BIOCONVERSION OF LIGNOCELLULOSIC BIOMASS TO BIOGAS

A thesis submitted to the

University of Petroleum and Energy Studies

For the award of

Doctor of Philosophy

In Science

BY

RAJAN SHARMA

December 2019

SUPERVISOR (s)

Dr. Shailey Singhal (Internal)

Dr.Avanish K. Tiwari(External)

School of Engineering
University of Petroleum & Energy Studies

Dehradun-248007 : Uttarakhand

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Professor
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Dr. Avanish K. Tiwari
Director
Centre for Renewable Energy & Sustainable Development
VIKALP (Nai Dishayen), New Delhi

UPES
UNIVERSITY WITH A PURPOSE

School of Engineering University
of Petroleum & Energy Studies

Dehradun-248007 : Uttarakhand

DECLARATION 2020

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

17/06/2020

Rajan sharma

SAP ID:500021617

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CERTIFICATE

I certify that Rajan Sharma has prepared his thesis entitled **"Bioconversion of Lignocellulosic Biomass to Biogas"**, for the award of PhD degree of the University of Petroleum & Energy Studies, under my guidance. He has carried out the work at the Department of Chemistry, University of Petroleum & Energy Studies.

Internal Supervisor

Dr. Shailey Singhal
Professor
Department of Chemistry
School of Engineering Studies
University of Petroleum & Energy Studies,
P.O Bidholi, Via Premnagar, Dehradun

DATE: 17/06/2020

CORPORATE OFFICE: 210, 2nd Floor, Okhla Industrial Estate, Phase III, New Delhi - 110 020, India.
T: +91 11 41730151/53, 46022691/5
F: +91 11 41730154

ENERGY ACRES: Bidholi Via Prem Nagar, Dehradun - 248 007 (Uttarakhand), India.
T: +91 135 2770137, 2776053/54/91, 2776201
F: +91 135 2776090/95

KNOWLEDGE ACRES: Kandoli Via Prem Nagar, Dehradun - 248 007 (Uttarakhand), India.
T: +91 8171979021/2/3, 7060111775

ENGINEERING | COMPUTER SCIENCE | DESIGN | BUSINESS | LAW | HEALTH SCIENCES

Centre for Renewable Energy & Sustainable Development

VIKALP (Nai Dishayen)
Reg. Office: A-1/266, Safdarjung Enclave, New Delhi - 110029
Tel. : 011 41053928 Fax: 011 26565923 Phone: +91.9810147350,
Website: www.vikalp.res.in

DATE: 28.12.2019

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External Supervisor

Dr. Avanish K. Tiwari
Director
Centre for Renewable Energy & sustainable Development
VIKALP (Nai Dishayen), New Delhi.

ABSTRACT

Depletion of fossil fuel and increase in environmental pollution at an alarming rate has motivated the researchers to look for the environmentally friendly as well as cost effective alternative sources of energy. Biomass is a renewable energy source developed from living or recently living plant and animal materials, which can be used as fuel. The main components present in biomass are polymers such as carbohydrate, protein, cellulose, lignin and fat. Biogas is produced when the biomass is anaerobically degraded by microorganisms. The process of anaerobic digestion (AD) takes place in four steps: hydrolysis, acidogenesis, acetogenesis, and methanogens. Biogas production from biomass is getting a lot of attention due to its easy availability and relatively simple biomass to energy conversion technology. Co-digestion of biomass with cattle dung is another promising method of converting biomass to energy through anaerobic digestion.

In most developing countries like India, China etc. The principal occupation of the people is crop production and the crop residues remaining after harvesting is a major challenge to deal with. These biomasses are lignocellulosic in nature as they contain cellulose, hemicellulose and lignin. They are not economically used; rather they are disposed off in the open environment or burnt, causing serious health problems and environmental pollution. Lignocellulosic biomasses are assessed for the use of anaerobic digestion with the objective of generating biogas from it and performing kinetic study on the produced biogas. The aim of the present study is to investigate the optimum pretreatment method and performance characteristics of anaerobic digestion of lignocellulosic biomass for biogas production in batch mode.

To assess the potentiality towards biogas production, three different types of biomasses were collected and characterized. Based on the results obtained from the characterization, three different lignocellulosic biomasses viz sugarcane bagasse, wheat straw and rice husk were selected, upon which small scale anaerobic digestion was performed. In this research, therefore, an optimal achievement of the lignocelluloses plant has been evaluated in the pretreatment impact (physical, chemical and biological) and multiple biogas manufacturing parameters. The pretreatment method focused on removal of lignin content by applying different alkaline and acid condition and then anaerobic digestion of pretreated biomass (WS, RH, and SB). The parameters considered for the analysis TS of biomass, temperature of substrate, C:N ratio and pH.

Biologically, Lignocellulose biomass gave maximum biogas yield followed by acid and alkaline treatment. Among thermal treatments, best results in the increase of methane formation were observed with the treatment of wheat straw followed by sugar cane bagasse and rice husk at 121°C & 120 minutes (19,8%,18%, and 13%, respectively). Acid pretreatment at optimized condition (30%, (60 minutes) and % increase in methane content is found maximum with anaerobic digestion of wheat straw (25%), sugarcane bagasse (20%) followed by rice husk (17%). Acid pretreatment has maximum impact on biomethanation of wheat straw biomass at optimized condition. Biological pretreatments performed with a fungal strain, improves methane production. The percentage increase in methane content after pretreatment with fungal strain is found maximum for wheat straw (34%), followed by sugarcane bagasse (30.2%) and rice husk (27.7%) respectively.

Findings also show that these biomasses have high volatile matter content (above 60%) and high fixed carbon content (above 10%) which make them potent for biogas production. Effect of total solid and particle size of biomass on biogas production was studied and it was found that with 8-9% of total solid and 0.355 mm of particle size, maximum amount of biogas can be produced. Effect of temperature on biogas production from lignocellulosic biomass was also studied at five different temperatures from 35°C to 55°C at a step of 5°C and it was found that with increase in temperature of the digestate from 40°C to 55°C, biogas production from substrates can be increased. It is also observed that in mesophilic condition, biogas generation is the highest at 35°C followed by 40°C.

Alongside the biogas delivered, AD additionally changes the additional feedstock into digestate that can be utilized as a compost which is high in nitrogen, potassium and phosphorus substance. The N (%) from spent slurry from anaerobic assimilation of biomass (WS, RH, SB) was in the scope of 0.93 to 0.98, most noteworthy P(%) and K(%) found from slurry of anaerobic processing of rice husk.

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Rajan Sharma

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ABBREVIATIONS

- AD: Anaerobic digestion
- BMP: Biochemical Methane Potential
- CD: Cellulose-degrading bacteria
- ASBR: Anaerobic Sequencing Batch Reactor
- HRT : Hydraulic Retention Time
- BOD: Biochemical oxygen demand
- CH4:Methane
- TPAD : Temperature-phased Anaerobic Digester
- RAS : Return Activated Sludge
- DNA : Deoxyribonucleic acid
- HRT : Hydraulic retention time
- OLR: Organic loading rate
- PCR: Polymerase chain reaction
- QPCR: Quantitative polymerase chain reaction
- SAO: Syntrophic acetate oxidation
- SRT: solid retention time
- TS: Total solids
- UASB: Upflow anaerobic sludge blanket
- VFA: Volatile fatty acids
- VS: Volatile solids

CHAPTER - 1

INTRODUCTION

1.1 BACKGROUND

Energy is an essential input for economic growth, social development and human development. In recent years, global energy demand has grown rapidly due to rising world populations and affluence, industrialization and improvement in the quality of life(Surendra et al., 2014; Perin et al., .2019). Worldwide, energy consumption has reached 524 QBtu in 2010, and is estimated to peak at 800 QBtu by 2040; corresponding to an average growth of 1.5% per year (EIA, 2013). Significantly, a large fraction of the world's total energy demands (more than 84%) is supported by non-renewable fossil resources such as coal, oil, and natural gas. These resources are not only limited in supply but also have adverse effects on the environment due to the emission of greenhouse gases (GHGs) into the atmosphere (EIA, 2013). Fossil fuels are the dominant source of primary energy because of their easy availability. Apart from their indigenous production, the majority of developing countries import crude oil to cope up with their increasing energy demand. Thus, a significant portion of their hard-earned export earnings is spent on the purchase of petroleum products. India is also a net energy importer and around 80% of the country's export earnings are directly spent on the purchase of petroleum products(Correa et al.,2019). There has been a sharp increase in the consumption pattern of petroleum products in India. The limited reserve of fossil fuel has been a matter of global concern as these are under threat of loss due to overexploitation. According to the World Energy Forum prediction, reserves of fossil fuels will exhaust in less than another ten decades.

The limited reserve of fossil fuel has been a matter of global concern as these are under threat of depletion due to overexploitation. Coal and natural gas are the two primary sources of power generation. Worsening environmental conditions have become an issue of ever-increasing worldwide public concern in present times. Currently, the combustion of fossil fuels is a significant source of emission of Carbon Dioxide (CO_2). There are efforts all around the globe to protect the environmental condition from further deterioration. Therefore, it is a need of today's world to concentrate on the renewable energy source to satisfy the demand, conserve our finite natural resources for the generations to come.

In India, there are various sources of renewable energy but the practice of traditional biomass is decades old(Patinvoh et al., 2017). However, traditional biomass is used mainly for cooking and heating and is characterized by the low efficiency of use and drudgery. The unsustainable extraction and use of traditional biomass energy lead to degradation of the local environment and forests, deforestation, and the consequent loss of forest products, soil erosion and loss of biodiversity, domestic air pollution affecting human health, etc. But the modern forms of biomass energy provide numerous environmental benefits. Of all the renewable energy sources, biomass (ligneous, herbaceous crops, agricultural and municipal wastes) is the largest, most diverse and most readily exploitable resource. Bio-energy technologies provide opportunities for the conversion of biomass into liquid and gaseous fuels as well as electricity. Use of biodegradable resources to produce biogas and thereafter use of that biogas for generating power and thermal applications has multiple benefits on environmental, social, economic aspects, etc.

Biomass energy could help to reduce the world's dependence on oil and fossil fuels**.** Bioenergy can play a significant role in mitigating global warming. This is because CO_2 released during combustion is used by the plant for photosynthesis and thus does not increase the net CO_2 in the atmosphere. Therefore, the use of renewable biomass (including energy crops and organic wastes) as an energy resource is not the only greener concering most pollutants, but its use represents a closing balance of carbon cycle with respect to atmospheric carbon dioxide.

Considering the importance of renewable energy,the Indian government has put Biogas as a renewable energy source, under the subject of The Ministry of New and Renewable Energy. It has implemented National Biogas Programmes for the dissemination and deployment of family/ small biogas plants in the remote, rural, semi-urban areas of the country. It helps in many ways by converting the biomass wastes into useful gains to the beneficiaries by providing clean power for various applications, thermal and cooking fuel, reductions in health hazards and mitigating emissions of Green House Gases (GHGs), combating climate change and simultaneously producing biogas slurry as nutrient- enriched organic fertilizer/manure as a by-product of the biogas generation/ production. The organic bio-manure/ fertilizer when applied in farming, contributes to higher crop production and yield and help in conserving the soil health and water. The Chhattisgarh government has decided to set up at least six biofuel plants to produce ethanol in the state. It will be the first state in India to use rice and paddy husk to producethe biofuel (Sharma, 2019).

Several possible technologies in the area of solar, wind and biomass have been discovered and popularized. Although the majority of renewable energy technologies are better eco-friendly as compared to conventional energy options, their adoption is very slow because of various reasons such as economic constraints, lack of supply and not user's friendly techniques, etc. Further, the uses of these technologies are still limited to the majority of the stable operations mainly due to technological limitations and pooreconomics.

Additionally, biofuels production along with byproducts, can not only provide new income but also employment opportunities in rural areas. Therefore 21st Century is looking for a shift to alternate industrial feedstock and green processes to produce these chemicals from renewable biomass resources. Lignocellulosic biomass conversion is the best alternative for bioenergy fuel. Lignocellulosic biomass is one of the largest renewable energy resource (Azevedo et al.,2019) It is an essential component of the primary food crops; it is the non-palatable segment of the plant, which is undertow and can be utilized for biogas production Transformation of these lignocellulosic residues into renewable fuel offers a prominent possibility in decreasing the use of nonrenewable fossil sources (oil, coal, and flammable gas) (Zhe et al., 2017). Biogas produced from waste substances is a promising sustainable power source utilized for generating electricity and heat. It is also used as vehicle fuel in numerous countries. In short, lignocellulosic biomass holds the way to supplying society's essential liquid transportation fuel without affecting the country's food supply. It is the best solution for cooking gas, bio CNG for the transportation sector, and power generation. Its practice will demotivate the use of fossil fuels and reduce the dependence of imported crude oil which will ultimately check the rapid exhaustion of fossil fuels and also will protect the environment save the cost of importing the petroleum product.

Lignocellulosic biomass comprises cellulose, hemicelluloses and lignin and can be utilized as raw material for biogas production (Hayn et al., 1993). Lignin protects cellulose and hemicellulose, make them more resistant to anaerobic digestion. A reasonable pretreatment technique is needed to strengthen the biodegradability of lignocelluloses materials. The pretreatment aims is to build up enzyme accessibility by improving the digestibility of cellulose(Surendra et al., 2018). The available lignocellulosic raw materials in India are cellulose-containing wastes, for instance, wheat husk, wheat straw, rice husk, rice straw, sugarcane bagasse, vegetable waste, and municipal waste. The major deposits amount to around 39.0 million metric tons or roughly 18-20 million metric loads of biodegradable cellulose (Paudel et al., 2017)**.** Fuels acquired from cellulosic biomass for example the woody, and normally unpalatable pieces of plant give an option in contrast to conventional power sources that support national economic development and environmental goals (Petersson et al., 2007). Besides, biogas from lignocelluloses may provide new job opportunities for local people in rural territories, which can make a great socio-economic impact (Wyman, 2005). Biomass sources utilized for power generation include rural and forest waste, municipal waste, and aquatic waste utilized for energy purposes (Surendra et al., 2018). Reasons behind the production of energy from crop and agro-waste is because bioenergy is a renewable type of energy form, which can be produced from lignocellulose upon need. This energy can be seen as more effectively accessible than fuel sources and may be produced using significantly involving low capital cost. In various cases, usage of biomass can add to handle environmental issues, for example, use of biomass produced due to eutroplication as raw material for producing biogas. Its use lead to the decrease of dependence upon petroleum- based energysources.

1.2 COMPOSITION AND PROPERTIES OFBIOGAS

Biogas is produced by anaerobic digestion of waste in anaerobic reactor. The most important fundamentals of biogas is methane and carbon dioxide. Apart from that, a trace amount of hydrogen sulfide, nitrogen, and hydrogen are there depending upon the variety of substrates used for producing the biogas. Table 1.1 suggest a general component of biogas.

Table 1.1General composition of biogas(Deublein and Steinhauser, 2008)

Compound	**Chemical Composition**	**(%)**
Methane	CH_4	55-70
Carbon dioxide	CO_2	44-28
Hydrogen sulfide	H_2S	1-2
Other gases	Water vapors	Traces

The most important part of the energy of biogas is the calorific value of its CH_4 content. This property of biogas makes it fit to be used as fuel for industry and domestic purposes.

Table 1.2 indicates the general propertied of biogas.

Table 1.2 General properties of biogas (Deublein and Steinhauser, 2008)

Properties	**Values**
Critical strain	75-89 bar
Critical heat	-82.5°C
Calorific value by CO_2	18.7 MJ/ m^3
Calorific value not including CO_2	26 MJ/ m^3

Density	1.0994 kg/m^3
Specific gravity	0.94
Viscosity	1.297x 10^{-5} kg/sec/m
Ignition temperature	700˚C
Energy substance	6.0-6.5 $kWhm^{-3}$

1.3 APPLICATIONS OFBIOGAS

Major constituents of biogas are methane with carbon dioxide. Methane is a prime constituent of natural gas. So, if somehow carbon dioxide can be removed from the biogas, it will be a source of immense energy that can be used effectively for different purposes like cooking, lighting, vehicle fuel, generation of electricity(Jeoh et al, 2017). After scrubbing the biogas it can be compressed and used as vehicle fuel like CNG and also it can be stored in cylinders and transported to other places.

1.4 ANAEROBIC DIGESTION PROCESS

Biogas can be synthesized by the method of anaerobic breakdown of organic matter for example biomass, manure, agricultural waste, industrial waste, etc. Anaerobic processing is a strategy where microorganisms decompose the biodegradable mass of the organic matter in the absence of oxygen to produce biogas (Curto et al 2019). The biomass breaks down to simpler substances during hydrolysis which is then acted by acidogenic bacteria, acetogenic bacteria followed by methanogenic bacteria. The reaction takes place through a significant number of steps with the help of the methanogens (Mahanta et al., 2006). The entire method of anaerobic digestion is partitioned into four noteworthy steps to perceive the framework appropriately. They are recognized as

- Hydrolysis of lignocellulosic biomass to solublecompound
- Acidogenesis ofsoluble compound & degrading to volatile fattyacids.
- Acetogenesis producing hydrogen, carbon dioxide and acetic acid derivation
- Methanogenesis creating biogas

As shown in Fig. 1.1, the digestion process starts with the hydrolysis of the biomass taken as input and breaks them down to insoluble simpler substances, which can be digested by bacteria. With the acidogenic bacteria, sugars and amino acids are converted into carbon dioxide, hydrogen, ammonia and organic acid. Then the acetogenic bacteria convert these organic acids into acetic acid along with additional ammonia, hydrogen, andcarbon dioxide. Finally, methanogen bacteria convert these products to methane and carbon dioxide. Anaerobic digestion produces biogas and prevent the production of foul smell and additionally produces manures with high nitrogen content (Mittal, 1996). The reaction that takes place in the process of methanogenesis is expressed in fig 1.1 (Mata-alvarez et al.,2000).

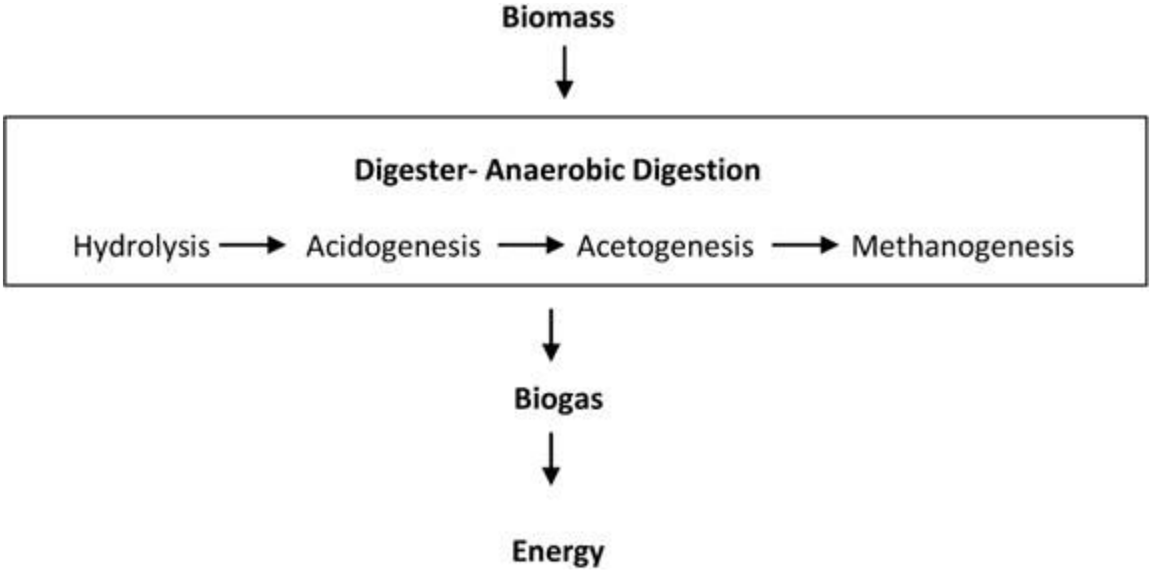


Fig. 1.1 Stages of an anaerobic digestion (Mata-alvarez et al., 2000)

1.5 TYPES OF BIOMETHANATIONPROCESS

Anaerobic digestion can be executed as a batch system or a continuous system. In a batch reactor biomass is added to the reactor at the start of the process. The reactor is then sealed for a period of the process. Since the batch processing is simple and requires fewer devices and lower levels of plan work, it is ordinarily a less expensive state of digestion as opposed to the continuous kind digesters. In continuous digestion the biomass slurry input isadded at regular intervals of time with the continuous removal ofwaste from theother side. The continuous digestion is better for production of biogas.

1.6 COMMONLY USED FEED MATERIALS FORBIOGAS PRODUCTION

Biogas producing from all different feed material is never equivalent. It shifts from substrate to substrate. Aside from that, there are numerous different parameter which affects the production of biogas. Some ofthemare C:N ofsubstrate utilized, temperature, pH value ofthe slurry, loading rate of input, hydraulic retention time, the toxicity of slurry, dilution and consistency of input feed and so on.The impact of agitation and added substances on biogas assembling is furthermore quite critical. Diverse types of material utilized as feed for bio-methanation can be classified as animal waste, plant waste, and domestic waste. Table1.3

Table 1.3 Various kind of feed material used for bio-methanation processes

Animal Wastes **[**Budiyono et al., 2010; Trujillo et al., 1991; Yusuf et al., 2011; Patil et al., 2011; Wong and Cheung, 1989]	**Plant wastes** [Mahnert et al., 2006; Das Ghatak and Mahanta, 2014]	**Domestic waste** [Viswanath et al., 1992; Knol et al., 1978]
Cattle dung	Grass clipping	Raw garbage
Rabbit wastes	Beanstalks	Bread
Horse manure	Bagasse	Potato tops
Pig manure	Cut Straw	Paper
Poultry manure	Peanut stalk and leaves	Kitchen vegetable scraps
Night soil	Wheat straw	Cabbage
Human urine	Rice straw	Tomato
Mixed slaughterhouse waste	Corn stalks	Fruit and vegetable waste

1.7 PRETREATMENT OFBIOMASS

1.7.1 Lignocellulose

Lignocellulose biomass comprises cellulose; hemicellulose and lignin (Jiang et al 2018). The plant cell wall is made up of cellulose which provides toughness and elasticity. The protective coat present outside the cell wall is lignin. A strengthening material which is available among cellulose and lignin is alluded to as hemicellulose. The diagram structure of cellulose is exhibited in Figure 1.2 however building block of lignin is showed up in Figure 1.3. In this figure (1.2), straight bundles appeared associated with lignin. The structure of lignin is then bound by particular hemicelluloses like xyloglucan, gelatin. Agricultural items, paper mash or tree industries alongside various agro essentially based exercises are sources oflignocellulosic biomass.

