl, and syringyl, based on their aromatic ring substitution pattern (Davin 2008). Davin also observed in his studies that there are inter-linkages between lignin and hemicellulose as well as between lignin and cellulose that keep the lignocellulosic structure together. These inter-linkages can be ester, ether, or glycosidic bonds. Lignin is extremely resistant to biodegradation due to these strong linkages (Davin 2008). According to Jimenez et al (1990), one of the biggest challenges in using lignocelluloses as a feedstock for biogas production is to open up and alter the lignin structure. High lignin content in the biomass has previously been connected to lower methane yields.

2.5.5 EXTRACTIVES

Extractives are non-cell wall components, which can be found in wood, bark and foliage. They are molecules with less than 40 carbon atoms per molecule (Yang and Jaakkola 2011). Kostamo et al. (2004) reported that extractives comprise about 1-5% of the wood and the most common extractives are resin acids, fatty acids, and sterols. Many of the extractives can be toxic to aquatic organisms and should be removed, for example, from the waste water of pulp and paper plants in order to avoid environmental pollution (Leiviska et al. 2009).

The availability of lignocellulosic materials is abundant worldwide (Zhou et al. 2011). Kuhad and Singh (1993) reported that lignocellulose accounts for approximately 90% of total global plant biomass production, which amounts to about 200 × 10^9 tons/year, where about 8–20 × 10^9 tons of the primary biomass remains potentially accessible. LB mainly comprises cellulose, hemicellulose and lignin together with traces of proteins, pectin, extractives and ash in which cellulose and hemicellulose entangled together and shielded by lignin (Chen 2014). This physically covered the utilizable cellulose and hemicellulose part from the degrading/hydrolysing enzymes (Isikgor and Becer 2015). Table 2.3 shows the lignocellulosic contents of three major plant groups.

Table 2.3 Composition of the lignocellulose groups (expressed as % of original dry matter).

Lignocellulosic Content	**Softwood (spruce) (%) Jin et al. (2006)**	**Hardwood (beech) (%) Jin et al. (2006)**	**Grass (switch grass) (%) Esteghlalian et al. (1997)**
Cellulose	44.7%	45.6	32.2
Hemicellulose	22.9	25.9	24.4
Lignin	30.6	23.8	23.2
Others	1.8	4.7	20.2

According to Bajpai (2016) polymers present in the LB forms hetero-matrix to different degree and varying composition and it depends on the species and growth age. The relative abundance of these polymers is a key determinant of the amount of methane that can be harnessed from a given biomass via AD (Kreuger et al. 2011). Consequently, this makes the hydrolysis stage of AD the rate-limiting step (Adekunle and Okolie 2015). Taherzadeh and Karimi (2008) stated that to exploit the potential of LB an appropriate pretreatment method is needed prior to AD in order to open up the recalcitrant structure and reduce the crystallinity of cellulose. Thus, the pretreatment method should be preferred to produce low amounts of fermentation inhibitors, like furfural and hydroxyl methylfurfural, as these may hinder the hydrolysis and further degradation (Baruah et al. 2018). Therefore, the success and suitability of different pretreatment methods should be assessed in terms of biogas yield. Many researchers have been working on pretreatment for AD still it’s in preliminary phase and requires to develop optimized pretreatment method for efficient biogas production. Table 2.4 shows biogas/methane yield from various lignocellulosic biomass wastes with different types of pretreatment process.

Table 2.4 Biogas production from lignocellulosic biomass

Sr.No	**Sources**	**Treatment**	**Methane/Biogas yield**	**Ref.**
1.	Switch-grass	Mulching, Alkalinization and Autoclaving	256.6 ± 8.2 mL g^{-1} VS	Frigon et al. (2012)
2.	Rice straw biomass	NaOH	74.1 L/kg VS	Chandra et al. (2012)
3.	Oat chopped/squashed -	No	193 mL g−1 VS	Dubrovskis andPlume(2017)
4.	Cotton Stalks	Hydrothermal pre-treatment,	0.184 Nm^3/kg-VS	Adl et al. (2012)
5.	Switchgrass	Alkali pre-treatment with the manganese peroxidase (MnP),	297.7 mL g^{-1} VS	Frigon et al. (2012)
6.	lipid-rich fat, oil, and grease waste	Algae biomass residue was co-digested	0.54 L CH4/g VS·d	Park and Li (2012)
7.	Oat husks	-	242 LN CH4 kg^{-1} VS	Kusch et al. (2011)
8.	Reed canary grass	Acid	8.26 L CH4 per *g*	Lakaniemi et al. (2011)
9.	Bagasse	Lime /Alkaline hydrogen peroxide	72.1 L methane/kg bagasse	Rabelo et al. (2011)
10.	Dried grass silage	NaOH	452.5 mL CH4/g VS	Xie et al. (2011)
11.	Grass silage, sugar beet tops and oat straw with cow manure	Semi-continuously fed laboratory continuously stirred tank reactors (CSTRs).	268, 229 and 213 L CH4 kg^{-1} VSadded	Lehtomaki et al. (2007)
12.	Mixed green leaf biomass	-	180 and 360 L biogas/kg TS	Jagadish et al. (1998)
13.	Fallen Leaves	Alkaline	81.8 L methane/kg VS	Niee et al. (2011)
14.	Grass Silage	-	0.141 to 0.204 m^3 CH4 /kg VS	Lehtomaki et al. (2008)
15.	Grass	-	0.15m^3 of Methane/kg of grass	Yu et al. (2002)
16.	Leaf Biomass	-	180 and 360 L biogas/kg TS	Jagadish et al. (1998)
17.	Gliricidia Leaves	-	165–180 mL CH4/g VS	Gunaseelan (1988)
18.	*Miscanthus giganteus*	Fenton	13.6 Ndm^3 biogas/kg TS	Karina et al. (2012)
19.	Grass	-	209 L Methane/kg VS	Owens et al. (1993)
20.	Leaves	-	123 L Methane/kg VS	Owens et al. (1993)
21.	Branches	-	134 L Methane/kg VS	Owens et al. (1993)
22.	Blend Of Grass, Leaves And Branches	-	143 L Methane/kg VS	Owens et al. (1993)
23.	Grass Silage Liquor	-	0.385 m^3 Methane kg^{-1} COD.	Abu-Dahrieh et al. (2011)
24.	Phragmites australis -	-	120 mL CH_4/g VS	Pelegrin and Holzem (2017)
25.	Rice straw	-	92 to 302 mL CH4/g VSadded	Rahnama et al. (2013)

2.6 PARAMETERS AFFECTING BIO-METHANATION PROCESSES

The key parameters that affect the reactions involved in AD process and biogas production are given and discussed below:

i. Feedstock and nutrients
ii. Carbon to nitrogen (C/N) ratio
iii. Temperature
iv. Total solids (TS)
v. Organic loading rate(OLR)
vi. Hydraulic retention time (HRT)
vii. Alkalinity
viii. pH
ix. Volatile fatty acids (VFAs)
x. Moisture
xi. Additives

2.6.1 EFFECT OF FEEDSTOCK AND NUTRIENT ON BIOGAS PRODUCTION

Biogas can be produced from different organic resources. Globally utilized feedstocks in anaerobic digesters consists of sewage sludge, waste water, municipal solid waste (MSW), assorted industrialized food wastes, slaughter house waste, animal dung and energy crops (Kougias and Angelidaki 2018).

To achieve an adequate growth of the microbes in AD reactor and to augment biomethanation, a proper nutrient is required. The nutrients essential for growth are the macronutrients, such as carbon, nitrogen, hydrogen, phosphorus, potassium and sulphur (Vavilin et al. 2008) and micro-nutrients such as cobalt, copper, iron, molybdenum, nickel, selenium, tungsten, and zinc, as well as vitamins (Schink 1997). The microbes prerequisite an energy source for their growth and activity with chemical compounds such as proteins, fats, or carbohydrates. Mao et al. (2015) had reported that microorganism uses carbon for cell structure, nitrogen for protein biosynthesis, and sulphur during the methanogenesis phase. According to Mittal (1996) micro nutrients or trace elements are also vital co-factors or parts of co-enzymes which are directly linked to the biochemistry of biogas production. They have also stated the mechanism of energy production in which the energy source is oxidized while electrons/protons are transferred through different intermediates and in the end to the electron acceptor energy is produced. Barun (2007) reported that the composition of biogas and methane yield is influenced by feedstock quality, type of AD reactor and retention time. According to Weiland (2010) studies fats produces higher methane yield but requires a longer retention time compared with proteins and carbohydrates due to low microbial availability. However, feedstock generally measured in TS/VS or in COD, which calculate the fraction of carbon-based material and it is easily practice and determine in the lab environment. The biogas yield varies significantly with different feedstock and it is entirely dependent on feedstock VS, fixed carbon, TS and C: N ratio (Weiland 2010).

2.6.2 ROLE OF CARBON AND NITROGEN ON BIOGAS YIELD

Carbon is the chief chemical elements in organic materials break down by microorganism to release methane and carbon-dioxide in the process. Microorganisms require carbohydrate as energy source and fixed quantity of nitrogen in feedstock to execute their metabolic function (Mittal, 1996). Nijaguna (2002) studied that the fermentative bacteria uses carbon 25 to 30 times as fast as nitrogen, therefore demanding the optimum C:N ratio of 25 to 30:1 and variation from this proportion slow down the process. Mittal (1996) reported a high C:N refers the nitrogen will be utilized before carbon is digested and conversely, a low C:N ratio means too much nitrogen in relation to carbon, which results in high ammonium accumulation tending to become toxic to anaerobic bacteria. Therefore an optimum mix of the input substrate should have the optimum C: N of 25:1 to 30:1. For optimizing the C:N proportion of agricultural residues, co-digestion with sewage sludge, animal dung or kitchen waste is recommended (Hagos et al. 2017).

For biogas production, feedstocks or substrates can be characterized as carbon-rich or nitrogen rich depend on the availability of carbon and nitrogen contents and further it benefits in fixing the mixing ratio of different feedstocks during co-digestion. Table 2.5 shows carbon to nitrogen ratio for a number of organic wastes.

Table 2.5 C:N (w/w) ratios in various organic wastes (Chandel and Sukumaran 2017)

Waste material	C/N (w/w)	Waste material	C/N (w/w)
Animal, Human Faeces		**Plant Waste**	
Cattle wastes	25	Wheat straw	12.8
Cattle dung/Buffalo dung	24	Rice straw	67
Poultry dropping	7.3	Corn stalks	53
Sheep wastes	29	Bean stalks	32
Pig Wastes	13	Peanut stalk and leaves	19
Horse wastes	25		
Urine	0.8	Rotted sawdust	200-500
Blood	3	Purslane	8
Slaughter house wastes	2	Amaranthus	11
Night soil	29	Cockstoot	19
Household Wastes		Lucerne	18
Raw garbage		Seaweed	79
Bread	25		
Potato tops	25		
Cabbage	26		
Tomato	12		
	128		

2.6.3 EFFECT OF TEMPERATURE ON BIOGAS PRODUCTION

According to Mittal (1996) and Weiland (2010) AD process can be operated over a wide temperature range i.e. psychophilic (11-25°C), mesophilic (35 to 40°C) and thermophilic (50 to 55°C) in which mesophilic and thermophilic have gained the most attention and recognition. Traditionally, AD was generally applied in the mesophilic temperature range. Labatut, Angenent, and Scott 2014 (2014) had reported that thermophilic process is more susceptible to inhibition, less stable and more rapidly leads to process failure. Shi et al. (2013) reported that thermophillic AD is increasingly promoted especially in lignocellulosic solid state anaerobic digestion (SS-AD) processes. This may be because the high operating temperature can simplify hydrolysis of crystalline cellulose (Frigon and Guiot 2010; Shi et al. 2013).

Temperature influences the growth and activity of microorganism in AD process as well as the separation of methane and carbon dioxide between liquid and gaseous phase's (Gerardi 2003). Mittal (1996) studied that it is essential to control the factors responsible for the change in temperature. Bushwell and Hatfield (1996) recognised that the environmental conditions promote anaerobic micro-organism in AD process for the biogas production.

David et al. (2018) conducted study on co-digestion of food waste and three different lignocellulosic wastes i.e. corn stover, prairie cord grass and unbleached paper for thermophilic AD to overcome the limits of digesting food waste alone (higher volatile fatty acid and C:N ratio problem). He had found that using an enriched thermophilic methanogenic consortium for 30 days of incubation at 60°C (100 rpm) with food and lignocellulosic waste combinations exhibited optimistic synergistic effects of co-digestion and highest methane yield of 305.45 L kg^{-1} VS. Lier (1995) infers the effect of temperature in the growth rate of methanogens in psychrophilic, mesophilic and thermophilic temperature which is shown in Fig. 2.4.

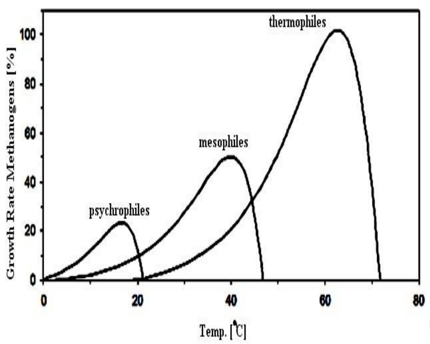


Fig. 2.2 Growth rate of methanogens under psychrophilic, mesophilic and thermophilic conditions (Lier 1995).

In Europe, small scale digesters are operated in psycrophilic temperatures without any additional heating however large scale anaerobic digesters are run at mesophilic or thermophilic conditions (Nielsen et al. 2017). In thermophilic reactor compared with mesophilic, the microbes shows 25-50% higher activity, resultant in higher methane productivity. Though, the microbial population at thermophilic conditions are more delicate to fluctuations in temperature or to different toxic compounds (Deublein and Steinhauser 2008). In contrast, mesophilic microorganism are stronger but with a lower reaction rate (Nielsen et al. 2017).

2.6.4 INFLUENCE OF TOTAL SOLID (TS) ON BIOGAS PRODUCTION

Many researchers had reported that TS of the feedstock plays an important role in production of biogas. Feedstock fed into a biogas digester comprises both solid matter and water. The solid materials consist of both volatile organic compounds and non-volatile or fixed solids. Only the volatile matter goes through digestion in anaerobic digestion process. The solid matter can be further divided into 70% of VS and 30% of fixed solids. TERI's (Tata Energy Research Institute) investigated and reported that fresh cattle waste i.e. cattle dung contains 20% TS matter and 80% of water. According to TERI (1987) TS between 8 and 10% is preferred in the feedstock for optimum biogas production. This appropriate value of TS is attained by formulating a homogenous mixture of 1:1 of cattle dung and water. On the other hand, for dry cattle dung, the quantity of water should be increased as a result to a desired consistency of the input i.e. 1:1.25 or 1:1.2. The quality of the dung (diluted or thick) effected or lowers the biogas production. It has been observed that in diluted cattle dung chances of settling down of solid particles are more which lowers the biogas production whereas for thick cattle dung the solid particles hinder the flow of gas produced at the bottom part of the digester (TERI 1987). Addition solid resources such as stones should be segregated from the feedstock before feeding into the anaerobic digester to avoid the loss of operative volume of the digester (Chandel and Sukumaran 2017). Pavan et al. (1997) reported that biogas and methane production efficiency is affected by TS content of dry waste in AD process. Abbassi-Guendouz et al. (2012) investigated that the total methane production reduced with TS contents increases from 10% to 25% in batch AD of cardboard under mesophilic conditions. Forster-Carneiro et al. (2008) showed that the biogas and methane production decreased while the total solids content increases from 20% to 30% in dry batch AD of food waste. Mahanta et al. (2004) had conducted experiment and observed that for cattle dung the maximum gas production was obtained with 8% TS.

2.6.5 EFFECT OF ORGANIC LOADING RATE (OLR) ON BIOGAS PRODUCTION

The amount of feedstock added per reactor volume and digestion time is defined as the OLR (Babaee and Shayegan 2011). Initially, AD process required a lower OLR in starting days, later while established and well-functioning processes can tackle a higher OLR (Babaee and Shayegan 2011). Schnurer and Jarvis (2010) reported thermophilic processes can handle 4-5 kg VS/m^3 /day, though mesophilic processes normally work at 2-3 kg VS/m^3 /day. Polizzi (2018) investigated in his study and reported that a higher ORL or overloading of the digester with addition of higher amount of degradable feedstock often leads to an inhibition caused by VFA accumulation. Subsequently, microorganism cannot stay alive in an acidic environment and biogas production will be stuck. Correspondingly, for low feeding conditions or low OLR, the gas production will be less because of ammonia accumulation henceforth alkaline environment prevails which is not a favourable state for the anaerobic bacteria (Weiland 2010). VS represent that portion of the organic-material solids that can be digested, while the remainder of the solids is fixed. The fixed solid and a part of the VS are non-biodegradables. According to Mattocks (1984) OLR can be influenced by the categories of biowastes fed into the digester because the same determines the level of biochemical activity that will occur in the digester.

2.6.6 EFFECT OF HYDRAULIC RETENTION TIME (HRT) ON BIOGAS PRODUCTION

HRT is an important parameter in AD process and can be defined as the digestion time required converting VS in the substrates into biogas (Shi et al. 2017). Generally the organic loading rate is higher than the conversion of substrate into methane and carbon dioxide, which shows that not all the feeding material is degraded (Chandel and Sukumaran 2017). The residues are made of the organic material that is not degraded, but also inorganic and inert material, as well as biomass from the microorganisms and salts and water (Shi et al. 2017). Shi et al. (2017) had reported that the HRT of ADs is generally 10-25 days or longer. Slowly degraded matters, like cellulose-containing crystalline materials or lignin, often need a longer HRT than easily degraded resources such as simple monomers of sugars. In general, a longer HRT is required when the ORL is high, in order to prevent a excessively low degradation. Schwede et al. (2016) investigated the influence of HRT on nutrient removal, settle ability and biogas production from integration of microalage from freshwater and activated sludge. They demonstrated that 6 and 4 days HRT are optimal for total nitrogen removal for microalgae and a rapid biogas production was observed within 9 days of incubation.

2.6.7 ROLE OF PH, VOLATILE FATTY ACIDS (VFAS) AND ALKALINITY ON BIOGAS PRODUCTION

The AD process functions within a pH range from 6.5 to 8 in which the microbial consortium can replicate and so degrade the available substrates (Cioabla 2012). Weiland (2010) reported that the AD process may be inhibited as results of the propagation of toxic intermediates below or above this range. The pH value in the AD process increases when there is ammonium accumulation (Calli et al. 2005), while the accumulation of VFA decreases the pH value (Gerardi 2003). VFAs are precursors for methane production but their accumulation may inhibit methanogenesis as a result of the drop in pH (Chen, Cheng, and Creamer 2008). The accumulation of VFA will not always result in a pH drop, due to the buffer capacity (alkalinity) of the system (Franke-Whittle et al. 2014). At low pH (3~5), the unprotonated VFAs may enter the microbial cell leading to the acidification of the cytoplasm (Wang et al. 2016). The pH in AD is usually governed by the liquid alkalinity, where feedstock characteristics directly influence the alkalinity through the formation of degradation products such as ammonium, bicarbonate, sulfides and phosphates (Gerardi 2003). Ammonium has a large influence on pH and alkalinity, hence protein rich biomass are associated with high alkalinity (Weiland 2010). pH is the measure of acidity or alkalinity of a solution. During anaerobic fermentation, microorganisms require a neutral or mildly alkaline environment for efficient gas production. A pH between 7 and 8.5 is optimum range for increased gas yield (Mittal 1996). pH is a very important variable and has a strong impact on degradation in AD process. It affects the growth rate of acetogenic and methanogenic microorganisms. Ammonia inhibition phenomena depend on pH and increases with increase in it (Yu et al. 2013).

2.6.8 EFFECT OF MOISTURE CONTENT (MC) ON BIOGAS PRODUCTION

The MC is an important environmental factor in enhancing biogas production. Lay et al. (1997) reported that water is essential to the AD process, since water is the media of all biochemical reactions, and the nutrients for the microorganisms must dissolve in water before they can be assimilated. Conversion of biomass to biogas with help of anaerobes takes place in the liquid phase and then transferred to the gas phase (Manan and Webb 2017). High MC may also assist microbial movement in the reactor matrix (Manan and Webb 2017). Moreover, MC is well known to influence the mass transfer in SS-AD as well as the balance between acidogenic bacteria and methanogenic bacteria (Yang et al. 2015). TS content are inversely proportional to moisture content, which infers higher TS and lower moisture in the system and this dimension influence the other parameters like ammonia and VFAs. Additionally, process improvement such as the addition of fresh water to the inoculum or inoculum recirculation with mixing aid has been reported in some studies (Yang et al. 2015).

2.6.9 EFFECT OF ADDITIVES ON BIOGAS PRODUCTION

Kumar et al. (1987) experimented to see the effect of additives on biogas production and found that 5% of commercial charcoal addition to cattle dung on dry weight basis increased the production of biogas by 17% and 35% in batch and continuous processes, respectively. Geeta et al. (1990) studied the effect of additives on biogas production. They used vermiculite, charcoal and lignin to bovine excreta as feed material and observed the increase in gas production by 15-30%.

2.6.10 INFLUENCE OF BIOFILM AS CARRIER ON BIOGAS PRODUCTION

Support media provide a surface on which microbial biofilms can attach and grow, increasing digester stability increasing the population of the microorganisms inside the digester (Sahar and Naz 2016). Biofilms form on the substrates and enhance the digestion increasing the OLR. This decreases COD (Chemical Oxygen Demand) leading to high rate of biogas production (Mshandete et al. 2008).

Andersson and Bjornsson (2002) investigated different support media and found that the use of straw as support media increases methane production more than glass or plastic carriers. Yang et al. (2004) reported that synthetic materials such as loofah sponge, rock wool and polyurethane foam have been found to be effective support media. A comparative study by Mshandete et al. (2008) looked into the effectiveness of waste sisal fibers, pumice and glass beads as biofilm carriers and found that sisal fibres was the most effective one among these materials. Pinho et al. (2004) reported that support media made of polyurethane matrices in batch reactor along with mechanical stirring improves biogas production.

Cuetos et al. (2017) had conducted AD batch experiments and found that addition of activated carbon to the residual poultry blood improved the digestion process due to its adsorption ability for ammonium. He has not identified any major enhancement in biogas production but observed improved incorporation of C3-C5 species, possibly signifying enhancement of syntrophic metabolism.

Studies suggest that effective biofilm carriers have a rough uneven surface with crevices, microscopic ridges and pores, which are good for attachment of bacteria. Biochar shows a rough, highly porous surface area, with both micro and macro structures that will allow organisms to adhere to their surface (Jaafar et al. 2015). He also reported that the size of the pore structure will determine whether biofilms develop only on the surface or also within the internal structure of the char. These characteristics make biochar a potential candidate as a cheap and available resource for microbial cell immobilization in anaerobic digesters (Lehmann et al. 2011). But in literature very few studies have been found on complete investigation on addition of optimum quantity of biochar or activated carbon with effective mechanism for biogas enhancement.

2.7 EFFECT OF CO-DIGESTION OF LIGNOCELLULOSIC BIOMASS ON BIOGAS PRODUCTION

Co-digestion is the simultaneous digestion of a similar mixture of two or more substrates (Ogunkunle, Olatunji, and Jo 2018). Conventionally, AD was a single substrate and utilized for single purpose treatment. In recent times, it has been realized that utilization of variety of substrates applied at the same time increased the stability of AD system (Tufaner and Avşar 2016). The most common situation is when a major amount of a main basic substrate (e.g. manure or sewage sludge) is mixed and digested together with minor amounts of a single, or a variety of additional substrate (Braun 2002). Furthermore, the combination of co-substrate results in positive synergestic effects to the system, such as a better balance (C/N ratio, pH, and moisture), dilution of potentially toxic compounds, and supplement of trace elements (Davidsson et al. 2008; Fernandez et al. 2005). Therefore, co-digestion is potential way to resolve the boundaries of mono-digestion, increase biogas production, develop waste recycling and contribute to environmental security (Wang et al. 2014). The studies on this subject have grown quickly in recent years. To overcome these limitations of mono-digestion, co-digestion was put forward and applied.

Biogas can be produced by a vast variety of organic substrates like cattle dung, agricultural biomass, food waste and municipal waste through the AD process but initially it has been started to use monodigestion viz; animal manure and sludge. However, worldwide it is accepted as a standard that manure has low methane yield and hence, co-digestion is realistic approach to obtain a more economical and feasible process. Co-digestion provides prospect to optimize the biogas yield and avoid AD system potential process imbalance through a suitable amalgamation of particular lignocellulosic biomasses (Li et al. 2013). The ideal co-substrate should have a high methane yield, be abundant, and enhance the biogas process (Li et al. 2013). Additionally, it should contribute to clean the solid waste and lower emission of GHG in the environment and not compete with food production because of increasing global population and scarcity of fertilized land. Lehtomaki, Huttunen, and Rintala (2007), Demirbas (2006), Kalia and Kanwar (1990) had worked on the co-digestion of animal dung with different plant materials and results and reactor type are illustrated in Table 2.6.

Table 2.6 Literature on effect of co-digestion of biomass on biogas production

Author	**Feed materials**	**Reactor type**	**Results**
Lehtomaki et al. (2007)	Sugar beets, oat and grass silage straw together with animal dung	semi-continuously fed continuously stirred tank reactors	The results showed that combination of animal dung and crops increased VS in the feedstock up to 40 %, resulting in methane yields of 0.268, 0.229 and 0.213 m3/kg VS than alone animal dung 0.155 m3/ kg VS
Lehtomaki et al. (2006)	cow dung and straw	continuously fed and stirred reactor	Digestion of cow dung and straw counting up to 30 % of VS added resulted in 0.213 m3/ kg VS methane in 20 days at 35ºC. Increasing the straw concentration to 40 % of VS added resulted in a reduced methane yield of only 0.157 m3/kg VS under similar conditions
Demirbas (2006)	Straw, kitchen waste and dung mixed in different combinations	Batch reactor	Highest biogas has been found with combination of kitchen waste: pig manure: rice straw with 0.4: 1.6:1 ratio, respectively.
Kalia and Kanwar (1990)	Mixtures of fresh and partially-decomposed Ageratum with cattle dung	Batch reactor	Fresh *Ageratum* mixed with cattle dung in a ratio of 3:2 did not produce any gas but similar mixtures of partially decomposed *Ageratum* and cattle dung yielded about 9% more biogas than that of pure cattle dung.

2.8 PRE-TREATMENT OF LIGNOCELLULOSIC BIOMASS

The bioconversion of LB to bio-energy in the form of methane via AD may be limited by its hydrolysis as the digestible cellulose and hemicelluloses are covered by a sheath of insoluble lignin (Weiland 2010). The ultimate goal of any pretreatment technology is to change the structure and composition of the feedstock to remove the obstacles of hydrolysis and therefore improve the rate of enzymatic hydrolysis and increase the yields of fermentable sugars from cellulose and hemicelluloses (Taherzadeh and Karimi 2008). Jonsson and Martin (2016) reported that some hydrolytic substances produced via certain pretreatments methods may be too toxic to the enzymatic biocatalyst and the anaerobic consortium which can lead to poor process yields, and even cessation of the AD process.

A number of pretreatment techniques have been studied meticulously proceeding to biogas productions from lignocellulosic feedstocks with respect to simplifying the biological degradation. Each of the treatment methods has its advantages and disadvantages. These pre-treatment methods can be divided into mechanical, thermal, chemical as well as, biological treatments or a combination of these techniques as it is shown in Table 2.7.

Table 2.7 Summary of pretreatment techniques investigated for enhancing biogas production from lignocellulosic materials.

Method	Processes	Change on biomass	Notable marks	References
Physical pretreatments	-Ball milling -Hammer milling -Colloid milling -High pressure steaming -Expansion -Pyrolysis -Gamma-ray irradiation -Electron beam irradiation -Microwave irradiation	-Increase in accessible surface area and size of pores -Partial depolymerization of lignin -Decrease in degree of polymerization and crystallinity of cellulose -Partial hydrolysis of hemicellulose	-Most of the methods are highly energy-Demanding -Most of them cannot remove the lignin -It is preferable not to use these methods for industrial applications -No chemicals are generally required for these methods	Barakat et al. (2014) Mais et al (2002) Tassinari et al. (1997) Park et al. (2010) Eskicioglu et al. (2007) Kumakura et al. (1983) Rusanowska et al. (2018)
Biological pretreatments	-Actinomycetes -Fungi	-Delignification -Reduction in degree of polymerization of hemicellulose and cellulose	- No chemical Requirement - Mild environmental Conditions - Very low treatment rate - Did not consider for commercial application	Ahring et al. (1996) Kurakake et al. (2007) Mishra et al. (2018)
Physico-chemical and chemical pretreatments	**Explosion:** -Steam explosion -Ammonia fiber explosion (AFEX) -CO_2 explosion -SO_2 explosion **Alkali:** -Sodium hydroxide -Ammonia -Ammonium sulphite **Gas:** -Chlorine dioxide -Nitrogen dioxide **Acid:** -Sulphuric acid -Hydrochloric acid -Phosphoric acid **Oxidising agents:** -H_2O_2 -Ozone **Cellulose solvents:** -Cadoxen Solvent extraction of lignin: -Ethanol-water extraction -Benzene-water extraction -Butanol-water extraction -Ethylene glycol extraction	-Delignification -Reduction in degree of polymerization and crystallinity of cellulose -Partial or complete hydrolysis of hemicellulose	-These methods are among the most effective and include the most promising processes for industrial applications - Usually rapid treatment rate - Typically need harsh conditions -There are chemical requirements	Emmel et al. (2003) Kim et al. (2006) Taherzadeh et al. (2008) Bjerre et al. (1996) Goel et al. (2003) Kumar and Sharma (2017) Bharathiraja et al. (2018) Geun, Pu, and Ragauskas (2017) Shirkavand et al. (2016)

Palmowski and Muller 2000 reported that during mechanical treatment milling is applied to cut the LB into smaller pieces which can effectively work on reducing the crystallinity and the degree of polymerization resulting in increasing available surface area for the attachment of degrading enzymes. Walpot (1986) reported that colloid mill and fibrillator can only work properly for wet materials, such as wet paper and paper pulps while roller mill, extruder and hammer mill are usually used for dry materials. Furthermore, mill ball can work on either dry or wet materials.

Thermal treatment refers to pre-treatment methods performed at higher temperatures (Ariunbaatar et al. 2014). The hemicelluloses part is the first to solubilize at temperatures above 150-180°C followed by the lignin part (Garrote et al. 1999). Gregg and Saddler (1996) reported that for the period of the breakdown of hemicelluloses acids will also be formed, which then will act as catalysts in the further break down and hydrolysis accelerating the solubilization of hemicellulose’s oligomers. However, Mok and Antal (1992) studied the risk of formation of inhibitory products, such as phenolic and heterocyclic compounds, furfural and HMF, especially in acidic conditions is elevated in heat pre-treatment. Gossett et al. (1982) reported that these inhibitory or toxic products have an adverse effect on the microorganisms during the subsequent bioconversion processes. Therefore, pre-treatment at temperatures of 250°C and above should be avoided due to production of unwanted products and pyrolysis reactions (Brownell et al.1986).

The steam explosion process another pretreatment method which is performed at high temperatures up to 240°C and pressure 33.4 bar and lasts for a few minutes. The pressure is released and biomass cools down quickly thereafter. The main purpose of this treatment is to get 80-100 % of the hemicellulose fraction solubilized making the cellulose fraction accessible to enzymatic hydrolysis (Grethlein and Converse 1991). The wet oxidation method has been widely used as an alternative to steam explosion. Wet oxidation is a form of hydrothermal treatment, where oxygen or air is used for the oxidation of dissolved or suspended components in water at temperatures above 120°C (e.g. 148-200°C) for a period of 30 min (Palonen et al. 2004). In industrial applications, wet air oxidation processes have been used to handle waste with high organic matter by oxidation of soluble or suspended matter, with oxygen in the aqueous phase at high temperatures (150-350ºC) and pressure (5-20 MPa)(Jorgensen et al. 2007). As a result of wet oxidation, cellulose is partly degraded, hemicelluloses are being hydrolyzed and lignin is subjected to oxidation and cleavage. All these alterations lead to an increase in the accessibility of the substrate to enzymatic hydrolysis (Schultz et al. 1984). Wet oxidation has proven a promising method for pre-treatment of lignocelluloses because it ruptures the crystalline structure of cellulose (Panagiotou and Olsson 2007) and has been a successful alternative for the treatment of wheat straw and hardwood (Schmidt and Thomsen 1998).

According to the literature reviewed, dilute acid treatment is among one of the most effective methods for LB (Digman et al. 2010). Dilute acid treatment typically carried out, either at high temperatures (T > 160°C) and continuous flow with low solids loading and short retention time (e.g. 5 min), or low temperatures (T ≤ 160°C) and batch process with high solids loading at longer retention time (e.g. 30-90 min) (Harmsen et al. 2010). Variety of acids i.e. dilute sulfuric acid, dilute nitric acid, dilute hydrochloric acid, dilute phosphoric acid, and peracetic acid reported in literature that have been applied to a wide range of feedstocks, including softwood, hardwood, herbaceous crops, agricultural

residues, wastepaper, and municipal solid waste. Among the acids, dilute sulfuric acid has been broadly applied due to its low cost and high effectiveness (Kumar and Sharma 2017). When dilute acid is added to the biomass and the mixture is kept at 160-220°C for a few minutes, this treatment offers decent performance in the breakdown of hemicelluloses recovering monomeric sugars and soluble oligomers from the cell wall into the hydrolyzate. Consequently, the removal of the hemicellulose fraction increases the porosity of the material enhancing the digestibility (Chen et al. 2011). However, lignin is not significantly removed in this process. Therefore, this method is more suited for biomass with low lignin content (Yang and Wyman 2008).

The drawback of this method is the risks of further degradation of hemicelluloses to furfural and hydroxymethyl furfural, which then have an inhibitory effect on the subsequent microbial processes (Kumar and Sharma 2017). Wyman and Abelson (1996) reported that concentrated acid pre-treatments are mainly performed at low temperatures using concentrated H_2SO_4 and HCl (30-72 %). These are powerful agents for cellulose hydrolysis and give high sugar hexose yield. However, highly concentrated acid causes corrosion. Thus, the material used to construct the reactor must be highly resistant to corrosion. In addition, the acid has to be recovered and the acid containing steam produced must be neutralized. Due to its high cost this pre-treatment method is not economically feasible for commercial use (Wyman and Abelson 1996).

Alkaline pre-treatment is one of the major chemical pre-treatment techniques used. This pre-treatment refers to application of various bases, including sodium hydroxide (Zhao et al. 2008; Sun, Lawther, and Banks 1995), potassium hydroxide (Brodeur et al. 2011), calcium hydroxide (lime) (Park et al. 2010) aqueous ammonia (Park et al. 2010) and ammonium hydroxide. Alkaline pre-treatment mainly results in delignification, together with solubilization of a remarkable amount of hemicelluloses. It is successful in removing acetyl and the various uronic acid substitutions on hemicelluloses which otherwise may decrease the accessibility of enzymes to hemicellulose and cellulose surfaces (Brodeur et al. 2011). Kumar and Sharma (2017) reported that the efficacy of alkaline treatment extensively depends on the properties of the lignocellulosic material treated and on the treatment conditions. Generally, alkaline pretreatment is more successful on the substrates with low lignin content such as hardwoods and agricultural residues than hardwoods with higher lignin content. Alkaline pre-treatment is based on saponification of intermolecular ester bonds cross linking lignin and hemicelluloses resulting in a decreased degree of polymerization (DP) and crystallinity, the disruption of the lignin structure and the separation of linkages present between hemicelluloses and lignin (Hendriks and Zeeman 2009). Among the different alkaline solutions investigated for the treatment of lignocelluloses, aqueous ammonia and lime (calcium hydroxide) pre-treatments are considered to be the most effective and inexpensive methods (Kumar and Wyman 2008). Pre-treatment with NaOH also shows great enhancement in the subsequent enzymatic saccharification phase by decreasing crystallinity and increasing the degree of polymerization (Kumar and Sharma 2017).

A new significant pretreatment method is oxidative pre-treatment which is carried out by the addition of an oxidating agent, such as hydrogen peroxide, ozone, oxygen or air. The main purpose of this pre-treatment is delignification. The oxidizing chemicals are highly reactive with the aromatic ring of lignin residues, and, consequently, the lignin polymer will be converted to carboxylic acids (Panagiotou and Olsson 2007). However, the formed acids can cause inhibition in the fermentation processes that follows, which means that these inhibitors have to be neutralized or removed before the substrate enters to the digester. Aside from delignification, the oxidative treatment also affects the hemicellulose fraction (Karimi and Taherzadeh 2008).

A significant drawback with mechanical, thermal and chemical pre-treatment techniques is the requirement of high energy input for an improved biomass conversion. Moreover, these methods are generally carried out using expensive instruments and chemicals. In contrast, utilizing microorganisms to enhance the biodegradability of organic matter and consequently methane production, offers advantages such as low-capital cost and low energy demand. In addition, these methods are environmentally friendly (Kumar and Sharma 2017). On the other hand, biological treatment methods require long resident times because the rate of the biological hydrolysis is usually very low (Sun and Cheng 2002).

Lignin is known as a major factor to determine the extent of biomass degradation in anaerobic conditions (Shi, Chinn, and Sharma-shivappa 2008), and biological pre-treatment methods have been considered as effective and cheap methods of delignification. Generally, improvement in methane production by fungi is explained by the disruption of cell wall structure. Additionally, lignin degradation also increases the surface area of the cellulose to develop its susceptibility to microbes and enzymes (Kumar et al. 2009). Microorganisms, such as brown, white and soft rot-fungi, are engaged to degrade hemicelluloses and lignin, but due to its high resistance only a very small amount of cellulose will be degraded (Sun and Cheng 2002).

Many researchers have been working on pretreatment methods for LB to identify the efficacy in degradation of LB structure and their positive effects on probable applications. However, there is no efficient, cost effective and environment friendly method is available. This is why it is vital to have sufficient research either in selecting the best pretreatment method or versed with strategies to alleviate such inhibition. The pretreatment methods employed during this thesis work were chosen with the aim to improve the overall process yield and rate of AD, while at the same time minimizing the risk of inhibition.

2.9 ENHANCEMENT OF BIOGAS PRODUCTION FROM LB AFTER PRE-TREATMENT

Nakamura et al. (2003) studied the effect of steam explosion on wood chips (Eucalyptus globules) at a pressure of 25 atm and a steaming time of 3 min prior to biogas production. The methane yield obtained was 0.194 m3/kg TS after the steam explosion treatment compared to untreated samples just 0.014 m3/kg TS.

Comparable results were achieved by Take et al. (2006) when steam explosion treatment was applied on wood, Japanese cedar chips and prior to biogas production. The treatment conditions were 4.51 MPa (258°C) for 5 min. The pretreated sample yielded 0.180 m3/kg TS methane, while the methane yield for untreated wood samples was almost zero.

Teghammar et al. (2010) investigated paper tube residuals as substrate for biogas production. Steam explosion treatment with the addition of sodium hydroxide and/or hydrogen peroxide has been applied to improve the biogas production. The best result was obtained after steam explosion at 220°C for 10 min and with the addition of both 2 % NaOH and 2 % H_2O. Treatment under these conditions resulted in an increase in the methane production by 17% i.e. 0.493 Nm3/kg VS methane was produced from the treated samples.

Mirahmadi et al. (2010) have performed alkaline pre-treatment using 7% wet weight NaOH on milled spruce and birch samples at different temperatures between -15 and 100°C. Anaerobic batch digestion assay was then performed at thermophilic conditions (55°C) for 30 days. Treatment of birch at 100°C led to a methane yield of 0.46 m3/kg VS, compared to 0.25 m3/kg VS for the untreated birch samples. On the other hand, the best results for spruce were obtained when the samples were treated with NaOH at 5°C resulting in a 74% improvement in the methane production. Furthermore, it was concluded that the use of alkaline treatment was more successful for hardwood (birch) than for softwood (spruce). There was, however, almost no destruction of lignin observed.

In a study of Sharma et al. (1988) the effect of particle size was investigated for wheat straw and rice straw through anaerobic batch digestions. Among five particle sizes (0.088, 0.40, 1.0, 6.0 and 30.0 mm), the maximum biogas yield was produced from raw materials with a particle size of 0.088 and 0.40 mm, both resulting in an almost equal quantity of gas production. It was observed that grinding below 0.40 mm would be uneconomical. Size reduction of wheat and rice straw produced 0.249 m3 CH_4/kg VS and 0.365 m3/kg VS, respectively, while using larger particle size (30.0 mm) yielded 0.162 and 0.241 m3 CH_4/kg VS from wheat straw and rice straw respectively.

Zhang et al. (1999) carried out experiments on conversion of rice straw into biogas using a high-rate AD system, SSAD system. They investigated the effect of different pre-treatments, i.e. mechanical, thermal and chemical using ammonia. The highest biogas yield was achieved when combining grinding to 10 mm particle size, heating at 110ºC and using 2% ammonia. The biogas yield of untreated whole rice straw was 0.38 m3/kg VS which increased to 0.47 m3/kg VS by applying this cobination of the treatment methods.

2.10 SUMMARY OF THE LITERATURE REVIEW

As observed from the literature review, AD has been proved useful, reliable, cost-effective, and suitable for household as well as large scale biogas application. An extensive literature review has been carried out on several aspects of biogas production processes, influence of different feedstock on biogas production, biofilm carrier, important parameters, different pretreatment methods and its effect on biogas generation, etc. It is observed that most of the experiments were performed on the first generation biomass whereas there is very limited work available for second generation biomass with optimized methodology. LB is most abundantly in all the geographic locations, which has enormous potential for biogas production but yet not exploited efficiently. LB has high cellulose and volatile matter content which is good source for bioenergy conversion but due to its recalcitrant structure it's difficult to hydrolyze by AD micro-organism easily. Hence, characterization of feedstock is important to ascertain it properly through suitable pretreatment route. Very few literatures have been found on characterization of LB which requires further research in terms of efficient biogas production. Mata-Alvarez et al. (2000) reported that utilization of co-substrate usually increases the biogas yields in AD due to positive synergisms established in the digestion medium and the missing resources/nutrients supplied by the co-substrates. In last decade, many researchers have reviewed the co-digestion of animal manure and LB but the optimum combination or mixing ratio is not quantified appropriately for enhancement of biogas production.

So far, many researchers have reported different kinds of pretreatment methods like acid, alkali, wet oxidation, milling and biological methods for the enhancement of biogas production. It is understood that the above mentioned pretreatment method gives encouraging results for breaking up the complex lignocellulosic structure. However, challenges remains in considering sustainable, eco-friendly and cost- effective pretreatment method. Continued research efforts are necessary towards the development of an advanced pretreatment method with fewer disadvantages in all the terms. The findings of Cuetos and co-workers (2017) reported the enhancement of C3-C5 species in AD process due addition of activated carbon which works as micro-organism carrier and ammonia adsorbent but not observed any significant improvement in biogas yield. It is observed from the literature that AD can be improved by using support media so as to retain the methanogenic bacteria for long time in the digester (Pinho et al. 2004). However, very little research information was found on the study on addition of activated carbon towards the same is hardly reported. Few of researchers reported that addition of biochar helps in enhancing the biogas production. But most of the work are in preliminary stages and requires a quantitative optimization to enhance the AD processes as well as methane yield.

In view of the above, in the present work attempt has been made to investigate the detail characterization, co-digestion and pretreatment of LB. Furthermore, experimental study has been conducted to identify the potential of LB for the enhancement of biogas production. In the following chapter (Chapter 3), the detail study on characterization of LB such as saw dust, bamboo dust, rice husk, maize, arecanut shell and duckweed from India and tropical countries is presented.

CHAPTER – 3

CHARACTERIZATION OF LIGNOCELLULOSIC BIOMASS

3.1 INTRODUCTION

Lignocellulosic biomass (LB) wastes are attractive bio-energy resources. They are particularly suited as feedstocks for small-scale decentralized community energy systems because they are locally abundant, able to create energy independence for local communities, and provide greatest sustainability benefits. Fundamental characterization of LB as a feedstock is required for biofuel and chemicals production, which demonstrate very different properties with respect to traditional fossil fuels and their products. Specifically, lignocellulosic resources are more reactive and have a higher volatility than coals. Though, all LB varies significantly in volatile matter content; even the similar kind of LB can change in composition based on the climatic conditions and seasonal deviation. LB feedstock composition determines the theoretical yield from a biochemical conversion, and can thereby have a significant impact on conversion process economics. From these details, the impact of LB characterization on the expanding biogas production can be seen. With knowledge of these complex feedstock compositions and its biochemical and thermal behaviour in conversion processes, yield can be predicted, mathematical models can be created to further understand products, economic analysis can be performed, and most importantly, technology can be formed to make biomass conversion more effectual, operational, and superior alternative for fossil fuel production. Hence present chapter is an attempt to characterize six types of LB abundantly available in India and other tropical countries to identify their potentiality for biogas production.

3.2 METHODOLOGY

A detailed physico-chemical characterization study has been done on bamboo dust, areca nut shell, maize, rice husk, duckweed and saw dust by performing proximate, ultimate analysis as well as calorific value. Determination of cellulose, hemicellulose and lignin content was also done to better understand LB structure by Van Soest method. Additionally, Fourier Transform Infrared (FTIR) spectroscopy, X-diffractometer (XRD) and Thermogravimetric Analysis (TGA) carried out to assess the characteristics with respect to anaerobic digestion which are considered to have a niche market at the small-scale in India. Above mentioned six LB was selected for this thesis work because hardly any literatures found on biogas production from these biomasses.

3.2.1 PREPARATION OF SAMPLES

Lignocellulosic biomass samples viz; bamboo dust, areca nut shell, rice husk, duckweed, maize and saw dust have been used in the present work. Bamboo dust, areca nut shell, rice husk, maize and saw dust were collected from agricultural fields in kamrup district, (Assam). Duckweed has been collected from nearby pond, Amingaon (26.1839° N, 91.6640° E), Assam. All the samples were oven dried and grounded in a high speed rotary cutting mill (0.5 ≤ mm ≤ 1.0) prior to characterization.

3.2.2 PROXIMATE ANALYSIS

Proximate analysis was conducted in triplicate ca. 2 g of sample, in accord with ASTM (American Society for Testing and Materials) protocol for total solids, moisture and ash present in biomass and ASTM Standard E872-82 was followed for volatile matter determination (ASTM, 2013). Fixed carbon was calculated by subtracting the summation of ash, volatile and moisture content from 100%.

3.2.2.1 DETERMINATION OF TOTAL SOLID CONTENT

TS content of feedstock are determined by following standard test methods ASTM E1756-08. Nearly 2 g of the sample biomasses are weighed in a pre-weighed porcelain crucible using an electronic balance with least count of 0.001 g. The samples are placed in the drying oven at 105±3°C for 3 hours. After cooling the samples to room temperature in desiccator, final weight of the dried samples with pre-weighed crucibles are recorded. The percentage of TS content of the sample biomasses are then calculated by using Eq. 3.1

$$\boldsymbol{TS\% = \frac{(mf-mt)}{(mi-mt)}\ x\ 100} \qquad \textbf{(3.1)}$$

where,

TS%= Mass percent of TS based on 105°C dry mass

m_t= tare mass of dried container

m_i = initial mass of container and biomass

m_f = final mass of container and biomass after drying at 105

3.2.2.2 DETERMINATION OF MOISTURE CONTENT

The moisture content of the feedstock is determined as follows: approximately 2 g of the sample biomasses are weighed in a pre-weighed porcelain crucible using an electronic balance with least count of 0.001 g. The samples are placed in the drying oven at 105±3°C for 3 hours. After cooling the samples to room temperature in desiccators, final weight of the dried samples with pre-weighed crucibles are recorded. The percentage of moisture content of the sample biomasses are then calculated by using the following relation.

$$\boldsymbol{M}\% = \frac{\boldsymbol{Ww-Wd}}{\boldsymbol{Ww}}\boldsymbol{x100} \qquad \textbf{(3.2)}$$

where,

M %= moisture content in % (wet basis)

W_w= weight of the wet sample in g

W_d= weight of the oven dried sample in g

3.2.2.3 DETERMINATION OF VOLATILE MATTER CONTENT

Volatile matter content is determined following standard test methods E872-82 (Reapproved 2006). Approximately 1 g of the oven dried sample used for determination of moisture content are further dried at 950±20°C and allowed to ignite for 7 minutes in pre-weighed porcelain crucible with lid. After cooling the samples to room temperature in desiccator, final weight of the cooled crucibles with lid containing the remaining of the burnt sample are recorded. The volatile solid content of the samples is calculated using Eq. 3.3 and 3.4.

$$\boldsymbol{Weight\ loss}\,\% = \frac{\boldsymbol{Wi-Wf}}{\boldsymbol{Wi-Wc}}\boldsymbol{x\ 100 = A}$$

(3.3)

where,

W_c = weight of crucible and cover in g

W_i = initial weight in g

W_f = final weight in g

$$\boldsymbol{Volatile\ matter}\% = \boldsymbol{A-B} \qquad \textbf{(3.4)}$$

where,

A = weight loss, %

B = moisture, %

3.2.2.4 DETERMINATION OF ASH CONTENT

Approximately 1 g of the oven dried sample used for determination of moisture content are further dried at 575±25°C and allowed to ignite completely for 3 hours in pre-weighed porcelain crucible followed ASTM E1755−01 (Reapproved 2015). After cooling the samples to room temperature in desiccator, final weight of the cooled crucibles containing the remaining of the burnt samples are recorded. The ash content of the samples is calculated using Eq. 3.5.

$$\boldsymbol{Ash}\% = \frac{\boldsymbol{Mash-Mcont}}{\boldsymbol{Mod-Mcont}}\boldsymbol{x\ 100} \qquad \textbf{(3.5)}$$

where,

Ash% = mass percentage of ash, based on 105°C oven-dried mass of the sample

M_{ash} = mass of ash and container in g

M_{cont} = tare mass of container in g

M_{od} = initial mass of 105°C oven dried sample and container

3.2.2.5 DETERMINATION OF FIXED CARBON CONTENT

The fixed carbon is a calculated value. It is the result of the summation of percentage moisture, ash and volatile matter subtracted from 100. All percentages shall be on the same moisture reference base.

$$\boldsymbol{Fixed\ carbon}\% = \mathbf{100} - (\boldsymbol{Moisture}\% + \boldsymbol{Ash}\% + \boldsymbol{Volatle\ matter}\%) \quad \mathbf{(3.6)}$$

3.2.3 ULTIMATE ANALYSIS

The elements C, H, N were analyzed using a CHN analyzer (Make: LECO, United States; Model: Micro TruSpec) using (1.0 ≤ mg ≤ 10). The determination range of the instrument for carbon is 50 ppm or 0.005% to 50%, for hydrogen is 200 ppm or 0.02% to 50% and for nitrogen it is 80 ppm or 0.08% to 100%.

3.2.4 HEATING VALUE

The higher heating values (HHV) of the samples were measured (in triplicate) by bomb calorimetry (Parr 1341 Plain Jacket, USA) using the procedure described in ASTM E711-87 standard manual (ASTM). Sample mass range was 1.0 ≤ mg ≤ 2.0).The calorific value of feed material is the amount of heat liberated when unit mass of fuel get completely burnt under the atmosphere of excess oxygen. It is expressed in MJ/kg. Calorific value of biomass is determined using isothermal bomb calorimeter. The biomass sample is placed in a totally enclosed vessel and is burnt at a constant volume in presence of excess oxygen by igniting electrically. The water equivalent of the bomb calorimeter is determined by burning a known amount of benzoic acid and the heat liberated during the combustion process is absorbed by a known amount of water. The calorific value of the sample is calculated using Eq. 3.7.

$$\boldsymbol{CV} = \frac{\boldsymbol{Wc\ x\ \Delta T}}{\boldsymbol{Ms}} \qquad \mathbf{(3.7)}$$

where,

CV = Heat of combustion of the biomass sample, MJ/kg

Wc = Water equivalent of the bomb calorimeter, MJ/°C

Wc =10.74×10^{-3} MJ/°C =2568.293 cal/°C

ΔT = Rise in temperature, °C

Ms = Mass of the biomass sample burnt, kg

3.2.5 LIGNOCELLULOSIC CONTENT

Lignin, cellulose and hemicellulose were determined by Fibra with unit (Pelican, Chennai, India) following Van Soest method (Van Soest and Wine 1968). The lignocellulosic content such cellulose, hemicellulose and lignin has been determined by neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) using the reflux apparatus. NDF is usually used to estimate the total lignocellulosic materials, while ADF is used to estimate the content of lignin and cellulose. Hemicellulose content can be determined by the percentage difference between NDF and ADF. Cellulose content can be determined by percentage difference between NDF and ADL.

3.2.6 THERMOGRAVIMETRIC ANALYSIS

TGA were performed on a Mettler TGA/SDTA 851 using sample weight in the range 3 ≤ mg ≤ 11. Samples were placed in an alumina crucible and heated in a nitrogen atmosphere between 10 and 700 °C at a rate of 10 °C min^{-1}, before being subjected to controlled slow pyrolysis. Pyrolysis represents the first stage of thermal decomposition for a number of fuel and processing applications, including combustion and gasification, along with the production of charcoal, and fine chemicals (Bridgewater 2003).

The reaction rate constant method was used to determine kinetics values. This has previously been shown to give good results for the early stages of pyrolysis in comparison to other models (Saddawi, Jones, and Williams 2010). It assumes that the reactions obey the Arrhenius equation

$$\boldsymbol{k = Aexp\left(\frac{Ea}{RT}\right)} \qquad \textbf{(3.8)}$$

where

k = experimental reaction rate constant

Ea = activation energy, in J mol^{-1}

A = frequency factor, in units of collisions sec^{-1}

R = universal gas constant, in J K^{-1} mol^{-1}

T = temperature, in Kelvin

The apparent first order rate constant (k) for each stage of mass loss per unit time was calculated over numerous small time period iterations from

$$\boldsymbol{k = -\frac{1}{(wi - w\infty)}\,x\,\frac{dw}{dt}} \qquad \textbf{(3.9)}$$

where

w_i = initial mass

w_∞ = terminal mass

dw = small change in weight

dt = small change in time

From these calculations of k, a plot of ln(k) versus 1/T (where T is the mid-range temperature of each iteration) was created. The slope of the trendline was used to determine E_a and A, with A being the ln(k) axis intercept and E_a being calculated by the slope value multiplied by R according to (3.8).

3.2.7 X-RAY DIFFRACTION

The XRD analysis was performed using Bruker D2 Phase, Germany using Cu Kα at 40 kV and 130 mA in the scanning angle of 10–40° at a scanning speed of 0.5° min^{-1}. To calculate the crystalline index (CI) of cellulose from the XRD spectra. The peak height method given by Segal et al was used to determine sample crystallinity (Segal et al. 1959). The crystallinity of the biomass samples was determined using equation given below

$$\boldsymbol{CI = \left(\frac{I002 - Iamorphus}{I002}\right) x\ 100} \qquad \textbf{(3.10)}$$

where,

CI= Crystallinity Index

I002 = Intensity at 22.8º

I amorphous = Intensity at 18º

3.2.8 FOURIER TRANSFORM INFRA-RED SPECTROSCOPY

All feedstock samples were analysed in solid state using a Shimadzu IR Affinity (Japan) instrument. A total of 30 scans were taken with a resolution of 4 cm^{-1}, between the range 400 to 4000 cm^{-1}. A background sample was taken prior to each analysis and deducted from the final results.

3.3 RESULTS AND DISCUSSION

3.3.1 PROXIMATE ANALYSIS

Chief among the parameters deciding the amount of biogas generated from substrate are its total solid content and % of volatile solid destruction (Nijaguna 2002). The total solid matter of biomass includes volatile organic matter and non-volatile organic matters. During anaerobic fermentation process volatile solids undergo digestion and non-volatile solids remain unaffected. The volatile content of bamboo dust, areca nut shell, rice husk, duckweed, maize, and saw dust was found to be 84.24±1.0%, 79.3±1.4%, 74.1±1.6, 84.45± 0.2%, 81.85±1.0% and 82.79±1.5% respectively. Duckweed has higher volatile content compared to other biomasses, which indicates its potentiality as ideal feedstock for gaseous combustible fuel. Volatile contents are a measure of the ease with which the biomass can be ignited and subsequently gasified, or oxidised, indicating the effective utilization of the biomass as energy source (McKendry 2002). The ash content of maize was found to be lowest which signifies that it has a lower non-biodegradable carbon and hence easy digestibility as ash content retards the enzymatic hydrolysis i.e. saccharification of biomass samples (Bin and Hongzhang 2010). The highest ash content was found in rice husk, which may be due to presence of silica (Arjmandi et al. 2015) followed by bamboo dust, saw dust respectively.

Moisture content is an important characteristic affecting the quality of biomass fuel. The moisture content of areca nut shell and rice husk was found to be lowest, which indicates their suitability for thermal conversion processes (moisture <50%), while saw dust and bamboo dust has higher moisture content, which renders them potential feedstock for bio-conversion processes like bioethanol production and anaerobic digestion (McKendry 2002).