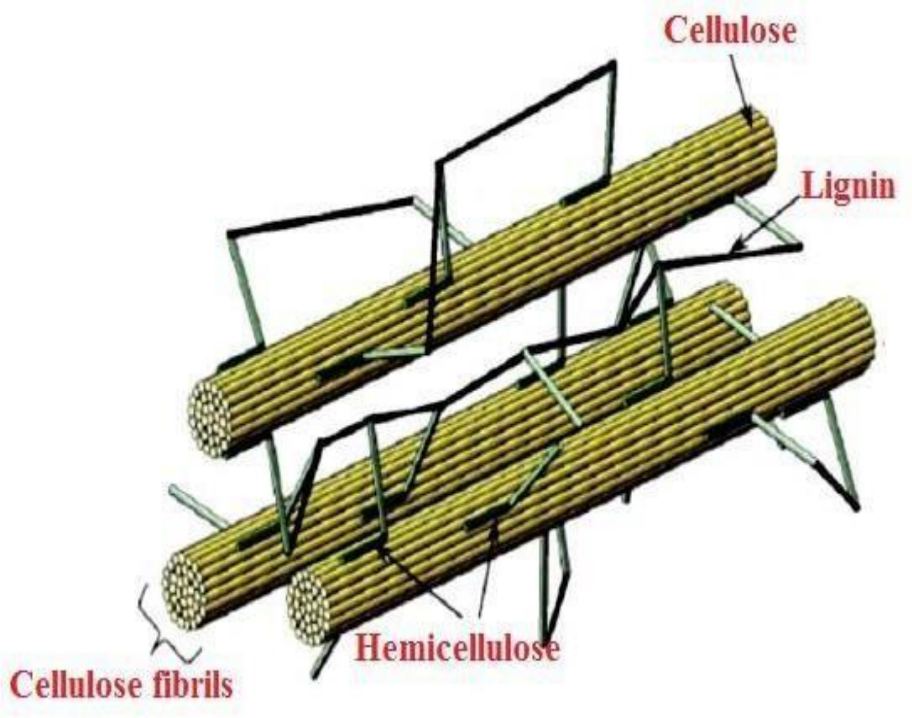
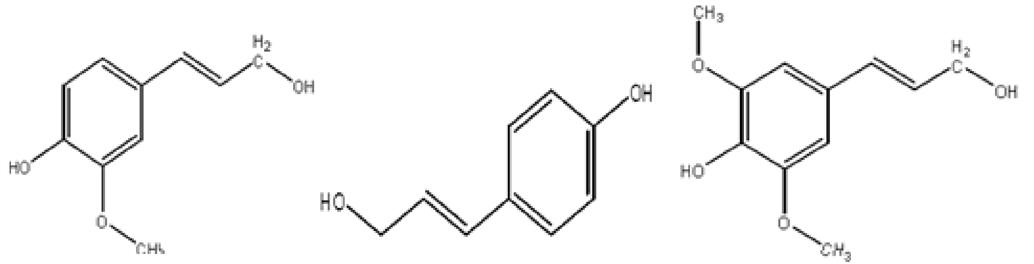


Fig. 1.2 Course of actionof cellulosic microfibrilsin plant cell dividers (Murphy and McCarthy, 2005)



p-coniferyl alcohol p-coumaryl alcohol p-sinapyl alcohol

Fig. 1.3 Building blocks of lignin (Wyman, 2003)

1.7.2 Pretreatment of lignocellulosicbiomass

Lignocellulosic biomass has more opposition to hydrolysis(Rajendran et al 2018). This is because of its remarkably well-ordered structure. This is the significant reason for its applications for the generationofvarious value presented stock i.e., ethanol, lactic acid, bio-oil, biogas are limited to some extent. In lignocellulose, sugar polymers are bounded with lignin with the help of hydrogen and covalent holding, which transforms into an unmanageable structure for hydrolysis and generation of value-added products such as biogas, etc (Kumari, et al 2018). In this manner, this structure of lignocellulosic biomass contains cellulose; hemicellulose and gelatin polymers join together and produce tough three- dimensional structure because of which hydrolysis is difficult (Perin et al.2019).The effect of pretreatment on the lignocellulosic biomass is shown in Figure 1.4.

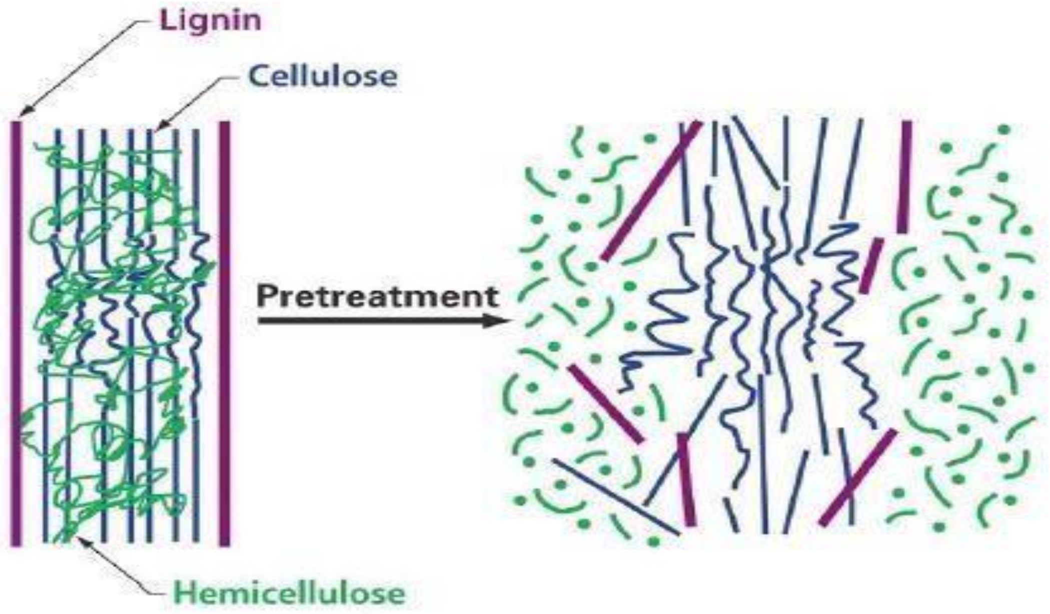


Fig. 1.4 Result of pretreatment on lignocellulosic biomass (Fernandes et al., 2007)

1.7.3 Utilization of lignocellulosic biomass for importance-additionalproducts

Lignocellulosic biomass is an inexhaustible valuable asset which is present in plant cell and can be utilized for energy production, to be utilized for various purposes (Veluchamy et al,2017). The effect of hydrolysis of lignocellulosic biomass is shown in Figure 1.5

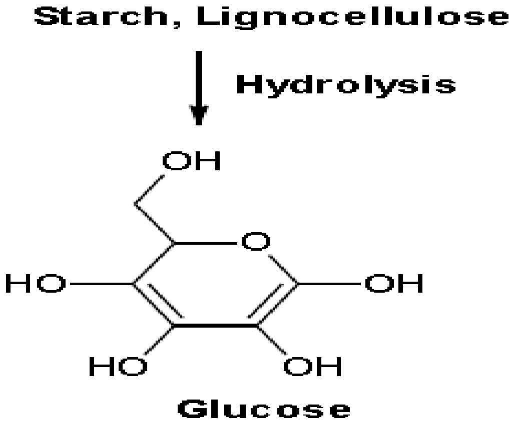


Fig. 1.5 Effect of hydrolysis of lignocellulosic biomass(Das et al., 2010)

But, it is essential to break the three- dimensional structure of polymer which is available in biomass into the less difficult composites for producing different types of significant items. The various types of significant worthincluded items fromlignocellulosic biomass are shown in Figure 1.6.

Fig. 1.6 Different value added products from lignocellulosic biomass(Huang et al., 2008)

Anaerobic processing is well- developed technology and is used worldwide for the production of bioenergy from different feedstock. These different sorts of substrate have high organic material of biodegradable nature which makes them a perfect biomass for anaerobic digestion process. Huge waste in farming and industrial level, can be used for producing biogas and manures.

1.8 MANURES

Bioslurry is a good fertilizer for crops and improves the soil fertility, soil structure and yields of crops. It is better than ordinary Farmyard manure (FYM) and may likewise lessen the utilization of chemical fertilizers. Bio slurry can be utilized to manufacture solid prolific soils for yield generation. Surely, bioslurry structure and substance settle with two- fold nitrogen content, which is not the same as Farmyard Manure (FYM). Likewise, the amount of the bio- slurry is additionally higher than normal FYM. Bioslurry contains helpful plant nutrients and more prominent measures of supplements, micronutrients than FYM. The results of bioslurry utility are similar to the results of the product of chemical fertilizers. In that capacity, bioslurry can be a genuine option in contrast to chemical fertilizers.

1.9 OBJECTIVES OF THE PRESENTSTUDY

The broad objective of the proposed study is the conversion of lignocellulosic biomass into biogas and its value-added products as an exercise to protect the environment. As per the literature survey, it was found that three lignocellulosic biomass named wheat straw, rice husk and sugarcane bagasse are in abundance in our country and can be a profitable step to increase energy generation in a developing country. The utilization of biomass for energy also decreases the gap generated due to energy demand and supply. The specific targets of the studyare:

- Optimizing the pretreatment prerequisites of agricultural biomass (wheat straw, rice husk and sugarcane bagasse) to causedelignification.
- To study the impact of reaction conditions (temp, pH, concentration, Nitrogenous substance) on biogasgeneration.
- Analysis ofoutlet slurry for fertilizer value.

1.10 ORGANIZATION OFTHESIS

Chapter 1 discusses the brief introduction about the biogas production and the feedstocks for biogas production. Chapter 2 discusses the various techniques for pretreatment of lignocellulose waste, selection of suitable pretreatment methods to our research and Anaerobic digestion of treated waste to biogas. Chapter 3 addresses the material and methodology to achieve the research objectives, namely designing conditions for our experiments. This involves culturing of Pretreatment of lignocellulose waste and the effect of different factors on biomethanation. The achieved targets are discussed in Chapter 4 wherein it will be explained how the researcher has implemented conditions in the pretreated biomass for bio methane production and manure production. The results will also be discussed the optimum for different levels of pretreated biomass which were studied and then will finalize the best optimum condition for specific biomass. Chapter 5, includes the summary of the final conclusion and findings for the research questions and problem statement, as well as give recommendations for future research.

CHAPTER -2
REVIEW OF LITERATURE

2.0 INTRODUCTON

The majority of fuels are derived from fossilized biomass but socioeconomic and environmental concerns are pushing towards an increasing contribution of recent biomass which is capable of closing the fixation/emission loop of CO_2 within ecological cycles. The potential of biomass is virtually enormous. It is because of this reason that the terrestrial net primary production corresponds approximately to 2000 EJ Y^{-1} (Krausmann et al., 2013). However, the effective potential of bioenergy is not easily quantifiable. It is dependent on many crucial assumptions such as the degree of sustainability, land availability, dietary habits that explain the wide range (Slade, Bauen, & Gross, 2014). The energetic reservoir built up by plants as net primary productivity serves to sustain the higher trophic levels of ecosystems, therefore diverting part of this energy flow out into human uses and has a profound impact on the environment and the distribution of resources (the estimated human appropriation is one- quarter of the theoretical net primary productivity). To circumvent or minimize the competition with other destinations, the production of bioenergy from wastes and residues is encouraged considering that its potential is deemed to be significant. At present, anaerobic digestion (AD) is one of the main technologies capable of transforming biodegradable substrates into a fuel, methane which is formed along with carbon dioxide in biogas (a mixture of about 60–65% CH4 and 35–40% CO_2) through a community of prokaryotic organisms. The complexity of the metabolic pathways leading to biogas from biopolymers is classically divided into four sequential steps: degradation of biopolymers into smaller molecules like monomers (hydrolysis), which are fermented principally into volatile fatty acids VFAs (acidogenesis), further digested into CH_3COOH, CO_2 and H_2 (acetogenesis) that are eventually converted in the ultimate products CH_4 and CO_2 (methanogenesis) (Fabbri & Torri,2016).

The hydrolysis step is rate- limiting due to the presence of complex polymers in biomass. Pretreatment is a process in which the biomass is made ready for microbial attack. This pretreatment can be physical operations such as mechanical commmunition, irradiation etc.; chemical treatment with alkali, acids, wet oxidation etc.; biological pretreatment, by fungi or enzymes; or a combination of these processes (Karuppiah & Azariah,2019).

Micro-anaerobic pretreatment is an efficient & cost-effective pretreatment method to meet the requirements for the industrial applications. Amin et al. (2017) concluded that the formation of cellulosic fiber for enzymatic attack, the prevention of the formation of inhibitors to the fermenting microorganisms and hydrolytic enzymes, reduced energy demand which finally reduced the cost of the feedstock. A recent update concerning developments in the field of pretreatment was developed. The improvement of the efficiency and cost limitations by combining both chemical and physical methods was found to be more beneficial as compared to both methods in individuals. Additionally, the chemical pretreatment can provide many disadvantages related to the extensive waste production and corrosion ofthe reactors (Marcin et al.,2019).

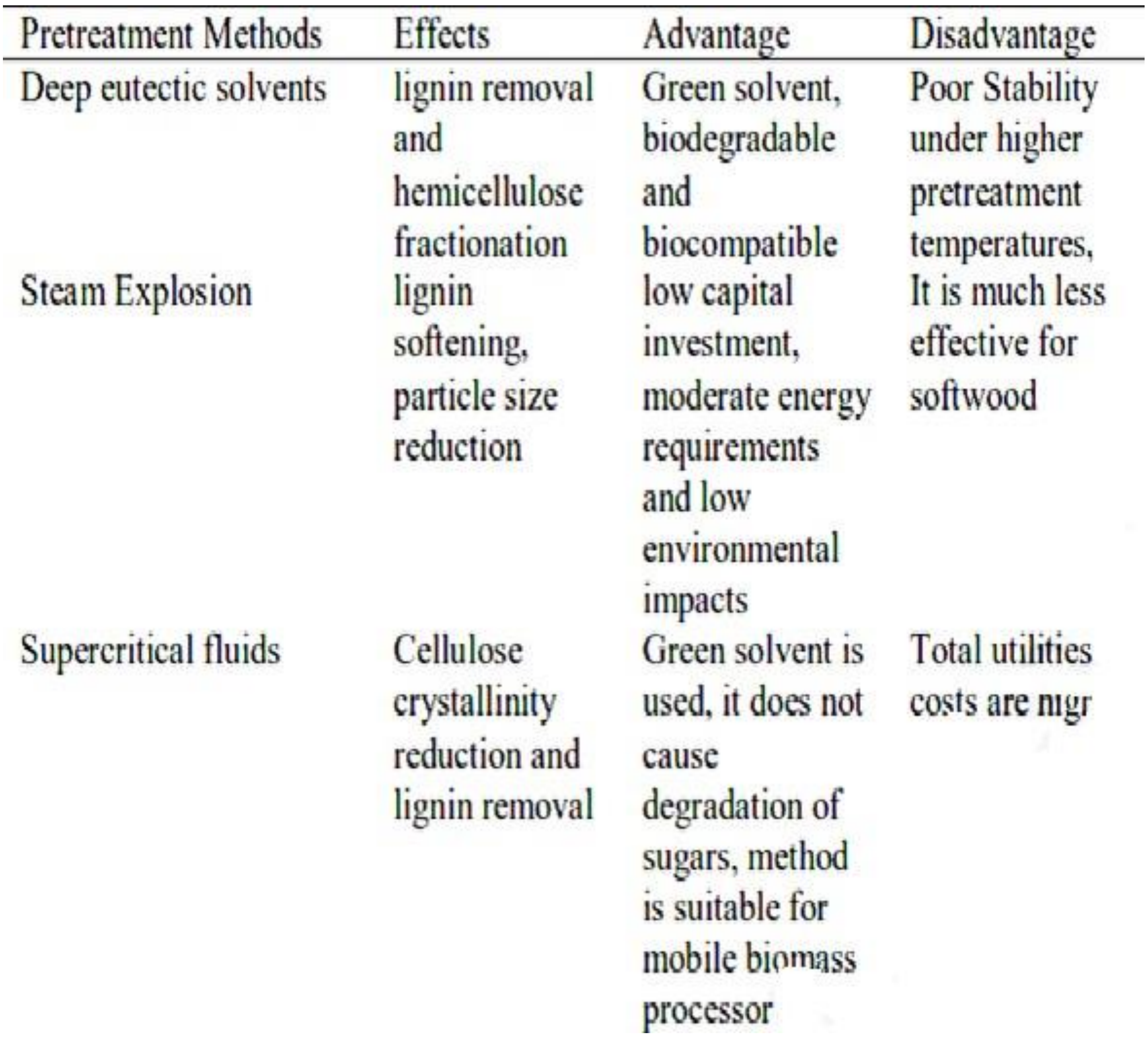
Considering different bioenergy resources, lignocellulosic has been recognized as the prime source of biofuels and other value-added products (Kumar, Singh, & Singh, 2008).This biomass is an abundant organic material that can be used for sustainable production of bioenergy and biofuels such as biogas (about 50-75% CH_4 and 25-50% CO_2) (Zheng, Zhao, Xu, & Li, 2014). Lignocellulosic wastes obtained from energy crops, wood and agricultural residues represent the most significant sources of sustainable biomass (Lin et al 2006; Kumar et al., 2008). It has been found that the plant photosynthesis accumulated around one hundred fifty billion stores of dry fabric every year with which about half is cellulose (Persson et al 1991). The lignocellulosic biomass from plants and residues of agricultural activities amounts to the biggest inexhaustible supply of fermentable sugars on earth, which is otherwise considered as agricultural waste(Azevedo et al,2019). Because of their easy availability, huge amount and sustainability, there has been an emerging interest of the scientific community in utilizing lignocellulosic wastes for the rebuilding of a lot of valuable stock and biomaterials (Pandey et al., 2000b; Howard et al., 2003; Das et al., 2010; Saha et al., 2005; Foyle et al., 2006; Tomas-Pejo et al., 2011; Mtui, 2008; Huang et al., 2008).

Agricultural residues, such as straws, nutshells, fruit shells, fruit seeds, plant stovers, green leaves, and molasses, are potential renewable energy resources. Many developing countries have a wide variety of agricultural residues in ample quantities. Large quantities of agricultural plant residues are produced annually worldwide but are vastly under-utilized (Demirbaş, 2001). Rice straw is an abundant and largely unused agricultural residue. Ninety percent of the world's rice production is in the developing countries of East and Southeast Asia where rice straw is utilized as a main feed for ruminants (Zhong et al., 2011). About 731 million tons of rice straw is produced annually, which is distributed in Africa (20.9 million tons), Asia (667.6 million tons), Europe (3.9 million tons), America (37.2 million tons), and Oceania (1.7 million tons). This amount of rice straw can potentially produce 205 billion liters of bioethanol per year, which is the largest amount recorded so far from a single biomass feedstock.But in general, half of the crop residues remain unused even though these are being used for purposes like animal feed, fuel for cooking, and house-heating energy for rural families. Underdeveloped conversion technologies prevent crop residues from becoming a practical option in energy production, and most agricultural residues are burnt in the field. This kind of disposal method has caused widespread environmental concerns as it contributes to air pollution(As in the case of Punjab, Haryana, UP,etc). It can't be denied that one of the reasons for the air pollution in Delhi since mid-October is thestubble burning ofcrops in Punjab and Haryana (Nirmal, 2019).

Dumping agricultural residues back into the field may reduce crop yields, increase foliar diseases, and degrade soil conditions (Siddique et al. 2017). Therefore, cost-effective technologies for corn straw, wheat straw, rice husk and sugarcane bagasse disposal and utilization must be developed.

Anaerobic digestion of organic waste and residues combines both sustainable treatment and renewable energy production. Lignocellulosic materials, are resistant to anaerobic digestion and can be converted into biogas, although only to low extents. The low susceptibility of these materials to conversion into biogas is attributed to their composition and structure. Lignocellulose is the complex and rigid matrix of plant cells; it is resistant to enzymatic attack because of the tight association between lignin, cellulose, and hemicellulose. Cellulose and hemicellulose can be degraded in biogas processes. However, lignin cannot be degraded under anaerobic conditions. Therefore, pretreatments, including the solubilization and biodegradation of the hemicellulosic and lignin components of the substrates, are necessary to facilitate biogas production by overcoming hydrolysis limitations (Nizami, Korres, & Murphy, 2009). Treatments facilitating the accessibility of holocellulose are necessary to increase the biogas potential of fibers such as corn stalks and wheat straw. Many treatments for increasing the biodegradability of lignocellulosic material have been reported.One of the objectives of this study also focuses onthis. (Sawatdeenarunat, Surendra, Takara, Oechsner, & Khanal, 2015).

The application of elected emerging technologies like ionizing & non-ionizing radiation; pulsed electrical field; ultrasound & high pressure; as a promising tool in the degradation of lignocellulosic biomass was studied and discussed in below table (Shady et al.,2018).



Pretreatment Methods	Effects	Advantage	Disadvantage
Deep eutectic solvents	lignin removal and hemicellulose fractionation	Green solvent, biodegradable and biocompatible	Poor Stability under higher pretreatment temperatures,
Steam Explosion	lignin softening, particle size reduction	low capital investment, moderate energy requirements and low environmental impacts	It is much less effective for softwood
Supercritical fluids	Cellulose crystallinity reduction and lignin removal	Green solvent is used, it does not cause degradation of sugars, method is suitable for mobile biomass processor	Total utilities costs are nigr

Table 2.1: Advantages and disadvantages of selected green chemistry pretreatmentmethods (Shady etal.,2018)

Bioenergy, especially biogas produced through the anaerobic digestion (AD) of renewable feedstocks, is considered to be one of the highly promising alternatives to fossil-derived energy due to several inherent and significant merits (Kaparaju, Serrano, Thomsen, Kongjan, & Angelidaki, 2009); Cheng et al., 2011). Because of its advantages over conventional fossil-derived resources, AD has been adopted and integrated into society over the last century, with thousands of full-scale plants currently in operation worldwide. AD is suitable for converting non-sterile, diverse, complex feedstocks into energy-rich biogas.

Many biodegradable feedstocks such as industrial wastewater, food wastes, animal manure, agri-wastes, sewage sludge, organic fraction of municipal solid waste, among others, have been employed as substrates for commercial biogas production. Such facilities illustrate the unique potential for bioremediation and waste stabilization with concurrent bioenergy production. More recently, lignocellulosic biomass, namely agri-residues and energy crops, have gained much attention as candidate feedstocks for producing bioenergy and biobased products. Unlike conventional bio-renewable feedstocks (i.e., sugar- and starch-based crops), lignocellulosic biomass does not directly compete with food or feed production. Moreover, high biomass yields even under low inputs of energy, water, fertilizers, and pesticides, make these crops ideal for biogas (and bioenergy) production (Azman, Khadem, Van Lier, Zeeman, & Plugge,2015).

As already discussed, the hydrolysis of lignocellulose often becomes the rate-limiting step during traditional AD. Several studies have focused on enhancing the digestibility of lignocellulosic biomass through physical, chemical, biological and hybrid pretreatments in the production of liquid fuels (primarily ethanol) via biochemical pathways (FitzPatrick et al., 2010; Takara and Khanal, 2011). Mechanical milling, steam explosion, hot water washing, acid and alkali pretreatments and ammonia fiber expansion, among others, have been employed as upstream unit operations to disrupt the complex structure of biomass, thereby increasing its porosity, removing lignin and/or hemicellulose, and reducing the overall crystallinity of the biomass structure to facilitate the biological conversion of biomass into bioenergy and biobased products (Monlau et al., 2013; Agbor et al., 2011). Many of these pretreatments, however, are economically and environmentally unfavorable due to the high cost of enzymes and the production of solid/liquid waste streams (Monlau et al.,2013). AD is the naturally occurring, biological pretreatment of organic substrates carried out by robust, mixed culture microbial communities in the absence of oxygen (Petersson, Thomsen, Hauggaard-Nielsen, & Thomsen, 2007). The consortium of microbes works synergistically to deconstruct recalcitrant biomass structures (like lignocellulose) into their respective fundamental components. In conventional bioprocessing strategies, the whole lignocellulosic feedstock is ground and fed into an anaerobic bioreactor to convert complex carbohydrates and organic matter into energy- rich biogas (Kratky & Jirout, 2011). Though effective, this approach is time - consuming and energy- intensive, consequently limiting its application for large-scale bioenergy production from dedicated energy crops. An insightful study conducted by Yuan et al. (2016) suggested that certain microorganisms present in the AD slurry may prefer specific biomass constituents over others (Sawatdeenarunat et al.,2015).

Biomass is a natural material made out of polymers that have enormous chains of carbon molecules connected to different macromolecules. The polymer backbone comprises of bonds connecting carbon with carbon or carbon with oxygen or once in a while with various components, for example, nitrogen or sulfur. It tends to be seen as congregations of some enormous atomic units. Lignocellulosic material comprises of the fundamentalthree abundant polymers namely cellulose (40-50%), hemicellulose (25-35%) and lignin (15-20%) which are interrelated. In the case of cellulose, the repeating unit is the glucan moiety, extremely an atom of glucose using one molecule of water, the unit is dependably a 5-carbon sugar, the xylose. Regardless, hemicellulose polymers are not straight chains as in the cellulose polymer. Some are extended and other monomer units have a point of view chains, withacetyl parties being extremely normal.

The lignin polymers are made out of phenyl propane subunits, a complex made up of different monomer through C•C and C•O bonds with the methoxy groups(Kumara et al., 2018). These bonds and complexes can be biotransformed by whole microbes or their enzymes by selectively degrading either of cellulose, hemicellulose or lignin. These three biopolymers are the main overall plant assets that can be successfully changed over to animal feed, bio stocks, biofuels, biochemicals, biomaterials, and biopower (Fernandes et al., 2007; Kaparaju and Felby, 2010; Cherubini and Stromman, 2011)

2.1 STRUCTURE AND PROPERTIES OF LIGNOCELLULOSESBIOMASS:

2.1.1 CELLULOSE

Cellulose is the β-1,4-polyacetal of cellobiose (4-O-β-D-glucopyranosyl-D-glucose) (Jiang, et al 2018). However, cellulose is normally considered as a polymer of glucose since cellobiose comprises of two molecules of glucose (Figure 2.1)

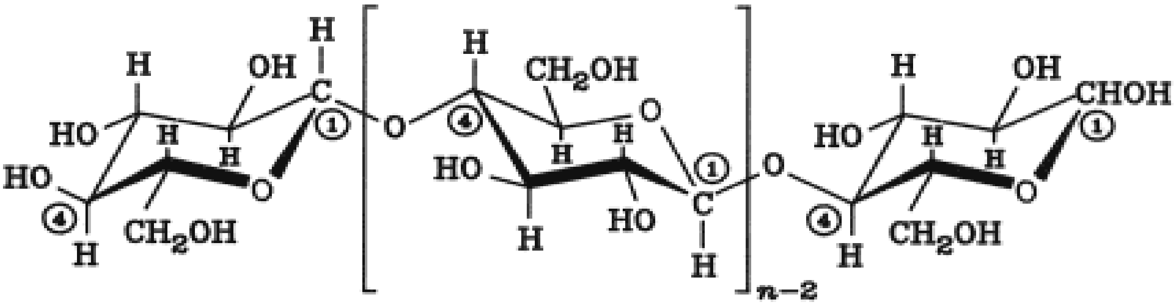


Fig. 2.1 Constitution of cellulose (R et al 2007).

The cellulose from wood has around 10,000 glycosyl units in chains that form the structure of fibrils, broad bundles of monomer, which are stabilized with the help of several strong intermolecular hydrogen bonds between hydroxyl molecules of bonded particles (Bon and Ferrara, 2008). Due to β-1, 4 linkage, cellulose is especially crystalline and quite resistant to degradation by microbes and/or their enzymes (Wyman, 2003; Gray et al., 2006). Cellulose is a remarkably hygroscopic material connecting with 8-14% water underneath regular atmospheric conditions (20°C, 60% relative wetness). Further, cellulose is insoluble in different solvents (including water) at low temperatures. The dissolvability of the polymer is directly related to the extent of hydrolysis accomplished. At higher temperatures, it will dissolve, as the bond dissociation energy is sufficient to break the hydrogen bonds that protected the crystalline structure of the atom. Cellulose,also,is dissolvable in mineral acids, accompanied by hydrolysis(Yuan,et al 2016). Higher cellulose degradation is achieved when this polymer swells due to the deterioration of the low atomic mass subunits (Krassig and Schurz, 2002). Solvents of cellulose that have been used in the present day or research center practice include cupri ethylene diamine (cuen) hydroxide or the cadmium tangled Cadoxen. Moreover, watery salt solution, for example, zinc chloride, degrades the structure of cellulose (Kirk-Otmer, 2001). Cellulose does not separate with temperature, yet its decomposition begins at 180°C (Thermowood Handbook, 2003).

2.1.2 LIGNIN

The lignin biopolymer is an amorphous, cross-connected and amazingly complex 3-D polymer of various phenylpropanoid subunits, polymerized with the help of the carbon–oxygen (C-O) and carbon-carbon (C-C) bonds (Figure 2.3). Lignin present between the external layers of the strands, leading to structural rigidity and holding the filaments of polysaccharides together (Davin and Lewis, 2005). Lignin is connected to hemicelluloses and cellulose, thus always resisting enzymatic attack of different fungi (Wood rotting white-root and brown-rot) and bacteria (Thomas et al., 2019). Generally, softwoods fuse additional lignin than hardwoods. Lignins have been classified into two classes, guaiacyl lignins and guaiacyl-syringyl lignins (Ahring et al., 2015). Even though that the fundamental basic factors in lignin have been for the most part explained, numerous variables of their science still remain to be discovered.

Lignin building subunits include *p*-coumaryl liquor, coniferyl liquor and sinapyl liquor (Howard et al., 2003; O'Connor et al., 2007Tomas-Pejo et al., 2008; Sanchez, 2009). Softwood lignin is made of coniferyl liquor units, while hardwood lignin is of coniferyl and sinapyl alcohol units (Pu Y et al., 2007). The main function of the lignin is to offer the plant basic help, impermeability, and opposition towards microbial attack and oxidative assistance (Fengel and Wegener, 1984; Hendriks and Zeeman, 2009). In lignocellulosic biomass, lignin is bonded closely to cellulose and hemicellulose and this strong affiliation impacts enzymatic degradation (Tomas-Pejoet al., 2008; Hendriks and Zeeman, 2009).

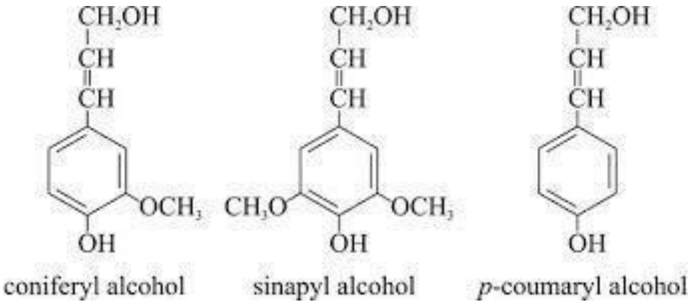


Fig. 2.2 Model structure of spruce lignin(Sanchez, 2009)

2.1.3 HEMICELLULOSE

Hemicellulose is a not well characterized and heterogeneous group of polysaccharides (copolymer of any of the monomers of glucose, galactose, mannose, xylose, arabinose and glucuronic damaging) (Figure 2.3). Hemicellulose incorporates the cellulose strands and is a hyperlink among cellulose and lignin (Siddique et al. 2017). Hemicelluloses are heterogeneous polymers of pentoses (xylose and arabinose), hexoses (for example mannose, glucose and galactose) and sugar acids (Hendriks and Zeeman, 2009; Girioet al., 2010). It includes an extraordinary chain xylan spine (β-1, 4 linkages) with anumber pieces of mannose, arabinose, galactose, glucuronic destructive and so on. Disparate cellulose, hemicelluloses are presently not artificially homogeneous. The level of branching and nature of the monomeric sugars in hemicellulose depend upon plant type (Laine, 2005; Gray et al., 2006; Albertsson et al., 2010). Depending upon sugar type, the hemicelluloses are alluded to as mannans, xylans or galactans. The C5 and C6 sugars, connected by means of 1-3, 1- 6, and 1-4 glycosidic bonds and in numerous events acetylated, structure a free, particularly hydrophilic structure that goes about as glue among cellulose and lignin (Bon and Ferrara, 2008). Hemicellulose differ from cellulose by different sugar units, by presenc of shorter chains and by branching the main chain which made structure less easier to hydrolyzein comparison to cellulose (Tomas-Pejoet al., 2008). The breakage results in their monomeric components containing glucose, mannose, galactose, xylose, arabinose and little proportions of rhamnose, glucuronic destructive,methylglucuroni destructive& galacturonic derivative.

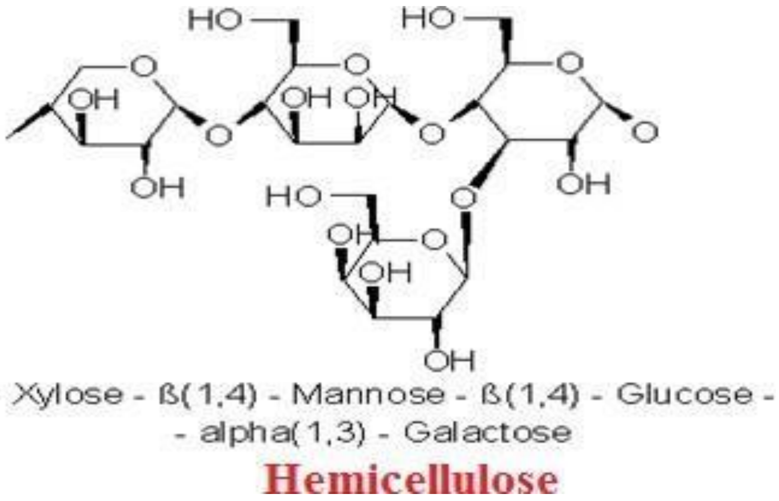


Fig. 2.3 Schematic portrayal of the hemicellulose back bone of arborescent plants(Bon and Ferrara, 2008)

Hardwood hemicelluloses mainly comprises of xylans however, softwood hemicelluloses contain glucomannans (Cao et al., 2017). Xylans are the most plenteous or abundant hemicelluloses. Xylans of many plant materials are heteropolysaccharides with homopolymeric back chain of 1, 4-related β-D-xylopyranose units. Xylanscan be derived from various plant sources, for example, grasses, grains, softwood and, hardwood. Other than xylose, xylans can in addition blend arabinose, glucuronic ruinous and acidic, ferulic and p-coumaric acids. The extent of polymerization of hardwood xylans (150-200) is higher than that of softwoods (Dahlmanet al., 2003; Tomas-Pejoet al., 2008; Alonso-Sandeet al., 2009; Scheller and Ulvskov, 2010). Further, of all the three segments: (Figure 2.6) cellulose, hemicellulose and lignin, the hemicelluloses are the most thermo-chemically sensitive. (Sweet and Winandy, 1999; Hendriks and Zeeman, 2009)

2.1.4 PRETREATMENT TECHNOLOGIES

Pretreatment refers to the disruption of outer protective lignin covering to fasten the cellulose hydrolysis by enzymes. The most important step in the formation of biofuel is pretreatment of the lignocellulosic biomass(Li et al. 2018). Pretreatment indicates the solubilization of cellulosic biomass. It makes the treated solid biomass more available for physical, chemical and biological treatment (Mosier et al., 2005b; Wyman et al., 2005; Demirbas A., 2005; Grayet al., 2006).The lignocellulosic complex is comprised cellulose and lignin connected through hemicellulose chains. Upgrades in pretreatment productivity and advancement of new enzymes require better comprehension of the components that decide the rate of enzymatic hydrolysis. Variables that are generally demonstrated to affect the enzymatic hydrolysis are cellulose crystallinity and level of polymerization.

The pretreatment is done to crush the lattice so as to diminish the confirmation of crystallinity of the cellulose and to increase the content of amorphous cellulose, for enzymatic attack (Sanchez & Cardona 2008). Objectives of a magnificent pretreatment system are:

(I) Formation of sugars immediately throughhydrolysis.

(II) Avoid loss and/ or degradation ofsugars formed

(III) To restrict development of inhibitoryitems.

(IV) To limit energy demand and to limit costs.

Physical, chemical, Physico-chemical and biological are the four fundamental sorts of pretreatment procedures utilized.Mix of these techniques are utilized in the pretreatment step. Pretreatment additionally influences the expense of the unique operational advances that is downstream expense , enzymatic hydrolysis rate and fermentation process variables factors (Carvalheiro et al., 2008; Taherzadeh and Karimi, 2008; Yang and Wyman, 2008; Hendriks and Zeeman, 2009 Alvira et al., 2010; Girio et al., 2010)

Table 2.2 Key factors for an effective pretreatment method for lignocellulosic biomass.

S. No	Key features for pretreatment of lignocellulosic biomass
1.	High yield from several crops, site, ages with harvesting times.
2.	Develop the nature of sugars or the ability to afterward form sugars by hydrolysis.
3.	Solid division highly digestible.
4.	Keep away from the degradation or loss of carbohydrates.
5.	Avoid the formation of byproducts that are inhibitory to the successive hydrolysis and fermentation processes.
6.	No necessity of size decrease.
7.	Process in normal size and modest price reactors.
8.	No production of solid-waste residue.
9.	Efficiency at low moisture content.
10.	Obtaining high sugar concentration.

11.	Fermentation compatibility of the pretreated substance.
12.	Cost-effectiveness.

During the most recent decade, countless assorted pretreatment innovations have been proposed, typically characterized into natural, physical, chemical, and Physico-chemical.

2.1.4.1 PHYSICAL PRETREATMENT

Mechanical pretreatment is used to reduce the particle size and crystallinity of lignocellulose to broaden the surface area and decrease the confirmation of polymerization (Palmowski and Muller, 1999 and Hendriks and Zeeman, 2009). Mechanical pretreatment advancements amplifies the digestibility of cellulose and hemicellulose in the lignocellulosic biomass. Decrease of biomassunderneath 20mm sizedemonstrates themagnificent mechanical exhibition (Sausaet al., 2004; Mtui, 2009). Physical treatment result in an expanded ethanol yield, low hydrolysis cost and no inhibitors were created (Hendriks and Zeeman, 2009; Hidenoet al., 2009). Mechanical pretreatments (chipping, crushing and processing) decrease cellulose crystallinity anyway require extreme energy and capital charges (Sun and Cheng, 2002; Sanchez and Cardona, 2008; Tomas-Pejoet al., 2008). Considering the high power necessities of processing and the constant ascent of the power costs, it is plausible that processing is by the by not financially achievable (Hendriks and Zeeman, 2009). Mechanical pretreatment decreases cellulosecrystallinity and improves the viability of downstream processing. Wet handling, dry preparing, vibratory ball preparing and weight handling are regularly done.The quality commitment for mechanical treatment of agricultural waste relies on the starter and remaining particle sizes, moisture content material and on waste (hardwood, softwood, stringy, etc) being overseen . Size reduction may similarly supply higher outcomes but some time it may impact problem to pretreatment and enzymatic hydrolysis. Size reduction can be one of the most operative means for increasing the enzymatic accessibility to lignocellulose. However, many of the physical methods for size reduction (milling, grinding, etc.) are not economically feasible because a veryhigh-energy input isrequired.

2.1.4.2 PHYSICOCHEMICAL PRETREATMENT

Combined Physical and chemical treatment structures are of significance in dissolving hemicellulose and modify of lignin formation, presenting a comprehensive accessibility of the cellulose for hydrolytic enzymes (Kumari, et al., 2018). The most advantageous physiochemical pretreatments join thermochemical prescriptions, for instance, steam impact, liquid warm water (lhw), smelling salts fiber impact (afex) and CO_2 impact. In these techniques, chipped biomass is made do with high pressure splashed steam, liquid smelling salts or CO_2, and a short time later the strain is all of a sudden decreased, making the biomass to undergo decompression (Hendriks and Zeeman, 2009; Mtui, 2009).

Steam impact is the most extensively used physico-chemical pretreatment for lignocellulosic biomass. In the process of steam pretreatment, the biomass is put in vessel and steamed at a high temperature (upto 240°C) for two to three minutes. After a set time, the steam is released and the biomass is immediately chilled off (Hendriks and Zeeman, 2009). The structure causes hemicellulose breakdown or separation and lignin change as a result of high temperature causing possible of cellulose hydrolysis (Mtui, 2009). Steam impact instead of various pretreatments, presents potential for lessening capital potential, altogether lower environmental impact, dynamically possible for energy efficiency, less formation of inhibitor and complete sugar recovery (Avellar and Glasser, 1998; Sun and Cheng, 2002; Tomas-Pejo et al., 2008). Steam explosion is a physicochemical pretreatment for deconstructing biomass (Ballesterosetal.,2002; Hamelinck etal., 2005). Nowadays, it is a best among pretreatment method for production of biogas.

The mechanical impacts are provoked on the grounds that the strain is at the same time diminished and strands are isolated since of the unstable decompression. In total with the halfway hemicelluloses hydrolysis and solubilization, the lignin is redistributed and somewhat disposed of from the fibre. Hemicellulose end will build compound openness to the cellulose microfibrils by utilizing uncovering the cellulose outside. Vapor blast fractionates the biomass in two divisions:

(I) A fluid portion rich in monomeric and oligomeric sugars regularly from hemicellulose solubilization.

(II) A solid element of absorbable cellulose and lignin. Steam blast mechanical ability has been accurately affirmed for biogas producing from a huge scope of uncooked materials.

Its fundamental disadvantages are strategy gear necessities (Oloffson et al., 2008), incomplete hemicellulose debasement and age of some harmful mixes got from sugar corruption over the span of pretreatment that might need to affect following hydrolysis and aging advances (Zaldivaret al., 2001; Olivaet al., 2003). The fundamental inhibitors are furan subordinates, vulnerable acids and phenolic mixes. The real furan subsidiaries are furfural and hmf got from debasement of pentoses and hexoses separately. Frail acids created for the term of pretreatment are generally acidic corrosive shaped from the acidic organizations existing in the hemicellulosic division and formic and levulinic acids . Wide scope of phenolic mixes are produced because of the lignin breakdown shifting broadly between select crude materials.

Other physic-synthetic pretreatment methodologies incorporate fluid high temp water pretreatment (Sun and Cheng, 2002; Mtui, 2009), smelling salts fiber/solidify blast (afex) (Dale and Moreira., 1982; Holtzapple et. Al., 1991 and Olofssonet al., 2008), ultrasound pretreatment (Mielenz, 2001; Nikolic et al., 2010), microwave pretreatment (Keshwani and Cheng, 2010), wet oxidation (Olsson et al., 2005; Kaparaju and Felby, 2010), and the utilization of supercritical liquids (Gao et al., 2010; Luterbacheret al., 2012).

2.1.4.3 CHEMICAL PRETREATMENT

Synthetic compounds going from oxidizing specialists, soluble base, acids and salts can be utilized to debase lignin, hemicellulose and cellulose from lignocellulosic structure (Mtui, 2009). Ground-breaking oxidizing venders, for example, ozone and hydrogen peroxide viably expel lignin and the reaction is done at room temperature (Sun and Cheng, 2002 and Mtui, 2009).

2.1.4.3.1 ALKALINE PRETREATMENT

Liquid lime or NaOH pretreatment is highly effective for sugarcane bagasse with lower temperatures than destructive pretreatments, regardless, in such cases the treatment times are hours long. For instance Chang et al., used lime with at 85°C for 3 h (Chang et al., 1998 and Pu et al., 2007). The solvent frameworks will by and large have increasingly unmistakable of degrading lignin and leaving cellulose and hemicellulose intact (Kim and Holtzapple, 2005; Kim and Lee, 2005; Teymouriet al., 2005; Gray et al., 2006 and Carvalheiro et al., 2008). The transfer of hemicellulose fabulously influences the degradability of cellulose. It is depicted to reason less sugar defilement than destructive pretreatment and it was shown to be more imperative charming on rustic stores than on woody substances (Kumar et al., 2009). The possible loss of fermentable sugars and some amassing of inhibitory blends ought to be taken as an idea to improve the pretreatment conditions. NaOH, KOH, $Ca(OH)_2$, and NH_4OH are the best additives for dissolvable pretreatments. NaOH causes swelling, extending the inside surface of cellulose and lessening the dimension of polymerization and crystallinity, which also prompts lignin shape unsettling influence (Taherzadeh and Karimi, 2008). NaOH has been proposed to make more noteworthy hardwood absorbability from 14% to 55% by using decreasing lignin content from 24-55% to 20% (Kumar et al., 2009). Moreover, pretreated switchgrass revealed a

course of action of pore advancement in the NaOH pretreatment growing the open floor an area to the proteins similarly as cutting down lignin content material (Nlewem and Thrash, 2010). Addition of an oxidant administrator (oxygen/ H_2O_2) to acid neutralizer pretreatment ($NaOH/Ca(OH)_2$) can improve the show by strategies for favoring lignin end (Carvalheiro et al., 2008). Upgrades for enzymatic hydrolysis have been in like manner reflected in extreme biogas delivering from pretreated with acid pretreatment method (Kumari,et al2018).

2.1.4.3.2 ACID PRETREATMENT

The acid treatment process is one of the most seasoned, simplest and most condition cordial procedures of producing biogas from biomass. The dilute acid is utilized to hydrolyze the biomass to sugars. The Liquid hydrolyzates are then neutralized and poisonous inhibitor for digestion are removed before anaerobic digestion of acid- treated biomass to biogas (Brennan et al., 1986).The hydrolysis is finished with weaken or vigorous acids. The goal is to solubilize the hemicellulose, and hence making cellulose higher available (Liu and Wyman, 2003; Hendriks and Zeeman, 2009). Itwill hydrolyze the hemicellulose part while leaving the cellulose and lignin unblemished in the leftover solids. The most well-known strategies utilize sulfuric corrosive, albeit other powerful acids have also been attempted (Liu et al., 2005; Lloyd andWyman, 2005; Mosier et al., 2005b; Gray et al., 2006). Weaken corrosive hydrolysis for the most part utilizes 0.4-2% H_2SO_4 at a temperature of 160-220°C to put off hemicelluloses and enrich cellulase assimilation of cellulose (Willforet al., 2005; Pu et al., 2007). Among all, weaken corrosive hydrolysis has been proficiently created, upgrading eminently the resulting arrangement of enzymatic hydrolysis (Esteghlalianet al., 1997; Ballesteros et al., 2007; Tomas-Pejoet al., 2008; Hsu et al., 2010; Parawira and Tekere, 2011; Kasthuriet al., 2012; Meinitaet al., 2012) because of the reality of their adequacy and reasonableness. These procedures have been connected in pilot pants and subsequently are near commercialization (Roparset al., 1992; Schell and Duff, 1996; Olofsson et al., 2008). Different research work on acid pretreatment (Campo et al., 2006 and Karimiet al., 2006) has found that 0.5% H_2SO_4 is most dependable for the restoration of wastes from vegetation and rice straw (Mtui,2009).

The increase of acid hydrolysis is the solubilization of hemicellulose and via this, making the cellulose more available for breakdown by enzymes in the digestion process. There is, on the different hand a danger on the association of flimsy corruption product where this carbon is lost for the transformation to ethanol. The buildup and precipitation of solubilized lignin is an unlucky response, as it diminishes digestibility (Cara et al., 2008; Hendriks and Zeeman, 2009; Rocha et al., 2009; Ferreira et al., 2010). Strong acid hydrolysis for biogas production is presently not alluring,since thereis probability of the development of inhibitory products. Weaken corrosive hydrolysis, anyway is regarded as one of the promising pretreatment techniques; for the reason that auxiliary responses all via the pretreatment can be disallowed in weaken corrosive pretreatment (Hendriks and Zeeman, 2009; Alviraet al., 2010).