Table 3.1 Proximate analysis and ultimate analysis of lignocellulosic biomass

Biomass Samples	**Proximate analysis (%)**					**Ultimate analysis (%)**		
	Total solids	Volatile matter	Moisture	Ash	Fixed carbon	C	H	N
Bamboo dust	88.24±1.0	84.24±1.0	11.76±1.0	3.79±0.6	0.21±1.4	47.5 ±0.1	5.84 ±0.3	1.05±0.9
Rice husk	91.2±1.0	74.1±1.6	8.8±1.2	9.8±1.0	7.3±0.3	19.8±0.2	2.76±0.5	1.16±0.1
Saw dust	86.77±1.0	82.79±1.5	13.23±1.5	3.51±0.5	0.47±1.0	48.5±0.5	6.40±0.6	1.10±0.4
Duckweed	78.24±1.0	84.45± 0.2	21.76± 0.2	3.79± 0.1	0.3± 0.4	47.44± 0.2	5.84± 0.1	5.0±0.1
Areca nut shell	92.3±1.0	79.3±1.4	7.7±1.0	3.1±1.1	9.9±1.0	43.8±0.3	7.1±0.1	1.54±0.1
Maize	88.49±1.0	81.85±1.0	11.51±1.0	2.64±1.0	4.0±1.0	45.5±1.0	6.05±1.0	1.29±1.0

3.3.2 ULTIMATE ANALYSIS

Table 3.1 shows the ultimate analysis results of the feed stocks in terms of carbon, nitrogen and hydrogen content. During combustion nitrogen is practically converted in gaseous N_2 and nitrogen oxides NO_x almost entirely, which is a main environmental impact of biomass burning. An insignificant quantity of N converts to ashes (Obernberger et al. 2006).

As seen in Table 3.1 all samples have N levels less than 1% which shows its lower impact on the environment. Saw dust has highest carbon content followed by bamboo dust, duckweed, maize, areca nut shell and rice husk. In thermo-chemical reaction, carbon and oxygen react during combustion in an exothermic reaction, generating CO_2 and H_2O. Thus, carbon contributes in a positive way to the fuel's HHV (Pedro et al. 2018). Excepting rice husk, the carbon values of all the other biomasses fit in the expected range (47% to 54%) signifying high energy density and hence suitability for both biochemical and thermochemical processes.

3.3.2 EVALUATION OF HEATING VALUE

The heating values of the biomass constituents were measured to assess total calorific values (Figure. 3.1). During this experiment it was observed that heating value of rice husk (19.06±1.0) was higher followed by maize (18.4± 0.3) saw dust (18.22±1.2), bamboo dust (17.63±1.0), duckweed (17.28± 0.4) and areca nut shell (17.11±1.4) respectively. The high calorific value of rice husk and maize indicates that these are comparatively the potential biomasses for thermochemical processes. Moreover the calorific value of areca nut shell in present study, i.e. 17.11 ± 1.4 MJ/Kg closely resembles the value (19 MJ/Kg) reported by Naik (2010).

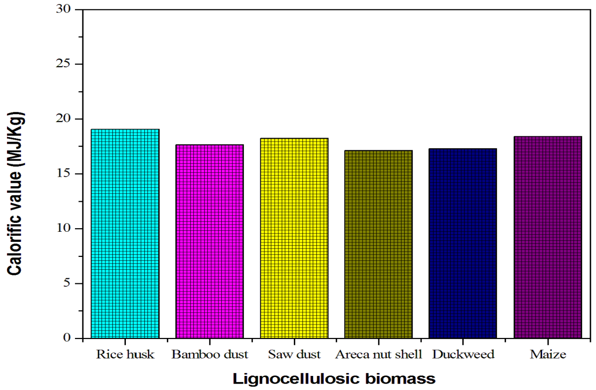


Figure 3.1 Calorific value of lignocellulosic biomass

3.3.3 FIBRE ESTIMATION BY VAN SOEST METHOD

The fibre analysis was performed to know cellulose, hemicellulose and lignin content in feedstock sample by Van Soest method chemically as shown in Figure 3.2. The cellulose content of bamboo dust, rice husk, saw dust, duckweed, areca nut shell and maize were found to be 48.9%, 35.6%, 54.9%, 55.2%, 45.9% and 47% respectively. Lignin content was found to be higher in bamboo dust 22 % compared to other biomasses. The cellulose to lignin proportions of biomass is important only in biochemical conversion processes. The biodegradability of cellulose is greater than that of lignin; hence the overall conversion of the carbon-containing plant material present as cellulose is greater than for plants with a higher proportion of lignin, a determining factor when selecting biomass plant species for biochemical processing (McKendry 2002). In case of lignocellulosic biomass, the lignin to cellulose ratio is normally used to define the degree of digestibility of the biomasses (Sharma 1988). From the Fig. 3.2 it has been observed that lignin to cellulose ratio is the highest in case of rice husk (0.51) followed by bamboo dust (0.45), areca nut shell (0.45), maize (0.30), duckweed (0.22) and saw dust (0.18) respectively.Therefore, some pretreatment will be required to hydrolyze lignin content for enhancing energy generation in biochemical processes like bioethanol and biogas production.

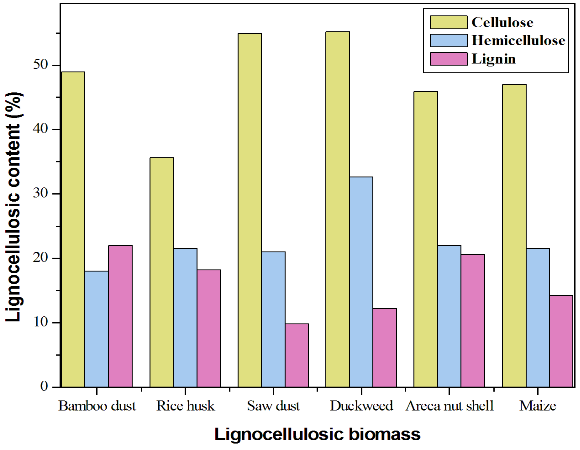
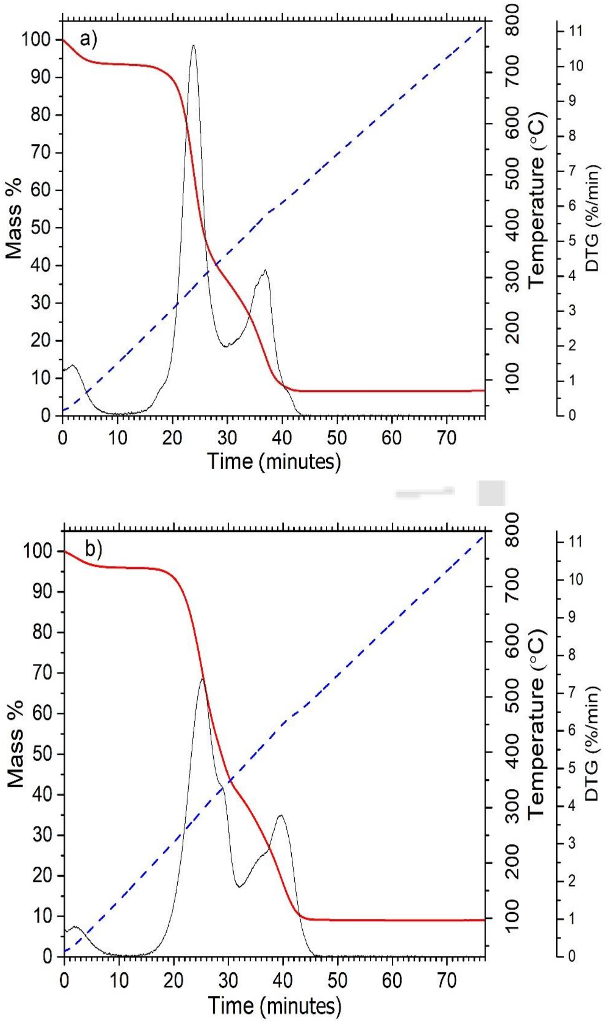
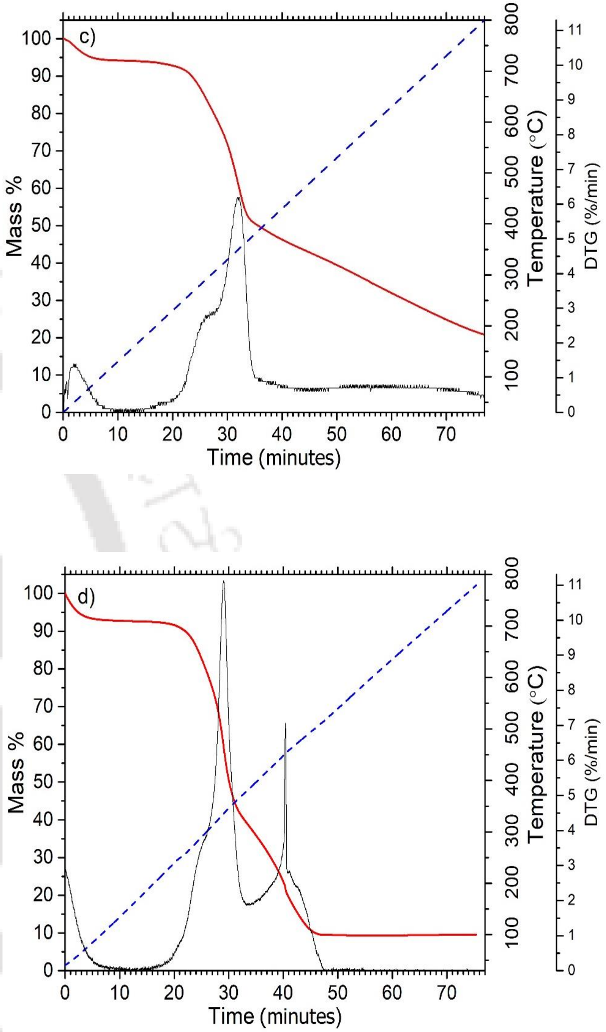


Figure 3.2 Lignocellulose composition of rice husk, bamboo dust, saw dust, arecanut shell, maize and areca nut shell

3.3.4 THERMOGRAVIMETRIC ANALYSIS

Thermogravimetric Analysis (TGA) curves showing mass loss as a function of temperature and time, along with Derivative Thermogravimetry (DTG) which reveals the rate of mass loss, are shown in Fig.3.3,a-e. Following moisture evolution, the relatively well-studied thermal decomposition curves of lignocellulose are evident with the DTG maxima (peaking at ca. 300°C) corresponding to the primary evolution of hemicellulose and cellulose (Jeguirim and Trouvē 2009). The loss of weight between 100-150 °C is associated with dehydration; water molecules adsorbed on the samples surface as well as those bound within the inner cells is released. The second stage of thermal decomposition of biomass samples occurs in the temperature range between 200 °C and 300 °C with maximum weight loss occurring at 220 to 350 °C, which may be attributed to volatilization of hemicelluloses (Meszaros et al. 2004). During this stage, the chemical bonds of different bio-polymers start breaking, with less thermally stable compounds decomposing first. The cellulose decomposition peak, which typically manifests itself in the temperature range of 350 to 380 °C, is probably overlapped with that of hemicelluloses (Yang 2007). That hemicellulose is the first to evolve can be seen with the DTG hemicellulose "shoulder" in Figure 3.3 c (rice husk) at ca. 20 min in comparison to (its absence in) the other sample (Fig.3.3b). Maize and saw dust (Fig.3.3b) shows an earlier DTG peak in comparison to the bamboo dust sample evidencing the presence of hemicellulose. These observations provide one reason for the higher biodegradability of maize, saw dust, arecanut shell in comparison to bamboo dust and rice husk. The main recalcitrant polymer for anaerobic digestion is lignin. Under thermolysis lignin is known to evolve over a wide range of temperatures, but its presence only becomes evident after hemicellulose and cellulose have evolved, as seen from Fig 3.3 a-e. These experiments reveal the presence of lignin in biomasses by the additional mass loss at T ≥ 350°C. Rice husk lost a further 21% of its volatile organic components at T ≥ 500°C and the TGA plots showed an almost constant rate of mass loss from 400 °C ≤ °C≤ 700, in contrast to many other woody biomass samples (Jeguirim and Trouve 2009). This phenomenon is seen to a lesser extent in the works of (Sharara and Sadaka 2014), and though not discussed, probable reasons for continued decomposition are related to the higher concentration of minerals in the sample relative to organic compounds, either through increased catalytic activity at these higher temperatures, or through the formation of more thermally stable organic compounds.





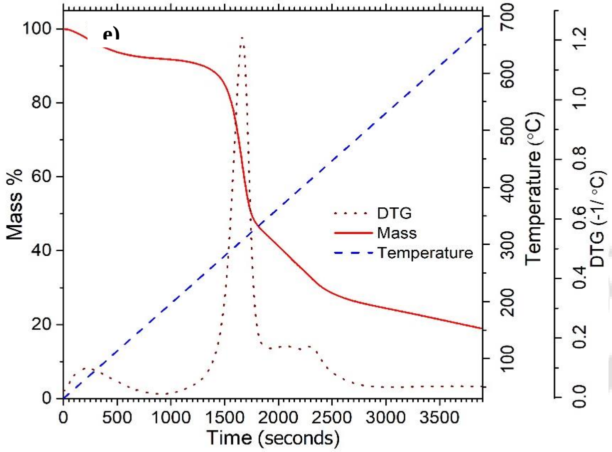


Figure 3.3 TGA and DTG curves as a function of time under a heating rate of 10°C min^{-1} and a nitrogen carrier gas flow: a) areca nut, b) bamboo dust, c) rice husk, d) saw dust e) maize

For kinetics analyses, demarcation of the reaction region associated with biomass pyrolysis can be subjective. In this study, and in accordance with the results obtained by Saddawi et al. (2011), the commencement of the reaction stage was chosen from the cusp of primary devolatisation and just after the inflexion point of mass loss as end point as shown most clearly from DTG. Only the primary decomposition stage was found to approximate to first order reaction kinetics. These exhibited a close linear fit (R^2), and all E_a values were high enough to suggest that the rates were still under kinetic control, as shown in Table 3.2.

Table 3.2 Kinetic parameters for primary pyrolysis of the biomass samples

Sample	**Kinetic Parameters**		
	E_a (kJ.mol^{-1})	**A_0 (sec^{-1})**	**R^2**
Areca nut	79.13	7.22×10^4	0.97
Bamboo dust	115.17	2.84×10^8	0.998
Rice husk	84.03	8.59×10^4	0.98
Saw dust	123.43	5.79×10^8	0.95
Maize	128.5	3.04×10^9	0.97

3.4 X-RAY DIFFRACTION

Figure 3.4 shows the XRD diffractogram of the three samples. Cellulose can be identified by the presence of peak at a 2Θ angle values around 18º and 22.8º, though with different intensities and curve width in the three samples. As expected the highest intensity and the sharpest peak occurred with the rice husk sample. The traditional two-phase cellulose model describes cellulose chains as containing both crystalline (ordered) and amorphous (less ordered) regions. A parameter termed the crystallinity index (CI) was used to describe the relative amount of crystalline material in cellulose (Park et al. 2010), with the maximum crystallinity observed in rice husk (43.71%) followed by arecanut shell (34.75%), maize (29.47%) and duckweed (20.46%) respectively. The rate of depolymerisation decreased with increase in crystalline index. In case of rice husk, the relatively higher CI is one of the major reasons of its recalcitrant nature compared to all other LB.

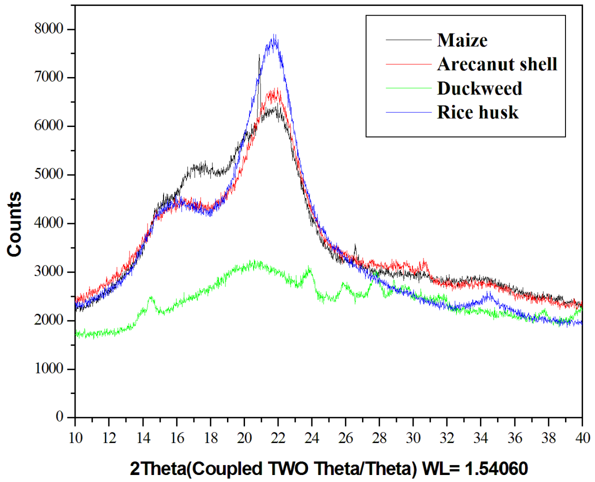


Figure 3.4 XRD profile of lignocellulosic biomass

3.5 FOURIER TRANSFORM INFRA-RED SPECTROSCOPY

The FTIR fingerprinting of lignocellulosic biomass samples are shown in Fig.3.5. The main peaks are attributed to the presence of different concentrations of lignin, hemicellulose and cellulose in the biomasses. Similar bands appeared with more or less different shapes and relative intensities for all the biomasses. The bands for lignin were highest in rice husk followed by bamboo dust, maize, areca nut shell, saw dust and duckweed respectively. The spectra indicate an aromatic structure stretch and vibration of biomasses (1458-1595 $^{cm-1}$) with numerous oxygen-containing functional groups: OH (3600- 3200 $^{cm-1}$), COOH (around 2600 $^{cm-1}$), C-O-(C) (~1158 $^{cm-1}$), and C-O-(H) (~1058 $^{cm-1}$). The most intense band of the spectra (1500- 1600 $^{cm-1}$) can be due to an aromatic skeletal mode, significantly intensified by the presence of oxygen containing polar substituents. In the fingerprint region, the bands at 1745, 1738 and 1720 cm^{-1} are assigned to C=C, C–O stretching or bending vibrations of different groups present in lignin (Popescu et al. 2007).The bands at 1462, 1425, and 1058 $^{cm-1}$ are characteristic of C–H, C–O deformation, bending or stretching vibrations of many groups in lignin and carbohydrates (Popescu et al. 2007).The hemicellulose peaks are showed in fig.3.5, in which bamboo dust and rice husk shows almost similar absorption followed by areca nut shell and saw dust. The rice husk and bamboo dust showed similar absorption amongst all the biomass samples in the range of 1700–1730 $^{cm-1}$ and 1378 $^{cm-1}$, which signified the similar density of hemicellulose in rice husk and bamboo dust followed by areca nut shell and saw dust respectively. Researchers proposed different peaks for representation of crystalinity and amorphous characters, attributed mainly by cellulose molecules in two wavelength (~1428 $^{cm-1}$ for crystalline and 893^{cm-1} for amorphous) (Ciolacu et al. 2011). The rice husk has highest crystalline cellulose (1428 $^{cm-1}$) followed by bamboo dust, maize, areca nut shell and saw dust respectively, which refers to its recalcitrant nature. In present study the assigned band of 893 $^{cm-1}$ wavelength for amorphous cellulose is absent in rice husk, bamboo dust, saw dust, maize, arecanut shell but present in duckweed biomass. Duckweed fingerprints is showing entirely low intensity for lignin, cellulose and hemicellulose which may predict higher rate of depolymerisation and ease in degradability. The bands at 1739, 1315, 1280, 1180, and 1060 $^{cm-1}$ are assigned to the characteristic bending or stretching of different groups from cellulose indicated in fig. 3.5. The other responsible bands for lignocellulosic biomass shown in fig. 3.5 and Table 3.3.

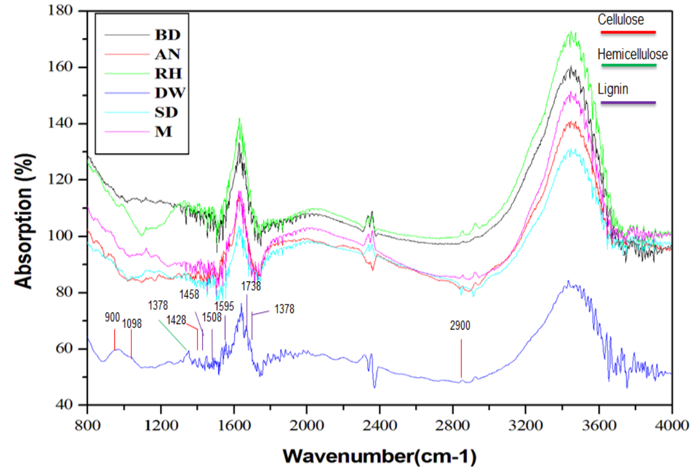


Figure 3.5 FTIR fingerprinting of lignocellulosic biomass; BD = Bamboo dust, AN= areca nut shell, RH= rice husk, M= Maize, DW= Duckweed and SD= saw dust

Table 3.3 The characteristic bands in FT-IR spectra and their assignment according to the literature data (Kumar et al. 2009)

Band position(cm^{-1})	Assignment
3348	O-H stretching (related to rupture of cellulose hydrogen bonds)
2900	C-H stretching (related to rupture of methyl/methylene group of

	cellulose)
1745	Carbonyl bonds (related to lignin side chain removal)
1738	C=O stretching due to carbohydrate linked with lignin
1720	Carboxylic acids/ester groups
1595	Aromatic ring stretch (related to lignin removal)
1508	Aromatic ring vibration (related to lignin removal)
1458	Aromatic ring vibration (related to lignin removal)
1428	Band of cellulose
1378	Band of hemicellulose
1260	Ester absorbance (related to removal of uronic acid)
1245	C=O absorption (resulting from acetyl groups cleavage)
1238	Hemicellulose–lignin linkage
1059	C=O stretching due to carbohydrate–lignin linkage
1098/900	Amorphous to crystalline cellulose ratio
900	Band of cellulose

3.5 SUMMARY OF THE CHAPTER

Six types of LB waste such as bamboo dust, areca nut shell, rice husk, duckweed, maize and saw dust were characterized by different analytical techniques such as FTIR and TGA analysis to ascertain the prospective for biogas production. The physico-chemical characterization of all six samples showed that saw dust and duckweed has high calorific value, devolatilization, cellulose and hemicellulose content, with low ash and lignin content and can be regarded as the most potential candidate for bio-chemical bioenergy production. The areca nut shell and maize has slightly lower VM, CV and cellulose content than duckweed and saw dust. The volatile content of bamboo dust was found to be highest among all six biomasses but its ash and lignin content is high, which indicate requirement of pretreatment procedures for biofuel production. Rice husk has high lignin and ash and low calorific value and volatile content, which makes it unsuitable feedstock for biogas production. The study of all these LB samples in the light of biofuel production ensures that these biomasses can be utilized for biogas production and may meet the demand of the second generation biofuel.

CHAPTER – 4

CO-DIGESTION OF LIGNOCELLULOSIC BIOMASS WITH CATTLE DUNG FOR ENHANCEMENT OF BIOGAS PRODUCTION

4.1 INTRODUCTION

Conventionally, animal manure is utilized as an ideal feedstock for biogas generation with reduction in environmental pollution of solid waste. Many research outcomes have been reported in this direction for last few decades. But uneven availability, inappropriate carbon to nitrogen (C/N) ratio, low pH, poor buffering capacity and high ammonia concentration (Mao et al. 2017) of animal manure/cattle dung attracted interest towards utilization of lignocellulosic biomass as a potential candidate for biogas production. Co-digestion of the same with cattle dung has been considered for future biogas production by a section of research communities in the present scenario. Hence present chapter is an attempt to investigate use of various lignocellulosic biomasses (LB) in different proportions with cattle dung for biogas production.

4.2 MATERIALS AND METHODS

4.2.1 PREPARATION OF SAMPLES

Biomass samples viz; rice husk, saw dust, maize and areca nut shell were procured from agricultural fields in kamrup district, Assam. Duckweed was collected from Amingaon village ponds (26.1839° N, 91.6640° E) nearby Indian Institute of Technology Guwahati (IITG) campus, Guwahati, Assam, India. All the lignocellulosic biomass samples were washed, dried and ground in the ball mill prior to proximate analysis. Proximate analysis i.e. total solids (TS), volatile solids (VS), carbon to nitrogen ratio (C:N), cellulose, hemicellulose and lignin of all the biomass were determined following ASTM and Van Soest method which is already mentioned in earlier chapter (Section 3.2). A known volume of fresh cattle dung (25 kg) was collected locally for the experiment. For batch experiment all the biomass samples were air dried and grounded in a high speed rotary cutting mill upto 2 – 5 mm size. Table 4.1 shows the proximate and ultimate analysis of selected lignocellulosic biomass for this experiment which already had been discussed in chapter 3 (Section 3.2).

Table 4.1 Composition of cattle dung, rice husk, areca nut shell, bamboo dust, saw dust and duckweed (w/w)

Constituents (%)	Cattle dung	Arecanut shell	Rice husk	Bamboo dust	Saw dust	Duckweed
Total solids	86.1± 0.4	92.3±1.0	91.2±1.0	88.24±1.0	86.77±1.0	78.24±1.0
Moisture	13.90± 0.4	8.8±1.2	7.7±1.0	11.76±1.0	13.23±1.5	21.76± 0.2
Volatile matter	62.2± 0.5	79.3±1.4	74.1±1.6	84.24±1.0	82.79±1.5	84.45± 0.2
Carbon	35.2± 0.1	43.8±0.3	3 9.8±0.2	47.5 ±0.1	48.5±0.5	47.44± 0.2
Nitrogen	1.55±0.3	1.54±0.1	1.16±0.1	1.05±0.9	1.10±0.4	5.0±0.1
Cellulose	48±0.1	47.8±0.3	35.6±0.1	48.9±0.3	54.9±0.9	55.2±0.2
Hemicellulose	24±0.4	31.3±0.1	21.5±0.9	18±0.1	21±0.5	32.6±0.6
Lignin	14.2±0.3	23.1±0.4	18.2±0.9	22±0.3	9.8±0.6	12.2±0.9

4.2.2 FEED PREPARATION AND EXPERIMENTAL SET UP FOR LAB SCALE BATCH BIOGAS PRODUCTION

To investigate the potential of all five lignocellulosic biomass as feedstock for biogas production on mix digestion three different ratios(w/v) of rice husk (RH), saw dust (SD), bamboo dust (BD) and duckweed (DW) with cattle dung slurry (CD) was taken for the experiment; LB:CD= 75:25, 50:50, and 90:10 respectively. These three combinations of each LB in batch reactor were compared with the cattle dung alone, which was kept as control. The experiments were carried out in batch type lab scale digesters of 1 Litre capacity shown in Fig. 4.1. The digesters were incubated at 38°C ± 2 in hot air oven with HRT of 55 days with occasional manual mixing. The gas produced from each digester was collected by the liquid (acidified brine water) displacement method in a graduated flask and measured daily. The brine solution is used in a 500 ml bottle to inhibit absorption of CO_2 by water. For this study 8-10% total solid was maintained for 55 days HRT. The pH value for all the samples were adjusted at 7.2 ± 1 using KOH solution. The produced biogas was collected in tedlar bag for further analysis of methane content by Gas Chromatography.

It has been reported that maximum biogas production can be achieved with C:N in the range of 20:1 to 30:1 (Nijaguna 2009). The C: N of the biomass considered in the present study varies widely from 9.5:1 to 45:1 as shown in Table 4.2. In case of cattle dung same is observed to be 22.7:1. In order to control the C:N in permissible limit for production of biogas effectively, the biomass considered were mixed with cattle dung with water to achieve in the range of 20:1 to 30:1. Aforementioned feed material prepared is mixed with water so as to control the TS to ~10 %. The volatile matter of cattle dung and LB mixture was determined by ASTM process and presented in Table 4.2.

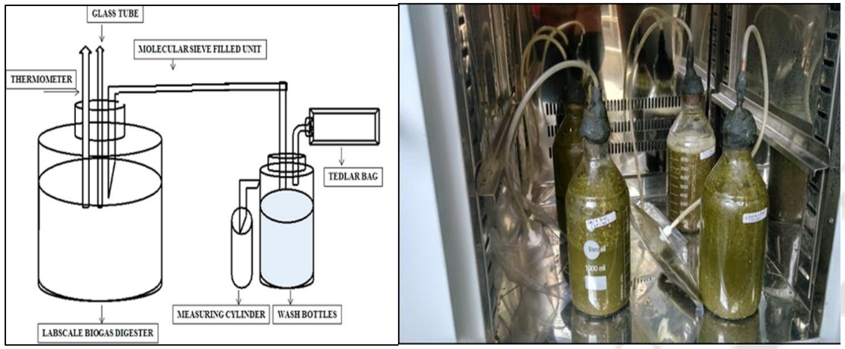


Figure 4.1 a) Schematic diagram and b) images of experimental setup

Table 4.2 Combination of cattle dung and lignocellulosic biomass

Feedstock combinations(w/v)	Total solids (%)	Volatile matter (%)	C:N
CD -100% (Control)	9.2±0.2	62.2± 0.9	22.70
RH (90) : CD(10)	10.2±0.5	73.5± 1.5	29.8
RH (75): CD(25)	10.1±0.3	72.9± 1.0	28.1
RH (50): CD(50)	9.1±0.1	71.6± 0.5	27.1
BD (90): CD (50)	10.5±0.1	83.1± 0.7	30.9
BD (75): CD (25)	10.3±0.2	82.7± 1.2	30.1
BD (50): CD (50)	9.4±0.5	81.9± 1.5	29.5
SD (90): CD (10)	10.9±0.1	81.8± 0.5	30.2
SD (75): CD (25)	10.1±0.2	81.1± 0.8	29.6
SD (50): CD (50)	9.9±0.3	80.4± 0.9	28.6
DW (90) : CD (10)	10.5±0.1	83.3± 1.2	19.5
DW (75) : CD (25)	10.3±0.1	82.5± 0.9	20.7
DW (50) : CD (50)	9.5±0.4	81.9± 0.5	21.1
AN (90): CD (10)	10.2±0.1	79.3±1.4	28.4
AN (75): CD (25)	9.5±0.1	78.7±1.4	27.6
AN (50): CD(50)	10.1±0.1	78.1±1.4	27.1

4.2.3 GAS CHROMATOGRAPHY

The biogas volume produced was measured by water displacement technique and the corresponding daily and cumulative gas volume was then calculated. Gas samples from each set up were collected weekly and analysed for their methane and carbon dioxide content using a gas chromatograph. Gas samples from each setup were collected in Tedlar® bags and immediately analysed in a gas chromatograph (Thermo Scientific Trace GC Ultra Gas Chromatograph, USA). All samples were analysed within 5 hours of sampling. Each bag was analysed three times and the average taken as the recorded measurement.