Natural acids, for example, maleic, fumaric or even acidic corrosive have been prescribed as determinations to inorganic acids. Natural acids don't increase debasement responses that have been depicted in corrosive hydrolysis, bringing about lower grouping of harmful mixes. Both maleic and fumaric acids have been contrasted and H_2SO_4 in enzymatic hydrolysis yields from wheat straw. Results affirmed that herbal acids can hydrolyze with inordinate yields in spite of the fact that fumaric corrosive used to be as soon as less high caliber than maleic corrosive. Moreover, notably less measure of furfural used to be once formed in the maleic and fumaric corrosive pre-treatment than H_2SO_4 in hydrolysis (Kootstraet al.,2009).

Organosolv pretreatment (Park et al., 2010; Obama et al., 2012), ozonolysis (Silverstein et al., 2007; Shatalov and Pereira, 2008 and Kumar et al., 2009), Ionic refreshments (as unpracticed solvents) (Olivier-Bourbigouet al., 2010; Fu and Mazza, 2011), and sulfite pretreatment (Zhu et al., 2009) are a variety of techniques utilized for synthetic pretreatment of lignocellulosic waste.

2.1.4.4 BIOLOGICAL PRETREATMENT

The biological lignocellulose treatment involves microbes or their microbial enzymes in the pretreatment of lignocellulosic/agricultural wastes (Li et al. 2018). Both bacteria and different species of fungus are utilized for biotreatment of lignocellulosic squander (Brémond et al,2018). Natural pretreatments utilize microorganisms much of the time dark colored, white and delicate decay fungus which debase lignin and hemicelluloses and next to no of cellulose, additional safe than the various methods (Sanchez, 2009). Fungal pretreatment of agricultural biomass is another strategy for the enhancement of biomethane content of biomass (Singhania et al., 2006). White, dark colored fungus have been utilized to debase lignin and hemicellulose in waste materials. The different white- rot fungi can be used to degrade lignin selectively (Siddique and Wahid, 2018). Such selective lignin-degrading fungi can be efficaciously used in microbial pretreatments. A few white- rot fungi, for example, *Phanerochaete chrysosporium, Ceriporia lacerata, Cyathuss tercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarius* and *Pleurotus ostreatus* have been studied for their exceptional high delignification effectiveness of different agro residues (Sun and Cheng,2002; Keller et al., 2003; Arora et al., 2005; Kumar et al., 2009; Mtui, 2009 and Shi et al., 2009; Tianet al., 2012; Wan and Li,2012).

Brown rot fungi as often as possible degrade cellulose while white and delicate rot strike both cellulose and lignin (Rudakiya et al, 2017). Dark colored contagious pretreatment has been as of now brought up as an exact methodology for enhancing the enzymatic hydrolysis yields of *P. radiate* and *Pinus sylvestris* attaining saccharification yields around 70% (Ray et al., 2010). For this situation, it was proposed that some natural acids discharged by way of potential of the utilized growths *Caniophora puteana* diminished the pH and depolymerized it to some extent. In general, such methods provide advantages, for example, low capital cost, low vitality, no artificial substances prerequisite, slight ecological stipulations, and no inhibitory enzyme mixes(Hosseini et al, 2019). The massive drawback to enhance natural approaches is the low chemical- based hydrolysis price by organic acid and bases (Sunand Cheng, 2002; Tomas-Pejoet al., 2008; Dashtban et al., 2009).

Bacterial pretreatment of lignocellulosic waste involves anaerobic and aerobic micro-organism(Kong et al, 2018). Anaerobic debasement makes use of familiar mesophillic, rumen derived microorganism (Han and Shin, 2002; Hu and Yu, 2005; Neveset al., 2006; Hu et al., 2008 and Mtui, 2009). In aerobic framework, *Actinomycetes* namely, *Streptomyces griseus* is the best- studied for the production of extracellular hydrolytic enzymes that degrade lignocellulose (Arora et al., 2005 and Mtui, 2009). *Escherichia coli* and *Klebsiella oxytoca* lines have been hereditarily designed to create microbial biocatalysts that produce bioethanol from lignocellulosic materials (Peterson et al., 2008 and Mtui, 2009). To go ahead, for cost-aggressive natural pretreatment of lignocellulose to improve the hydrolysis, and, in the end, improve biogas yields, it is vital to proceed to peruse and looking at additional growths for their abilityto delignifythe plant fabric hastily and effectively (Figure 2.4).

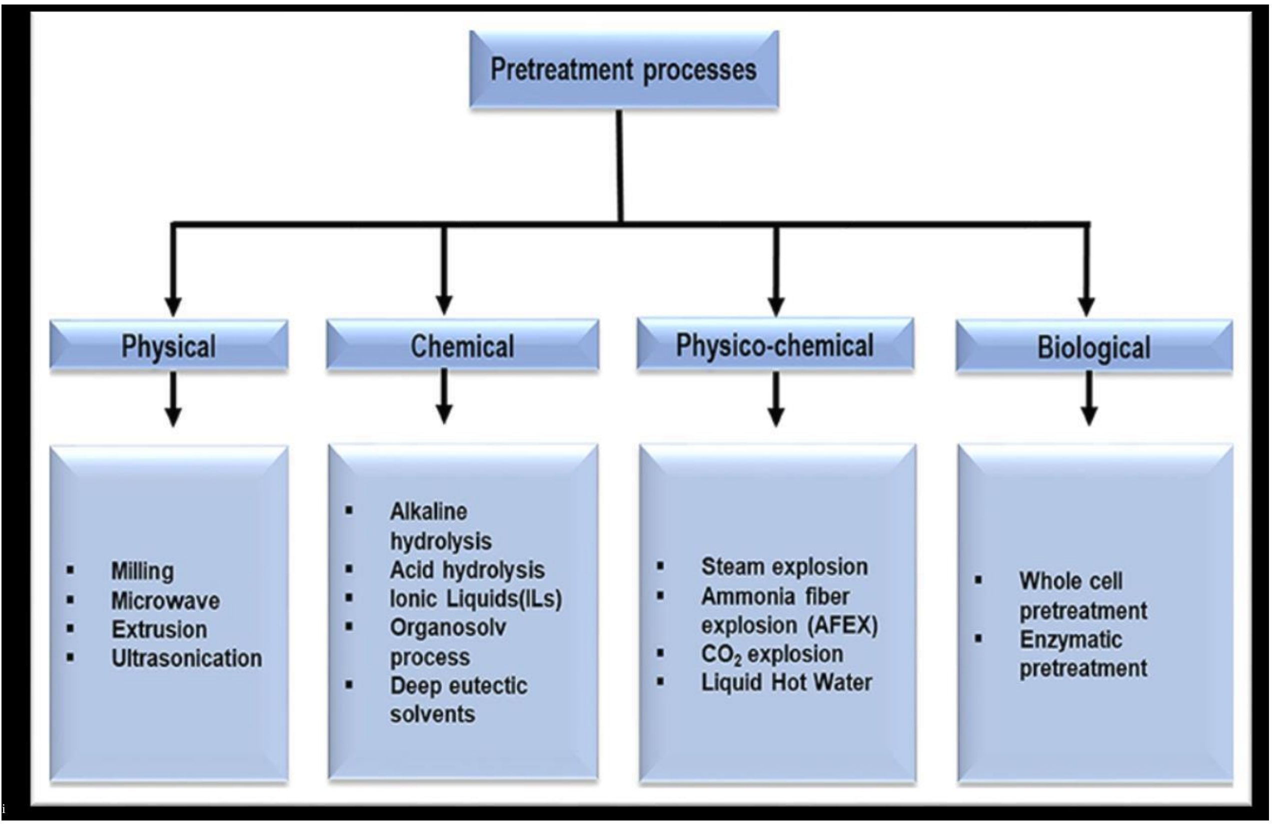


Fig. 2.4 A rundown ofdifferent strategies utilizedinthe pretreatment of lignocellulosic squanders (Mtui,2009).

2.2 FACTORS AFFECTING BIO-METHANATIONPROCESSES

2.2.1 EFFECT OF TEMPERATURE ON BIOGAS PRODUCTION

The bacterial metabolism to produce biogas mainly relies on anaerobic fermentation condition. Consequently, it becomes necessary to control the growth parameters to optimize bacterial growth and metabolism (Martin et al, 2008). Temperature is recognized to be one of the significant components for proficient anaerobic processing of biomass (Rodriguez et al,2018). The authors have identified the anaerobic microscopic organisms and the prerequisites that increase methane generation at some stage in the Thirties (Mahanta et al., 2004). Lusk, 1998 focused on several varieties of digesters with inner warming and observed that biogas technology was best working in mesophilic temperature conditions (30°C-45°C). Another study also suggested that thermophilic situation (45°C-60°C) improves the metabolic rates and explicit development rate of microscopic organisms in comparison to the mesophilic condition (Lier, 1995). Additionally, Mackie and Bryant, (1990) observed four times higher biogas generation with steers fertilizer when contrasted with that of in the mesophilic conditions. Thermophilic adjustment of biogas digesters at 55°C temperature is genuinely conservative as it delivers higher production of biogas (Vindis et al., 2009). In any case,

anaerobic assimilation in the mesophilic conditions is progressively basic when contrasted with that in thermophilic conditions because of the decrease in- process stability, dewatering properties and prerequisites of enormous measure of energy for warming the substrate inside the digester. Vindis et al., (2009) distinguished the methane framing smaller scale life forms and announced that methanogenic microscopic organisms are better and diverse at both in mesophilic and thermophilic temperature regime. Varel et al., (1976) studied thermophilic methane production from steers waste and found that the methane generation was the highest at 60°C. The procedure was started easily and biogas generation was quick without any lag or delay. The test perceptions demonstrate that methanogenesis was fast with a higher stacking rate and shorter maintenance time.

Vindis et al., (2009) examined both the thermophilic and mesophilic anaerobic absorption of three distinct kinds of assortments for biogas creation and its quality in the two methodologies and found that methane content of biogas acquired from thermophilic assimilation was higher than that of the mesophilic processing by 2%. They revealed that the thermophilic anaerobic digestion was two times quicker than mesophilic degradation (Maj et al, 2018).

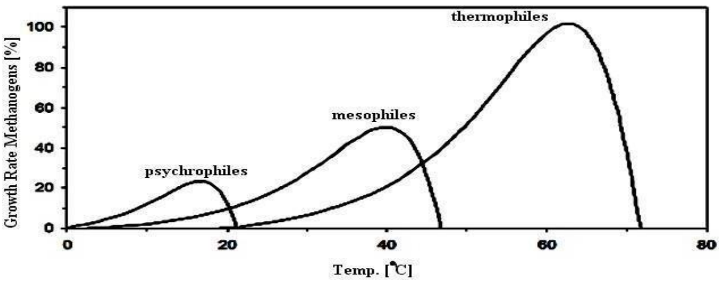


Fig. 2.5 Growth price of methanogens under psychrophilic, mesophilic and thermophilic conditions [Liar et al., 1995]

Lier et al., (1995) observed the growth rate of methanogenic microscopic organisms in thermophilic, mesophilic and psychrophilic stipulations as demonstrated in Fig. 2.5. It is evident from Fig. 2.5 that the growth rate of methanogenic microscopic organisms is highest for thermophilic circumstances pursued by mesophilic and psychrophilic conditions, respectively.

Sambo et al., (1995) demonstrated the result of temperature on biogas generation and showed that biogas production maximum at 50°C than 60°C and 40°C, separately. Mahanta et al., (2004) probed the impact of temperature variation on anaerobic absorption of steers waste and biogas production was once best at 35°C followedby 45°C, 40°C and 30°C, separately.

2.2.2 EFFECT OF FEED MATERIAL ON BIOGAS PRODUCTION

Diverse feed materials have different carbon content, cellulose content material or C:N proportion because of which biogas yield contrasts widely with feed material. Busch et al., 2009, Kalra and Panwar, 1986, Somayaji and Khanna, 1994 have utilized crop residues like maize, grass, sugar stick, husk and straw, rice and wheat straw for creation of biogas. It used to be seen that biogas produced from maize, grass and sugarcane has high methane (>72%) and low H_2S production (<100 ppm). It was further observed that the biogas producing with straw is 456% more noteworthy than that with the husk without anyone else and 167% additional than the mix of straw and husk. The husk has little gas potential.

A few studies show the impact of one of a kind foods grown from the ground as feed texture on biogas creation at extraordinary HRT and saw that with these kinds of feedstock have a higher rate of gas production rate even at low retention time (Prema Viswanath et al., 1992, Bardiya et al., 1996, Kalia et al., 1992).

Mähnert et al, 2005 investigated three fresh and ensiled grass species in lab-scale batch tests at 35°C and articulated the production of the right amount of biogas. Methane content in the biogas was found to be in the range of 50-58%. Table 2.1 presents the primary discoveries of a portion of the researcher on biogas yield with feedmaterials.

Table 2.3 Literature on various feed material and their results

Authors	Feed Materials used	Results

Busch et al., 2009	maize, grass, sugar cane	The process was extraordinarily stable and no malfunction had been detected so far. The biogas got has high methane (72%) and low H_2S concentration (100 ppm).
Kalra et al., 1986	husk and straw	The straw aloneproduced 45.6% greater fuel than the husk alone and 16.7% extra than the mixture. The husk has very small gasoline practicable.
Mähnert et al., 2005	fresh and ensiled grass species	Biogas was in the range of 0.65-0.86 m^3/kg VS

2.2.3 EFFECT OF CO-DIGESTION OF BIOMASS ON BIOGAS PRODUCTION

Different analysts have worked on co-digestion of biomass with cow dung and with other kinds of feed substances (Li et al., 2009; Hills and Roberts, 1981). Jingqing et al., 2013 revealed that when kitchen waste: pig excrement: rice straw was in the extent of 0.4:1.6:1, biogas production was resolved to be the most astounding. Somayaji and Khanna, 1994 dealt with rice and wheat straw one by one blended with dairy cattle manure in an elite offer and saw that biogas production was highest when rice straw was 100% and wheat straw was once 40% with cows compost as substrate. Additionally Kalia and Kanwar, 1990 worked on blends of new and incompletely disintegrated ageratum and found that somewhat decayed ageratum and cattle dung in 3:2 proportions yielded about 9% more biogas than that of unadulterated cattle dung. It is found that when biomass is co-processed with various feed material in fitting rate biogas production can be broadened fundamentally.

Table 2.3 exhibits a portion ofthe takes a shot at co-processing of biomass with various feed material in batch mode gain fromliterature.

Table 2.4 Literature on effect of co-digestion of biomass on biogas production

Author	Feed Materials used	Results

Hills and Roberts,1981	Fresh dairy manure and chopped field crop residues	The methane generation per unit COD from new dairy fertilizer was upgraded by expansion of hacked field crop deposits, up to a point of confinement of non-lignin carbon to nitrogen proportion of 30:1.
Li et al., 2010	Food waste with dairy manure	The gas fabrication rate was enhanced by 0.8-0.5 times as compared to the absorption with dairy fertilizer by yourself.
Jingqing et al.,2013	Rice straw with kitchen waste and pig manure	When kitchen squander: pig dung: rice straw was 0.4:1.6:1, biogas production was the highest
Somayaji and Khanna, 1994	Rice and wheat straw in cattle dung	Maximum gas production at 100% rice straw and 40% wheat straw substitution in cattle dung
Kalia and Kanwar,1990	Mixtures of fresh and partially-decomposed Ageratum	New ageratum blended with cows manure in a proportion of 3:2 did not create anygas but rather comparable blends of somewhat decayed Ageratum and steers compost yielded about 9% more biogas than that of unadulterated steers excrement.

2.2.4 EFFECT OF CARBON AND NITROGEN RATIO ON BIOGAS YIELD

Carbon is the principal chemical matter in natural wastes digested through microorganism to produce methane and carbondioxide in the process(Konstantinis et al,2018). Microorganisms additionally require a sure amount of nitrogen in feed to function their characteristic (Mittal, 1996). The fermentative bacteria makes use of carbon 25 to 30 times as speedy as nitrogen, as a result necessitating the finest C:N of 25 to 30:1. Deviation from this ratio slows down the system (Nijaguna, 2002). An excessive C:N skill that the nitrogen will be exhausted earlier than carbon is digested and conversely, a low C:N ratio means to an awful lot nitrogen in relation to carbon, which consequences in high ammonium concentrations tending to end up toxic to anaerobic micro organism (Mittal, 1996). Therefore the most efficient mix of the input is vital to get the optimal C:N of 25:1 to30:1. Hilland Roberts (1981) mentioned the activity of digesters is most when the C:N ratio

of the input feed was as soon as between 25:1 to 30:1. Table 2.4 indicates the C:N current in more than a few feed material.

Table 2.5 C: N ratios in various organic wastes (Nijaguna, 2002)

Animal Wastes	**C:N**	**Plant wastes**	**C:N**	**Domestic waste and refuse**	**C:N**
Cow-dung	25:1	Grass clipping	19:1	Raw garbage	25:1
Sheep manure	20.1:1	Hay	18:1	Bread	20:1
Horse manure/mule	25:1	Corn stalks	60:1	Potato tops	25:1
Pig manure	14.4:1	Cut straw	48:1	Kitchen vegetable scraps	16:1
Poultry manure/pigeon waste	5.2:1	Sea weeds	19:1	Rags	12:1
Night soil	8:1	Peanut stalk and Leaves	19:1	Household dirt	41:1
Farmyard manure (average)	14:1	Rice straw	67:1	Cabbage	12:1
Human urine	0.8:1	Bean stalks	32:1	Tomato	128:1

2.2.5 EFFECT OF LOADING RATE (LR) ON BIOGAS PRODUCTION

The rate at which biomass is supplied to the digester is termed as loading rate (LR) and is expressed in of kg of volatilesolids per m³of digester potential every day. The formation of gas depends on LR. The span of the digester also thus depends upon the loading, which in turn depends upon dilution, retention time, and temperature of processing.The diverse loading rate can be obtained by means of both changing the concentration of the solids in the influent or by varying the flow of inlet slurry in a digester. As studied by Mohanrao, 1975 loading rate of biomass dependent on night soil should be 1.04 to 2.23 kg VS per m³ of digester limit. He prescribed more loading rate for ambient temperatures. Cara et al., 2000 concentrated the impacts of adaptation of loading rate on biogas generation and saw that a hundred kg of interchange day loading delivered higher biogas as opposed to 50 kg of consistently loading. They furthermore presumed that for exact size of the plant, the choicest expense of loading rate will create most fuel and past that change in loading rate will now not make greater the fuel production proportionately

2.2.6 ROLE OF pH ON BIOGAS PRODUCTION

pH is a very significant variable in anaerobic digestion. The pH value has a strong influence on the degradation process. It impacts the growth fee of acetogen and methanogen microorganisms. Ammonia inhibition phenomena rely on pH and increases with amplification in it (Campos and Flotats, 2003).pH is the proportion of sharpness or alkalinity of the reactor system. Amid anaerobic aging, microorganisms require a fair-minded or somewhat antacid surroundings for effective fuel generation. A pH somewhere in the range of 7 and 8.5 is an ideal range for extended fuel yield (Mittal, 1996).

Fig. 2.6 suggests that when substrate concentration is much less than 20 mM/L, specific boom rate is the best possible with pH=6.0 followed by way of pH=6.5 and pH=7.0. With amplification in substrate concentration,the precise boom charge of microorganisms come to be greater with make bigger in pH from pH 6.0 to pH 7.0.

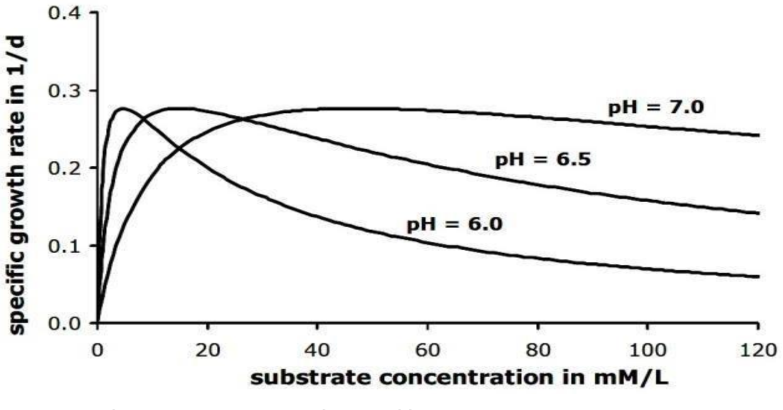


Fig. 2.6 Maximum specific growth rate for different pH values (Andrews and Graef, 1971)

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

HRT is characterized as the fundamental time spent with the aid of the records slurry inner the digester before it leaves it. HRT shifts between 20-120 days relying on the outline and running circumstance of the digester (Koszel et al, 2015). HRT taken in tropical territories like India is typically 40-50 days. HRT in bloodless climatic countries like the U.K., Canada, and China is taken to be one hundred days (Mittal, 1996).

Linke, 2006 presented that gas production varies with HRT as per the following relationship

$$P = \frac{HRT.k}{HRT\ .k1}$$

Where

p = y/y_m = biogas yield concerning maximum yield

y= gas production at any HRT

y_m= maximum gas production

k = the first- order kinetic constant

Figure. 2.7 present the biogas production with HRT for different kinetic constants. Biogas yield increases with HRT for all the values of kinetic constant. Moreover, increase in kinetic constant increases biogas yield for a specific HRT

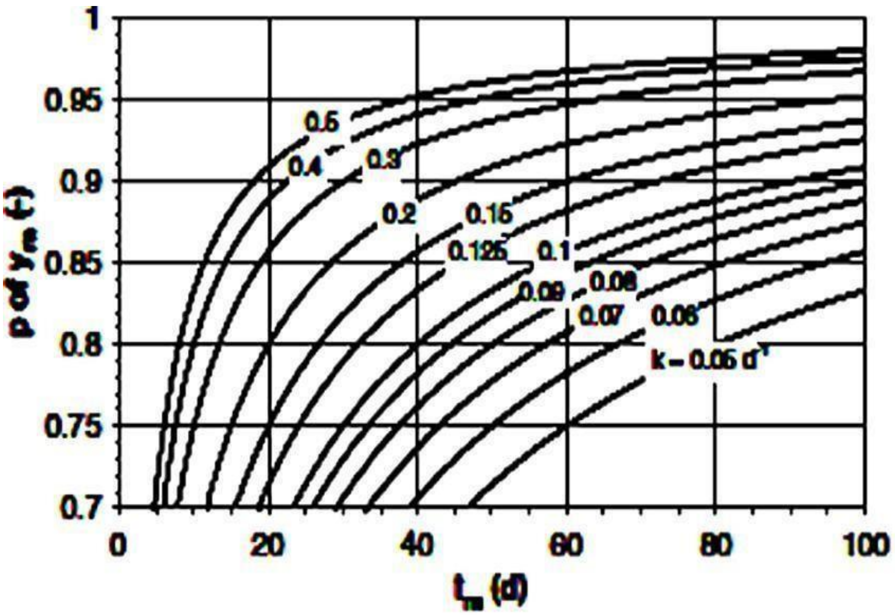


Figure. 2.7 Present the biogas production with HRT for different kinetic constants

2.2.8 EFFECT OF MECHANICAL STIRRING AND AGITATION ON BIOGAS YIELD

A mechanical stirrer helps the diffusion of the microorganism throughout the slurry, for this reason exposing them homogenously to the undigested material (Karim and Hoffmann, 2005). Further, it helps in mixing the clean organic material and nutrients with the slurry already retained in the digester. Stirring also helps in better temperature distribution enabling the microbial communities to thrive (Burton and Turner, 2003; Ward et al., 2008). Agitation helps in the launch of gas foam spellbound in the intermediate and prevents sludge of denser organic material on the bottom of the reactors nicely as stopping the formation of scum layers (Mahanta et al., 2005). Raheman et al., (2012) observed that mixing enhances the overall digestion charge through enhancing the mass switch fluxes. The stirring improves the particle suspension and will increase the solubility of the solid natural count number in suspension in the digestate (Pinho et al.,2004).