4.3 RESULTS AND DISCUSSION

4.3.1 EFFECT OF C: N RATIO AND TS ON SELECTING LIGNOCELLULOSIC BIOMASS AND CATTLE DUNG PROPORTIONS

The C: N ratio and TS value of three different combinations of rice husk, areca nut shell, bamboo dust, duckweed and saw dust with cattle dung is shown in Table 4.2. Carbon: Nitrogen ratio was high for the lignocellulosic biomass so it has been balanced with addition of water and cattle dung in appropriate quantity. In anaerobic digestion process C: N ratio is essential for the growth of microorganism. Carbon is attained from carbohydrates and utilized by microorganism for the energy and nitrogen obtained from proteins needed for building up of the cell structure (Mittal 1996). However, proper C/N ratio does play a significant role in the anaerobic digestion process. Biomethanation process generally measured C/N ratio ranging from 20:1 to 30:1(Das and Mandal 2016). Effect of C: N ratio reflects on biogas production volume and rate from lignocellulosic biomass and cattle dung in a batch experiments. The highest biogas production has been observed with combination of LB: CD 50:50. The similar pattern has been observed for all three combinations of each biomass. The biogas production found to be low in combination of LB: CD 90:10 and their C: N ratio found between 30.1 – 25.5. Rice husk, saw dust and bamboo dust show lowest gas production with C: N ratio of 29.8, 30.2 and 30.9 respectively. The reason could be high carbon to nitrogen ratio with low amounts of nitrogen which would be unfavourable for microorganisms due to deficiency of amino acids, nitrates and proteins (Wang et al. 2014). On the other hand, DW: CD 90:10 has given lowest biogas production compare to DW: CD 75:25 and DW:CD 50:50. The probable reason could be a low C/N ratio which indicates high amounts of nitrogen in duckweed which would again be detrimental because of ammonia toxicity. It has been observed from Table 4.2 that C/N ratio for the CD control is 22.70 which are considered to be optimum.

4.3.2 DAILY BIOGAS PRODUCTION FROM LIGNOCELLULOSIC BIOMASS AND CATTLE DUNG CO-DIGESTION AT DIFFERENT PROPORTION

The hydraulic retention time (HRT) of 55 days gives diversified results for different lignocellulosic biomass and cow dung proportion. It was observed that initially biogas production was slow and after 14 days to about 45– 50 days, it was significant. Afterwards, the biogas production was reduced due to unavailability of nutrients and volatile matter which affects the growth of microbes leading to their lower population. The daily biogas production by the co-digestion of rice husk, bamboo dust, saw dust, areca nut shell, duckweed with cattle dung for 55 days HRT (anaerobic digestion) was calculated under different mixing ratios and shown in fig. 4.2 to fig 4.6 respectively. Biogas production was first observed within initial seven days from incubation in case of the digester containing only cattle dung as the feedstock. This may be due to the presence of higher amount of easily degradable organic carbon fractions in the fresh cattle dung. Presence of labile carbon sources in cattle dung could be easily hydrolysed by acedogenic and acetogenic bacteria, which provide nutrient substrate to methanogenic bacteria for methanogensis. Animal dung has been reported to contain a good level of both macro and micronutrients (Seppala et al. 2013). On the other hand RH, SD, AN, BD and DW being a lignocellulosic biomass contains lignin and crystalline cellulose, which needs more time to get broken down into more balanced carbon moieties and thus gets converted in to precursors for methanogenesis (Yadav et al. 2016). Hence, biogas production in the rest of the digesters containing a mixture of RH, SD, AN, BD and DW with CD started after 14 days incubation (DAI). In case of the digester with CD alone, the gas production decreased rapidly after 35 days while with the other all combinations; DW:CD, RH:CD, SD:CD, AN:CD and BD:CD, the gas production sustained till 55 DAI. It has been observed that the log phase was higher for the mixed feedstock (35 DAI) compared to cattle dung alone (28 DAI). Samples from the mixing ratios of DW/CD90:10, DW/CD75:25, DW/CD 50:50 and CD 100:0 were measured highest among all biomass, and their peak yield values were 20, 559, 610, and 510mL/d on the 35th day respectively Fig. (4.2).

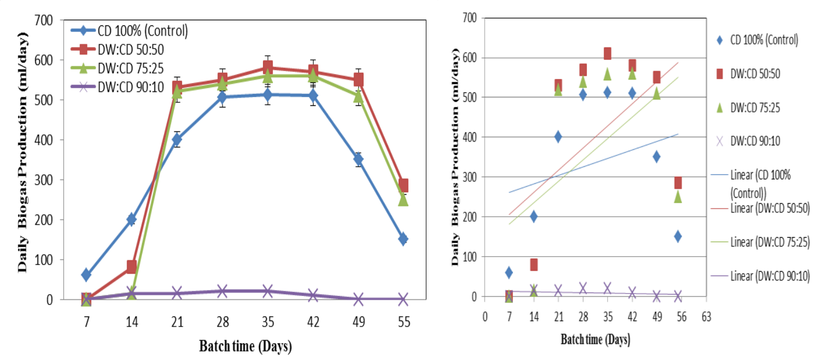


Figure 4.2 Daily biogas production profiles of duckweed (DW) and cattle dung (CD) combinations

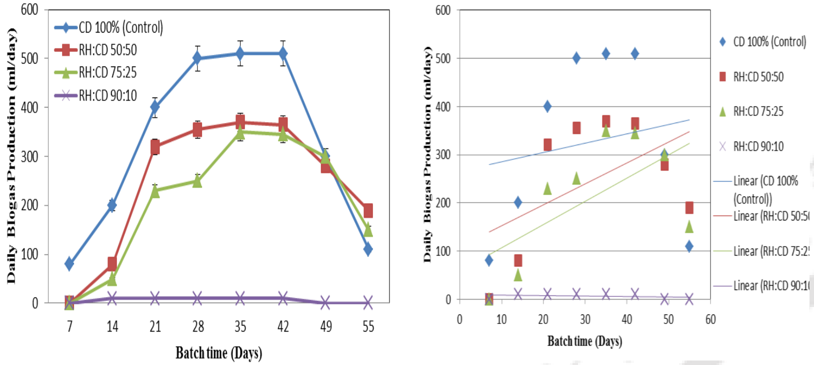


Figure 4.3 Daily biogas production profiles of rice husk (RH) and cattle dung (CD) combinations

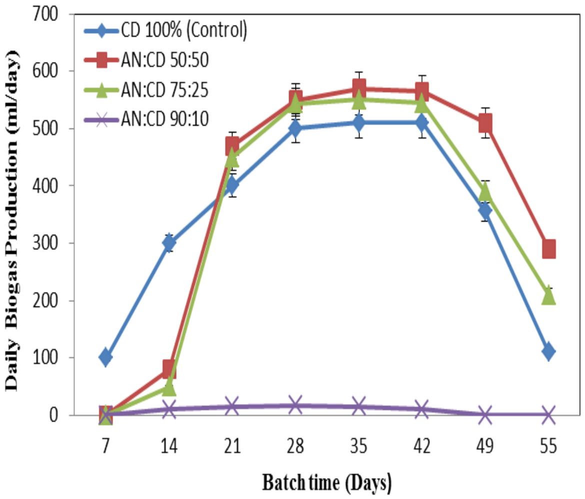


Figure 4.4 Daily biogas production profiles of arecanut shell (AN) and cattle dung (CD) combinations

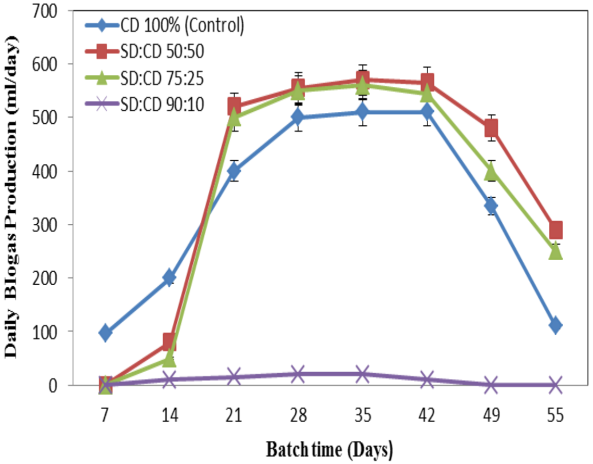


Figure 4.5 Daily biogas production profiles of saw dust (SD) and cattle dung (CD) combinations

The LB: CD (90:10) combination shows lowest biogas production for all the biomass samples due to presence of higher lignin which plays the role of adhesive for the cross-linking between cellulose and hemicellulose to form a rigid three-dimensional structure of the cell wall. Lignin shield is difficult to breakdown by the AD microorganism and consequently not able to access to cellulose and hemicellulose for conversion into biogas. The cattle dung added as inoculum which presents in very small quantity (10%) for this combination of LB: CD (90:10), which means less hydrolytic and fermentative microbial population and eventually leads to ceasing of anaerobic digestion process.

Figure 4.3 depicts the daily biogas production profile of rice husk and cattle dung; it has been found that the production is very less in all the combination compare to other lignocellulosic biomass combination. The RH: CD (50:50) shows substantial biomethanation but RH: CD (90:10) very poor biogas production. The probable reason might be the presence of higher lignin and silica which makes it difficult to decompose by AD microorganism (Arjmandi et al. 2015).

The combination of SD: CD and AN: CD exhibits similar biogas production pattern with different combination. The similar highest biogas production peaks observed in SD: CD 50:50 and AN: CD 50:50 with 570 and 565 on 35th and 42nd day respectively. On the other hand, bamboo dusts with cattle dung combination shows different biogas production pattern. Initially, biogas production starts slowly and gradually increases in later days of the experiment. The BD: CD profile reveals that lignocellulosic biomass takes time to hydrolyse the lignocellulose content. Previous studies have shown that pre-treatment is required which can increase the initial methane production rate and this in turn depends primarily on the type of substrate (lignocellulosic biomass) and type of pre-treatment methods (Kumar and Sharma 2017). Kobayashi, Take, and Asada (2004) reported that methane could not be produced from raw bamboo, but methane production can be enhanced through steam explosion.

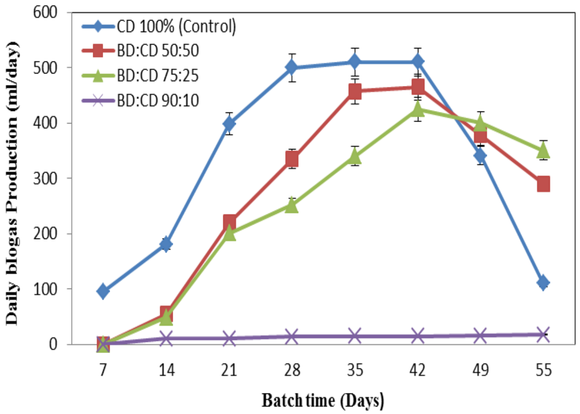


Figure 4.6 Daily biogas production profiles of bamboo dust (BD) and cattle dung (CD) combinations

4.3.3 CUMULATIVE BIOGAS PRODUCTION FROM LIGNOCELLULOSIC BIOMASS AND CATTLE DUNG CO-DIGESTION AT DIFFERENT PROPORTION

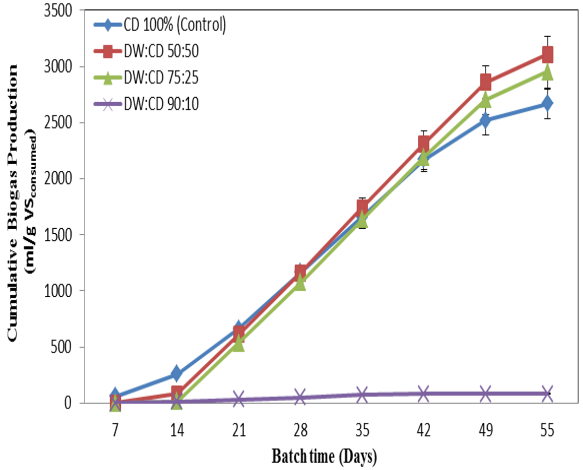


Figure 4.7 Cumulative biogas productions from duckweed (DW) and cattle dung (CD)

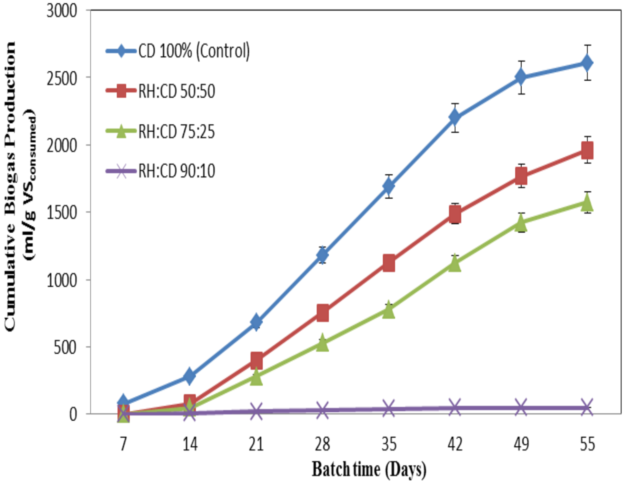


Figure 4.8 Cumulative biogas productions from rice husk (RH) and cattle dung (CD)

The cumulative biogas production has been found highest in duckweed, rice husk, arecanut shell, saw dust and bamboo dust with cattle dung combination with 50:50 which indicates that the optimum ratio for co-digestion of LB and CD is 1:1. The highest biogas production was observed in LB/CD (50:50) combination, which may be due to more simple degradable monomers in cattle dung that can easily converted into soluble monomer; followed by acid conversion with the help of acedongenic bacteria. Fermentative bacteria degrade these molecules to volatile fatty acids and to ammonia; methane formation occurs in which the acids are converted to biogas (Heyer et al. 2015). Therefore in whole 55 days HRT this combination LB/CD (50:50) gave highest biogas production, initially volatile matter of cattle dung was utilized by microbes present in the AD process and it created the appropriate environment for the system which later on leads to faster degradation cellulose and lignin present in the LB. The very low biogas production for LB: CD at 90:10 combinations may be due to the presence of lignin and crystalline cellulose, which takes time to get hydrolysed and hence unable to provide sufficient nutrition to methanogenic bacteria which in turn suppress a microbial growth and inhibit methanogenesis process. Figure 4.7 depicts the biogas production for CD (100%), DW/CD (90:10), (75:25) and (50:50) are 11620, 305, 11695, and 12070 mL, respectively.

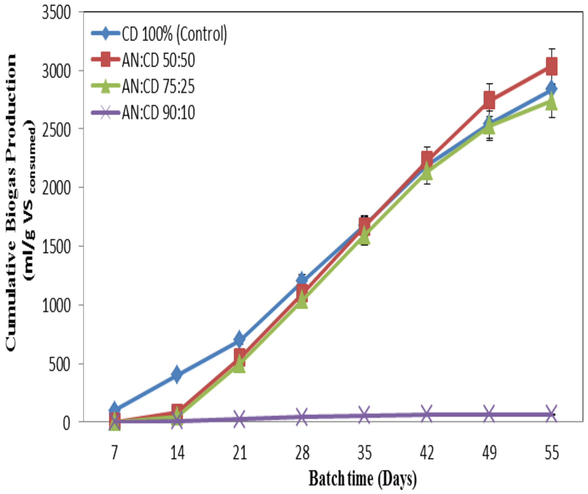


Figure 4.9 Cumulative biogas productions from arecanut shell (AN) and cattle dung (CD)

DW: CD (50:50) shows highest cumulative biogas production followed by SD:CD, AN:CD, BD:CD and RH:CD and are 11680ml, 11415 ml, 7649ml and 7580 ml respectively. Many researchers have reported duckweed as good supplement for higher methane content and biogas production. Clark (1996) reported that duckweed helps in enhancing the methane content in anaerobic digestion. He investigated experiments on batch digester and found that without duckweed it took 40 days to reach peak methane production compared to 15 days when duckweed was added and, an increase in gas production of about 44% in the semi-continuous digesters was observed. Rice husk and bamboo dust exhibited lower biogas production than cattle dung which shows its recalcitrant behaviour and higher lignin content which is in agreement to proximate analysis results. Therefore, biomass pretreatment prior to anaerobic digestion is usually required to reduce structural and compositional impediments of lignocellulosic biomass and expose the polymer chains of cellulose and hemicellulose to microbial breakdown so as to increase the rate of biomass degradation and biogas yield.

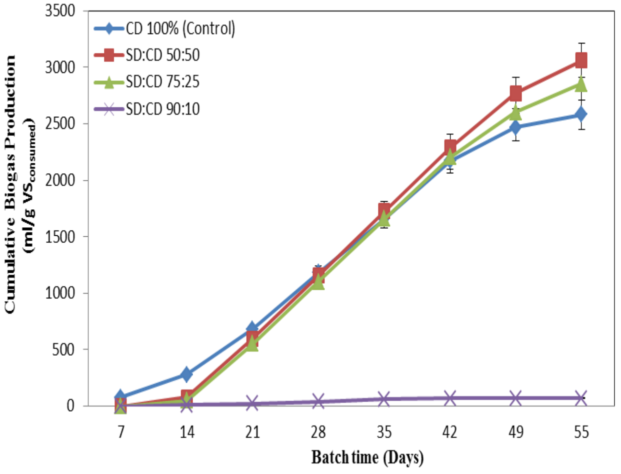


Figure 4.10 Cumulative biogas productions from saw dust (SD) and cattle dung (CD)

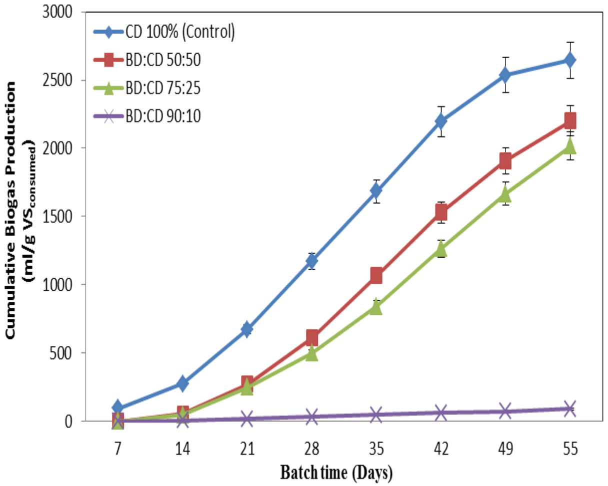


Figure 4.11 Cumulative biogas productions from bamboo dust (BD) and cattle dung (CD)

4.3.4 BIOGAS QUALITY FROM LIGNOCELLULOSIC BIOMASS AND CATTLE DUNG CO-DIGESTION AT DIFFERENT PROPORTION

Figure 4.12 represents the methane concentration profile of the biogas from cattle dung and mixed feedstock (LB and CD). Yadav et al. (2016) also reported the similar and highest methane content of 64.3% from CD alone followed by the biogas from the mixed feedstock, DW: CD=50:50 (63.8%) and DW: CD=75:25 (63.5%). In the AD process, the methanogenic phase is characterized by presenting methane concentration at a 50 to 60% level with a decrease in the concentration of carboxylic acids and as a result increases in pH of the environment (Barlaz, Ham, and Schaefer 1989). The high methane content in the biogas from CD may be attributed to the optimum C: N ratio compared to LB.

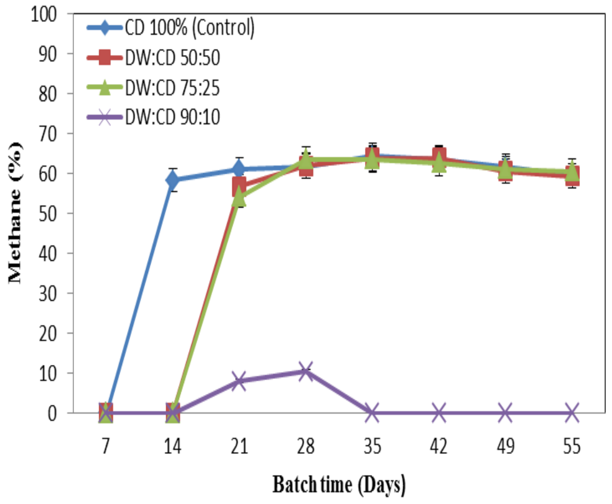


Fig. 4.12 Methane content of duckweed (DW) and cattle dung (CD) combinations

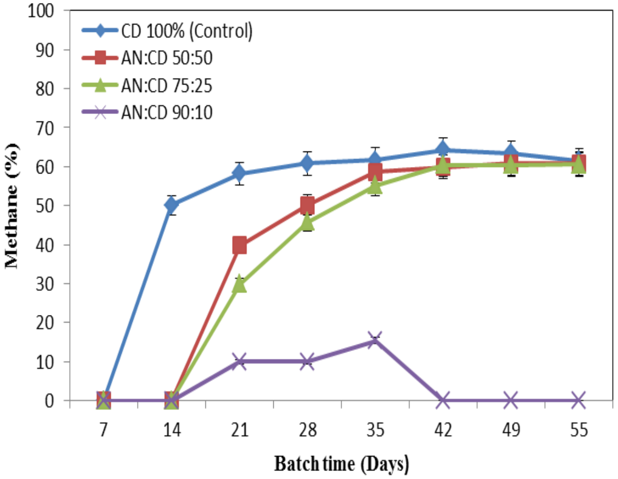
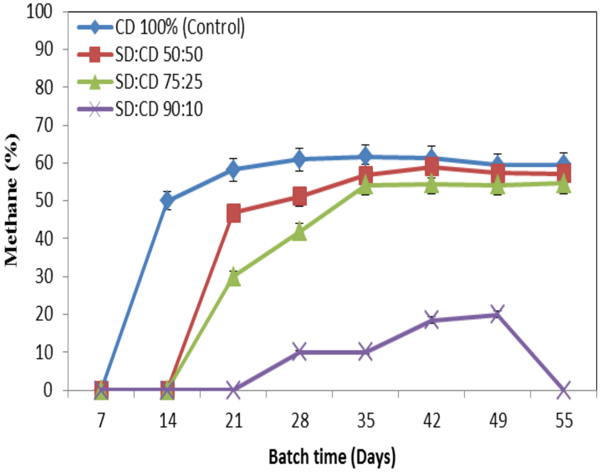
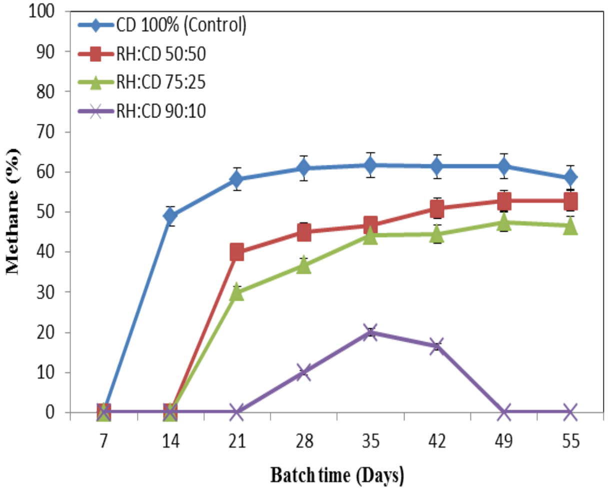


Fig. 4.13 Methane content of areca nut shell (AN) and cattle dung (CD) combinations

In all the lignocellulosic biomass viz; duckweed, arecanut shell, rice husk, saw dust and bamboo dust it was observed that initially for about 11–15 days, the methane content was low after which it starts increasing with time for 35–50 days, till the gas production automatically falls. Maximum methane content, 63.4–57%, was obtained for the combination of 50 (LB):50 (CD) and not much difference was observed between the values obtained for other combination involving 75% or 90%.

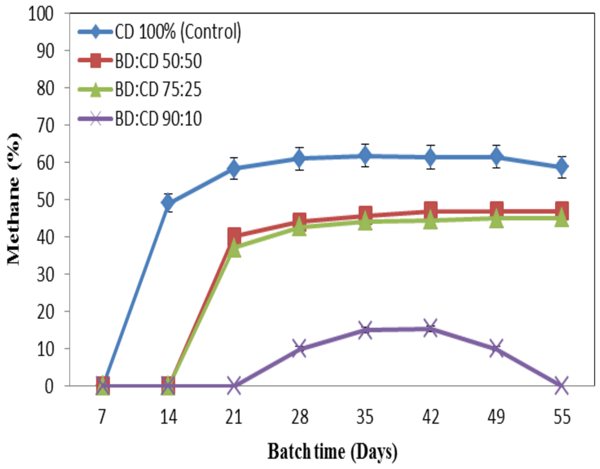


4.14 Methane content of saw dust (SD) and cattle dung (CD) combinations



4.15 Methane content of rice husk (RH) and cattle dung (CD) combinations

Bamboo dust and rice husk displayed different trend and less methane concentration then cattle dung alone, arecanut shell, saw dust and duckweed combination. RH:CD 50:50 and BD 50:50 shows delayed methane production but it gradually increases with days (DAI) but RH:CD 75:25 and BD:CD 75:25 exhibited low methane concentration in the range of 30-46%. The results are in agreement to the available literature, Vivekanandan and Kamaraj (2011) studied no substantial biogas production from 50% weight of raw rice chaff (without boiled) with 50% of cow dung due to high percentage of lignin in raw rice chaff. Similarly, Kobayashi et al. (2004) reported that methane could not be produced from raw bamboo, but methane production can be enhanced through steam explosion. He investigated in his study that the maximum quantity of methane produced (215 mL), was obtained from 1 g of exploded bamboo at a steam pressure of 3.53 MPa and a steaming time of 5 min. Therefore, pretreatment is perquisite for utilization of lignocellulosic biomass as a feedstock for biomethanation.



4.16 Methane content of bamboo dust (BD) and cattle dung (CD) combinations

4.3.5 TOTAL BIOGAS VOLUME FROM LIGNOCELLULOSIC BIOMASS AND CATTLE DUNG CO-DIGESTION AT DIFFERENT PROPORTION

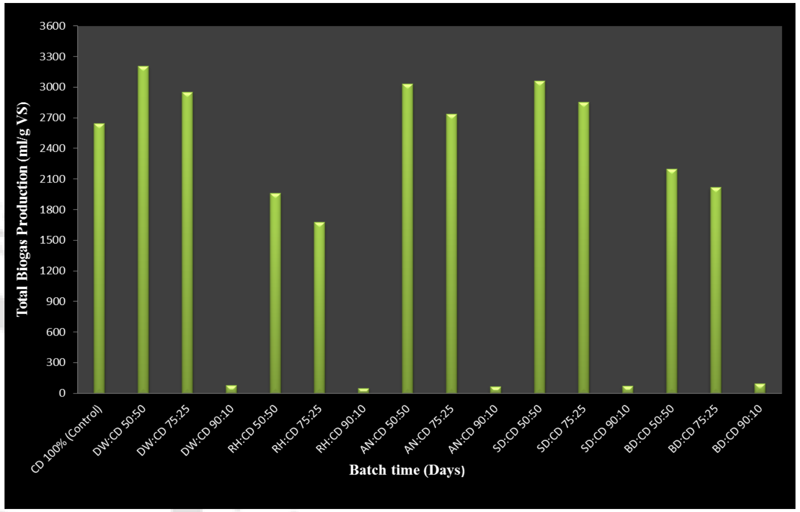


Fig. 4.17 Total biogas production of duckweed (DW), arecanut shell (AN), saw dust (SD), bamboo dust (BD), rice husk (RH) with cattle dung(CD) in different proportions

The effect of 55 day hydraulic retention time (HRT) gave varying results for different proportions designed. It was observed that initially biogas production was slow and later after 14 days to about 45– 50 days; it was significant. Subsequently, the rate decreased because of the reduction in nutrients, which might have affected the growth of bacteria thus affecting the biogas production. Figure 4.17 depicts the total volume of biogas produced per gram VS for different combination considered. Maximum biogas volume of 11870± 7.0 ml/g VS was obtained with treatment 50 (DW): 50 (CD), i.e., combination containing 50% duckweed and 50% cattle dung with TS maintained at ~10% in the anaerobic digester. The increase in the proportion of raw LB from 50%, 75% to 90% was unfavourable as depicted by the decrease in biogas volume obtained (Fig. 4.17). The biogas volume with LB:CD (50:50) combinations produces higher biogas compared to other combinations. This was followed by lignocellulosic biomass of different combinations; 75 (DW): 25(CD), 50 (AN): 50 (CD), 50 (SD): 50 (CD), 75 (SD): 25 (CD),

75 (AN): 25 (CD), 100 (CD), 50 (BD): 50(CD), 50 (RH): 50(CD), 75 (BD): 25(CD), 75 (RH): 25 (CD). It has been found that 90:10 combinations with duckweed, rice husk, arecanut shell, bamboo dust and saw dust exhibited very poor biogas production in all the biomass. It specifies that AD microorganism would not able to decompose lignocellulosic biomass alone with less inoculum and consequently the fermentative and methanogenic bacteria not able to survive due to deficiency of proper nutrients for their growth. Recently, Polizzi, Alatriste-mondragón, and Munz (2018) showed that comparative quantities of proteins, lipids and total carbohydrates strongly influence the potential methane yield from the given substrates since three macro-compounds have different degradation kinetics and specific methane yield. The potential methane yield of a given substrates strongly depends on the relative content in terms of carbohydrates, proteins and lipids. The addition of cattle dung percentage to 25% and 50% with lignocellulosic biomass helps to enhance the microbial population and availability of nutrients in the digester thus promoting the growth of hydrolytic, fermentative and methanogenic bacteria and thereby leading to an increase in the biogas production. It was observed that in all the cases, LB: CD 50:50 gave significantly higher results as compared to raw LB. Also and LB: CD 75:25 gave better biogas production compared to cattle dung alone and lignocellulosic biomass. It has been found that the total biogas volume of DW: CD 50:50, SD: CD and AN: CD increased by 6.38%, 4.68%, 2.3% and decreased by 31.45%, 32.07% in BD: CD 50:50, RH:CD 50:50 respectively from CD 100% alone. These findings suggest the requirement of pre-treatment of lignocellulosic biomass prior to its introduction into anaerobic digestion reactor for enhancement and accelerating the biomethanation process. Nevertheless, further appropriate pretreatment technique studies would be required to validate this observation.

4.4 **SUMMARY OF THIS CHAPTER**

This work investigated the potential of using duckweed, arecanut shell, saw dust, bamboo dust and rice husk widely available lignocellulosic plant material as a feedstock for biogas production. Results shows cumulative biogas and methane concentration for a set of biomass materials digested separately in different ratio of LB and CD. The LB: CD; 50:50 exhibited highest biogas production compared to other combinations. Based on the total gas production volume during a HRT of 55 days, the optimum mixing for co-digestion of DW, RH, SD, AN, BD and CD has been found to be at 1:1 ratio. Co-digestion generally resulted in improved cumulative biogas yields compared to mono digestion. The combination of duckweed, arecanut shell and saw dust with 50% and 75% with cattle dung exhibited higher cumulative biogas volume than cattle dung alone. In other hand rice husk and bamboo dust combination showed lower cumulative biogas volume compare to cattle dung alone. Methane content of the biogas from co-digested feedstock is comparable to the biogas from cattle dung alone. Biogas and methane production was furthermore significantly higher early (0–7 days) in cattle dung alone and to some degree late (above 14 days) in the lignocellulosic biomass digestion process during co-digestion. However, based on this study due to presence of recalcitrant lignin the biogas and methane yield is not encouraging. To enhance or exploit lignocellulosic biomass fully a thorough study on pretreatment has been conducted and presented in the next chapter.

CHAPTER – 5

PRETREATMENT METHODS FOR LIGNIN DEPOLYMERIZATION

5.1 INTRODUCTION

Lignocelluloic Biomass (LB) is attaining increased industrial application due to its abundance and benefits as substrates for valuable chemical production. Apart from the cost effectiveness and low environmental impact, LB also represents a crucial option as a biogas feedstock. As discussed in Chapter 1 and 2 concerning scarcity and problems associated with animal manure/cattle dung attraction shifted towards LB which is a potential and viable feedstock for biogas production. Generally, LB has to process through different physicochemical or biological routes to hydrolyze higher complex moieties into lower sugar moieties in order to fully exploit by microbial population to convert into useful products or biofuels. As discussed in earlier chapters LB consists of cellulose (25%–50%), hemicelluloses (20%–35%), lignin (10%–30%). Cellulose is a ß-1, 4-linked linear polymer of glucose units, has a highly crystalline structure and a high degree of polymerization. In comparison, hemicellulose is an amorphous hetero polymer, and is thus comparatively easier to hydrolyse into simple sugars. Lignin consists of phenyl propanoid units covalently linked to hemicelluloses. Lignocellulose structure is arranged in such way that lignin is shielded the cellulose and hemicellulose inside and because of its recalcitrant properties, it is more difficult to process, extract, hydrolyze, or react. Pretreatment is required to breakdown the recalcitrant lignin shield to expose cellulose and hemicellulose and to improve the bioconversion process.

This chapter contains two sections. First section describes the pretreatments of biomass with various physico, chemical and thermochemical method. A study was conducted to explore and compare its effects against various pretreatment methods and observed changes in biomass compositions, biomass structures and ease of degradability with priority to lignin breakdown has been presented in this work. Second section illustrated a novel plasma assisted pretreatment method for LB in AD process. A novel plasma reactor has been designed and tested to investigate pretreatment efficiency on maize for the enhancement of biogas production in labscale AD reactors.

5.2 Part I- MATERIALS AND METHODS

Rice husk (RH), saw dust (SD), arecanut shell (AN), duckweed (DW) and maize were selected for this work. Various pretreatment methods has been adopted and described below:

- Alkali pretreatment – 2%, 5% and 7% (w/v) NaOH
- Alkali with autoclave- 2%, 5% and 7% (w/v) NaOH with autoclave
- Banana peel ash –pH-11.2
- Plasma pretreatment

5.2.1 PRETREATMENT OF LB WITH PHYSICO-CHEMICAL METHODS

The RH, AN shell and SD were selected for physic-chemical pretreatment work. The RH and AN were collected from Kamrup, Guwahati, Assam, India. SD was procured from a

local furniture factory near IIT Guwahati, Assam, India. The AN shell was cut into 2.5 – 4 mm pieces using a cutter, while the SD and RH were used without any mechanical communition. All the LB samples were grounded to powder with ball milling prior to analytical analysis. All prepared biomass viz; RH, AN and SD has been pretreated with three different alkali pretreatment, banana peel ash (BPA) solution, sodium hydroxide and sodium hydroxide with autoclave respectively.

5.2.1.1 BANANA PEEL ASH SOLUTION PROCESSING

The banana peels were collected and air dried at 45º C for a week, till it lost its yellow color. The peels were then burnt on a flame till it turned into ash in aerobic condition. The formed banana peel ash thereafter was crushed into a fine powder and stored in dry conditions at 25º C. To prepare the banana peel ash (BPA) solution, 5 grams of ash was taken and mixed with 500 ml of distilled water. The amount of ash chosen was in 1:1 ratio to the biomass taken for pretreatment. The solution was allowed to settle and the supernatant liquid was filtered with the help of filter paper to get the BPA solution. The pH of the solution was measured using a pH meter (Eutech Instruments, ThermoFisher Scientific, USA).

5.2.1.2 BPA SOLUTION PRETREATMENT

In each case, the amount of RH, SD and AN sample was taken 5 g and they were soaked in the BPA solution in 100 ml beakers. A control has been also taken, using only biomass soaked in distilled water. The amount of liquid solution taken in each case was 100 ml. The contents were completely mixed to ensure maximum contact. The pH of the supernatant solution was measured after every 5 days, with mixing after every 2 days. The beakers were kept in the laboratory at ambient temperature (30 ± 2° C) for 14 days.

5.2.1.4 SODIUM HYDROXIDE PRETREATMENT

For sodium hydroxide (NaOH) treatment, the raw samples were treated with 2%, 5% and 7% (w/v) NaOH solution and incubated for 14 days at room temperature. The NaOH utilized for the experiment was of analytical grade (Merck).The RH, SD and AN sample was taken 5 g each and the pretreatment solution taken was 100 ml. The contents were then completely mixed to ensure maximum contact. The beakers were kept in the laboratory at ambient temperature (30 ± 2° C).

5.2.1.5 SODIUM HYDROXIDE WITH AUTOCLAVE PRETREATMENT

The biomass samples of RH, AN and SD were treated with 2%, 5% and 7% (w/v) NaOH solution and incubated for 24 hrs. at room temperature and then autoclaved at 121° C, 15psi for 20 minutes. Impulsive depressurization of autoclave valve was done after completion of pretreatment.

After the pretreatment using BPA solution, NaOH, NaOH with autoclave and maleic acid, the samples were filtered through filter paper. The biomass residues were washed with water and acetone and oven dried at 105° C for 3 hrs.

5.2.2 ANALYTICAL ANALYSIS

5.2.2.1 FIBRE ANALYSIS (VAN SOEST METHOD)

The fibre analysis involves the characterization of RH, SD and AN. Cellulose, hemicellulose and lignin were determined by Van Soest’s method using Pelican Fibrewith FES 02 R analyser (Pelican Instruments, Chennai, India).

5.2.2.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY

Fourier transform infrared spectroscopy (FTIR) spectra were obtained using a Shimadzu IR Affinity (Shimadzu, Japan) by mixing 1 % sample with finely ground and dried KBr (Sigma Aldritch, USA). A total of 30 scans were taken with a resolution of 4 cm^{-1}. The scans were recorded between 400 cm^{-1} to 4000 cm^{-1}.

5.2.2.3 THERMOGRAVIMETRIC ANALYSIS

Thermogravimetry provides a rapid method for determining the temperature assisted degradation profile of a material and the kinetics of its thermal decomposition. Thermogravimetric analysis (TGA) measurements were performed on STA 7200 Integrated Thermal Gravimetric Analyzer (Hitachi, Japan). Approximately 5 mg of the prepared sample was placed in platinum pan and heated in nitrogen atmosphere (100ml/ min) between 40 to 800° C at a rate of 10° C min^{-1}.

5.3 RESULTS AND DISCUSSION

5.3.1 PRETREATMENT OF LB SAMPLES WITH ALKALINE, ACID AND NATURAL BASE METHODS

An evident physical and chemical change has been observed in all lignocellulosic biomass after pretreatment with NaOH, NaOH with autoclave and BPA solution.

5.3.1.1 COMPARATIVE ASSESSMENT OF BPA, SODIUM HYDROXIDE PRETREATMENT AND SODIUM HYDROXIDE WITH AUTOCLAVE BY VAN SOEST METHOD

Pretreatment affected the lignin carbohydrate complex (LCC) which resulted in the cleavage of the bonds which are responsible for the recalcitrant structure of LB. Solubilization of lignin and some amounts of cellulose and hemicellulose were also observed. The mechanism of alkaline pretreatment is in which saponification of intermolecular ester bonds crosslinking occurs with xylan hemicelluloses and lignin (Sun et al. 2016). In alkaline hydrolysis, it has been observed that the biomass turn out to be swollen, and thus, increased surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (Behera et al. 2014).

The changes included substantial fibre swelling in case of 5% and 7% (w/v)NaOH while 2% (w/v) NaOH and BPA solution pretreatment resulted in milder fibre swelling and degradation. It was seen that BPA solution treatment reduced the percentage of lignin in the selected biomass but to varying degrees. Figure 5.1 presented the effect of pretreatment on RH lignocellulosic contents with different concentration of alkaline solution in the form of NaOH, banana peel ash and NaOH with autoclave. RH proved to be the most difficult to degrade which could be attributed to the high percentage of lignin in RH. Since BPA solution is a mild alkali compared to NaOH, the amount of fibre swelling in the case of BPA solution was not high.

Higher NaOH concentrations did not necessarily lead to higher lignin removal. About 5% (w/v) NaOH was the optimum for all biomass pretreatment as seen in Figure 5.2 and 7% (w/v) NaOH led to a higher hemicellulose removal while the percentage of lignin did not change appreciably. The amount of hemicellulose degradation in 7% (w/v) NaOH was highest in SD (44.33%), while it was 39% for AN shell and 11.22% in the case of RH. In all cases, it has been seen that NaOH and BPA solution pretreatment was effective in reducing the lignin content of the biomass. BPA solution was responsible in the degradation of 26.57% of the initial lignin content for the case of SD (Fig 5.3). Its effect on RH was not as potent as explained previously and only 12.59% of the initial lignin was removed. In the case of AN, BPA solution pretreatment resulted in the lowering of the initial lignin content by 20.4% (Fig. 5.4). When NaOH was used for pretreatment, in all the cases, a 2% (w/v) solution was effective to a much lesser degree in comparison to a 5% and a 7% (w/v) solution. The lowering of the initial lignin content was 19.2% for SD, 11.5% for RH and 8.8% for AN when a 2% (w/v) NaOH solution was used. Using the 5% and 7% (w/v) NaOH solution significantly increased the amount of depolymerized/solubilized lignin. For the most optimum case of 5% (w/v) NaOH solution, the percentage of lignin was lowered by 33.6% for SD, 45.9% for RH and 32.1% for AN shell. The effect was most significant in the case of RH and it was observed that outer layers of the RH peeled off and particles became more brittle. So, it could be concluded that the particle size did not play a significant role when higher concentrations of NaOH were used, but in the case of 2% (w/v) NaOH and BPA solution, the particle size proved to be a major disadvantage in lignin degradation. The pretreatment techniques employed also degradation a very small portion of cellulose but left most of it intact. It is possible that the crystallinity of the cellulose changed after pretreatment, as it has been reported by several other authors.

In other hand, autoclave assisted alkali pretreatment was found to be most effective in lignin removal compare to BPA solution and sodium hydroxide respectively. The amount of lignin reduction was found highest in RH followed by areaca nut shell and SD respectively with 7%(w/v) NaOH with autoclave pretreatment. Autoclaving pretreatment increases surface area by breaking closed structure of the LB, which may be formed due to lignin removal (Obeng, Premjet, and Premjet 2019). Additionally, autoclave with alkaline with 2%, 5% and 7% (w/v) pretreatment increases the delignification efficiency and decreased the pretreatment incubation time. One interesting observation has been found that the BPA solution was more effective in reducing the lignin content of the biomass samples than the 2% (w/v) NaOH with autoclave. The amount of lignin removal from BPA is almost similar to 2% (w/v) NaOH with autoclave for all the biomasses. Its effects were not as potent when compared to a 5% or 7% NaOH with autoclave but BPA solution has the added advantage of not being as corrosive and has lesser impact on downstream processes. BPA solution also does not affect the cellulose and hemicellulose content as much as NaOH, leaving a higher percentage of digestible material. Higher concentrations of BPA, longer residence times, higher temperature and pressure might also be useful in making the process more effective in lignin removal. For autoclave-assisted NaOH pretreatment, the pretreated arecanut shell biomass samples by using 7% (w/v) NaOH gave the highest cellulose content by increasing cellulose content around 79%. It has been also found that autoclave with alkaline pretreatment reduced the hemicellulose content of the biomass. About 7 % (w/v) NaOH with autoclave pretreatment reduced about 53.6%, 55.5% and 71.28 in AN, SD and RH respectively. The overall porosity of the lignocellulosic materials also increases during alkali with autoclave pretreatment. However, BPA pretreatment does not remove the hemicellulose through hydrolysis.

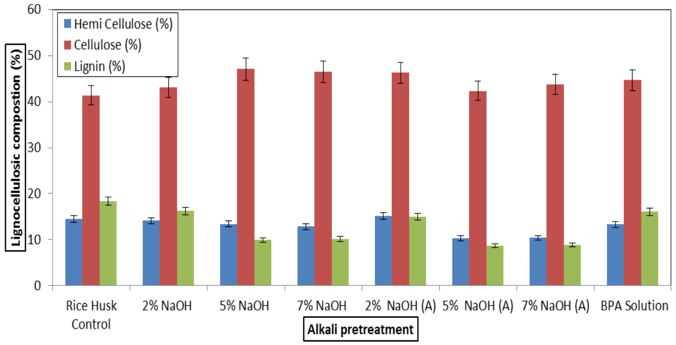


Figure 5.1 Alkali pretreatment of RH with 2%, 5%, 7% (w/v) NaOH, 2%, 5%, 7% (w/v) NaOH with autoclave and BPA solution

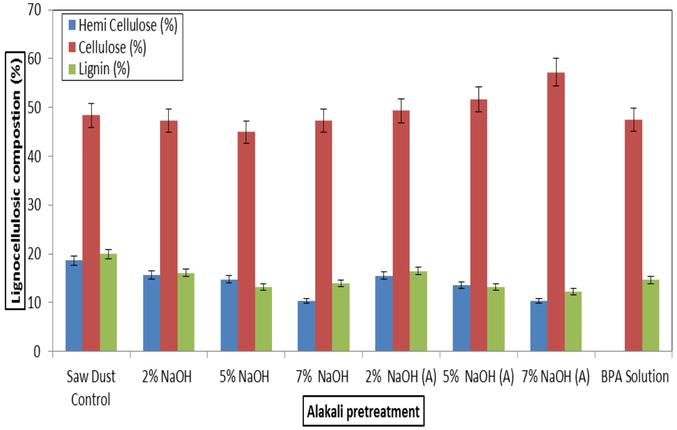


Figure 5.2 Alkali pretreatment of SD with 2%, 5%, 7% (w/v) NaOH, 2%, 5%, 7% (w/v) NaOH with autoclave and BPA solution

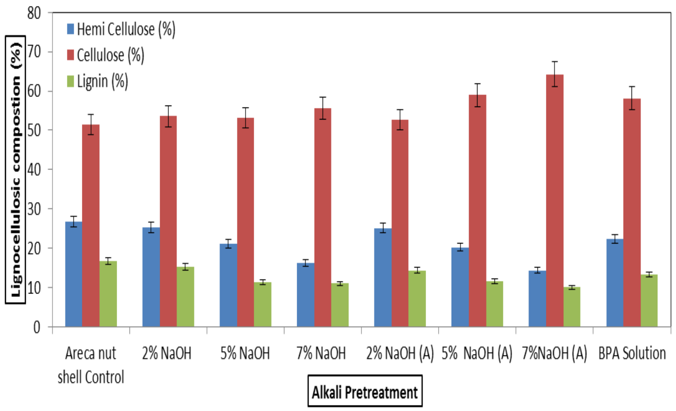


Figure 5.3 Alkali pretreatment of AN with 2%, 5%, 7% (w/v) NaOH, 2%, 5%, 7% (w/v) NaOH with autoclave and BPA solution

5.3.1.2 COMPARATIVE ASSESSMENT OF BPA, SODIUM HYDROXIDE PRETREATMENT AND SODIUM HYDROXIDE WITH AUTOCLAVE BY THERMOGRAVIMETRIC ANALYSIS

The TGA curves of raw RH, SD and AN were plotted in order to compare 2%, 5%, 7% (w/v) NaOH, 2%, 5%, 7% (w/v) NaOH with autoclave and BPA solution pretreated LB samples. It has been reported that the initiation of lowering of the curve is due to the loss of weight due to the volatilization of hemicelluloses (150-350 °C), cellulose (260-350 °C) and lignin (till 300-500 °C) (Yadav et al. 2016). The degradation of lignin at temperatures below 300 °C can be attributed to breakage of bonds with low activation energy and high temperatures of above 300° C, results in the cleavage of more energetically stable bonds. The major degradation of biomass samples observed at around 250 °C, 318 °C and 430 °C respectively, which signified the degradation of structural components of the biomass. The value of weight loss at the region between 250 °C and 350 °C signifies the amount of hemicellulose and cellulose respectively, the lignin degradation started at around 400 °C and above. The rate of change of weight tends to become very low after 500-550 °C to 800 °C, since the remaining biomass contains more complex and thermally stable structures. In all the TGA graphs of RH, SD and AN similar pattern has been observed where 7% NaOH with autoclave shows highest lignin degradation and BPA solution pre-treated samples was found to be degraded faster than the 2 % (w/v) NaOH but the rate of degradation was lesser than 5% and 7 % (w/v) NaOH and autoclaved pre-treated samples. This showed that the BPA solution was more effective in destabilizing the biomass samples than the 2% (w/v) NaOH pretreated samples. The results were also in agreement with the data obtained from the fibre analysis and FTIR spectra. The detail thermal decomposition curves of the biomass samples are illustrated in fig. 5.5, 5.6 and 5.7 respectively.

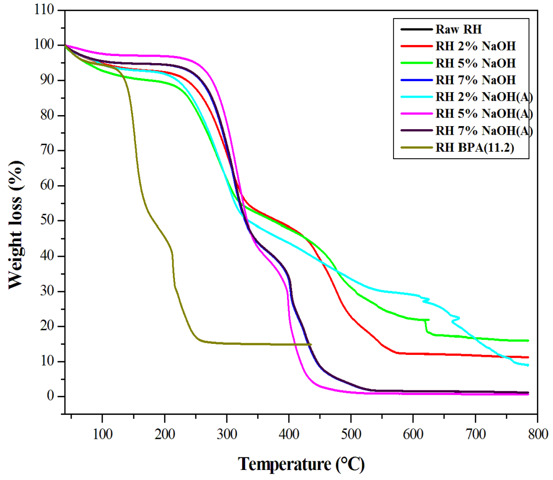


Figure 5.4 TGA profile alkali pretreatment of RH with 2%, 5%, 7% NaOH, 2%, 5%, 7% NaOH with autoclave and BPA solution

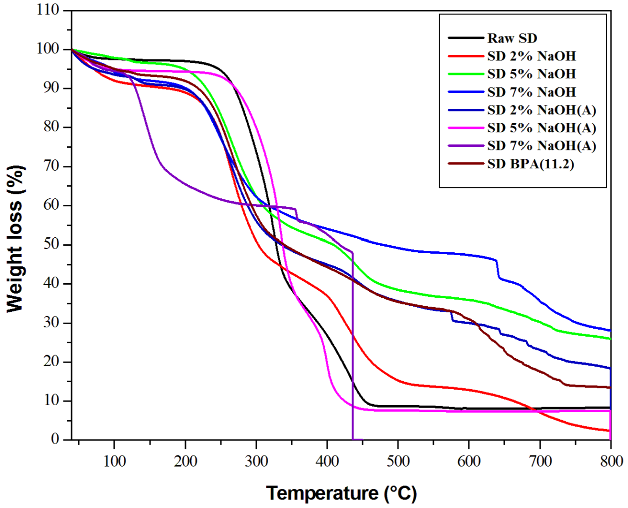


Figure 5.5 TGA profile alkali pretreatment of SD with 2%, 5%, 7% NaOH, 2%, 5%, 7% NaOH with autoclave and BPA solution

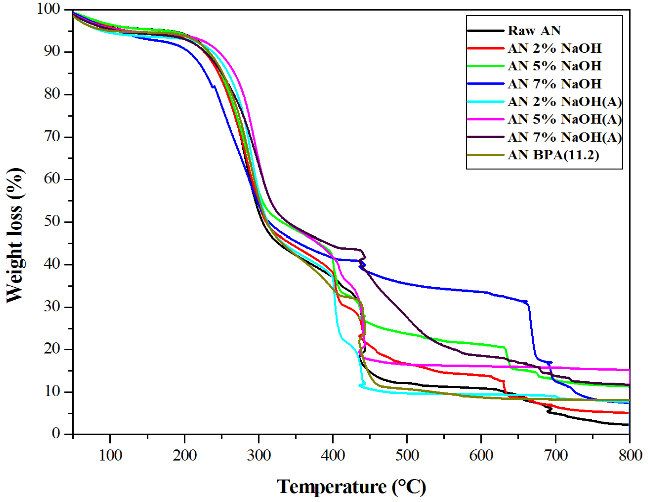


Figure 5.6 TGA profile alkali pretreatment of AN with 2%, 5%, 7% NaOH, 2%, 5%, 7% NaOH with autoclave and BPA solution

5.3.1.3 COMPARATIVE ASSESSMENT OF BPA, SODIUM HYDROXIDE PRETREATMENT AND SODIUM HYDROXIDE WITH AUTOCLAVE BY FOURIER TRANSFORM INFRARED ANALYSIS

FTIR Spectroscopy showed that the functional groups associated with lignin were changed after pretreatment with NaOH, NaOH with autoclave and BPA Solution. Figure 5.9, 5.10 and 5.11 shows FTIR spectrogram of RH, SD and AN respectively. The changes fall into one of the 3 categories a) disappearance of bands, b) decrease of band intensity/decrease in the number of functional groups and c) Appearance of new bands. The bands which are associated with lignin, indicated in the FTIR spectra of AN and RH, had lower intensities after pretreatment. The lowest decrease in band intensity was observed in the case of 2% (w/v) NaOH while 5% and 7% (w/v) NaOH resulted in a much higher decrease in the band intensities. However, the difference was not very noticeable when biomass samples pretreated using 5% and 7% (w/v) NaOH were compared. BPA solution pretreatment resulted in a higher decrease in band intensity than 2% (w/v) NaOH but not as much as 5% and 7% (w/v) NaOH. It was noticed that in the case of RH, the FTIR spectra of 2% NaOH and BPA solution pretreatment are quite similar while the differences in intensities are more noticeable in the case of the FTIR spectra of AN husk which can be attributed to the higher particle size of RH when it was subjected to BPA solution pretreatment. The lower particle size of SD and AN would result in a larger surface area with better contact with the pretreatment solution. This was possibly why SD and AN showed more changes in the band intensities than RH when they were pretreated using BPA solution.

The decrease in band intensity of AN and RH at 1741 cm^{-1} and 1740 cm^{-1} respectively, which is associated with carbonyl (C=O) stretching, is an indication of disruption of the lignin side chain. It was seen that the intensity reduction for AN after 2% (w/v) NaOH pretreatment at 1741 cm^{-1} was almost negligible. The effect of BPA solution was noticeable with a higher decrease in the peak intensity than 2% (w/v) NaOH pretreatment. 5% and 7% (w/v) NaOH pretreatment was able to disrupt the lignin side chains for AN to a much greater degree which resulted in the highest decrease in the band intensity, but the decrease in intensity was similar in both cases. In the case of RH, the decrease in band intensity gradually increased as the concentration of NaOH, with 7% (w/v) NaOH having the highest decrease in band intensity. It seemed like BPA solution pretreatment was quite effective in the removal of lignin side chains for RH since the decrease in the intensity was comparable to 7% (w/v) NaOH pretreatment. The band intensity at 1721 cm^{-1} is associated with hemicellulose acetyl and uronic ester linkages in lignin along with ester-hemicellulose feurilic and p-coumaric acid carboxylic groups. The effects were nearly similar as the band at 1741 cm^{-1}, except disappearance of the band in the case of 7%(w/v) NaOH for AN, which is an indication of stronger effects on uronic ester groups in lignin as the concentration of alkali increased. Also, BPA solution was not as effective in this case; however, the decrease in intensity was more than 2% and 5 % (w/v) NaOH pretreatment. The band at 1639 cm^{-1} is associated with C=O stretching vibration of the conjugated carbonyl of lignin. In the case of AN, the highest decrease in intensity at 1639 cm^{-1} was observed in the case of 5% (w/v) NaOH while in the case of RH, the band intensity was the lowest for 7% (w/v) NaOH. This was probably due to the difference in the arrangement of microfibers and the difference in the population of the bonds in RH and AN husk. The band at 1595 cm^{-1} is indicative of aromatic ring stretch and the change/shift in intensity is generally attributed to removal of lignin in lignocellulosic biomass. In AN, BPA solution pretreatment was able to reduce the intensity at 1595 cm^{-1} to a higher degree than pretreatment with 2% (w/v) NaOH. The wavelength band

observed in AN and RH at 1466 cm^{-1} and 1465 cm^{-1} respectively, associated with the aliphatic part of lignin. The intensities were seen to decrease the most for AN with 7% (w/v) NaOH and the least for 2% (w/v) NaOH. The effect of BPA Solution was fairly moderate in AN, however higher compared to 2% (w/v) NaOH pretreatment. The effects were also seen to be similar in the case of RH. In both cases, 5% (w/v) NaOH pretreatment resulted in the lowest relative intensity when compared to the control. The band at 1360 cm^{-1} for AN and 1362 cm^{-1} for RH corresponds to aliphatic C-H stretching in methyl and phenolic functional groups. The bands decreased in intensity upon pretreatment due to its reaction with the alkali. The increase in concentration resulted in the disappearance of the band at 5% (w/v) NaOH in the case of RH. The effect of BPA solution effect on the phenolic groups in lignin was also prominent when compared to the control sample and the sample pretreated using 2% (w/v) NaOH. In the case of AN, the intensity of the band gradually decreased as the concentration increased but it did not completely disappear. However, it was seen that the decrease in 5% (w/v) NaOH was higher when compared to 7% (w/v) NaOH pretreatment. Finally, the prominent band at 1260 cm^{-1} for AN and 1262 cm^{-1} for RH is indicative of methoxyl stretching in lignin and xylan. The nucleophilic reaction of methoxyl with the alkali (BPA Solution and NaOH) was the most probable reason for the decrease in the relative intensity of the band. In the case of AN, the trend in the intensity value was Untreated>2% NaOH>BPA Solution>5% NaOH>7% NaOH, which means that 7% (w/v) NaOH pretreatment had the most potent effect on the methoxyl group in AN. However, in the case of RH, it was seen that 2% (w/v) NaOH and BPA solution had no appreciable effect on the methoxyl group when compared to the control, while 5% and 7% (w/v) NaOH resulted in a high, but very similar decrease in intensity levels.