2.2.9 EFFECT OFADDITIVES ON BIOGAS PRODUCTION

Kumar et al., 1987 experimented to observe the effect of additive components on biogas manufacturing and showed that 5% of commercial charcoal addition to cattle dung on dry weight foundation improved the production of biogas by using capability of 17% and 35% in batch and non-stop processes, respectively. Geeta et al., (1986) studied the result of components on biogas production. They used vermiculite, charcoal, and lignin to bovine excreta as feed fabric and determined the expand in gasoline production by 15-30%.

2.2.10 EFFECT OF TOXICITY ON BIOGASPRODUCTION

A little volume of mineral particles (for example sodium, potassium, calcium, magnesium, ammonium, and sulfur) are expected to decrease the rate of growth and development of microbes, However, a similar mineral particles in overwhelming focuses produce harmful impact restraining the expansion of microorganisms. Some metals (for example copper, nickel, chromium, zinc, lead, and so forth.) in lower or micro concentrations increase the ordinary rate of growth and development of microorganisms (Youngsukkasem et al.,2013).

2.2.11 INFLUENCE OF TOTAL SOLID (TS) ONBIOGAS PRODUCTION

Many researchers had stated that TS of the feedstock performs a necessary position in the production of biogas. Fresh cattle wastes consist of 20% TS and 80% water. TS again incorporates 70% unstable solids and 30% constant solids. Since, 8 to 10% TS in feed is required to yield the most desirable amount of gasoline via anaerobic digestion, new dairy cattle compost is diluted with water in 1:1 proportion. Whenever required this proportion can contrast from 1:1.25 to 1:2 proportions [TERI, 1987]. Smith, 2009 expressed that the presentation of digesters containing dairy compost and order crop deposits is the most when the TS of the slurry was 8%

2.2.12 BIOSLURRY ASMANURES

Bioslurry can be used as fertilizer directly or added to the composting of other organic materials (Koszel et al,2015). Bioslurry is an already digested source of animal waste, and if urine (animal and/or human) is added, more nitrogen is added to the bioslurry, which can speed up the compost-making process. The bioslurry may contain 93% water and 7% of dry matter, of which 4.5% is organic matter and 2.5% is non-organic matter. Furthermore, bioslurry contains phosphorus, potassium, zinc, iron, manganese and copper, of which the latter has become a limiting factor in many soils. Bioslurry can be used to build healthy fertile soils for crop production. Indeed, bioslurry form and content stabilizes with double nitrogen content, which is different from Farmyard Manure (FYM). Furthermore, the quantity of the bioslurry is also higher than regular FYM. Bioslurry contains readily available plant nutrients and higher amounts of nutrients, micronutrients than FYM, and composted manure. The effects of bioslurry application are comparable to the effects of the application of chemical fertilizers. As such, bioslurry can be a serious alternative to chemical fertilisers (Warnars, 2013). The bioslurry can be applied:

(1) as a foliar fertilizer, being sprayed onto the crops;

(2) in liquid form(diluted) onto the rootsor;

(3) in dry and composted form (combined with irrigation techniques so that crops have sufficient water).

Bioslurry is an easily available form of compost compared to traditional compost. It is also attractive to mix with vegetative waste compost. Bio-slurry increases crop revenues by an average of 25% (Warnars, 2012). Seeds treated with bioslurry have given better germination rates (Gurung, 1998). With regards to tillage, note that the immediate incorporation of pig bioslurry through tillage would increase the N value and favor greater N/P fertilizer value. From experience, it is generally suggested to apply the bioslurry at a rate of 10 to 20 tons/ha in irrigated areas and 5 tons/ha in dry farming to have a significant increase in yields (Warnars, 2012). Bioslurry can be used for the following:

(1) as a basal manure and as a foliar application or spray;

(2) as a plaguerepellent;

(3) to increase soil fertility (cation exchange capacity), and improve the soil structure and water retaining capacity;

(4) to prevent soilerosion;

(5) to treat seeds for higher germination, disease resistance, better yields, improved coloration of fruits and vegetables, and tenderness and taste ofleafy vegetables;

(6) to increase the feed value of fodder with low proteincontent;

(7) for concentrated feed for cattle, pig, and fish and the productionofearthworms and algae;

(8) for the production of vitamin B12 and amino acids for animal growth. Also, it contains enzymes which stimulate hunger for more food intake and better nourishment of animals;

(9) as a means to increase the quality and quantity of organic fertilizer production at the farm levels;

(10) as manure for pot grown flowers andvegetables;

(11) to increase the availability of nutrients for soil micro-flora like nitrogen- fixing and phosphor solubilizingorganisms;

(12) to reduce and recycle the use of phosphate, a non-renewable source which is being depleted globally;

(13) to reduce wastewater, water pollution, greenhouse gas emissions and noxious odor;

(14) to reduce weed growth and to reduce attractiveness to insects or flies;

(15) bioslurry reduces the number of pathogens through sanitation and as such, it is almost pathogen free.

2.3 SUMMARY

Different methods such as physical, thermal, chemical, and biological were studied. Alkaline and biological treatment has shown more impact on delignification . It has been shown that most of the experiments were carried out on cattle dung whereas there are plenty of lignocellulosic bio-wastes with high cellulose content material. A very few studies have been done on biogas production from lignocellulosic biomasses. These studies are preliminary in nature and development. Based on literature it is found that wheat straw, rice husk and sugarcane bagasse show good potential for biomethantion as per their characteristic studies. Anaerobic digestion can be a useful process for the conversion of lignocellulose to biogas and manure .The parameters affecting biogas yield were studied .

CHAPTER - 3

CHARACTERIZATION OF BIOMASS

3.0 INTRODUCTION

Lignocellulosic biomass is recognized to be important feed material for the production of biogas. They are available in plenty throughout the world. Cellulose, hemicellulose, and lignin are the three most important elements of lignocellulosic biomass(Raven et al., 1992), crystallinity being due to the presence of cellulose. Hemicellulose is located between the micro-and macro fibrils of cellulose.. Fig. **3.1** shows the fiber content material present in thebiomass.

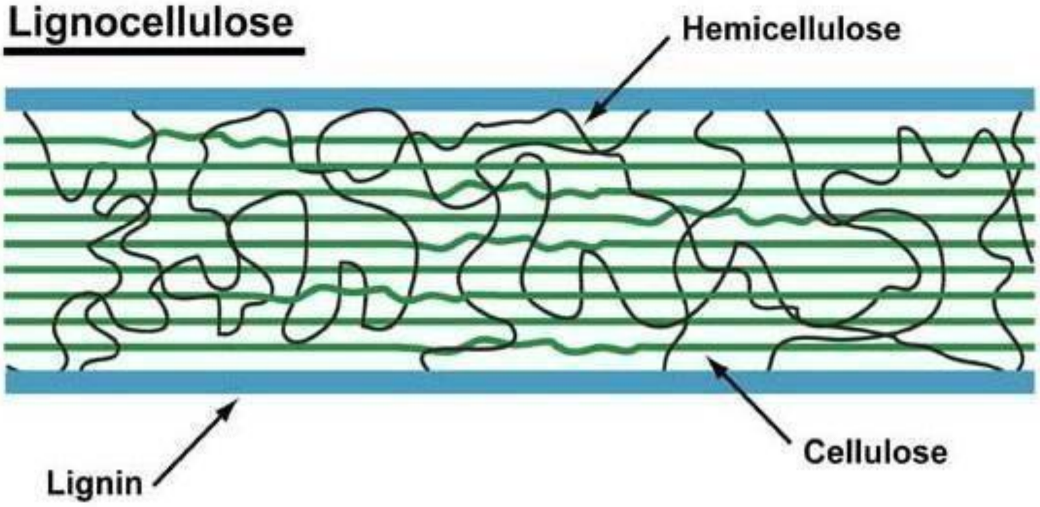


Fig. 3.1 Fiber constituents in biomass [Faulon and Carlson, 1994]

Lignin is responsible for the structure of the matrix in which cellulose & hemicellulose is present (Faulon and Carlson, 1994). Approximately 44% of the fermentable materials are shielded by lignin (Robbins et al., 1979). Due to poor degradation of lignin in anaerobic conditions, the velocity and degree of digestion of lignocellulosic material become incomplete (Fan et al., 1988). The lignocellulose is used for the generation of biogas, If lignocellulose is exposed in open environment, its breakdown will cause pollution and also have an effect on health. Proper identification and characterization of the specific biomass is essential to estimate the biogas production. Hence, identification and characterization of biomass plays a very important role to determine the biogas production potential. In the present study, a total of five biomass available in the Dehradun district of Uttarakhand,India, are collected and characterized. Based on the characterization and supporting literature, three different biomasses are identified from the group, for subsequent study.

3.1 SAMPLE PREPARATION

The agro residues (Rice husk, Wheat Straw, Sugarcane Bagasse) were collected from the Dehradun Region of India and the samples were washed. For proximate analysis sugarcane bagasse, rice husk, wheat straw, were chopped. All the samples of the biomasses were dried in a hot air oven for 24 hours and ground by ball milling and passing through the sieve and used for estimation of COD and calorific value

3.2 CHARACTERIZATION OF BIOMASSFEEDSTOCK

Three different biomasses used for characterization were (1) rice husk (2) sugarcane bagasse (3) Wheat Straw. These biomasses were identified based on the supporting data from the literature as well as their abundance in and around the Dehradun city (Uttarakhand) India. The characterization of the above mentioned lignocellulosic biomass was carried out by performing proximate as well as ultimate analysis. Determination of chemical oxygen demand,the calorific value of the biomass was also performed on the biomass to check their potentiality for biogas production. Limited scientific literature is available on this lignocellulosic biomass. The present work of biogas production from agro wastes was consolidated by studying the following parameters during an experiment in feedstock and fresh cattle dung(used as inoculum).

1. Moisture content
2. Volatile Mattercontent
3. Ashcontent
4. Total Solidcontent
5. Lignin
6. Cellulose
7. Hemicellulose
8. pH
9. Elementalanalysis
10. Chemical OxygenDemand

The details of the method are given below:

3.2.1 PROXIMATE ANALYSIS

Moisture content , Volatile matter content and, ash content were determined by proximate analysis.

3.2.1.1 MOISTURE CONTENT

For determination of moisture content, 2gmof biomass samples was taken in a porcelain crucible, using an electronic balance.It was then dried in an oven at 105±3°Cfor 3 hours and cooled in desiccators to room temperature.The final weight of the dried sample was noted after cooling to room temperature. The moisture content of biomass is characterized because the amount of water per unit mass of dry biomass affects its heating value. High moisture indicates low heating value since heat is necessary to evaporate the moisture. The % of the moisture content present in samples was calculated by using the following formula:

$\%M = W_2 - W_3 / W_2 - WW_1 \times 100$ **3.1**

Where: %M= moisture content (in percentage)

W_1 = weight of the empty crucible

W_2 = weight of the crucible and sample

W_3 = Finalweight ofthe crucible and sample(after heating in ovenand cooling)

The details ofexperiment are given in Appendix -1

3.2.1.2 VOLATILE MATTERCONTENT

The determination of Volatile substance content was done by methods E872-82(**REF**). Approximately 1 g of the oven- dried sample was used for the volatile matter determination. This sample was heated at 950±20°C and allowed to ignite in a muffle furnace for seven minutes in pre-weighed porcelain crucible with lid. The sample was cooled at room temperature using desiccator. The final weight was recorded.

Volatile matter $\% = W_2 - W_4 / W_2 - W_1 \times 100$ .... **(3.2)**

Here

W_1 = weight of the empty crucible

W_2 = weight of the crucible and sample

W_3 = final weight of the crucible and sample(after heating in oven and cooling)

W_4 = weight of crucible and sample(after heating in muffle furnace at 975°C & cooling)

The details of experiment are given in Appendix -1

3.2.1.3 ASH CONTENT

This sample was heated in a muffle furnace at 575±25°C, for 3 hours in a pre-weighed porcelain crucible. After this, the sample was cooled to room temperature in desiccator. The crucible was finally weighed after cooling to room temperature by using the equation 3.3 given below:

$$\text{Percentage of ash content}(\%\text{ash}) = \frac{(W_5 - W_1)}{(W_2 - W_1)} \times 100 \ldots\ldots \mathbf{(3.3)}$$

Here:

W_1 = weight of the empty crucible

W_2 = weight of the crucible and sample

W_5 = final weight of crucible and sample(after heating in muffle furnace at 575°C & cooling)

The details of the experiment are given in Appendix -1

3.2.1.4 FIXED CARBON CONTENT

The fixed carbon in the sample is a measure of its combustible part responsible for energy production . The fixed carbon content in the sample was determined by equation(3.4) :

Percentages of Fixed carbon= 100 – (Moisture content + ash percentage + Percentages of volatile matter) .... **(3.4)**

The details of the experiment are given in Appendix -1

3.2.1.5 TOTAL SOLID (TS) CONTENT

Total solid content was determined by ASTM E1756-08(Hamelinck et al., 2005) method. 2 grams of biomass sample was weighed in a porcelain crucible using and dried in a hot air oven that is drying oven at 105±3°C for 3 hours. After cooling the final weight was noted. The total solid content present in sample biomass was calculated by using the equation given below: eq 3.5.

Where

Equation

$$\text{Total solid} = \frac{(m_{f1} - m_t) \times 100}{(m_{i1} - m_t)} \quad \textbf{(3.5)}$$

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

m_t= tare mass of dried container

m_{i1} = initial mass of container and biomass

m_{f1} = final mass of container and biomass after drying at 105

The details of the experiment are given in Appendix -1

3.2.1.6 ULTIMATE ANALYSIS

This ultimate analysis determines the weight percentage of basic elements present in biomass including C, H, N, O, and S.These analyses were done to determine the elemental composition of biomass using the CHNS Analyzer.

3.2.1.7 CALORIFIC VALUE OF FEEDMATERIALS

The calorific value of samples is the quantity of heat liberated on burning its unit mass completely in thepresence of oxygen. It is expressed in MJ/kg,or MJ/g or another appropriate unit similarly. It was determined using isothermal bomb calorimeter. The bimass sample was placed in a enclosed vessel and burnt at a constant volume in the presence of excess oxygen by igniting electrically.The water equivalent of the bomb calorimeter was determined by burning the known amount of benzoic acid in a calorimeter and the amount of heat liberated by the combustion process by a known amount of water. After that the calorific value were determined by using the equation: Eq. (3.6)

$$C = \frac{W_C \times \Delta T}{M_s} \quad \textbf{(3.6)}$$

where

C_v = Heat of burning of the biomass sample, MJ/kg **(3.6)**

W_c= equivalent of water the bomb Calorimeter, MJ/°C

$W_c = 10.74 \times 10^{-3}$ MJ/°C

=2568.293 cal/°C

ΔT = Rise in temperature, °C

M_s = Mass of the biomass sample burnt, kg

The details of experiment are given in Appendix -1

3.2.1.8 FIBRE ANALYSES OFBIOMASS

The fiber content of lignocellulosic biomass like hemicellulose, lignin and cellulose were analysed by determining NDF (neutral detergent fibre), ADF (acid detergent fiber) and ADL (acid detergent lignin). This analysis was done by using the reflux condenser apparatus (Goering, et al. 1970). Neutral detergent was used for the determination of the total lignocellulosic material, whereas acid detergent fibre was used for the separating fiber and lignin. On the other hand hemicellulose was calculated as different percentages of neutral detergent fiber and acid detergent fiber. The content of the cellulose was determined by the percentage difference between neutral detergent fiber & acid detergent lignin.

3.3 PROXIMATE ANALYSIS

The biogas production was carried out using three different lignocellulosic materials that is sugarcane bagasse, wheat straw, and rice husk. Characterization of fresh cattle dung was also carried out. Fresh material of the samples was used present in triplicates and analyzed for the volatile matter content, moisture content, ash, and total solid. These parameters are the main indicator of potentiality for biogas production. The physiochemical properties of the feed material obtained from the analysis have been discussed in the comingsection.

Fig. 3.2 showsthe results ofthis moisture content in different biomass.

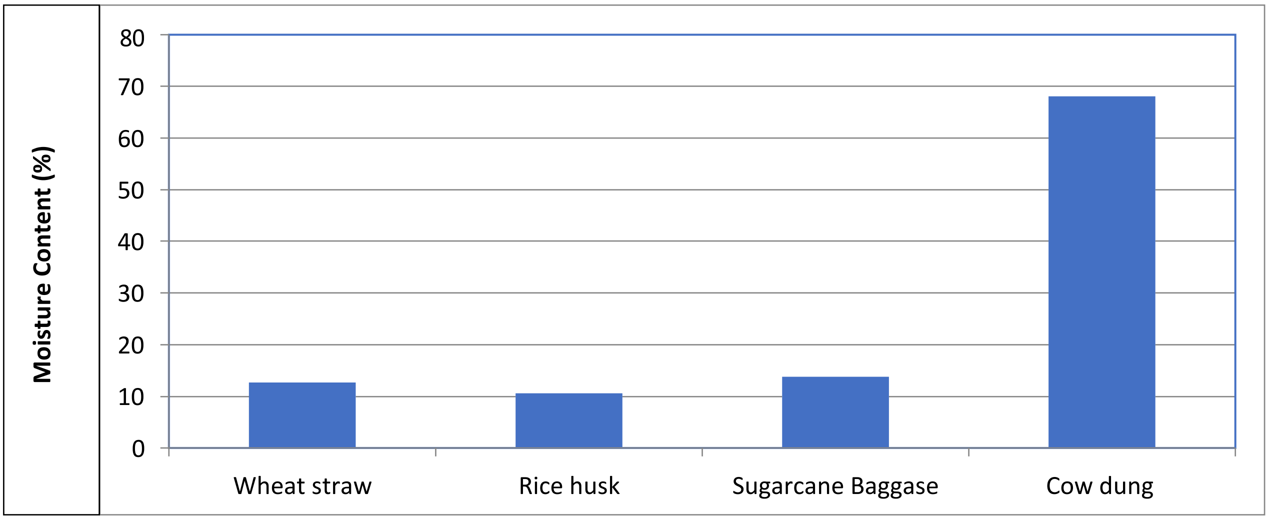


Fig. 3.2 Moisture content of biomass

It was observed that for rice husk and wheat straw, sugarcane bagasse, moisture content ranged from 10-14%. For fresh cattle dung, moisture percentage varied from 65–82%.

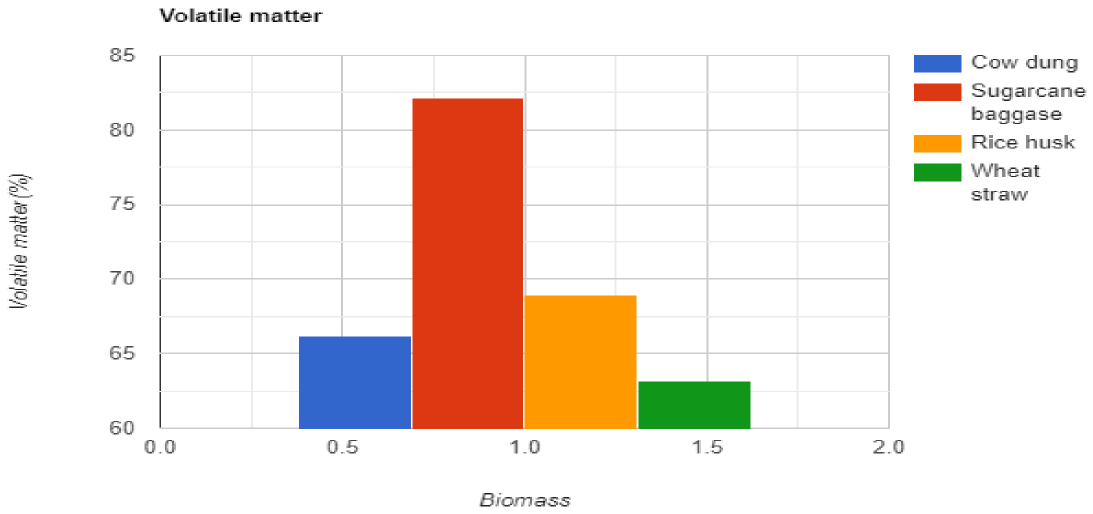


Fig. 3.3 Volatile matter content of biomass

Fig. 3.3 shows the volatile matter content in the lignocellulosic biomasses. It was observed that volatile matter contents of the biomasses were quite good and ranged from 63-83% which is in agreement with early literature (Miskam et al., 2009). High volatile matter content indicates good potential for biogas production. The importance of the VM (Volatile matter) and carbon lies in the fact that they are responsible for how easily the biomass can be dissolved.The ash content of biomass is the non-volatile inorganic matter which remains after subjecting it to a high decomposition temperature 500˚C-550˚C. Lower the ash content better is the biomass fuel production. It is observed from the data in Fig. 3.4 that rice husk has got very high ash content (14.10%), followed by wheat straw (5.46%). Elephant grass and banana leaves (dry as well as green) contain ash content approximately 13-14%. Sugarcane bagasse contains less than 5% ash content. Ash content of sundried cattle dung is found to be 12%.The ash content of biomass is shown in fig 3.4

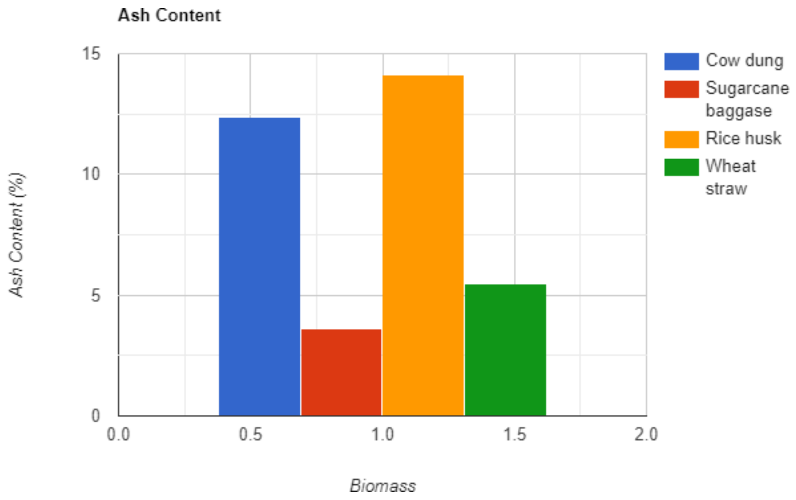


Fig. 3.4 Ash content of biomass

The fixed carbon content of biomass is shown in Fig. 3.5. It is observed that the fixed carbon content of rice husk, sugarcane bagasse, wheat straw, fall in the range of 10-15%, whereas in case of cattle dung, fixed carbon varies ranges from 19-26%. High fixed carbon indicates good potential for biogas production.