The band at 1680 cm^{-1} in the FTIR Spectra of SD corresponds to unconjugated C=O stretching of lignin in woody Biomass. BPA Solution was able to reduce the relative intensity significantly of the band in comparison to the control. 5% (w/v) NaOH and 7% (w/v) NaOH pretreatment was able to decrease the band intensity even further due to the increase in alkaline behaviour. The band at 1499 cm^{-1} can be associated with aromatic ring vibration in lignin (Xu, 2013). The decrease in band intensity was almost similar in 2% (w/v) NaOH, 5% (w/v) NaOH and BPA Solution while 7% (w/v) NaOH pretreatment resulted in the highest decrease in the band intensity. The band at 1427 cm^{-1} is indicative of C-H along with aromatic skeletal in plane deformation and stretching in woody biomass. BPA Solution pretreatment was effective in decreasing the population of the bonds more than the pretreatment using 2% (w/v) NaOH; however, 7% (w/v) NaOH resulted in the highest reduction of band intensity when compared to the control. The band at 1360 cm^{-1} for SD decreased in intensity upon pretreatment due to its reaction with NaOH and BPA solution. The increase in concentration resulted in the gradual reduction of the band intensity, which was the lowest when 7% (w/v) NaOH solution was used for pretreatment. The band at 1328 cm^{-1} is associated with C-O of the syringil ring in woody biomass. It seemed as if pretreatment was not able to disrupt the C-O bonds to a large degree in SD as indicated by the minimal reduction in band intensity at 1328 cm^{-1}. The effects were almost negligible in the case of 2% (w/v) NaOH and BPA Solution while there was only a small change in the intensity upon 5% (w/v) and 7% (w/v) NaOH pretreatment. The band at 1262 cm^{-1} is indicative of methoxyl stretching in lignin and xylan in SD. The decrease in intensity was quite similar for 2% (w/v), 5% (w/v) NaOH and BPA Solution, which meant that the effectiveness of nucleophilic reaction of BPA solution with methoxyl was comparable to its reaction with 2% (w/v) and 5% (w/v) NaOH. The effect of 7% (w/v) NaOH with autoclave was better than all the aforementioned cases.

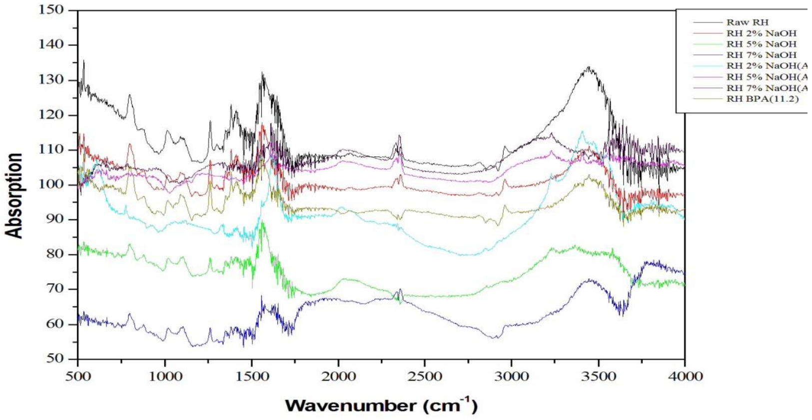


Figure 5.7 FTIR spectrogram of alkali pretreatment of RH with 2%, 5%, 7% (w/v) NaOH, 2%, 5%, 7% (w/v) NaOH with autoclave and BPA solution

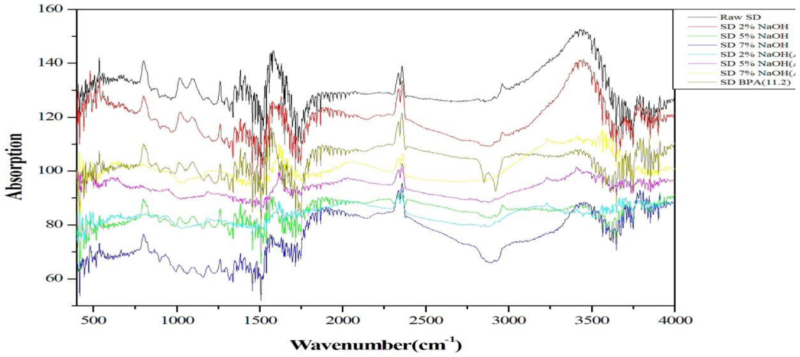


Figure 5.8 FTIR spectrogram of alkali pretreatment of SD with 2%, 5%, 7% NaOH, 2%, 5%, 7% NaOH with autoclave and BPA solution

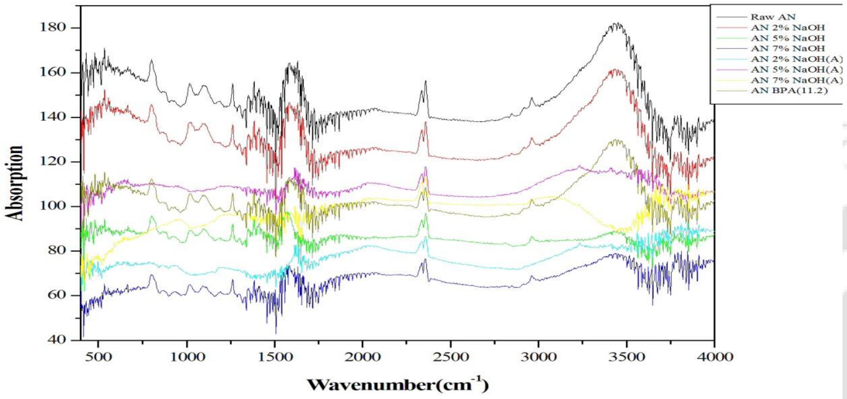


Figure 5.9 FTIR spectrogram of alkali pretreatment of AN with 2%, 5%, 7% (w/v) NaOH, 2%, 5%, 7% (w/v) NaOH with autoclave and BPA solution.

5.4 PART II- METHODS AND MATERIALS- PLASMA ASSISTED PRETREATMENT AND BIOGAS PRODUCTION FROM MAIZE

5.4.1 FEEDSTOCK PREPARATION AND CHARACTERISATION

Maize (*Zea mays*) was grown outdoors by STW Ltd, Stoke Bardoloph, UK. It was harvested as whole and mature plants in the autumn, chopped to ~6 mm size and stored on-site in clamps. Random samples from these clamps were collected and dried at 105°C in a laboratory oven for two hours. They were then stored and used for experimentation. Time between harvest and experimentation was 12 to 18 months. Prior to the experiments, maize was oven dried overnight and shredded to ~1-2 mm size using a cutting mill (pulverisette 15, Fritsch GmbH). The shredded maize was characterised using the standard methods for the examination of water and wastewater for total and volatile solids determination (APHA 1999). Analysis of random oven-dried maize samples (in triplicate) indicated 97.81% total solids and 96.55 % of volatile solids from a total mass. Further analysis of the dried maize samples to obtain accurate data regarding the lignocellulosic composition of biomass sample was performed by is Celignis Analytical, ROI, following analytical protocols by the National Renewable Energy Laboratory (NREL) of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy for ash in biomass, extractives in biomass and structural carbohydrates and lignin in biomass (Sluiter et al. 2005, Sluiter et al. 2005 and Sluiter et al. 2008). The results of the analysis are shown in Table 5.1.

Table 5.1 Characterisation analysis of maize

Sugars	**Percentage (%)**	**Lignin and others**	**Percentage (%)**
Glucan	15.29	Klason Lignin	9.09
Xylan	9.37	Acid Soluble Lignin	1.94
Mannan	0.29	Extractives	32.63
Arabinan	1.76	Starch	19.24
Galactan	0.65	Ash	4.34
Rhamnan	0.07	Moisture	5.34
Total Sugars	**27.42**	**Total Lignin and others**	**67.24**

5.4.2 DBD MICROBUBBLE-PLASMA REACTOR

Design details and characterization of the plasma-microbubble reactor used in this study is presented elsewhere (Wright et al. 2018a, b, c). The pretreatment reactor mainly consists of two sections: the plasma module and the reaction tank as shown in figure 5.10. Air is supplied to the plasma chamber at 1.5 SLPM via a mass flow controller (PR4000B, MKS Inc). The porous nickel membrane separating two main sections acts as the ground electrode for the plasma discharge as well as the sparger for microbubble production. This facilitates the immediate transfer of short-lived RONS from the plasma to the liquid phase through the gas-liquid interface of the growing microbubbles. The microbubbles produced from the nickel membrane have a diameter of ~550 µm (Wright et al. 2018c) and provide sufficient surface to volume ratio for efficient mass transfer. The reaction tank has a volume of 3 L, and the height and the internal diameter are 300 mm and 152 mm respectively. The bubbles that rises through the draft tube attached to the reaction tank induce convective flow, removing the need for mechanical mixing and ensuring a homogeneous suspension of biomass within the reaction tank. This is particularly important for maize suspensions as larger particles tend to settle at the bottom of the tank reducing the uniformity of the treatment. The plasma was powered by a full-bridge resonant power supply operating at 21 kHz with a sinusoidal voltage of 16 kV_{pp}. The power supply was modulated at 45% duty cycle, the optimum value found during the characterization of the reactor for maximum ozone generation, which corresponds to a power of draw of 18.6 W.

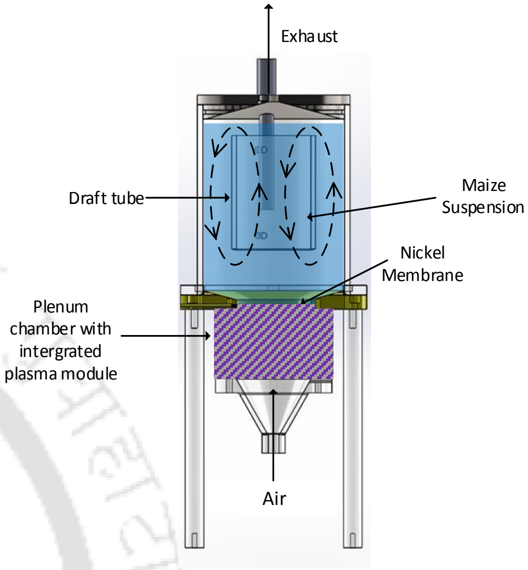


Figure 5.10 The DBD microbubble-plasma reactor used for pre-treatment of maize

5.4.3 MAIZE PRETREATMENT

Maize suspensions for FTIR analysis and SEM imaging were prepared by adding 20 g of dried, milled maize to 2.7 L of either phosphoric acid at pH 3 (Sigma Aldrich Ltd), sodium hydroxide at pH 9 (Sigma Aldrich Ltd) or tap water at pH 7.4. For feeding AD reactors, suspensions were prepared by adding 20 g of maize in 390 ml of tap water. All suspensions were pre-treated in the plasma-microbubble reactor for 2 hours at 45% duty cycle. Following the pretreatment, maize suspensions were fed to AD reactors as either unwashed or washed to determine the effect of inhibitory compounds produced during the pretreatment process. For preparation of the washed feed, maize was first separated from the suspension by filtration using a Buchner funnel and filter paper (413, VWR), washed with de-ionised water and dried in an oven for 12 hours at 60 ^{o}C. 20 g of this dry material was re-suspended in 450 ml of tap water before introducing to the AD reactors. Unwashed samples were prepared by adding 60 ml of water to the pretreated suspensions (to make total volume 450 ml) followed by neutralisation with NaOH. Note that all maize feeding suspensions contained 20 g maize (raw; plasma pretreated, washed; and plasma pretreated, unwashed) in 450 mL of tap water. This ratio was maintained so that the total solids and volatile solids are in line with that of sewage sludge. 450 mL of sewage sludge was used for the control test. This was in preference to using a standardised organic loading rate based on total and volatile solids, since inherent mass loss was associated with the plasma treated washed sample.

5.4.4 ANAEROBIC DIGESTION WITH CONTINUOUS FEED

The anaerobic digesters used in this study were 10 L glass reactors with a 9 L working volume. They were subjected to continuous automated stirring at a rate of 100 rpm, and a constant ambient temperature of 37° C (±2°C) was maintained throughout the study. Two access ports located at the top of each reactor enabled feeding of the units and continuous monitoring of the gaseous output using Ritter Milli Gascounters (MGC-1, V3.4 PMMA). Biogas composition (CH_4, CO_2, O_2, and H_2S) was measured using a handheld gas analyser (Gas Data GFM-416, UK) via an access port in the gas line.

All four AD reactors were first incubated with sewage sludge acquired from the Wanlip AD plant, Leicester, UK, and fed over a period of 15 consecutive days prior to this study. They were then fed simultaneously for over a period of 25 days with sewage sludge, untreated maize suspension, pretreated unwashed maize suspension (Unwashed) and pretreated washed maize suspension (Washed) separately. In all cases, the reactors were

fed once a day from Monday to Friday, with no feeding at the weekends. Immediately prior to feeding, 450 mL of digestate was extracted for analysis through a manual stop cock situated at the base of the reactor.

5.4.5 ANAEROBIC DIGESTION – SINGLE BATCH FEED

The Biochemical Methane Potential (BMP) methodology followed Strömberg et al. 2014. Experiments were repeated in triplicate for each sample set using an array of 1L continuously stirred anaerobic digesters, along with three control reactors (inoculum only). All reactors were subject to the same stirring intensity (through a top-mounted gearbox), while placed in an electrically heated water bath set to 38°C. The water bath had a sealed lid to contain heat and reduce evaporative losses. This unit was part of a kit supplied by Anaero Technology, UK. The reactors were made of opaque HDPE, each with an independent gas line that was diverted through a translucent communal water-filled array of individual flow cells (Anaero, UK) each of which was in turn connected to its own 5 L Tedlar bag (by Essvial, US, part 1212-7000-GD).

Prior to the experiments, all reactors were filled with 650 mL of sewage sludge (inoculum), acquired from Wanlip, UK. All, except the control reactor, were then fed with maize in a ratio with respect to the inoculum of 1:4 based on volatile solid (VS) content. This equated to 3.85± 0.1 g for raw and washed maize, and 9.07 ± 0.2 g for unwashed maize. Where plasma treated maize samples were used for feeding, these were prepared 24 hours before and kept refrigerated in covered flasks for five hours prior to the experiments, whereupon they were acclimatised to room temperature.

The duration of the batch experiment was 35 days. During this time gas output data was logged onto a personal computer via an Arduino 2560 microcontroller. Microsoft Excel was then used to correct the biogas product to a N.ml/g_{vs} basis, discounting residual biogas from the inoculum taken as an average of the three samples. Biogas data was not corrected for water vapour. The Tedlar bags were evacuated on days 7, 14, and 35 using a handheld gas analyser (Gas Data GFM-416, UK), which simultaneously provided gas composition values.

5.4.6 DIGESTATE ANALYSES

Digestate from the continuous feed experiments was analysed three times per week, on alternate days. The amount of ammonium nitrogen and Volatile Fatty Acids (VFA) were both determined by UV spectroscopy (Haach Lange DR 3900, Germany), using chemical kits LCK 303 and LCK 365 respectively. The Ripley method was used to determine the ratio of VFA: TA (Total Alkalinity) (Ripley, Boyle, and Converse 1986), with 0.1 M HCl as the titrant. A Mettler Delta 340 was used to measure pH.

5.4.7 SCANNING ELECTRON MICROSCOPY

Pretreated and raw samples were analysed with SEM by sputter coating (SC7640, Quorum Tecnologies Ltd) with a gold palladium source for 180 s with 25 mA providing a 5 – 10nm thick conductive layer. Analyses were completed using a Leo (Zeiss) 1530-VP FEGSEM. Imaging was carried out at 20 kV accelerating voltage with an aperture of 30 µm and observed in standard secondary electron mode.

5.4.8 ATTENUATED TOTAL REFLECTION - FOURIER TRANSFORM INFRARED SPECTROSCOPY (ATR-FTIR)

Samples were first ground to a homogenous powder before measuring the attenuated total reflection. Samples were then placed in a sample holder (GS10800-x, Quest Ltd) mounted with an infrared spectrometer (IRAffinity-1, Schimadzu Inc). A total of 64 scans were taken with a resolution of 4 cm^{-1}, between the ranges of 400 to 4000 cm^{-1}. A background scan was also taken prior to each analysis and deducted from the final result.

5.5 RESULTS AND DISCUSSION- PLASMA ASSISTED PRETREATMENT AND BIOGAS PRODUCTION FROM MAIZE

5.5.1 REACTOR OPTIMISATION

As pH of the pretreatment solution influences the treatment efficacy (Wright, Marsh, et al. 2018), this effect was first studied by pretreating maize under acidic, basic and neutral pH conditions and carrying out FTIR analysis. An FTIR spectrum provided information about changes to the chemical structure that happens during the pretreatment process. A raw maize sample analysed with FTIR is shown in Figure 5.1.

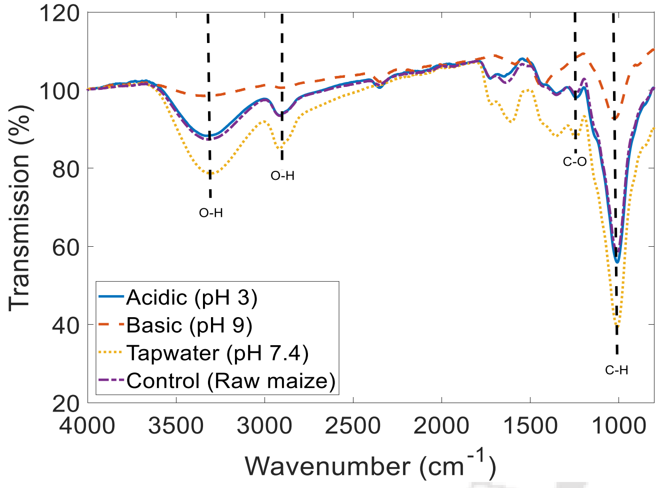


Figure 5.11 The normalised FTIR spectrum of maize suspensions pretreated at different values of pH.

The most pronounced peaks were observed at 3300 cm^{-1}, 2890 cm^{-1} and 1020 cm^{-1} which corresponds to the intermolecular and intramolecular bonds O-H and C-H respectively (Singh et al. 2000). Further peaks can be identified at 1230 cm^{-1} which corresponds to C-O bonds and indicate damages to the cellulose structure (Souza-Correîa et al. 2013). These changes to the transmission intensity observed in FTIR spectrum provides a measure of the effectiveness of the treatment with a large change in peak from that of the raw maize indicating a more effective treatment. The largest deviation from the raw samples is observed for maize pretreated under alkaline conditions. This spectrum differs from that of the other pretreatment conditions tested and there is a noticeable reduction in C-O peaks. This suggests excessive damage to the samples (Souza-Correîa et al. 2013); hence alkaline conditions were avoided in preparing maize for AD. Pretreatment carried out with both tap water and acidic conditions were shown to be effective. Reactive nitrogen species (RNS) species produced from the plasma such as HNO_3 and ONOOH create acidic conditions in tap water producing an environment similar to that of the treatment carried out at low pH (Wright et al. 2018c).

Pretreated maize samples were further analysed for morphological changes using SEM micrographs and the results are shown in Figure 5.12. Micrographs of the raw maize (Figure 5.12(a) and 5.12 (b)) show that the outer surfaces of the fibres are relatively undamaged. Despite the variation in samples collected from different parts of the maize plant, the number density of cracks and pores on the raw untreated surface is relatively low. The samples pretreated under acidic conditions and in tap water show similar micrographs. Here the maize strands contain large open pores and has a flaky appearance to the surface; hence preferential for digestion as this could potentially aid microbial access to the centre of the fibres (Travaini et al. 2016). For maize pretreated under basic conditions, a completely different surface structure is observed. In this case, the surfaces remain relatively smooth with small pores and pitting visible on the surface. The combination of FTIR and SEM analysis suggest that plasma-microbubble pretreatment under alkaline conditions could be overly aggressive for the maize and possibly damage the cellulose structure. Therefore, tap water was selected as the treatment medium. This has the added benefit of not requiring addition of chemicals for pretreatment, reducing the cost of the treatment as well as overcoming the issues in recovery of chemicals used.

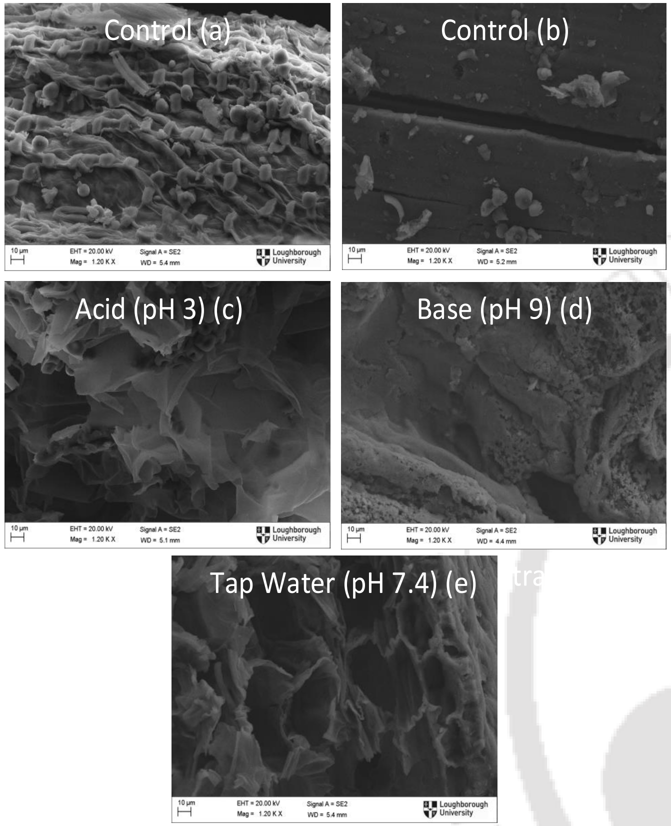


Figure 5.12 SEM images of maize samples following pretreatment at different pH. (a),(b) Raw maize (control) (c) Maize pretreated under acidic conditions: pH=3 (d) Maize pretreated under alkaline conditions: pH=9 (e) Maize pretreated in tap water.

5.5.2 CONTINUOUS FEEDING

Figure 5.13 shows stability parameters (VFA, Ripley's Ratio, ammonium and pH) for the duration of the experiment for 4 reactors, measured in fresh digestate samples.

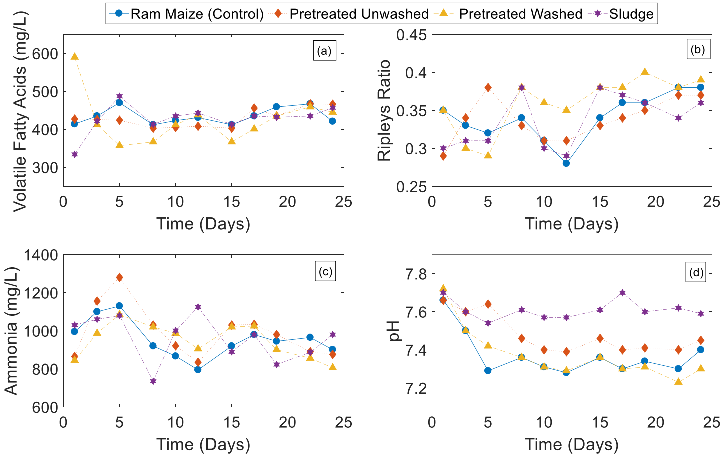


Figure 5.13 Stability parameters of the AD.

In the AD process, VFA concentration is a very good indicator of the degradation status (Fernández, Sánchez, and Font 2005). Volatile fatty acids are intermediate product of anaerobic biodegradation of maize, and resulting in the eventual production of H_2, CH_4 and CO_2. The determination of VFAs is an essential prerequisite to determine the impact of pre-treatment on metabolic activities under anaerobic conditions. Start-up of an AD process is sometimes characterised by a slight instabilities in VFA, when losing the balance of the hydrolytic microbes, fermentative microbes, and methanogens (Hartmann and Ahring 2005), which is followed by the stabilization of the active processes of acidification and methanogenesis. As seen in Figure 5.13 (a) after the short stabilisation period, the concentrations of VFAs in raw maize, plasma treated unwashed maize, and control were ranging between 350 - 500 mg/L indicating a stable process. This stability was also confirmed by the pH values. The optimal pH range of 7.3 – 7.7 was achieved and maintained throughout the experiment, which provided good condition for microorganism to thrive and this resulted in stable biogas productions (Figure 5.13 (b)).

VFA/alkalinity ratio (Ripley's ratio) was a feasible criterion to estimate the stability of the fermentation system (Duan et al. 2012). The following critical values are used for this parameter: <0.4, digestion system should be relatively stable; 0.4–0.8, a sign of instability; and >0.8, significant instability (Callaghan et al. 2002). In all the AD reactors, VFA/alkalinity ratio was optimal in the range of 0.3–0.4 (Figure 5.13c). Similarly, ammonium concentration for all reactors was stable throughout, after the initial stabilisation process, with concentrations ranging between 800 and 1130 mg/L, as shown in Figure 5.13 (c).

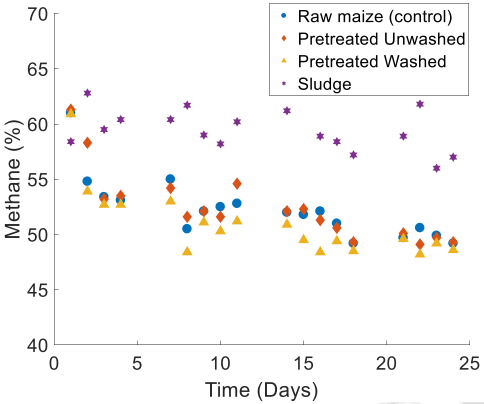


Figure 2.14 Analysis of the methane output from the AD reactors during continuous daily feeding.

Methane percentage in biogas produced from the AD reactors during the continuous feeding regime is shown in Figure 2.14. The reactor fed with sewage sludge produced the highest methane percentage (~60%) compared to that of the other reactors fed with maize. Sewage sludge is relatively easier to be digested by the AD process and is the feed that AD microorganisms are accustomed to prior to the experiments. On the other hand, pretreated maize is unlikely to undergo complete hydrolysis during pretreatment, and the complex nature of this lignocellulosic substrate make it difficult for microbial attack. Eastman and Ferguson, 1981 stated that it is indeed the hydrolysis of particles, rather than soluble matter that is rate limiting in AD. Therefore, the methane percentage dropped to ~50% immediately after switching to maize suspensions regardless of whether the suspensions were pretreated or not.

Having established the health of the reactors, quantification of the cumulative biogas volume (Figure 5.15 (a)) and methane volume (Figure 5.15 (b)) can be considered. All profiles exhibited a gradual rise in cumulative output corresponding to the daily feeding of each reactor, with weekends identifiable by temporary plateaux. The cumulative biogas output for all reactors increased at the same rate regardless of the feed material, and the slight differences (up to 10% after 25 days) could be due to minor variation of the AD cultures or conditions within the reactor. However, cumulative methane volume shows a noticeable difference from ~day 4 onwards and shows ~20% variation after 25 days between sludge fed reactor and the reactor fed with pretreated, washed maize. In the daily feeding regime, sewage sludge outperformed maize fed reactors with respect to the quality of biogas produced primarily due to the digestibility of the feed in addition to providing sufficient nutrients required for optimal operation. Lignocellulosic biomass and energy crops (such as maize) are often rich in carbohydrates, but low in nitrogen (Li et al. 2015). This could lead to lack of micro and macro nutrients required for the associated micro-organism during the AD process particularly in continuous operation over a long-time period. The slightly weaker performance observed with the pretreated washed maize compared to the unwashed maize (13% less) could be due to removal of starch during the washing process. Maize contains a significant starch content (19%) and a fraction of unbound starch could be entrained with the washing liquid, reducing the easily accessible digestible material in the feed.