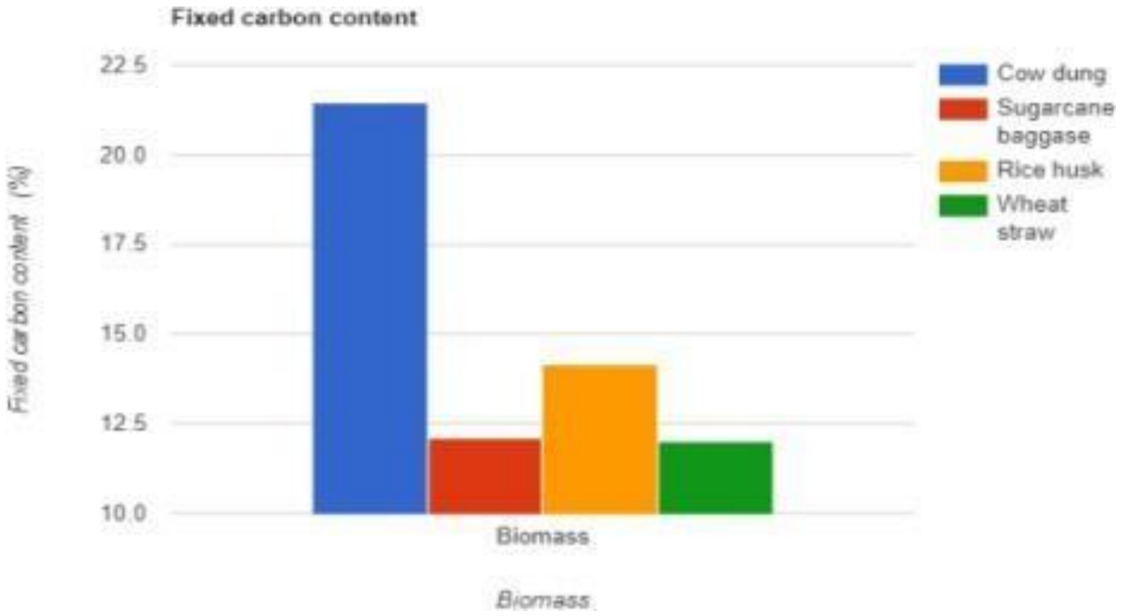


Fig. 3.5 Fixed carbon content of biomass

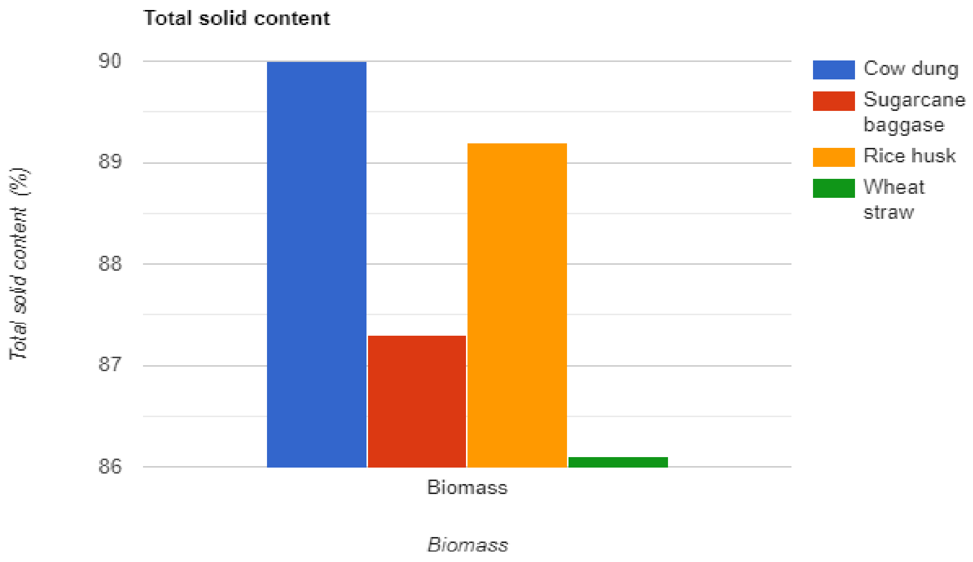


Fig. 3.6 Total solid content of biomass

Total solid(TS) is an important parameter for biogas production. Roberts, & Hills,1981 studied that in the case of cattle dung,the best biogas yield was obtained when the total solid is 8%. Budiyono et al., 2010 evaluated that TS content in cattle dung is 7.4 and 9.2% give the best result for digestibility. TS mainly consist of organic and inorganic matter present in biogas. Fig. 3.6 shows the TS content of the biomasses. From the analysis, it was found that TS of rice husk, sugarcane bagasse, wheat straw, ranges from 85-90%.

Table 3.1 shows the evaluation of proximate analysis data that mentioned the material and literature data. It is observed that the data obtained from the proximate analysis of the feed material in this work is approximately similar to the most ofthe available review/literature.

Table 3.1 Comparison of results of proximate analysis of the feed material and literature

Biomass type	Present work			(MJ/kg)	Literature data				(MJ/kg)	Literature
	FC	ASH	VM		FC	ASH	VM	M		
Cattle Dung	21.45±0.40	12.35±0.21	66.2±0.43	14.676±0.01	19.3	19.3	46.4	15	11.4	Roy et al., 2010
Sugar. Bagasse	12.10±0.15	3.60±0.10	82.17±0.02	18.26±0.24	12.4	2.1	85.5	--	--	(Jiang et al 2018)
					15.8	2.9	84.2	--	--	(Jiang et al 2018)
					13.15	3.2	83.66			Yin C.Y., 2011
Rice Husk	14.13±0.30	14.10±30	68.96±0.36	14.01±0.01	19.2	18	62.8	--	--	Miles et al., 1995
					13.1	0.8	73.8	12.3	13.36	Vijay et al., 2009
					18.4	23.5	81.6	--	--	(Rudakiya et al, 2017)
					16.95	21.24	61.81			Channiwala and Parikh, 2002
Wheat Straw	12.03±0.25	5.46±0.24	63.15±0.27	12.73±0.31	15.6	20.1	64.3	--	--	Miles et al., 1995
					16.55	12.64	65.23	5.58	14.40	Li et al., 2009
					13.91	20.38	65.7			Channiwala and Parikh, 2002

3.4 ULTIMATE ANALYSIS(UA)

The UA of biomass was done for studying the chemical composition of the biomass. Table 3.2 shows the comparison of the ultimate analysis of the lignocellulosic biomass obtained from the present work and the literature. The data analysis was done by ASTM and elements analysis (CHNSO) was determined by a Flash 2000.

Table 3.2 Comparison of results of ultimate analysis of the feed material and literature

Substrate	Present study				Literature value				
Feed Material	C (%)	N (%)	O (%)	Si (%)	C (%	H (%	N (%	O (%	Literature
Cattle Dung	32.5 ±0.20	1.7± 0.10	50.42 ±0.17	0.01± 0.005	33.33	--	1.68	--	Raheman and Mondal, 2012
					31.6	5.18	6.12	37.8	Roy et al., 2010
S. Bagasse	47.39 ±0.16	0.63 ±0.14	44.78 ±0.05	--	43.8	5.8	0.4	47.1	Kirubakaran et al., 2009
					49.8	6.0	0.2	43.9	Miles et al., 1995
Rice Husk	42.19 ±0.23	0.455 ±0.11	38.75 ±0. 18	--	38.9	5.1	0.6	32.0	Kirubakaran et al., 2009
					49.3	6.1	0.8	43.7	Veluchamy et al,2017).
Wheat Straw	40.17 ±0.26	0.984 ±0.006	34.58 ±0.36	--	36.9	5.0	0.4	37.9	Kirubakaran et al., 2009
					50.1	5.7	1.0	43	Miles et al., 1995

3.4.1 CALORIFIC VALUE OFBIOMASS

The data (Fig. 3.7) shows that the calorific values of the lignocellulosic biomass are quite good, which ranges from 12 to 19 MJ/kg. It is the highest in the case of sugarcane bagasse (18.26 MJ/kg) followed by rice husk (14.01 MJ/kg), wheat straw (12.73 MJ/kg).Calorific value of cow dung 14.676 MJ/kg.((Dhungana et al., 2012)

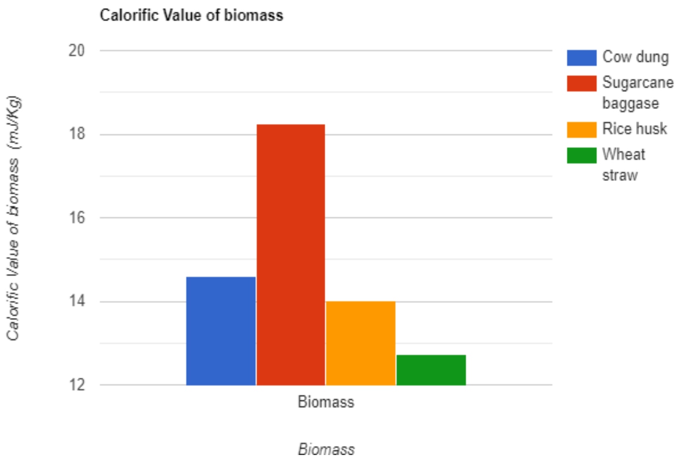


Fig. 3.7 Calorific value of biomass

3.4.2 FIBRE ANALYSIS

Figure 3.8-3.11 elaborate on the outcomes of fiber evaluation cellulose content (%),hemicellulose content (%),Lignin content (%) and lignin to cellulose ratio.

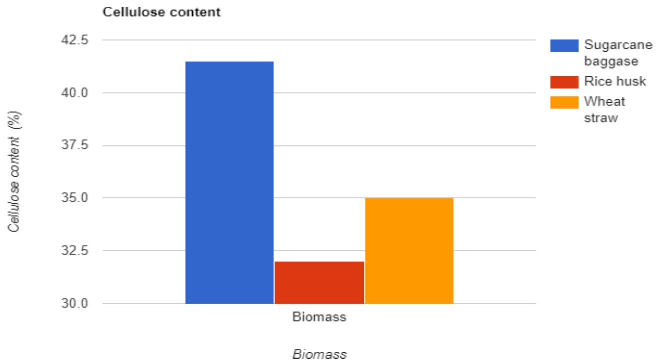


Fig. 3.8 Cellulose content (%) of biomass

Figure 3.8 shows the cellulose content of the above- mentioned biomass. Higher cellulose content indicates good potential for biomethanation (Veluchamy et al, 2017). Sugarcane bagasse has the highest cellulose content (41.5%) followed by wheat straw (35%)and Rice husk (32%)cellulose content .Fig.3.9 shows the hemicellulose content of lignocellulosic biomass. It is seen that all of the biomass has got a high value of hemicellulose content.

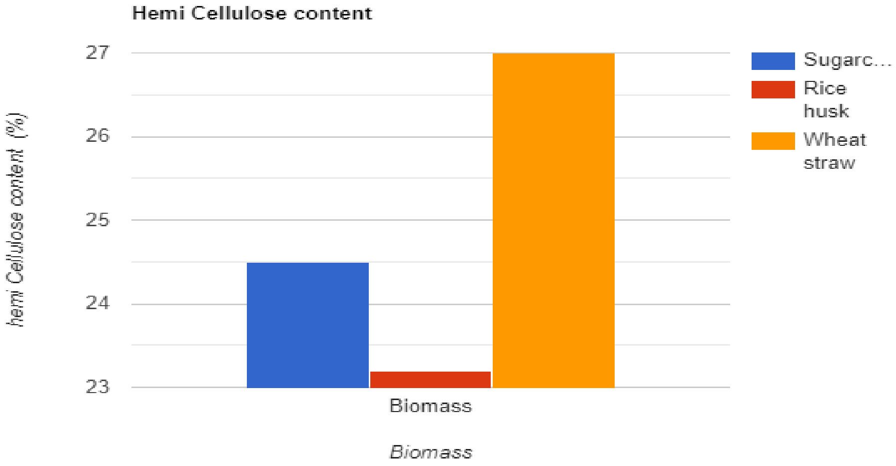


Fig. 3.9 Hemicellulose content (%) of biomass

Sugarcane bagasse has hemicellulose content in the range of 24.5%. Rice husk and wheat straw present 23.2%, 27% hemicellulose content respectively. Fig. 3.10 shows the lignin content (%) of the above- mentioned biomass. It indicates that the superior the lignin content lower is the digestibility of the biomass in anaerobic conditions. It binds the cellulose and hemicellulose content in an intense cross- link. Therefore it is required to break the lignin before using the biomass for fermentation or biomethanation.

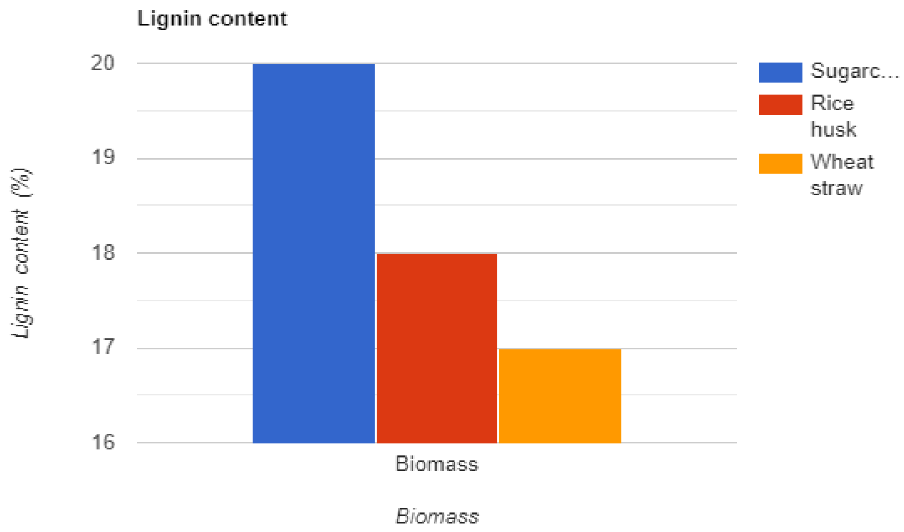


Fig. 3.10 Lignin content (%) of biomass

Rice husk is having a considerable amount of lignin (18%). whereas for sugarcane bagasse lignin content is 20% and for wheat straw is 17%(Fig 3.10) . Cattle dung has got very little lignin content in the range of 3-8%. Lignocellulosic biomass gives a unique and sustainable beneficial aid for environmentally invulnerable natural fuels and chemicals. However, the digestibility of lignocellulosic biomass is low owing to structural elements such as lignin content, crystallinity etc. In case of lignocellulosic biomass the lignin to cellulose ratio is typically used to outline the degree of digestibility of the biomasses(Sharma, 1988).

From the Fig. 3.11 it is clear that lignin to cellulose ratio is the highest in the case of rice husk (0.562) followed by wheat straw (0.491), sugarcane bagasse (0.481) respectively. The estimate for the characterization of biomass is given in Appendix I.

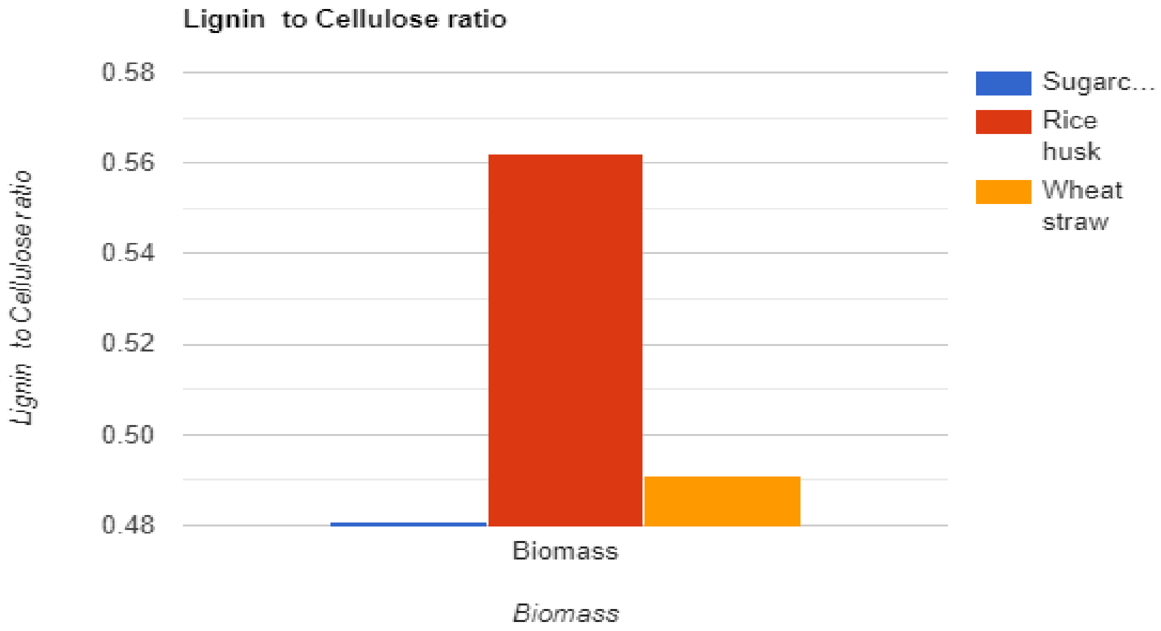


Fig. 3.11 Lignin to cellulose ratio (%) of biomass

3.5 SELECTION OF LIGNOCELLULOSIC BIOMASS FOR PRESENT STUDY

Based on the results of characterization, feed materials were selected for carrying out the biomethanation process. The selection criteria were based on the fact that the chosen feed material should have good volatile substance and carbon content, high calorific value, less ash content, and an acceptable percentage of cellulose content. The three feed materials selected for carrying out biomethanation were sugarcane bagasse, rice straw and rice husk. All of them have more than 60% volatile matter content, more than 10% fixed carbon content, more than 14 MJ/kg calorific value and good cellulose content which makes them good potential for biogas production. Ash content of sugarcane bagasse are less than 5%, whereas rice husk and wheat straw and, rice husk have approximately 15% and 20% ash content. Since the lignin content of this biomass is high, pretreatment of these biomasses was carried out using physical, chemical, and biological which eventually helps in increasing their digestibility.

3.6 SUMMARY

Analysis of the lignocellulosic biomass was carried out by performing proximate analysis, ultimate analysis, determining the calorific value, and estimating the fiber composition of the biomass. It is observed from the results that lignocellulosic biomass have a very high amount of volatile substance content, carbon content, and high calorific value which make them a potential sources of renewable energy. Furthermost of the biomass have a very high amount of lignin content too that resist the decomposition of cellulose content of the biomass. As a result, the fermentation of lignocellulosic biomass takes a long period as compared to other non-lignocellulosic biomass. To avoid the delay in the hydrolysis process of fermentation, the pretreatment of biomass is done. Pretreatment of biomass breaks the lignin of the biomass beforehand thus exposing the cellulose of the biomass which makes it easier for the bacteria to decompose the biomass effectively thus avoiding delay in the hydrolysis process. Subsequently, chapter 4 presents the pretreatment of biomass and anaerobic digestion for the production ofbiogas.

CHAPTER - 4

RESEARCH METHODOLOGY

4.0 INTRODUCTION

The bioconversion of lignocellulose based agricultural waste includes three significant stages that at last lead to the creation of biofuel. The stages incorporate

1. Pretreatment
2. Hydrolysis of cellulose to fermentable sugars
3. Anaerobic digestionofsugars to yield the purified biogas

Pretreatment is required to experience perfection of hydrolysis of lignocellulose biomass to discharge high concentration fermentable sugars (Veluchamy et al, 2017). Real sorts of pretreatment incorporate physical, chemical, physicochemical and enzymatic procedures and after pretreatment, the cellulose is broken down to glucose by microbial hydrolysis, which is digested to yield biogas with the help ofmicroorganisms.

Methane production from organic waste is always a better way of utilizing biomass as compared to produce ethanol worldwide(Chandra 2012). In terms of yield/input proportion, it is an effective process as all components (carbohydrates, Protein) of biomass can be converted to biogas as compare to bioethanol production in which only sugar unit breakdowns to ethanol by a microorganism (Gerardi, 2003). In this process, most of the biomass substances (starches, fats, and proteins) in anaerobic assimilation are changed into products, which then changes to biogas (CH_4, CO_2) with the assistance of various sortsofanaerobic smaller scale life forms (Mtui, 2009).

Horticulture isn't just significant from the financial perspective. Even farming waste, for example, wheat straw, rice straw, can assume a significant job. Specifically, lignocellulosic biomass harvests waste has immense unutilized energy generation potential. A gauge demonstrates that the worldwide yearly generation of wheat straw biomass remained at 681.92 million heaps from 225.437 million hectares of all-out developed areas in 12 months 2009 (Duke, 1983). A large amount of this wheat straw has been used as animal feed, some of the above stay unutilized or is singed in open condition, which is an unsustainable and non-recommendable practice. The grasp elicitation has confirmed that cellulosic biofuel innovation is very needful and considerable for the biofuel’s advent to succeed over the long haul reasonably. Further, it has been discovered that biogas-to-power is among innovations having expanding creation potential on criteria-based assessment of low carbon control advances. Consequently, biogas can contribute to carbon impartial power. Kaparaju (2010) suggested that the use of wheat straw for biogas creation or multi-fuel generation is the most effectiveprocedure when compared to the generation of mono fuel, for example, bio-ethanol. Hence, numerous biofuels generation from wheat straw can increment the effectiveness in material and vitality and can be a progressively affordable procedure for biomass use.

Contemporary mechanical advancement and fast pace of urbanization have created a need for different renewable sources of energy which can be A sustainable root to meet the energy demand of the world. Biogas produced from biomass is an important ecological financial enhancer advantage and can be considered as a safe and cleanest gaseous fuel option in contrast to non-renewable energy sources. Noteworthy, advances have been made at lab scale towards the biogas formation from cellulosic biomass. Many hurdles make the vaporous fuel program fruitless. At industrial scale the (enzymatic) hydrolysis of lignocellulose is restricted by a few elements. The lignin shapes a defensive covering, which shields cellulose and hemicelluloses from degradation by microscopic organisms. To utilize biomass effectively, pretreatment is required for depolymerization of lignin before the conversion of cellulose, and hemicellulose into simple sugars. Pretreatment advances based on various physical, chemical and biological techniques have been designed, which modify the structure of lignocelluloses and expel lignin. Pre-treatment will in general upgrade the biodegradability of most substrates and in this way increment the quality and amount of biogas which can be utilized for developing energy need. The uncovered complex starches, for example, cellulose and hemicelluloses are then hydrolyzed to fermentable sugars. A successful pretreatment procedure must have the following preferences:

- It should protect and decrystallize the celluloses.
- It should depolymerize hemicelluloses.
- It should limit the formation of inhibitors which oppose the hydrolysis of starches.
- It should have low energy input.
- It should be cost- effective.

Many physical, chemical and microbial pretreatment techniques have been accounted for improving bioconversion of lignocellulosic materials (Sun S. et al., 2016). The lower hydrolysis rates during the traditional AD process results in higher hydraulic retention time (HRT) in the digester and bigger digester volume, comprise the prime downsides of the customary AD (Sakuragi; et al. (2018). Pretreatment of biomass upgrades the AD, with lower retention time and with causing higher biogas creation (Oladi; et al. 2017)

Acid pretreatment of biomass requires its minimum amount, but the process is operated at very high temperatures, requires high energy input (Liu and Wyman, 2003; Hendriks and Zeeman, 2009). The presence of acids at high temperatures can be destructive, and hence, the procedure requires explicit response vessel which have to be impervious to these conditions. Corrosive treatment creates inhibitors that must be expelled and thus downstream handling expenses are high.

The alkaline pretreatment includes the use of calcium, sodium, and potassium hydroxides giving higher yields of diminishing sugars with biomass having low lignin content (Singhania et al., 2006).Although lime and different hydroxides are economical, downstream processing expenses are high. The immense use of water for washing salts of calcium and sodium, which are joined into the biomass are hard to expel. Likewise, the procedure additionally creates inhibitors during the depolymerization of lignin which must be expelled before the hydrolysis step.

The advancements in different pretreatment methods viz thermal, chemical, mechanical, biological & and their combination efficiently enhance the biodegradability of biomass. Broad research has been conveyed all through the world to set up the best monetarily feasible pretreatment innovation to upgrade the absorbability of biomass (Nair et al., 2015).

4.1 MATERIAL & METHODOLOGY

4.1.1 GLASSWARE

Glassware used in the experimental work included shown below**:**

Sr. No.	**Glassware**
1.	Conical Flask
2.	2 L Beaker
3.	Spatula
4.	Test tube
5.	Crucible

4.1.2 EQUIPMENT

The equipment used in the experiment is given below in table 4.1.