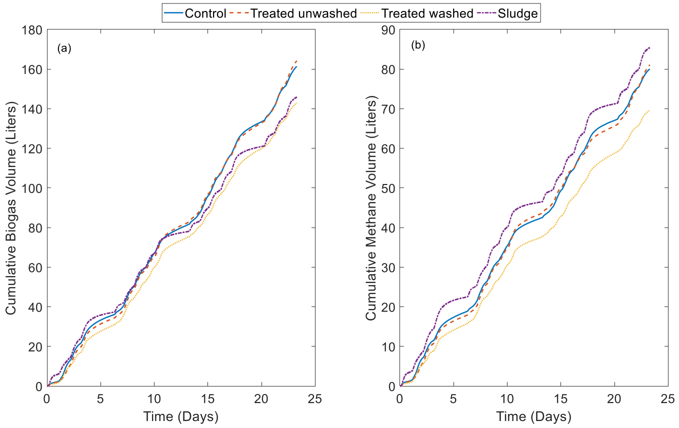


Figure 5.15 Cumulative gaseous outputs from the AD reactor (a) Cumulative biogas volume (b) Cumulative methane volume

Whilst the SEM micrographs and FTIR analysis demonstrated that pretreatment has changed the maize chemical structure, it shows no significant effect on the biogas production in the continuous feeding experiments. As the reactors are fed on a daily basis, it is probable that easily digestible material contained in the maize (especially starch) is sufficent to maintain AD process and the pretreated lignocellulosic material remains relatively unused. To validate this conjecture, biogas production from the AD reactors following a single batch feed was studied.

5.5.3 SINGLE BATCH FED REACTORS

Cumulative biogas production from the AD reactors that were fed once at the start of the experiments is shown in Figure 5.16. The AD reactor fed with maize prepared by bubbling the suspension with air produced significantly lower biogas volume compared to that of the reactor fed with raw maize (dry). This result provides sufficent evidence to confirm that significant proportion of unbound starch present in maize was removed during the bubbling period and digestible materials within maize structure is not available for the microorganisms as the feed sample was not pretreated with reactive species from the plasma. The cumulative biogas volume profiles for all feed samples (except the bubble treated with no plasma) shows sharp increase up to 5-10 days followed by plateauing behaviour. This result is expected as less digestible material is available within the batch reactors with time as anaerobic digestion continues. However, it is interesting to observe that plasma pretreated, washed maize feed produce 18% more cumulative biogas than raw maize fed reactors while plasma pretreated, unwashed samples produce 29% less cumulative biogas compared to raw maize after 35 days. For the reactor fed with pretreated and washed maize, digestibility has been improved by the pretreatment; hence even though starch has been removed in the washing step, this feed produced the highest cumulative biogas volume. Underperformance by the pretreated unwashed maize could be due to inhibitory compounds generated during the pretreatment process. This feed still contains starch, but it was not sufficent to reach the biogas output produced by either raw feed or pretreated washed feed in the long run. As the purpose of washing is to remove any potential inhibiting biocidal components from maize, it is possible that once the starch has been exhausted, microorganisms may be inhibited by the by-products of lignin break down produced during the pretreatment (Panneerselvam et al. 2013).

One of the main implications of this result is that it is necessary to remove the inhibitory by-products generated during the pretreatment, but starch loss should be minimized to obtain the maximum benefits. This is particularly important for AD reactors operated with a long residence time and fed with organic matter rich with lignocellulosic materials and higher unbound starch content. Alternatively, it may be possible to recover the unbound starch prior to pretreatment and then reintroduce to the suspension before feeding the AD reactors.

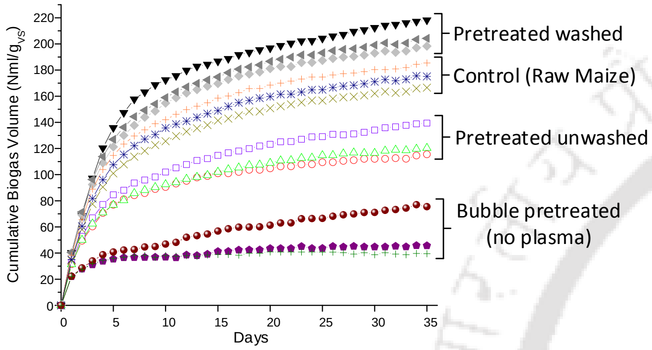


Figure 5.16 Cumulative biogas productions from batch BMP experiments

Table 5.2 Methane and carbon dioxide in biogas from batch BMP experiments for samples accumulated between days 7 and 14.

Sample	U	W	R
CH_4 (%)	53.6 ±2	49.8 ±4	49.5 ±5
CO_2 (%)	16.7 ±0.4	18.6 ±0.6	19.0 ±1

U- plasma pretreated, unwashed maize; W- plasma pretreated, washed maize; R- raw maize

Methane and carbon dioxide concentrations in the biogas samples collected between Day 7 and Day 14 are shown in Table 5.2 . Gas analysis for maize feed suspension pretreated by bubbling (with no plasma) was not attainable due to the low volume of gas produced by this reactor. H_2S and O_2 concentrations are also not shown in Table 5.2 . H_2S was only present in the raw maize samples at a concentration of 15 ± 10 ppm. O_2 was however present in all biogas samples collected during the batch study (on Days 7, 14, and 35) and the concentrations varied between 5 and 10%. This can be attributed to residual air in the headspace in the sample taken at Day 7. However, this does not provide an explanation for the O_2 present in the second (Day 14) and the final sample (Day 35) as anaerobic conditions were maintained in the AD reactors. These O_2 concentrations measured in the latter stages can be attributed to algal respiration within the flow cell reservoir. By the end of the experiments, the reservoir had a visible orange tinge, and analyses of the water (using a HQ40d Multi with LD0101 probe) revealed dissolved oxygen levels of 0.56 mg.L^{-1} $\sigma = 0.03$; in comparison to initial Purite water which contained 6.50 mg.L^{-1}. This limitation of the methodology could perhaps be addressed by either a biocide addition and/or using an opaque material for the flow cell container instead of a translucent cell. According to table 5.2, the quality of biogas, i.e. the methane percentage, does not vary much between the raw, pretreated washed and pretreated unwashed samples suggesting that it is the volume of biogas that is affected by the plasma-microbubble pretreatment.

Following these experiments, a preliminary study was performed by separating the starch-rich pretreatment liquid just before the washing process and then reintroducing to the AD reactor with the pretreated washed maize. A cumulative biogas volume of 813.42 (Nml/g$_{vs}$) was recorded after 35 days which is 4 times higher than that of the unseparated washed result presented in Figure 5.16. This clearly highlights the importance of preserving the soluble fraction of the digestible material in the pretreatment process, if washing is required post-treatment. Further work is required to establish the plasma-microbubble pretreatment approach presented here to demonstrate its feasibility: especially scaling up and techno economic analysis.

5.5.4 ENERGY BALANCE

Based on the observed DBD reactor power consumption of 18.6W, energy consumption per treatment of 20g sample was determined as 3.72×10^{-2} kWh, or 1.86 kWh.kg^{-1}. Using the energy density of CH_4 (taken as 35.8 J.ml^{-1}), multiplied by the percentage of CH_4 in the BMP biogas (Table 5.2) and the total biogas produced (Figure 5.16), an energy balance for the total process was then obtained. The total amount of energy attainable from the biogas produced by the three samples (corrected to 20g) was found to be 0.20 kWh (Washed maize), 0.13 kWh (Unwashed maize), and 0.17 kWh (Raw maize). This then gave a net maximum process efficiency of $3.5 \leq \eta \leq 5.4$. Of note however is that this calculation excludes the energy input to heat the BMP reactors, and also excludes intrinsic efficiency losses associated with ultimately converting the biogas to useful energy.

5.6 SUMMARY OF THE CHAPTER

In the present study investigation performed on pretreatment of LB with physico, chemical, thermochemical and natural base method with aim of exposure of cellulose moieties in biomass for breakdown of lignocellulose. Saw dust, arecanut shell and rice husk were pretreated with various alkali methods and were characterized by TGA, FTIR and Fibre analyses to observe the effect of pretreatment on structure and constitution of biomass. Banana peel ash solution (BPA solution) is a natural base and a potential source of potassium of 78.10mg/g. It has a very high pH. In all the cases, it was observed that BPA solution aided in the removal of lignin more than a solution of 2% NaOH from the LB. The fibre analysis results revealed that the BPA solution was able to degrade lignin up to 26.57% in saw dust, 12.59% in rice husk and 20.4% in areca nut shell. Based on the obtained data, we predict that this BPA pretreatment will decrease the required amount and cost of chemicals and physico thermo-chemical treatment. The lignin reduction with BPA Solution has been found to be highest in saw dust and the lowest for rice husk. BPA Solution was also able to selectively degrade more lignin while it had an almost negligible impact on the cellulose and hemicellulose contents. The optimum results in lignin removal was observed with a 7% NaOH and 7% NaOH with autoclave in all the selected LB, which is in agreement with literature but its application in rural areas is prudent due to its cost, disposal and its interference with downstream processes because of high pH levels. BPA Solution, being a milder and a much more inexpensive alkali, would probably be a more suitable alternative for rural areas. The spent liquor could be used as a potassium rich fertilizer. An increase in the pretreatment time and BPA Solution concentration may be able to degrade the lignin further and could probably lead to a shorter hydraulic residence time in the digester.

A novel lignocellulosic biomass pretreatment reactor has been designed and tested to investigate pretreatment efficiency on maize biomass to explore the plasma pretreatment biomass feedstock for the enhancement of biomethanation in labscale anaerobic digestion reactors. The reactor was designed to optimise the transfer of highly oxidative species produced by dielectric barrier discharge plasma to the liquid phase immediately after generation, by arranging close proximity of the plasma to the gas-liquid interface of microbubbles. The reactor produced a range of reactive oxygen species and reactive nitrogen species, and the rate of production depended on the power source duty cycle and the temperature of the plasma. Ozone and other oxidative species were dispersed efficiently using energy efficient microbubbles produced by fluidic oscillations. Reactive oxygen and nitrogen species (RONS) were transferred from a DBD plasma to maize suspensions using microbubbles and the pretreatment effect on maize structure was demonstrated using ATR-FTIR and SEM analysis.

Additionally, this study presents pretreatment of maize using a microbubble-enhanced DBD plasma reactor for improving biogas yield from anaerobic digestion. Continuous daily feeding regime did not produce a considerable improvement to the biogas output. However, AD reactors operated in batch mode with a single feed at the start showed 18% increase in cumulative biogas after 35 days compared to that of raw maize fed reactors, if the pretreated material is washed. Unwashed pretreated material produced 29% less cumulative biogas, potentially due to inhibitory compounds produced during the pretreatment. This pretreatment approach could be useful in improving the performance of AD reactors operated with a long residence time and fed with material that contains high lignocellulosic content, but the starch loss should be minimised in the washing process for maximum biogas output.

CHAPTER – 6

STUDY ON EFFECT OF ADDITION OF ACTIVATED CARBON FOR ENHANCEMENT OF BIOGAS AND METHANE

6.1 INTRODUCTION

Biogas is a well-known form of a renewable energy and fuel source that can be utilized in various applications like gas engines, microturbines, electricity generation, and fuel cells for continuous energy production (https://en.wikipedia.org/wiki/Biogas). As discussed in chapter 1, biogas basically comprises 55–70 % combustible methane and 35–40% non-combustible carbon dioxide in addition to smaller amounts of other gases such as oxygen 0-1%, nitrogen 1%, siloxanes 0– 0.02%, halogenated hydrocarbons 0.6%, hydrogen sulfide 0.005%–2%, and water vapours 5–10%) (Rashed, Mamun, and Torii 2017; Nijaguna 2001; Chandel and Sukumaran 2017). Many researchers have observed that methane does not produce any soot particles or other pollutants during combustion and considered as a very clean fuel. On the other hand, CO_2 is the incombustible part of biogas which lowers its calorific value. Calorific value can be amplified by removing carbon dioxide from the biogas. Therefore, there is an enormous need for removal of CO_2

from biogas. The purifying process of methane enhancement from biogas generates many new prospects for its utilization but up gradation may increases the process cost. Hence, it is important to develop an enhancement technique or method by addition of some materials which can increase the methane content in biogas.

Recently researchers have been working on the addition of carbon materials in the AD process to supplement hydrolysis rate of the substrate by allowing an alternative route for electron transport (Martinez et al. 2017). Lin et al. (2018) reported that activated carbon enhances the biogas and biomethane with decreasing startup time in the AD process. The probability of microbial resistant to organic loading is increased with the addition of activated carbon (Satayev et al. 2017). Additionally, Satayev et al. (2017) also reported that mesoporous structure is more developed due to the addition of activated carbon which is complimentary for the colonization of specific bacteria, which leads to increased syntrophic associations between bacteria and methanogens. Therefore, the AD process supplemented with activated carbon would be economically effectual for the production of biomethanation.

Hansen, Angelidaki, and Ahring (1999) enlightened the advantages of utilizing activated carbon for its adsorption capacity of inhibitory compounds. On the other hand, recent studies proved that its capability for enhancing AD performance is dependent on the combination of different factors (Cuetos et al. 2017). The combination of factors like the transport of electrons and removal of inhibitory compounds by adsorption phenomena has diverted to the extensive search for alternative resources with similar qualities (Martinez et al. 2017). The report mentioned above was proved by Cuetos et al. (2017) who studied the digestion of poultry blood. The poultry blood waste contains a high amount of carbon and protein concentration which makes it difficult to degrade and causes a rapid accretion of VFAs and ammonium under anaerobic environment, causing complete ceasing of the process. The granular activated carbon (GAC) has been added with blood as sole substrate in the AD process which resulted in better assimilation of C3–C5 acid forms, signifying that augmentation in syntrophic metabolism may have taken place. GAC addition may act as a conductive material, helps in the adsorption of ammonium and posing defence to the bacterial population by preventive mass transfer of toxic compounds (Cuetos et al. 2017). Capson-Tojo et al. (2017) had investigated study on the effect of the addition of GAC on digestion of food waste in AD process and had been found that the presence of carbon material like GAC supports the growth of archaea and syntrophic bacteria signifying that collaborations between these microorganisms were enhanced with the predominance of hydrogenotrophic methanogens. However, hardly any literature has been reported for the improvement of bio-methane quality from lignocellulosic biomass (LB) as a feedstock. Most of the work is in preliminary stages and requires a quantitative optimization to enhance the AD processes as well as methane production.

This chapter investigates the effects of activated carbon addition on anaerobic digestion process to find out activated carbon efficacy in the enhancement of biogas and methane production. Present work had been carried out in the Department of Architecture, Civil and Building Engineering, Loughborough University, UK. The possible ability of activated carbon to enhance biogas and methane was addressed and the results obtained from AD batch and continuous stirring tank reactor (CSTR) were found to be the same. The addition of AC enhances the biogas and methane concentration from sewage sludge and maize (LB).

6.2 MATERIALS AND METHODS

The present section demonstrates the materials and methodology adopted to perform this study. The laboratory scale batch and continuous stirng tank reactor CSTR experimentation has been done to know the efficacy of addition of AC in AD process.

6.2.1 FEEDSTOCK PREPARATION

Powdered activated carbon made from Sigma –Aldrich (Darco G-60) high-purity powder has been used for this work. Activated carbon is a form of amorphous carbonaceous materials with high porosity and large surface area. Due to its high adsorptive capacities, AC is widely used in wastewater treatment to eliminate color, odor, taste and antibiotics through physical adsorption (Zhang et al. 2016). AC was autoclaved and dried prior the experiment. The properties of activated carbon (AC) are given in Table 6.1.

Table 6.1 Activated carbon properties (Sigma –Aldrich (Darco G-60))

Activated carbon	Properties
Particle size (mesh)	100-325
Surface area (m2/g, approx.)	600
Pore volume (ml/g, dry basis)	0.95
Moisture (%, max.)	12
pH (water extract, approx.)	6-8
Ash (%)	3.5
Water solubles (%, max.)	0.5
Tamped bulk density (lb/cu ft)	25
Fe (ppm, max.)	200

Sewage sludge was used as feedstock in all four AD reactors and it was obtained from Wanlip plant, Leicester, UK. For inoculum the Wanlip digestate has been used as starter and run for 7 days prior experiment has been started.

Maize (*Zea mays*) was grown outdoors by STW Ltd, Stoke Bardoloph, UK. It was harvested as whole and mature plants in the autumn, chopped to ~6 mm size and stored on-site in clamps. Random samples from these clamps were collected and dried at 105°C in a laboratory oven for two hours. They were then stored and used for experimentation. Time between harvest and experimentation was 12 to 18 months. Prior to the experiments, maize was oven dried overnight and shredded to ~1-2 mm size using a cutting mill (pulverisette 15, Fritsch GmbH). The shredded maize was characterised using the standard methods for the examination of water and wastewater for total and volatile solids determination (APHA 1999). Analysis of random oven-dried maize samples (in triplicate) indicated 97.11% total solids and 96.05 % of volatile solids from a total mass.

6.2.2 EFFECT OF ACTIVATED CARBON ON BIOGAS PRODUCTION WITH SINGLE BATCH FEED

The single batch feed experimental setup shown in figure 6.1 includes four anaerobic reactors. They are 1liter small lab bioreactors, with a glass top and sealed anaerobically. Each reactor filled with sewage sludge with volume of 800 ml and outlet connected to measuring cylinder. Biogas was measured through water displacement method on daily basis. Sewage sludge (obtained from Wanlip plant, UK) was used as feedstock in all four AD reactors. For inoculum the Wanlip digestate has been used as starter and run for 7 days prior experiment has been started. At the bottom, magnetic stirrer was placed and kept at 100 rpm to provide continuous stirring to AD reactors. For this study AC has been used in 3 different concentrations 1%, 5% and 10% (w/v) respectively. All the reactors are fed with sewage sludge and first one is kept control without AC and other three were with respective amount of AC 1%, 5% and 10% (w/v) and applied to batch type anaerobic digesters in laboratory scale anaerobic digester for the experiments at 37±2 °C for 25 days HRT.

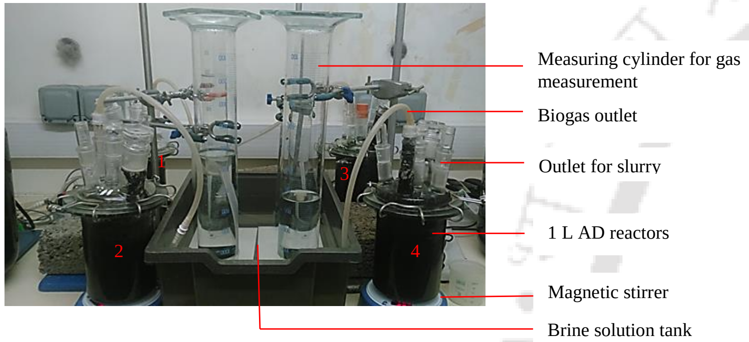


Fig 6.1 Experimental setup for single feed batch AD reactors for biogas production with activated carbon AC 1%, 5% and 10% (w/v).

6.2.3 EFFECT OF ACTIVATED CARBON ON BIOGAS PRODUCTION WITH CONTINUOUS FEED

The anaerobic digesters used in this study were 10 L glass reactors with a 9 L working volume. They were subjected to continuous automated stirring at a rate of 100 rpm, and a constant ambient temperature of 37° C (±2°C) was maintained throughout the study. Biogas composition (CH_4, CO_2, O_2, and H_2S) was measured using a handheld gas analyser (Gas Data GFM-416, UK) via an access port (Fig. 6.2). Two access ports located at the top of each reactor enabled feeding of the units and continuous monitoring of the gaseous output using Ritter Milli Gas counters (MGC-1, V3.4 PMMA) shown in Figure 6.3.

All four AD reactors were first incubated with sewage sludge attained from the Wanlip AD plant, Leicester, UK, and fed over a period of 15 consecutive days prior to this study. They were then fed simultaneously for over a period of 25 days with sewage sludge, 1% AC (w/v) with sewage sludge, raw maize suspension, and 1% AC (w/v) with maize suspension separately. In all cases, the reactors were fed in week days with no feeding at the weekends. Immediately prior to feeding, 450 mL of digestate was extracted for analysis through a manual stop cock situated at the base of the reactor.

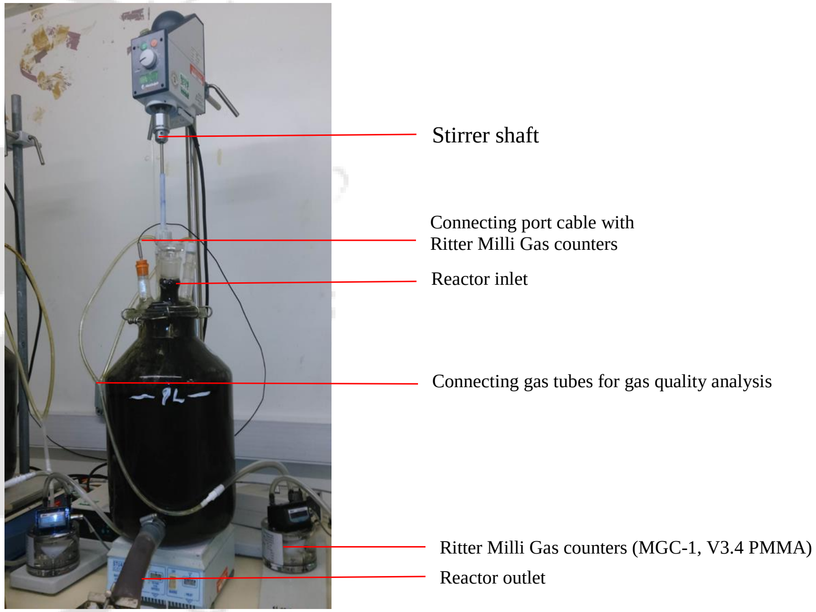


Fig. 6.2 Experimental AD bioreactor for biogas production in continuous stirring tank reactor (CSTR) fed system

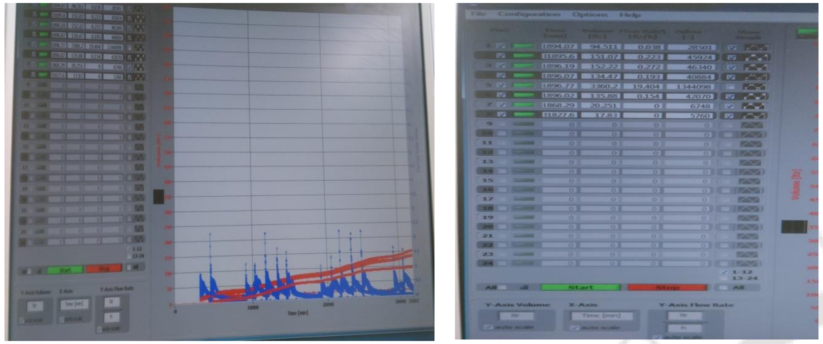


Fig. 6.3 Rigamo software for collection of biogas production data from Ritter Milli Gas counters (MGC-1, V3.4 PMMA)

6.2.4 DIGESTATE ANALYSES

Digestate from the single and continuous feed experiments was analysed. The amount of ammonium nitrogen and VFA were both determined by UV spectroscopy (Haach Lange DR 3900, Germany), using chemical kits LCK 303 and LCK 365 respectively. The Ripley method was used to determine the ratio of VFA: TA (Total Alkalinity) (Ripley, Boyle, and Converse 1986), with 0.1 M HCl as the titrant. A Mettler Delta 340 was used to measure pH.

6.3 RESULTS AND DISCUSSION

6.3.1 BIOGAS BATCH EXPERIMENTS WITH ACTIVATED CARBON TREATMENTS

The effects of addition of AC in three different ratios viz; 1%, 5% and 10 % (w/v) compared with control were studied in the batch experiment for 25 days HRT. The volume and the composition of the biogas were determined and the characteristics of the digestate after the experiment were studied. The cumulative biogas production shown in fig. 6.4 and the values have been observed that 5262 ml/day in control, 7345 ml/day in 1% (w/v) AC, 5617 ml/day in 5% and 2603 ml/day in AC 10 %(w/v). AC 1% (w/v) had higher cumulative biogas production compared with 5% (w/v) AC and 10%(w/v) AC treatment. It has been observed that Control had started producing biogas production earlier compared to other AC treatments but after 16th day, AC 1%(w/v) has taken the lead and was producing highest cumulative biogas production compared to others. The probable reason could be the addition of additional carbon substrate which enhanced the microbial population thus higher biogas production. On the other hand, in the beginning AC 1% (w/v) and AC 5% (w/v) shown almost similar biogas production pattern but later after 16th day the biogas production with AC 5% (w/v) started decreasing but over the whole experiment it seems that it had slightly better biogas production than control (Fig. 6.4). It has been found that AC 10% (w/v) shown lowest cumulative biogas production compared to others may be due to higher carbon sources which increase the VFA concentration. Subsequently, the microbial population started decreasing due to VFA accumulation and toxicity, which leads to ceasing of the AD system. From the result it can be concluded that excessively AC with substrate can be detrimental effect on biogas production.

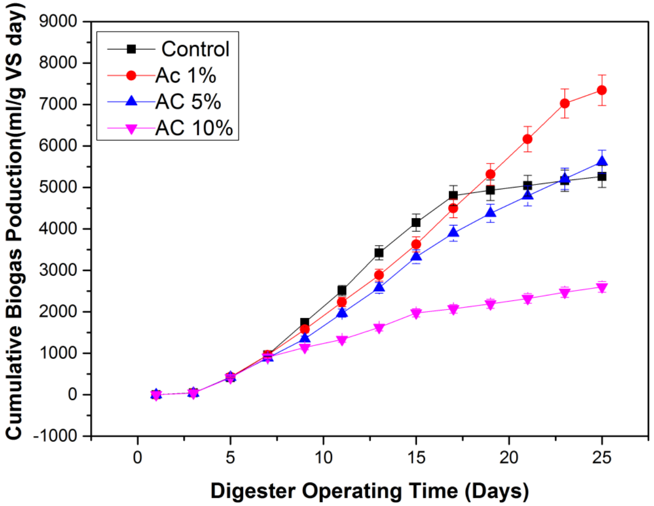


Fig. 6.4 Cumulative biogas production of AC1%, AC5%, AC10% (w/v) and control (sludge) in batch experiment (AC=activated carbon)

Table 6.2 Feedstock characteristics before and after the AC treatment with control

Digestate characteristics	**Before AD Process**				**After AD Process**			
	Control	**AC 1%**	**AC 5%**	**AC 10%**	**Control**	**AC 1%**	**AC 5%**	**AC 10%**
VS	68.6±0.1	71.06±0.2	82.9±0.1	90.1±0.1	23.4±0.2	22.1±0.2	45.3±0.3	55.8±0.5
TS	4.82±0.2	5.12±0.3	5.7±0.1	5.9±0.2	3.2±0.3	4.3±0.2	4.8±0.2	5.1±0.2
VFA	285±0.1	403±0.2	456±0.3	489±0.1	360±0.1	412±0.1	405±0.1	218±0.1
Ammonia	1280±0.4	1170±0.1	1100±0.3	1023±0.1	812±0.1	542±0.1	567±0.1	276±0.1
pH	7.35±0.5	7.64±0.2	8.35±0.1	8.67±0.1	8.7±0.2	8.6±0.1	8.8±0.2	8.8±0.2

TS and VS in AC 1%, AC 5 % and AC 10 % were higher due to additional carbon source in digestate (before and after). Interestingly, it has been found that the ammonia was lower than control which exhibits the quality of ammonia adsorption of AC reported by Cuteos et al. (2017). The pH of all four reactors is around 7.35 to 8.8. The biogas production had been started on 3rd day of incubation with equal amount of biogas. It has been observed that the biogas production in first seven days is similar in all four reactors but in the later days of the experiment changes occurs was evident. It's worth mentioning that in the present experiment biogas started producing on 3rd day with sewage sludge but in the case of cattle dung it was started producing on 7th day, which is demonstrated in section 4.3.2. Figure 6.5 infers the daily biogas production of control and all three AC treatments. It has been found that control had higher biogas production in initial days of experiment but after 15th day it started decreasing possibly due to exhaustion of substrate by microorganism. In contrast, AC 1% (w/v) shows higher biogas production after 13th day of incubation till the end of the experiment. Lu et al. 2016 reported that biochar enriches micro-organism cultures and biochar and activated carbon are considered as the source of carbon enrichment. Consequently, this batch experiment indicated that AC 1% (w/v) is optimum addition value of activated carbon for the enhancement of biogas production in 1L batch AD process.

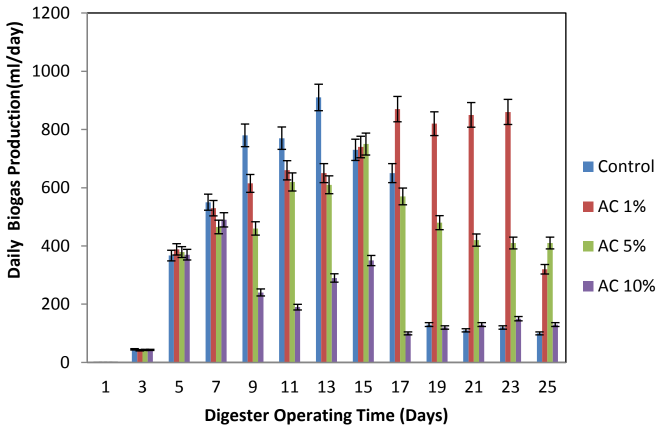


Fig. 6.5 Daily biogas production of AC1%, AC5%, AC10% (w/v) treatment and control(sludge) in batch experiment (AC= activated carbon)

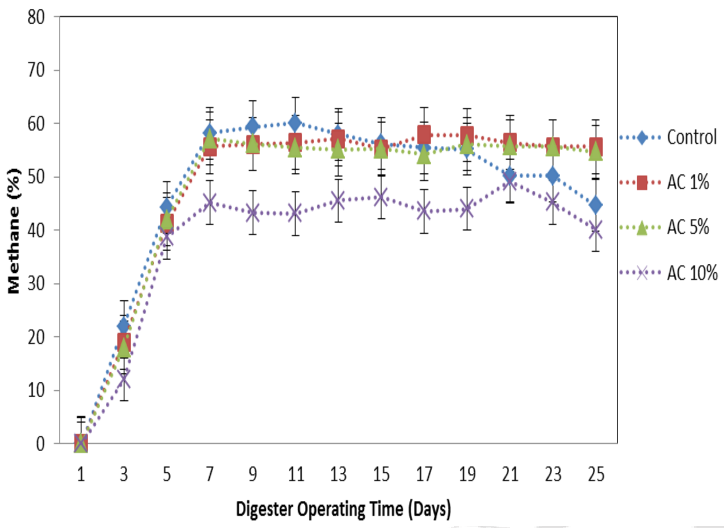


Fig. 6.6 Methane concentration of AC 1%, AC 5%, AC 10% (w/v) treatment and control (sludge) in batch experiment (AC= activated carbon)

Figure 6.6 represents the methane (volume of methane with respect to per Kg of biogas) concentration profile of the biogas from control and AC treatments. The highest methane content of 60.1% was observed from the control followed by the AC 1% (57.9%), AC 5% (57. 1%) and AC 10% (44%) respectively. Although it has been perceived that the methane content of AC 1% and 5% are uniform throughout the experiment between 51-57%.The results from this experiment suggested that the activated carbon does not affect the methane production but overall cumulative biogas production in AC 1% and AC 5% treated reactors was higher in comparison to the control reactors. The methane values are quite close to each other therefore extensive or more insightful investigation is required to check the efficiency of addition of AC on biogas and methane production enhancement. Figure 6.7 demonstrate the daily biogas and methane production with time for each AC treatments.

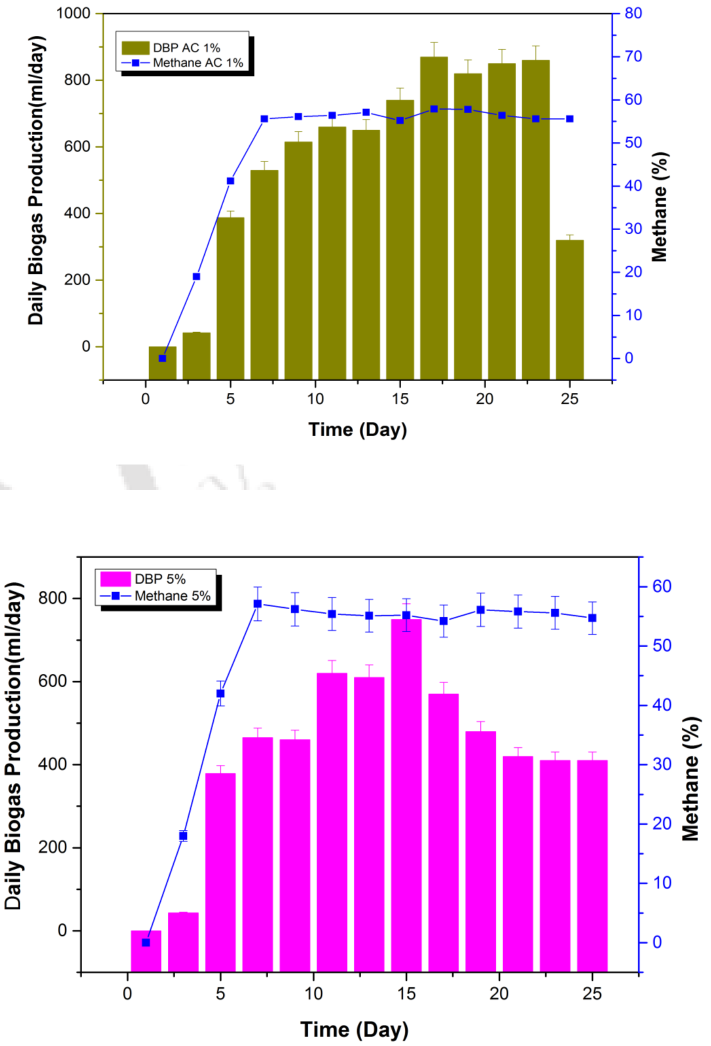


Fig. 6.7 Daily biogas production and methane conc. profile with time of AC 1% (w/v) and AC 5% (w/v) treatment (DBP= daily biogas production and AC= activated carbon)

6.3.2 BIOGAS EXPERIMENTS IN CONTINOUS STIRRING TANK REACTOR (CSTR) WITH ACTIVATED CARBON TREATMENTS

The overall result obtained from the CSTR experiment displays the biogas and methane production from AC 1% (w/v) , maize (alone), AC 1% (w/v) with maize in comparison with control(sludge alone). It has been observed that the combination of AC 1% (w/v) with maize given higher biogas production compared with the other feed stocks (Fig.6.8). Among the various feedstocks, the highest biogas production was obtained with AC 1%(w/v) with maize (161 L kg^{-1}VS). The probable reason could be LB and energy crops (such as maize) are often rich in carbohydrates (https://en.wikipedia.org/wiki/Energy_crop) and provided extra carbon source with AC 1% to microorganism present in the AD process. AC 1% had given second highest biogas production (127 L kg^{-1}VS) due to absence of lignocellulose materials which can be easily accessed by microorganism/enzymes present in the AD process. It indicates the significance of AC for the enhancement of biogas production. AC has highly porous surface area, with both micro and macro structures that will allow microorganisms to adhere to their surface and develop biofilms, not only on the surface but also within the internal structure. Raw maize (110 L kg^{-1}VS) shows lowest biogas production due to the presence of lignocellulosic materials which is difficult to hydrolyse by AD micro biome leads to lesser biogas production.

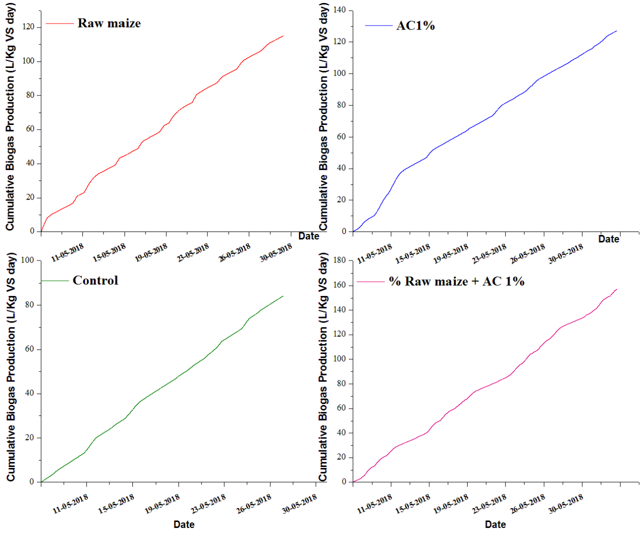
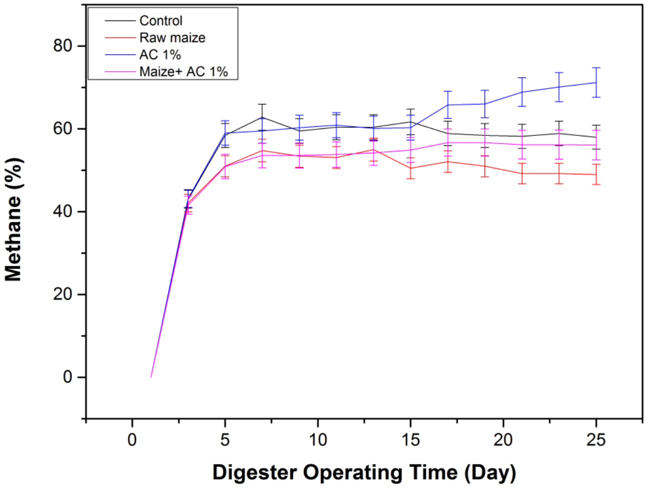


Fig. 6.8 Cumulative biogas production from control, raw maize, AC 1% (w/v) and raw maize with AC 1%

Figure 6.9 represents the methane concentration profile of the biogas produced from AC 1%, AC 1% with maize, raw maize and control. Initially the similar pattern of methane has been observed in raw maize and AC 1% with maize but after 14th day AC1% with maize displays slightly higher methane content between 41.6- 56.7%. AC 1% has been given highest methane content followed by control, AC1% with maize and raw maize respectively. The reason could be the complex nature of these lignocellulosic and cellulosic substrates, which makes microbial enzymatic attack difficult. Eastman and Ferguson 1981 stated that it is indeed the hydrolysis of particles, rather than soluble matter that is rate limiting in AD. Raw maize and AC 1% with maize have shown uniform methane content, it's between ~42-54% with less fluctuation until 13th day but later, straight drop of methane content has been observed with raw maize. In contrast, AC 1% with maize exhibited higher biomethane content (50-60%) due to support of activated carbon which helps in breeding of more microbial population to breakdown lignocellulosic content with efficacy in the AD system.



6.9 Methane profile of continuous stirring tank reactor (CSTR) experiment from control, raw maize, AC 1% (w/v) and raw maize with AC 1% (w/v)

6.3.3 COMPARATIVE ASSESSMENT OF THE RESULTS OF BIOGAS PRODUCTION IN THIS STUDY WITH LITERATURE

The results obtained in this study were compared with other reports in terms of biogas production and methane % with respect to raw biomass (Table 6.3). It could be inferred from the data that methane yield with respect to raw biomass in the present study is at par with the previous reports. The results of the current study are remarkable despite the use of plasma DBD for biomass hydrolysis. This result can be attributed to the physical treatment of DBD plasma, which breakdowns the lignin present in the maize and makes cellulose accessible to the AD micro-organism which shows higher biogas production.

Table 6.3 Comparison of the results of present study with published literature.

Lignocellulosic biomass	**Co-digestion**	**Pre-treatment**	**Methane yield (ml CH_4 /g VS)**	**Reference**
Phragmites australis	-	-	120	Pelegrin and Holzem (2017)
Barley	-	milled	314.8	Himanshu et al. (2017)
Winter wheat	-	silage	269.2– 327.6	Herrmann et al. (2016)
Sunflowers	-	Silage	210– 286.1	Herrmann et al. (2016)
Rice straw	-	Urea addition	434.2	Haryanto el a. (2018)
Oat straw	-	Acid/alkaline/enzymatic	360	Gomez-Tovar et al. (2012)
Ensiled meadow grass	-	Physico-chemical	372	Tsapekos et al. (2015)
Sugar beet		Silage	350.4– 399.4	Herrmann et al. (2016)
Meadow grass	-	No	297	**Hernández-Beltrán** et al. (2019)
Cotton stalk	-	3% H2O2 8% Ca(OH)2	192.4	Zhang et al. 2018
Maize	Sludge	silage	330	Klimiuk et al.(2010)
Raw maize	-	No	315	This study
Maize	-	Plasma DBD (washed feedstock)	345	This study
Maize	-	Plasma DBD (unwashed feedstock)	400	This study

6.3.4 COST ANALYSIS OF ACTIVATED CARBON ADDITION FOR BIOGAS PRODUCTION EXPERIMENT

Plasma pretreatment experimental studies for biogas production have been performed and the obtained results verified its higher efficiency towards lignincellulosic structural break down but economic analysis is limiting factor for laboratory scale experiments. Hence, it has been considered solely for research purpose. The other experiment with addition of activated carbon offers so many advantages over other pretreatment process with higher proficiency towards methane production which is already shown in secion 6.3.2. This section elaborates in brief, on how to conduct an economic assessment and details shown in Annexure A.

The economic analysis for addition of activated carbon to enhance biogas production experiment was performed based on fixed cost, cost of interest, repair cost and variable cost mainly of electricity, feedstock and labor cost. Depending on the purpose of the biogas production cost calculation can be either stated as annual cost or as cost per experiment. In this case cost analysis was performed on annual basis (300days/year). The break-even point of for sludge, sludge with AC 1% (w/v), raw maize and raw maize with AC1% (w/v) is 3.6 year, 1.2 year, 1.6 year and 0.8 year respectively. Addition of 1% (w/v) AC with maize treatment shows least break even point which indicates more economic viability.

6.4 SUMMARY

The addition of AC to an anaerobic co-digestion process simultaneously enhanced methane production while the activated carbon concentration is very important. The highest cumulative biogas production has been found with AC 1% (w/v)compare to AC 5% (w/v) and AC 10% (w/v) in 1L batch experiment. In CSTR experiment it has been observed that addition of AC 1% (w/v) with maize enhanced the biogas as well as methane content. AC likely increased the relative abundance of archaea and altered the dominant species of methanogens, allowing methanogenesis through acetoclastic and hydrogenotropic pathways using multiple archaeal species (Zhang et al. 2019). Membrane transport pathways also became more prominent with AC. This study proves that modest addition of AC in AD system not only improved biogas and methane production alone but it also supports the most recalcitrant LB for faster hydrolysis with higher efficiency. Chapter 7 reviews the results achieved from the experimental study discussed in previous chapters and evaluate the conclusions and scope for the future work.

CHAPTER - 7

CONCLUSIONS AND SCOPE FOR FUTURE WORK

7.1 INTRODUCTION

Recent fuel-development strategies to reduce oil dependency, mitigate greenhouse gas emissions, and utilize domestic resources have generated interest in the search for alternative sources of fuel supplies. Biogas is one of the prospective alternatives identified so far which is economically feasible as it has the lowest financial input of output energy and unlimited in potential and eco-friendly in nature. Among the alternative energy source, Biogas is the most mature in terms of years of use and number of unit installed in India. No negative or limited environmental side effects have been reported because biogas can be produced from all types of “green” biomass. The whole plant is used for biogas unlike diesel fuel or bioethanol, where only the seeds are used. Biogas can be utilized for cooking, electricity and heat production and its upgraded and purified biomethane can be used as vehicle fuel. Alternatively, lignocellulose biomass (LB) such as plant residues, represent the most encouraging renewable organic feedstock for biogas production, as they available plentiful and production does not compete for cultivable land. Inopportunely LB is recalcitrant in nature due to presence of lignocellulose which is difficult to hydrolyse by microbial degradation. The present work mainly focuses on biogas production from utilization of lignocellulosic biomass as feedstock. The major conclusions are presented in this chapter. Moreover, future scope has been stated in which present thesis contribution can be utilized as stepping step.

7.2 CONCLUSIONS

Based on the detailed characterization done on six types of LB, further co-digestion study has been performed with cattle dung to identify its potential for biogas production. The results demonstrated a good amount of volatile solids and cellulose content in LB which is majorly responsible for biogas or any fuel production, even though LB comprises a reasonable amount of lignin which is difficult to hydrolyze by enzymes and microorganisms present in the AD process. Therefore, in the present study different kinds of pretreatment methods were performed to find out the optimum amount of degradation or changes in lignocellulosic biomass structure. The pretreatment methods utilized in the present work are alkali, physico-chemical, acid and dielectric barrier discharge (DBD) plasma-assisted treatment. Afterwards, analyses of effect of pretreatment methods on LB structure such conversion of cellulose and lignin was identified. Subsequently, selected pretreatment method has been utilized for biogas production to know its efficacy on enhancement of biogas and methane production. Furthermore, investigation has been performed on biogas and methane enrichment by addition of activated carbon in sewage sludge and maize as feedstock in batch and continuous stirring tank reactor (CSTR) AD process. The obtained results are found to be encouraging and have potential for the enhancement of biogas as well as methane concentration.

7.2.1 CHARACTERIZATION OF LIGNOCELLULOSIC BIOMASS

To achieve aim and objectives of the present work six types of LB waste such as bamboo dust, areca nut shell, rice husk, duckweed, maize and saw dust were characterized by different analytical techniques such as FTIR and TGA analysis to ascertain the prospective for biogas production. The volatile matter content in saw dust, bamboo dust, areca nut shell, rice husk, duckweed, and maize was found to be 82.79±1.5 %, 84.24±1.0 %, 79.3±1.4 %, 74.1±1.6%, 84.4±1.0% and 92.24±1.0% respectively, indicating their potential for bio-energy production by anaerobic digestion. The calorific values of all the biomasses were relatively high in the range of 17 MJ/kg to 22 MJ/kg, evidencing their general potential. The physico-chemical characterization of all six samples showed that saw dust and duckweed has high calorific value, cellulose and hemicellulose content, with low ash and lignin content and can be regarded as the most potential candidate for bio-chemical bioenergy production. The areca nut shell and maize has slightly lower volatile matter, calorific value and cellulose content than duckweed and saw dust. Fourier infrared spectroscopy examination of the rice husk revealed the presence of highest crystalline cellulose (λ = 1428 cm-1) followed by bamboo dust, areca nut shell, maize, saw dust and duckweed respectively. The volatile content of bamboo dust was found to be highest among all six biomasses but its ash and lignin content is high, which indicate requirement of pretreatment procedures for biofuel production. Rice husk has high lignin and ash and low calorific value and volatile content, which makes it unsuitable feedstock for biogas production. The study of all these LB materials in the light of biofuel production confirms that these biomasses can be employed for biogas production and may meet the demand of the LB, although some will be more effective than others.

7.2.2 CO-DIGESTION OF LIGNOCELLULOSIC BIOMASS WITH CATTLE DUNG FOR ENHANCEMENT OF BIOGAS PRODUCTION

This work investigated the co-digestion potential of using abundantly available global LB plant material such as duckweed, arecanut shell, saw dust, bamboo dust and rice husk with cattle dung (CD) as a feedstock for biogas production.

1. The results obtained with combination of LB: CD; 50:50 exhibited highest biogas production compared to other combinations.
2. Based on the cumulative biogas production volume during a HRT of 55 days, the optimum mixing for co-digestion of DW, RH, SD, AN, BD and CD has been found to be at 1:1 ratio.
3. The combination of duckweed, arecanut shell and saw dust with 50% and 75% with cattle dung exhibited higher cumulative biogas volume than cattle dung alone.
4. In other hand rice husk and bamboo dust combination showed lower cumulative biogas volume compare to cattle dung alone.
5. Methane content of the biogas from co-digested feedstock is equivalent to the biogas from cattle dung alone.
6. Biogas and methane production was furthermore significantly early (0–7 days) in cattle dung alone and to some degree late (above 14 days) in the lignocellulosic biomass digestion process during co-digestion.

Co-digestion generally resulted in improved cumulative biogas yields compared to mono digestion. However, based on this study due to presence of recalcitrant lignin the biogas and methane yield is not very encouraging and required pretreatment.

7.2.3 PRETREATMENT METHODS FOR LIGNIN DEPOLYMERIZATION

In the present study investigation performed on pretreatment of LB with physico, chemical, thermochemical and natural base method with aim of exposure of cellulose moieties in biomass for breakdown of lignocellulose

1. The optimum results in lignin removal were observed with a 7% (w/v) NaOH and 7% (w/v) NaOH with autoclave in all the selected LB, which is in agreement with literature.
2. Banana peel ash solution (BPA solution) is a natural base and a potential source of potassium of 78.10mg/g. It has a very high pH. In all the cases, it was observed that BPA solution aided in the removal of lignin more than a solution of 2% (w/v) NaOH from the LB.
3. The fibre analysis results revealed that the BPA solution was able to degrade lignin up to 26.57% in saw dust, 12.59% in rice husk and 20.4% in areca nut shell. Based on the obtained data, we predict that this BPA pretreatment will decrease the required amount and cost of chemicals and physico thermo-chemical treatment.
4. The lignin reduction with BPA Solution has been found to be highest in saw dust and the lowest for rice husk.
5. BPA Solution was also able to selectively degrade more lignin while it had an almost negligible impact on the cellulose and hemicellulose contents.
6. BPA Solution, being a milder and a much more inexpensive alkali, would probably be a more suitable alternative for rural areas. The spent liquor could be used as a potassium rich fertilizer. An increase in the pretreatment time and BPA Solution concentration may be able to degrade the lignin further and could probably lead to a shorter hydraulic residence time in the digester.

7. A novel lignocellulosic biomass pretreatment reactor has been designed and tested to investigate pretreatment efficiency on maize biomass to explore the plasma pretreatment biomass feedstock for the enhancement of biomethanation in labscale anaerobic digestion reactors.
8. Ozone and other oxidative species were dispersed efficiently using energy efficient microbubbles produced by fluidic oscillations. Reactive oxygen and nitrogen species (RONS) were transferred from DBD plasma to maize suspensions using microbubbles and the pretreatment effect on maize structure was demonstrated using ATR-FTIR and SEM analysis.
9. Additionally, this study presents pretreatment of maize using a microbubble-enhanced DBD plasma reactor and utilized as feedstock for improving biogas yield from anaerobic digestion.
10. Continuous daily feeding regime did not produce a considerable improvement to the biogas output.
11. AD reactors operated in batch mode with a single feed at the start showed 18% increase in cumulative biogas after 35 days compared to that of raw maize fed reactors, if the pretreated maize is washed.
12. Unwashed pretreated maize produced 29% less cumulative biogas, potentially due to inhibitory compounds produced during the pretreatment.
13. The plasma-assisted pretreatment approach could be useful in improving the performance of AD reactors operated with a long residence time and fed with material that contains high lignocellulosic content, but the starch loss should be minimised in the washing process for maximum biogas output.

7.2.4 EFFECT OF ADDITION OF ACTIVATED CARBON FOR ENHANCEMENT OF BIOGAS AND METHANE

This work investigates the effects of activated carbon addition on anaerobic digestion were studied to find out could carbon additives enhance the methane production. The highest cumulative biogas production has been found with AC 1% (w/v) compare to AC 5% (w/v) and AC 10% (w/v) in 1L batch experiment. An addition of 1% (w/v) activated carbon has given highest biogas yield 6365ml followed by 5%(w/v), 0% (w/v)and 10% (w/v); 5530ml, 5275ml and 2410 respectively. Methane content was also found to be high in 1% activated carbon in later days of the experiment. The addition of AC to an anaerobic co-digestion process simultaneously enhanced methane production while the activated carbon concentration is very important. In CSTR experiment it has been observed that addition of AC 1% (w/v) alone with sewage sludge and with maize enhanced the biogas as well as methane content. This study proves that addition of AC in AD system not only improved biogas and methane production alone but it also support the most recalcitrant LB for faster hydrolysis with higher efficiency.

7.3 SCOPE OF FUTURE WORK

In the present investigation, characterization, co-digestion, pretreatment of lignocellulosic biomass, and their potentiality for enhancement of biogas production were explored. Addition to this, study had been conducted to recognize the effect of addition of activated carbon for the improvement of biogas and methane concentration. The important findings of the research have been highlighted in the section 7.2. However, there still remains plentiful opportunities for future study, relevant to this area of research, which can promote augment the expanse of lignocellulosic biomass as alternative fuel. Some of the scopes and suggestions for further studies are discussed here.

In the present investigation, biogas production from lignocellulosic biomass mixed with bio waste is studied in small scale laboratory set-ups. So in future the same experiment can be repeated for medium as well as for large scale studies and a relationship can be developed among the biogas production in small, medium and large scale.

- With the characterization studies of complex LB compositions, its biochemical and thermal behaviour in conversion processes, yield can be anticipated, mathematical models can be designed or developed to further understand products, economic analysis can be achieved, and most significantly, technology can be formed to make biomass conversion more effectual, effective, and implementation can be done in large scale.
- Further studies can be done on pretreatment method to make it more cost effect, desirable, eco-friendly and easy to implement in rural and large scale AD reactor.
- Remote sensing apps can be added in AD reactor to monitor and control it from far distance. It also helps to better understand biochemical process and extraction of intermediate product like acetic acid in AD process.
- In this work enhancement of biogas and methane has been observed by addition of AC. Furthermore, this work can expand and utilized for immobilization of microorganism consortium for the enrichment of methane and simultaneously lignin depolymerisation in the AD reactor.
- During AD process many valuable intermediate chemicals are formed like acetic acid,methanol and ammonia etc. can be extracted for further applications.

- The activated carbon can be substituted by biochar which has similar qualities to AC for biogas enhancement. It is very cheap option as well as improves biogas quality and digestate as fertilizer.
- Extensive study can be investigated on microbial quantum metabolism.

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ANNEXURE- A

Total biogas production costs comprises of fixed and variable cost as given below

$$C_{BP} = C_F + C_V \qquad (A.1)$$

where,

C_{BP} = Total biogas production cost

C_F= Fixed cost

C_v= Variable cost

To determine the biogas production cost the following three steps are considered

1. Define realistic assumptions(realistic assumption cost is given in Table A.1)
2. Determine variable cost
3. Determine the fixed cost component

Table –A.1 Realistic assumption for the biogas production experiment

	Sludge	Raw maize	Activated carbon 1% with sludge	Activated carbon1% with raw maize
Anaerobic reactor service life	10 Years	10 Years	10 Years	10 Years
Anaerobic reactor cost	Rs. 10,000	Rs. 10,000	Rs. 10,000	Rs. 10,000
Interest rate	8%	8%	8%	8%
Capacity per reactor	9L	9 L	9L	9L
Digestion time (Experiment)	25days	25days	25days	25days
Reactor utilization	300 days/year	300days/year	300 days/year	300 days/year
Total feedstock for the experiment (on the basis of VS %)	15.82kg	20.76 kg	18.07 kg	23.01kg
Total feedstock per year (on the basis of VS %)	189.84kg/year	249.12 kg/year	216.84 kg/year	276.12 kg/year

Total biogas production per experiment	0.086 m^3	0.11 m^3	0.13 m^3	0.16 m^3
Biogas price	Rs. 15/L	Rs. 15/L	Rs. 15/L	Rs. 15/L
Price of feedstock	Rs. 2847/year	Rs.3737/year	Rs.4848/year	Rs.5737/year
Labor charge/year	Rs. 9,000	Rs. 9,000	Rs. 9,000	Rs. 9,000
Electricity consumed in anaerobic bioreactor	Rs. 300/year	Rs. 300/year	Rs. 300/year	Rs. 300/year
Repair & maintenance for anaerobic bio reactor	4%	4%	4%	4%
*Volatile solids =VS %, Sludge VS%= 77.3% and Raw maize VS%= 96.1%				

VARIABLE COSTS

The variable cost or operating cost consists of the cost items those only occur when the experiment is actually being running, namely cost of labor, feedstock, and other minor cost items.

$$C_{var} = C_{feedstocks} + C_{electricity} + C_{labor} + C_{Vothers} \tag{A.2}$$

where,

C_{var}= Variable cost [Rs/kg]

$C_{feedstock}$= Feedstock cost [Rs/kg]

C_{labor}= Labpr cost [Rs/hr]

$C_{vothers}$= other operating cost [Rs/kg]

FIXED COST

The fixed cost mainly consists of investment costs for a system and depends highly on bioreactor.

$$C\,fix = \frac{Cdepr + Crepair + Cinterest + Cothers}{U} \tag{A.3}$$

where,

C_{fix}= Fixed cost [Rs/kg]

C_{depr}= Annual depreciation [Rs/year]

C_{repair}= Annual repair cost [Rs/year]

$C_{interest}$= Annual cost of interest [Rs/year]

U=Annual utilization [kg/year]

Break-even Point

The break-even point in biogas production experiment per year can be calculated as follows:

$$BEP = \frac{CF}{Cp} \tag{A.4}$$

where,

BEP= Break even point

CF= capital cost

Cp=annual profit

Table A.2 Cost analysis of biogas production experiment with addition of activated charcoal (AC)

	Sludge(alone)	Raw maize	Sludge + AC 1 %	Raw maize+AC1%
Total Fixed Cost (Rs)	10,000	10,000	10,000	10,000
Total Variable Cost (Rs)	13,547	13,437	14,548	15,437
Total revenue generated (Rs)	15,300	19,800	22,860	28,980
Break even point (year)	3.6	1.6	1.2	0.8

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