Table 4.1 Inventory of Equipment

Sr. No.	Equipment Name	Make	Specification
1.	Weighing Balance	Shimadzu analytical balance	0.0.1 gm to 200gm
2.	Muffle Furnace	Bionics	Room temp to 1200
3.	Hot air oven	Bionics	Room temp to 300
4.	UV-Visible Spectrophotometer	Shimadzu	200nm to 700nm
5.	CHNS Analyzer	Nucon	Heavy metal
6	Gas Analyser	Thermo	Porapac Q

4.1.3 CHEMICALS AND STANDARDSOLUTION:

The chemical used during different pretreatment method are as follow in table 4.2.

Table 4.2 List of Chemical:

Chemical	Manufacture	Country
Hydrochloric acid and Sulphuric acid	Himedia	India
Sodium Hydroxide	Oxford lab. Chem.	
Urea	Sigma	USA
Calcium Hydroxide		
Lignin	Sigma	USA

Standard solution:

Standard lignin tests of 1 and 10 ppm were set up by dissolving the required amount of pure lignin (Rankem) in a suitable volume of distilled water.

4.1.4 INOCULUM

The inoculum stands for microbial culture used for biomethanation. The inoculum used in this research was taken from the mesophilic anaerobic digester plant situated at the University of Petroleum and Energy Studies, Dehradun, Uttrakhand. The pretreated biomass mixed with cow dung as inoculum for anaerobic digestion in ratio biomass to cow dung(1:2) The inoculum was kept in an oven at 35°C (mesophilic temperature) in different storage containers. The inoculum that was used after a few days is defined in this studyas fresh inoculum.

4.1.5 BIOMASS (RICEHUSK, SUGARCANE BAGASSE, WHEAT STRAW)

Sugarcane bagasse Rice Husk, Wheat Straw as waste was gathered from nearby market of Dehradun, Uttarakhand. This was then mechanically broken down using physical treatment methods and separated using sieve shaker to get particles with size across under 0.35 mm. or equivalent to 1 mm was gathered for further use. The molecule size of 1 to 2 mm has been prescribed for the hydrolysis of lignocellulosic biomass. The samples were packed in sealed packets kept at room temperature.

4.1.6 DESIGNING LAB SCALE REACTOR FORBIO-PROCESSING

A 10L-Batch bioreactor furnished with pH probe, stirrer, sampling port, and temperature controller was designed in the present study. The working volume of the bioreactor was 8L. The fermenters were mounted on stage shakers at 140 rpm. The fermenters were maintained at stable temperature (set at 30ºC), and temperatures were kept up utilizing warming tapes folded over the fermenters. Biogas creation was gathered in gas - impermeable Tedlar packs. Biogas volume was estimated by the solution displacement method.

I. The setup for the gathering the gas over water consisted of a digestor where the anaerobic digestion(where anaerobic digestion takes place) takes place and a gas accumulation compartment loaded up with water and a calibrated volumetric cylindrical tube. The gas produced was gathered by connecting one end of a hose to the response holder .The quantity of gas formed was measured withrespect to water displaced.

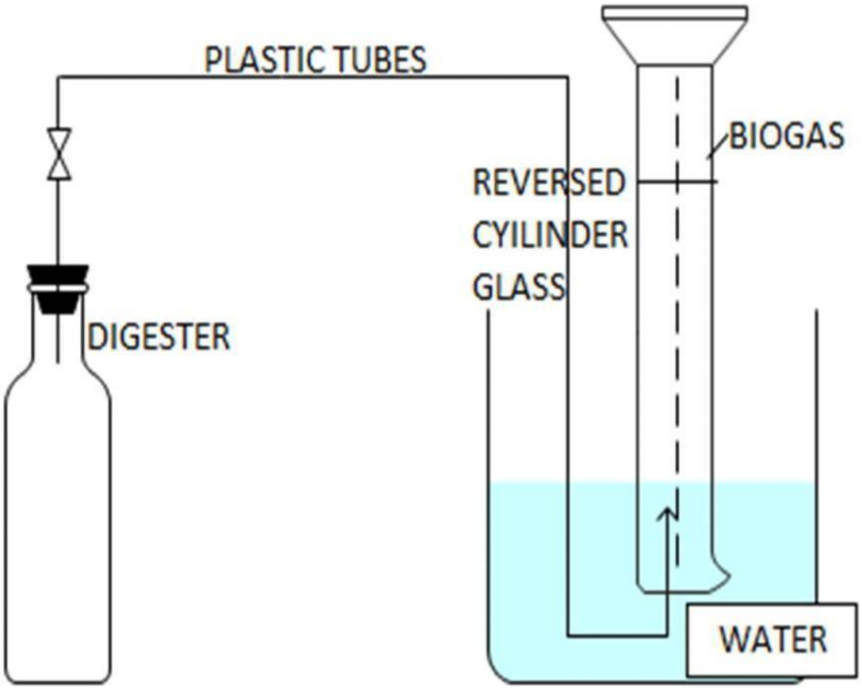


Fig. 4.1 REACTOR DESIGN

4.2 EXPERIMENTAL PROCEDURE &METHODOLOGY

The experimental sequence comprised of three phases:

- The first phase focused onthe pre-treatment of lignocellulose biomass (WS, RH, SB).
- The second phase included batch anaerobic digestion tests carried out according to the Biomethane potential protocol **(**Tomas-Pejoet r4 al., r4 2008**)** .
  1. Anaerobic digestion of untreated biomass (WS, RH, SB) with cow dung as a source of microbial inoculum.
  2. Anaerobic digestion ofpretreated biomass (WS, RH, SB) with cow dung.
  3. Optimization of treated biomass for various parameter affecting biogas production
- The third phase involved the analysis ofslurry as manure.

4.2.1 BIOGAS PRODUCTION POTENTIAL OF BIOMASS (WS, RH, SB)

Biochemical methane potential (BMP) test is broadly used as a test for assessing the production of biogas creation from a given substrate by the use of anaerobic digestion. The BMP test is typically operated for 20 to 30 days or until biogas production turns into steady at a low rate.The BMP test was used out to resolve the methane potential of Biomass (WS, RH, and SB). Reactors of 1000 ml capacities and Duran bottles were used. All reactors preposition with nitrogen gas for 3 minutes, and then sealed with rubber stoppers and kept in a shaker incubator. All BMP assay has been conducted in the replica at 37˚C for 30 days. The substrates to the inoculum had been mixed at 1:2 on a VS basis. Each bottle had a working quantity of 500 mL. The biogas extent produced from each digester was once measured once daily. Furthermore, some reactors acquired only cow dung as a control.

- In the first phase of experimental set-up I was carried out using biomass (WS, RH, SB),in which proximate and ultimate analysis was done to assess the impact onthe biogas yield.
- The second phase of alkali acid and thermal pre-treatment of biomass samples (Experimental set-up II) was carried out using different percentages of NaOH solutions (w/w) and sulphuric acid at a temperature of 121°C and time of30 to 180 minutes.
- The third phase was employed to optimize pre-treatment condition for NaOH concentration (1–3.0%), temperature (121°C) and reaction time (60−180 minutes), Sulphuric acid concentration (1%, 30%, 40%, 60%, 72%), temperature (121°C), and reaction time (60−180 minutes); thermal pretreatment also carried under period. All pre- treatment conditions are reported in Table4.3

Table 4.3 Pretreatment variables for Biomass

	Chemicalpretreatment									Biological pretreatment
	Acid					Alkali				
	1%	30%	40%	60%	72%	1%	2%	3%	Neutral Medium	Fungi
Variable	30 minute 60 minute 120 minute 180 minuet	30 minute 60 minute 120 minute 180 minute	30 minute 60 minute 120 minute 180 minute	30 minute 60 minute 120 minute 180 minute	30 minute 60 minute 120 minute 180 minute	30 minute 60 minute 120 minute 180 minute	30 minute 60 minute 120 minute 180 minute	30 minute 60 minute 120 minute 180 minute	30 minute 60 minute 120 minute 180 minute	*P. chrysosporium* *A. niger* *A. flavus* *C. contiment* *H. solani*

4.2.2 PRETREATMENT OFBIOMASS

Pretreatment of lignocellulose biomass was done by:

- Physico-chemical method
- Chemical method
- Biological Method

4.2.3 BIO-DIGESTION OF PRETREATEDBIOMASS

Experiments was carried out with digestion of treated wheat straw, rice husk with sugarcane bagasse at mesophilic temperature of 35-40^{o}C and mixing was aided by a mechanical stirrer. Quality & quantity of biogas produced was studied at different reactor operational conditions (Temp; pH; solid to liquid ratio,C:N ratio) Quantitative and qualitative analysis of biogas produced was done by water displacement method and GCrespectively.

4.2.4 ANALYSIS OFMANURE

The slurrycollected from the bioreactor was recycled back to the reactor and leftover slurrywas examined for its N: P: Kratio**.**

4.2.5 PRETREATMENT OFBIOMASS

4.2.5.1 PREPARATION OF STANDARD LIGNINSOLUTION

Standard lignin tests of 1 and 10 ppm were set up by a dissolving small amount of lignin (Rankem) in a suitable volume of distilled water.

4.2.5.2 PHYSICAL PRE-TREATMENT

All the straw samples were grounded to acquire a 1 mm and stored at room temperature. This sample was characterized as untreated biomass.

4.2.5.3 ACID TREATMENT OFBIOMASS

Pretreatment of biomass(WS, RH, SB) for the removal of lignin content in lignocellulose was done by acidic treatment (variable groupings of H_2SO_4. (Alizadeh et al., 2005) in an autoclave for various time at 121°C and 15 psi), biomass was treated with H_2SO_4 for different time frame at 121°C and 15 psi. The sample was separated and the filtrate was analyzed using a UV-Visible spectrophotometer. The residue was treated similarly for the second crop of the filtrate, which further was studied similarly under UV Vis. Allthe setups were completed as per the first one up to a similar level ofsuccessive steps

4.2.5.4 THERMAL HYDROLYSIS

Biomass (WS, RH, SB) was treated in the presence of water for various time in an autoclave at 121°C and 15 psi. The biomass blend was separated and absorbance of the filtrate was noted at 205 nm.The same methodology was repeated sequentially multiple times with residues from the first, second, and third stages. Similar treatments were done at varioustimeframes.

4.2.5.5 ALKALINE HYDROLYSIS

Biomass (WS, RH, SB) was treated with sodium hydroxide in the presence of water for various time frames in an autoclave at 121°C and 15 psi. Soluble pre-treatment was connected utilizing NaOH going in focus from 1.0% to 3% (w/v). Biomass(WS, RH &SB) was blended with NaOH arrangements at a proportion of 1:10 (1g of biomass in 10 mL NaOH arrangement), and put into an autoclave. The treated biomass was washed with deionized water, maintained to pH 7, and packed in a plastic holder at 4˚C.

The reaction blend was separated and absorbance of the filtrate was noted at 205nm.The same method was repeated multiple times with residues from the first, second, and third stages. Residue from the third stage was treated in the presence of the basic condition in an autoclave for a time of 2hr at 121°C and 15 psi and the absorbance of the filtrate was noted.

4.2.6 PRODUCTION OFBIOGAS

Treated biomass (WS, RH, SB) after pretreatment (with both of the above techniques) was exposed to batch type bio-reactor at various procedure parameters, for example, temperature, pH, total solid conc,C:N proportion for biogas generation independently. Gas was gathered in biogas expansion using gas spout of digester and examined on gas chromatograph sampler injector with infusion. The various blends of gases (STD1-60% CH_4, 35% CO_2 and 5% H_2; STD 2-30% CH_4, 60% CO_2 and 10% H_2; STD 3-90% CH_4, 9% CO_2, 1% H_2)(Busch et al., 2009) were served to decide the standard curve.

Fig. 4.2 Batch stirred tank reactor with water displacement

4.2.7 YIELD OFBIOGAS

The totalproductionof biogas in mlwas resolute byusing the water dislocation method. This is used for the calculation of% yield in each experiment. % yield is calculated as:

$$\% \ \textbf{Biogas yield}(\text{Tiehm et al 2001}) = \frac{\textbf{Total Biogas Produced}}{\textbf{Total amount of feedstock}}$$

4.2.8 PRETREATMENT OF WHEAT STRAW, RICE HUSK AND SUGARCANE BAGASSE

Delignification of the biomass (WS, RH, SB) was done by chemical and biological strategies. Substrate treatment was done in the presence of acid, soluble base, or thermal (Table 4.4-4.9).The Substrate was treated in the presence both of acid in an autoclave for 1 h at 121° C and 15 psi weight) or base (1%, 2%, and 3% arrangements of sodium hydroxide in an autoclave or the neutral medium at various times spans in an autoclave. Biological treatment of the biomass was completed within the sight of five distinct growths, viz., *Aspergillus niger, Aspergillus flavus, Cladosporium contiment*, *Helminthosporium solani,* and *Phaenerochate chrysosporium*. The solutions were filtered and the filtrates were contemplated under UV-Visible spectrophotometer at 205 nm.

Table 4.4 Delignification of Wheat straw under chemical treatment.

		Pretreatment agent								
		1% acid	30% acid	40% acid	60% Acid	72% Acid	1% alkali	2% alkali	3% alkali	Neutral
Absorbance	30 Minute	1.045	3.205	3	4.065	5.56	1.543	1.765	1.826	0.845
	60 Minute	3.42	6.308	6.02	5.879	3.038	1.983	2.112	2.245	1.453
	120 Minute	2.205	4.97	4.58	4.894	2.816	2.745	3.123	3.024	2.212
	180 Minute	1.564	3.657	3.256	3.294	1.536	1.564	2.768	2.897	1.879

Table 4.5 Delignification of wheat straw under biological treatment.

Fungi

	Aspergillus niger	*Aspergillus flavus*	*Cladosporium contiment*	*Helminutethosporium solani*	*Phaenerochate chrysosporium*
Absorbance	10	9	8	7	10

Table 4.6 Delignification of Rice Husk under chemical treatment

		Pretreatment agent								
		1% acid	30% acid	40% Acid	60% acid	72% Acid	1% Alkali	2% alkali	3% alkali	Neutral
Absorbance	30 minute	0.923	2.325	2.987	3.254	4.524	1.452	1.632	1.754	0.698
	60 minute	2.012	4..125	4.568	4.984	3.012	1.874	2.658	2.984	1.235
	120 minute	2.231	4.145	4.301	4.452	1.985	2.789	2.969	3.210	1.963
	180 minute	1.925	3.487	3.846	2.984	1.489	1.021	2.857	2.967	1.725

Table 4.7 Delignification of Rice husk under biologicaltreatment

Fungi

	Aspergillus Niger	*Aspergillus flavus*	*Cladosporium contiment*	*Helminutethosporium solani*	*Phaenerochate chrysosporium*
Absorbance	9	8	7	6	9

Table 4.8 Delignification of Sugarcane Bagasse under chemical treatment.

		Pretreatment agent								
		1% acid	30% acid	40% acid	60% acid	72% Acid	1% alkali	2% alkali	3% alkali	Neutral
Absorbance	30 minute	1.452	2.452	2.678	3.564	3.102	1.654	1.985	1.854	0.754
	60 minute	1.954	3.254	3.568	4.784	3.214	1.985	2.735	2.845	1.324
	120 minute	2.231	3.945	4.101	4.265	1.854	2.654	2.986	3.145	1.987
	180 minute	1.895	3.386	3.446	2.845	1.632	1.421	2.800	2.984	1.884

Table 4.9 Delignification of Sugarcane Bagasse below biological treatment.

	Fungi				
	Aspergillus Niger	*Aspergillus flavus*	*Cladosporium Contiment*	*Helminutethosporium solani*	*Phaenerochate chrysosporium*
Absorbance	10	8	7	9	10

4.2.9 BIO-METHANATION

Substrate was subjected to lab scales Stirred Tank Reactor in batch mode (BSTR) for the biomethanation under mesophilic prerequisites (Fig. 4.2). The methane content estimated after anaerobic digestion of pretreated (chemical and biological) biomass in the batch reactor is shown in tables 4.10-4.15. The optimized conditions for biomethane generation from biomass (WS, RH & SB) is shown in table 4.21. Pretreatment impact on the delignification of biomass is cited below fig 4.3.The GC Chromatogram shown in Appendixv.

Table 4.10 Methane content in biogas after chemical treatment of WS

		1% Acid	30% acid	40% acid	60% Acid	72% Acid	1% alkali	2% alkali	3% alkali	Neutral
Methane content (%)	30 minute	22	58	55	63	70	28	30	30	12
	60 minute	59	80	75	72	55	32	56	57	48
	120 minute	40	65	64	65	53	60	75	72	72
	180 minute	31	62	58	58	31	23	60	63	53

Table 4.11 Methane content in biogas after biological treatment of WS

	Fungi				
	A.niger	*A.flavus*	*C.contiment*	*H.solani*	*P. chrysosporium*
Methane content (%)	90	88	85	81	92

Table 4.12 Methane content in biogas after chemical treatment of RH

		1% Acid	30% acid	40% acid	60% Acid	72% Acid	1% alkali	2% alkali	3% alkali	Neutral
Methane content (%)	30 minute	24	60	48	58	68	30	34	35	15
	60 minute	57	73	71	70	58	31	64	59	55
	120 minute	51	63	68	67	54	62	72	71	69
	180 minute	30	59	58	56	29	28	61	62	53

Table 4.13 Methane content in biogas after biological treatment of RH

	Fungi				
	A.niger	*A.flavus*	*C.contiment*	*H.solani*	*P. chrysosporium*
Methane content (%)	84	81	82	79	83

Table 4.14 Methane content in biogas after chemical treatment of SB

		1% Acid	30% acid	40% acid	60% Acid	72% Acid	1% alkali	2% alkali	3% alkali	Neutral
Methane content (%)	30 minute	30	68	50	55	62	32	37	39	24
	60 minute	62	75	73	72	61	38	71	66	60
	120 minute	55	65	69	70	60	64	76	70	71
	180 minute	31	62	63	59	40	29	63	60	62

Table 4.15 Methane content in biogas after biological treatment of SB

	Fungi				
	A.niger	*A.flavus*	*C.contiment*	*H.solani*	*P. chrysosporium*
Methane content (%)	86	83	81	80	84

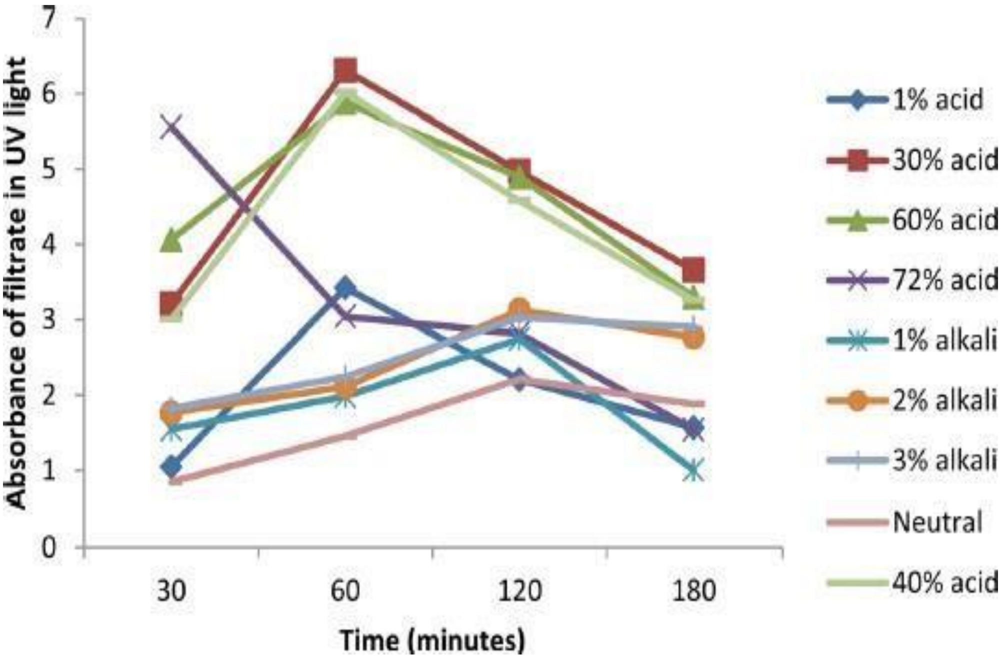


Fig. 4.3 Effect of chemical Treatment

4.2.9.1 EFFECT OF TOTAL SOLID CONCENTRATION ON METHANE CONTENT (ml) OF BIOMASS

From table 4.16 below it is found that a total solid concentration of 8% gave maximum biogas production in three different biomass substrates. At optimum total solid concentration of slurry, the sugarcane bagasse produces maximum biogas (340 ml) followed by wheat straw(335 ml) and rice husk (320ml) respectively. The effect of total solid concentration is shown in fig 4.4 & 4.5.

Table 4.16 EFFECT OF TOTAL SOLID CONC ON METHANE

Solid Conc (%)	Biogas (ml) RICE HUSK	Biogas (ml) WHEAT STRAW	Biogas (ml) SUGARCANE BAGASSE
6	240±1.6	270±1.8	290±3.1
8	320±1.4	335±2.8	340±2.4
10	260±2.5	280±2.1	285±2.7

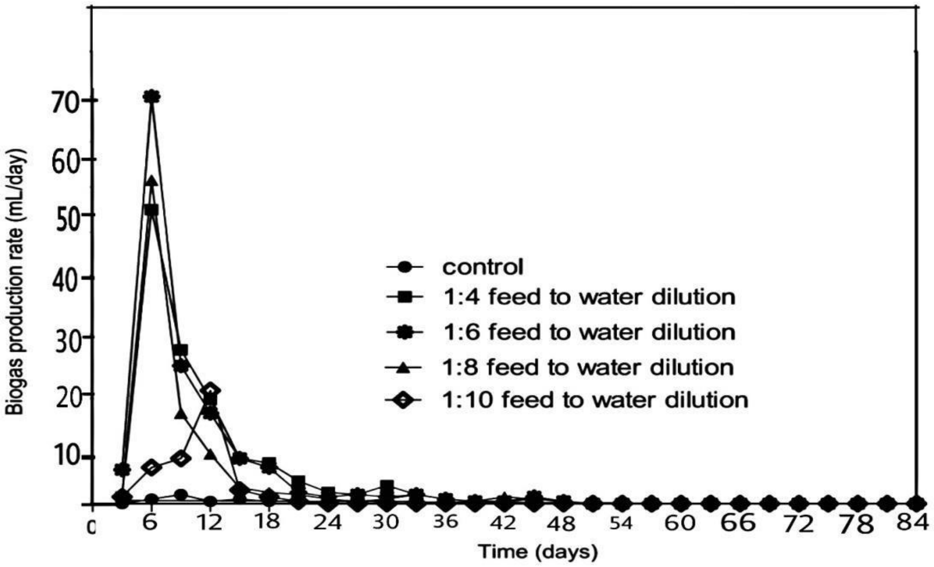


Fig. 4.4 Effect of total solid on biogas (ml/day) production from pretreated and untreated

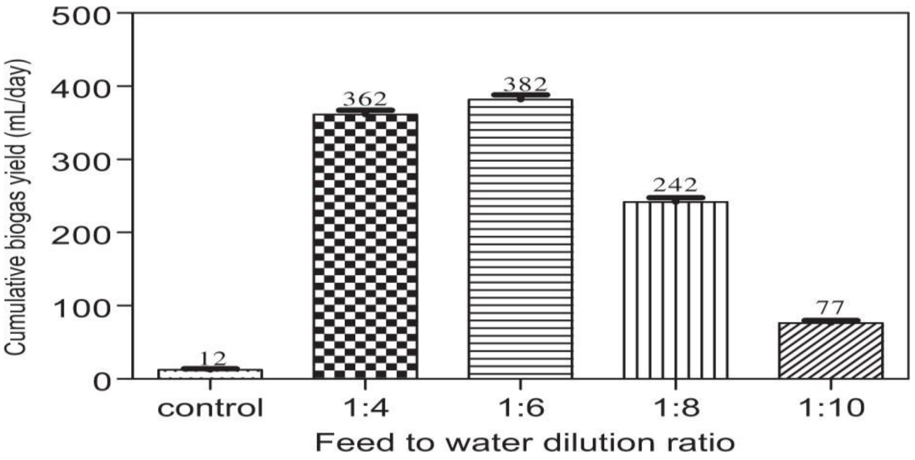


Fig. 4.5 Biogas yield on feed to water dilution ratio

4.2.9.2 EFFECT OF NITROGENOUS SUBSTANCE ON BIOGAS YEILD

It is important to maintain the proper ratio of feedstock for proficient plant activity so the C:N proportion in feed stays in optimum range. It is commonly observed that during anaerobic digestion. Microorganisms use carbon 25–30 times quicker than nitrogen**(**Busch et al., 2009**).** Subsequently to this ,a standard C:N ratio of 30:1 is achieved by mixing nitrogen rich sources with feed stock.The impact of C:N ratio is shown in table 4.17 and fig 4.6.

Table 4.17 Impact of C: N ratio on Biogas yield

Carbon to Nitrogen Ratio	WS Biogas (ml)	RH Biogas (ml)	SB Biogas (ml)
20	**250±1.8**	**210±1.6**	**270±2.6**
25	**300±2.4**	**280±1.9**	**298±2.7**
30	**325±3.1**	**301±3.4**	**340±2.3**
35	**290±2.1**	**270±1.5**	**293±1.6**

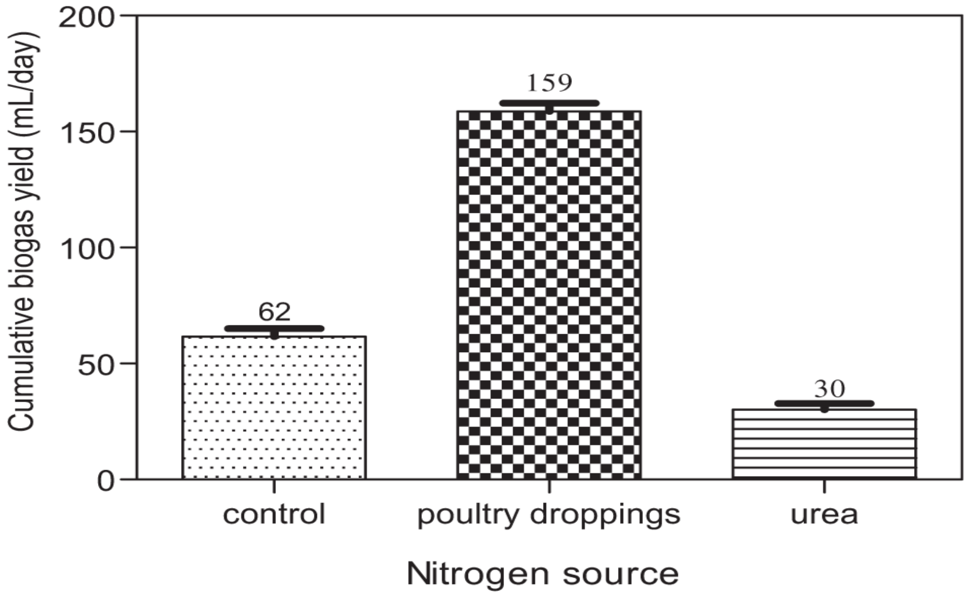


Fig. 4.6 Nitrogen content on biogas yield

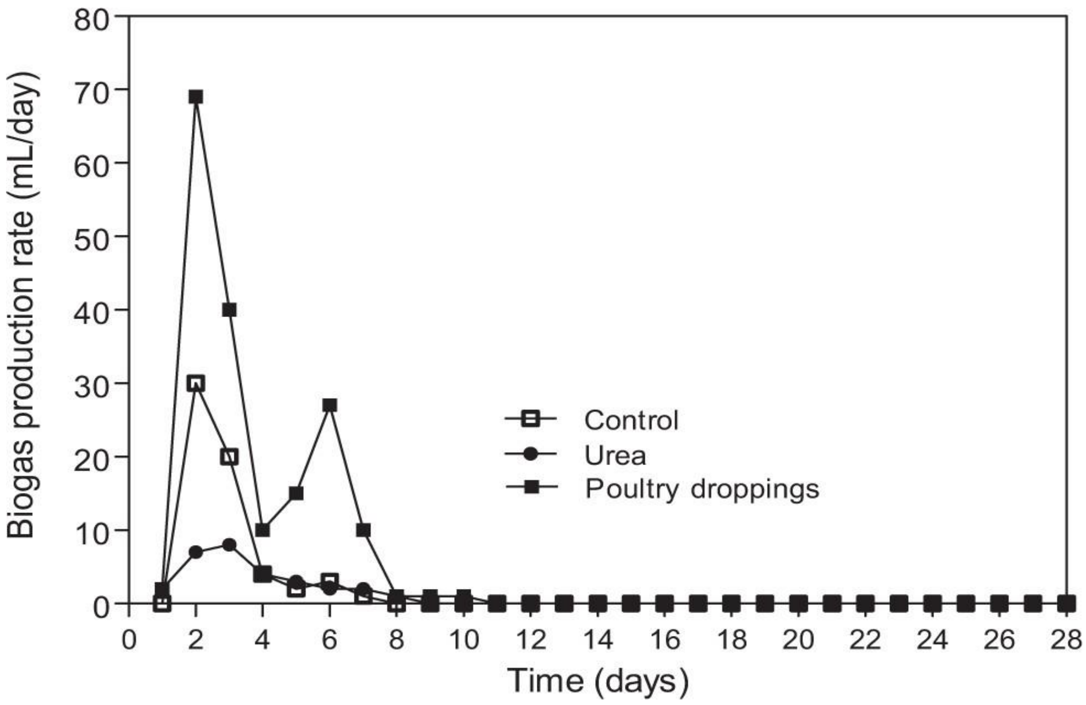


Fig. 4.7 Effect of temperature on biogas production

4.2.9.3 EFFECT OF pH ON BIOMETHANATION OF BIOMASS

The pH is defined as the negative logarithm of its H^+ion . It is a measure of the substrate's acidity or alkalinity. A digester works well at a pH of 7.0 or above, marginally under alkaline conditions (Mittal, 1996). The methanogenic microorganisms are extremely sensitive to pH changes and are dynamic just in the limited pH run (between 6.8 to 8.5) though the acidogenic microscopic organisms can make due in as low a pH as 5.5 (Nijaguna, 2002). An automated pH meter was utilized to study the pH of the digestion slurry. The pH meter was standardized utilizing solution of pH 4.0, 7.0 and 10.0. An appropriate pH between 6.8–7.2 was maintained using buffer solutiton to increase the methane production rate. The quantity of carbon dioxide and volatile fatty acids affect the pH of the digester substance. For an anaerobic digestion to go faster than normal,the Concentration of volatile fatty acid needs to be below 2000 mg/l. It can be seen from table 4.18 that a pH of 7 is favorable for maximum biogas generation from different pretreatedbiomass.

Table 4.18 EFFECT OF pH ON BIOMETHANATION OF BIOMASS

pH	Biogas (ml) RICE HUSK	Biogas (ml) WHEAT STRAW	Biogas (ml) SUGARCANE BAGASSE
4	**120±1.9**	**200±1.9**	**126±3.1**
7	**180±2.0**	**310±2.8**	**290±3.4**
9	**160±2.1**	**180±2.4**	**166±2.9**

4.2.9.4 EFFECT OF TEMPERATURE ON BIOMETHANATION OF BIOMASS

The biogas production is highest at the optimum temperature(35ºC). An ideal temperature for the survival of thermophilic and mesophilic microscopic organisms are 55ºC and 35ºC, respectively This, thus, renders different natural material biodegradability levels that impact the biogas creation sum and quality, accomplishing the most extreme at 35ºC. From table 4.19 it can be seen that a mesophillic temperature of 35 ºC is best suited for biogas generation from different biomass. Biomethanation is found maximum from anaerobic digestion ofwheat straw (330ml) followed by digestion of sugarcane bagasse (310ml) and rice husk (205ml). Incubation temperature surround by the digester affects the biogas creation process. There are distinctive temperature ranges throughout which anaerobic aging can be completed: psychrophilic thermophilic (50º-60ºC). Nonetheless, anaerobes are most dynamic in the mesophilic & thermophilic temperature run. The distance end to end of the maturation phase is subject totemperature.

Table 4.19 EFFECT OF TEMPERATURE ON BIOMETHANATION OF BIOMASS

Temp	Biogas (ml) RICE HUSK	Biogas (ml) WHEAT STRAW	Biogas (ml) SUGARCANE BAGASSE
30	**140±2.5**	**230±2.4**	**146±2.1**
35	**205±3.4**	**330±3.5**	**310±2.9**
45	**170±3.1**	**195±2.4**	**165±3.6**

4.2.10 BIOSLURRY ASMANURE

Bioslurry is used to improve soil fertility, soil structure, and crop productivity, and it may be used as an excellent fertilizer(Warnars, 2012). Table 4.20 shows the NPK value ofspent slurry from thedigestor of different biomass. Analysis shows that NPK value was found comparable to the slurry obtained after digestion ofrice husk, sugarcane bagasse, and wheat straw.

Table 4.20 Analysis of NPK value of slurry from digestor

S.No	N(%)	P(%)	K(%)
Cow Dung	**1.932±0.03**	**0.83±0.04**	**1.54±0.05**
Rice Husk	**1.986±0.04**	**0.96±0.01**	**1.73±0.01**
Wheat Straw	**1.980±0.01**	**0.93±0.02**	**1.70±0.02**
Sugarcane Bagasse	**1.975±0.07**	**0.90±0.06**	**1.63±0.08**

4.3 SUMMARY

Delignification of WS, RH and SB with acidic and biological methods was persistently checked by using UV-VIS spectrophotometer. Degree of expulsion of lignin during different conditions has been presented in tables 4.4-4.10 and figure 4.3. Results demonstrate that all the treatment strategies prompted the obvious expulsion of lignin content from the substrate, however it was maximum in case of fungal attack. In acidic conditions, pretreatment was completed in the presence of varied sulfuric acid starts from(1% to 72%); optimum concentration of sulphuric acid being 30% H_2SO_4 causing maximum expulsion of lignin. Acid pretreatment accomplished high response rates and altogether improved cellulose hydrolysis (Jeonget al., 2016). The relation between methane yield and the measurement of lignin is one where less lignin influenced the expanded methane yield (Sindhu et al., 2016; Bhatia et al., 2017b). Acid treatment demonstrates an appropriate strategy for delignification and biomethanation of biogas from biomass(Bharathiraja et al.,2018).

The alkali pre-treatment utilizing 2% NaOH had the impact of separating hydrogen bond associations which can act to solubilize lignin. Although it is most ordinarily utilized technique for the pretreatment of lignocellulosic materials yet by this strategy, lignin is evacuated uniquely to an constrained degree and is redistributed on the fiber surfaces because of softening and depolymerization responses.

Delignification in the presence of dilute NaOH was lesser than acid which is in concurrence with literature**(**Vindis etal., 2009**).** Lesser delignification during basic hydrolysis is due to reason that soluble base expels acetyl and other group substituted on hemicellulose, decreasing the availability of hemicellulose and cellulose to catalysts. Dilute NaOH treatment of lignocellulosic materials cause swelling driving an expansion in inner surface region. Thisdecline in the level of polymerization and crystallinity caused partitionof auxiliary linkages among lignin and starches, and finally breaking of the lignin structure (Ko et al., 2015; Li et al., 2018)). Pretreatment under thermal conditions in neutral medium result in least degree of delignification (Abdul et al.,2016).

Biological treatment is a safe and condition amicable strategy for delignification. Containing higher C/N proportion (30:1), growths are equipped for debasing any lignocellulosic material. White-spoil growths are best for biological pretreatment of lignocellulosic materials. Among different contagious species, *P. chrysosporium* (white decay parasites) caused maximum extreme delignification, followed by *A. niger, A. flavus, C. contiment,* and *H. solani.* . The white-decay organism *P. chrysosporium* discharges three sorts of extracellular phenoloxidases, specifically, lignin peroxidase (LiP), manganese peroxidase and laccase in charge of starting the depolymerization of lignin (Zhang et al., 2016).The effect of biological pretreatment on delignification is shown in fig4.8.

Methane content in biogas production under different conditions is related to the degree of delignification. Measurement of methane in biogas obtained from pretreated substrate underdifferent conditions (best condition browsed for all the pretreatment techniques) is shown in Figure 4.8. Expanded generation of biogas after the treatment of substrates with rot-fungi is because of the way that the organisms need to utilize the lignocellulosic squandersas substrate for its development. Additional cell compounds and enzymes delivered by organisms are responsible for separating the lignocellulose present in the biomass. These chemicals transformed waste into simpler structures, which is basic for the development and nourishment of the organisms.

The optimum pretreatment condition for WS,RH, SB are appeared table 4.21, which shows that sulphuric acid treatment at 30%(60 minutes) gave highest methane content (% in biogas) in wheat straw (80%) followed by Sugarcane bagasse (75%) and Rice Husk (73%). Alkali treatment 2% Sodium Hydroxide(for 120 minute) yieldedvaried amounts of methane in biogas: Wheat straw(75%) Rice husk (72%) and sugarcane bagasse(76%) by .Lignocellulosic waste are fantastic wellsprings of biogas satisfying two significant purposes, one administration of the loss to be discarded, and second creation of biogas ready to fulfill the need of vitality to an impressive degree. The finest feed to water weakening proportion was 1:8 w/v, which give the biogas way of 340 mL in sugarcane bagasse. The best biogas yields of 310 mL/day in wheat straw were accounted for pH 7. Maximum biogas yield 340 mL in sugarcane bagasse got at Nitrogenous substance 30 .High NPK substance estimation of slurry was acquired after anaerobic processing of treated Rice Husk. Pretreatment of Wheat Straw produce biogas with methane substance ranges from 80% (Acid Treatment),75% (Alkaline Treatment), 72% (Thermal Treatment) and 92% (Fungal Treatment). The ideal condition for anaerobic assimilation was 8% TS , 7 (pH) focus at the temperature of 35°C. High NPK substance estimation of slurry was gotten after anaerobic absorption of treated WS. Pretreatment of Sugarcane Bagasse produce biogas with methane ranges from 75% (Acid Treatment),76%(Alkaline Treatment),71% (Thermal Treatment)and, 86% (Fungal Treatment). Anaerobic digestion pretreated rice husk yield biomethane content at optimum parameter of 73% (Acid Treatment), 72% (Alkaline Treatment), 69% (Thermal Treatment), and 83% (Fungal Treatment). The comparative biomethantion content is shown in fig 4.9-4.12 The maximum output of methane content was found after the biological treatment of wheat straw biomass at optimum condition.

Table 4.21 OPTIMIZED PRETREATMENT CONDITION (WS, RH & SB)

S. No.	**PRETREATMENT**	**OPTIMIZED PRETREATMENT CONDITION**	**Lignin estimation (%)**			**W.S Methane content (%) in Biogas**	**R.H Methane content (%) in Biogas**	**S.B Methane content (%) in Biogas**
			WS	**RH**	**SB**			
1.	Acid	30% (60 minute) Sulphuric acid	6.308±0.003	4.125±0.006	3.254±0.002	80±0.8	73±0.9	75±0.4
2.	Alkali	2%Sodium Hydroxide(120 minute)	3.123±0.002	2.969±0.001	2.986±0.004	75±0.6	72±0.2	76±0.1

3.	Thermal	Water (120 Minute)	2.212±0.007	1.963±0.004	1.987±0.005	72±0.7	69±0.6	71±0.4
4.	Biological	*P. chrysosporium*	10±0.1	9±0.2	10±0.3	92±0.7	83±0.6	86±0.3
5	Standard	Without Pretreatment	0.9±0.15	0.8±0.26	1.0±0	60±0.5	58±0.3	64±0.4

Thermal treatment Water(120 Minuteutes) gave max yield in wheat straw (72%) trailed by sugarcane bagasse (71%) and rice husk(69%). The pre-treatment seemed to affect the cellulose content, causing decreases in crystallinity due to expansion in water- soluble extractives, mainly cellobiose and glucose created from the cellulose. At high temp water- soluble extractives water solubles mostly comprise of sugar, gelatin, starch, and inorganics, which deliver methane. The percentage increase in biomethane content due to different pretreatment is shown in fig 4.13.

Baesd on results, pretreatment of lignocellulosic waste can be guaranteed and endorsed as a profitable technique for improving the amount of biogas, especially under biological treatments Henceforth, before exposing to anaerobic assimilation, the waste needs to be dealt for delignification under appropriate condition to build the productivity of the procedure. Biogas, the outcome of anaerobic digestion through suitable pretreatment is a potential future fuel, able to impact the economy of the nation .

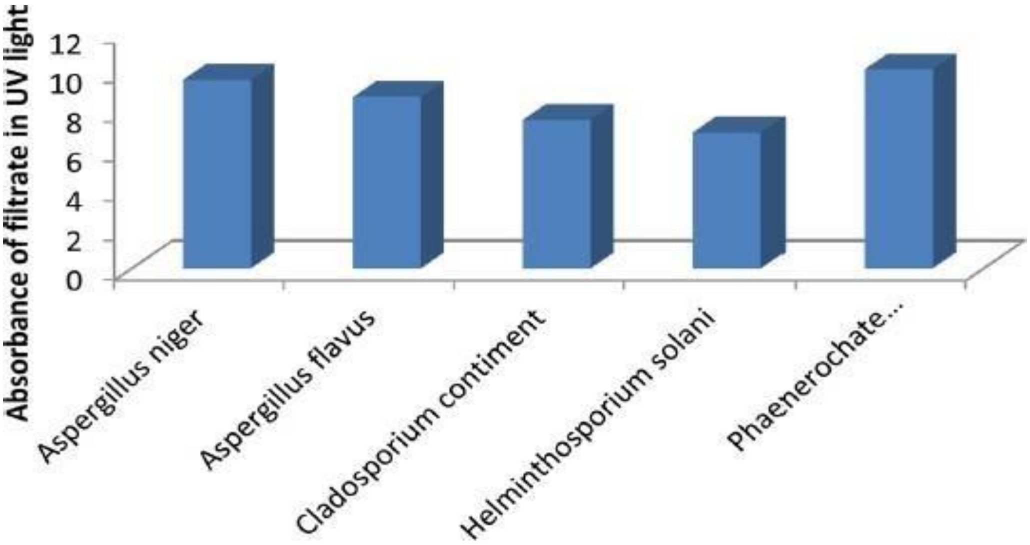


Fig. 4.8 Effect of Biological Treatment

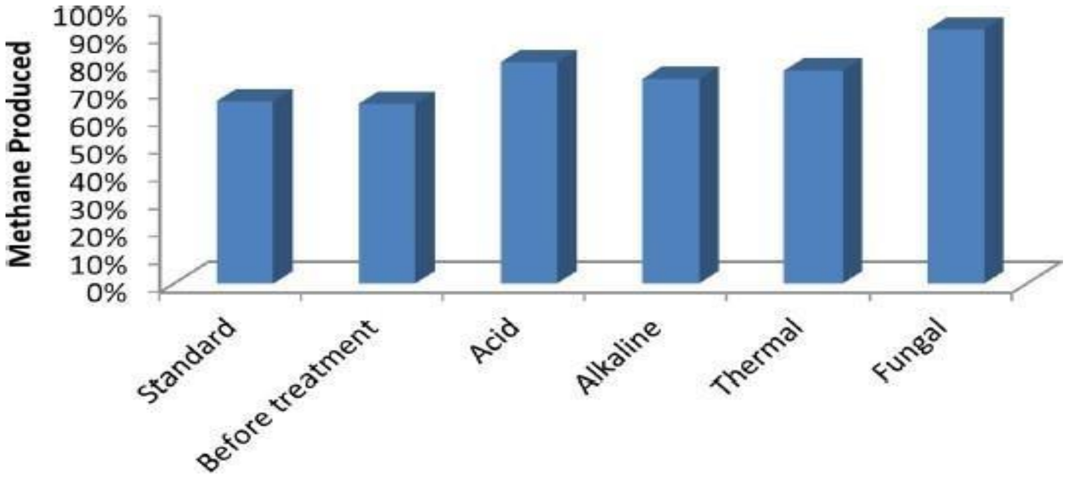


Fig. 4.9 Comparative biomethane content of wheat straw

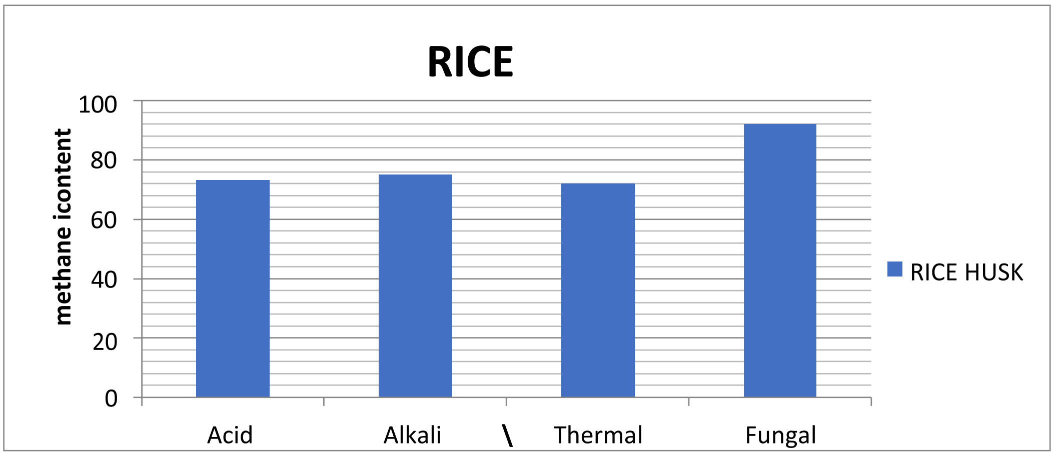


Fig. 4.10 Comparative biomethane content of Rice husk

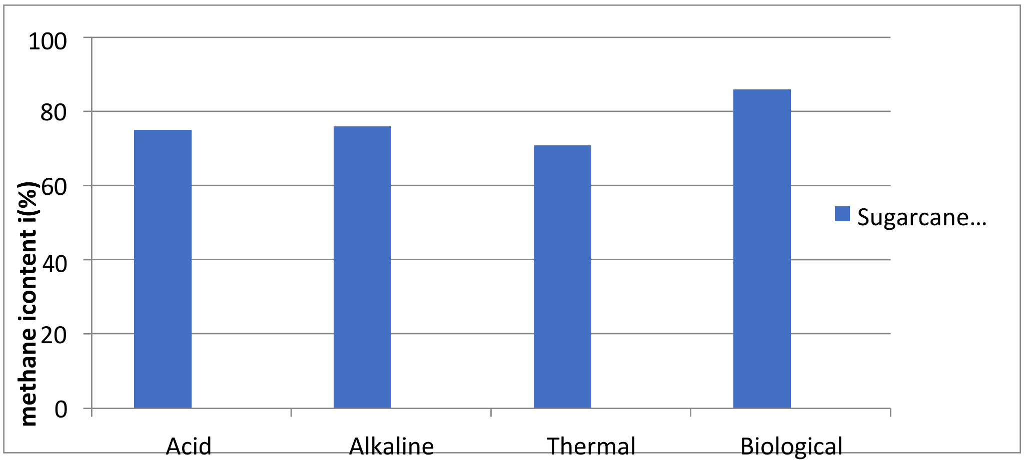


Fig. 4.11 Biomethane content of Sugarcane Bagasse

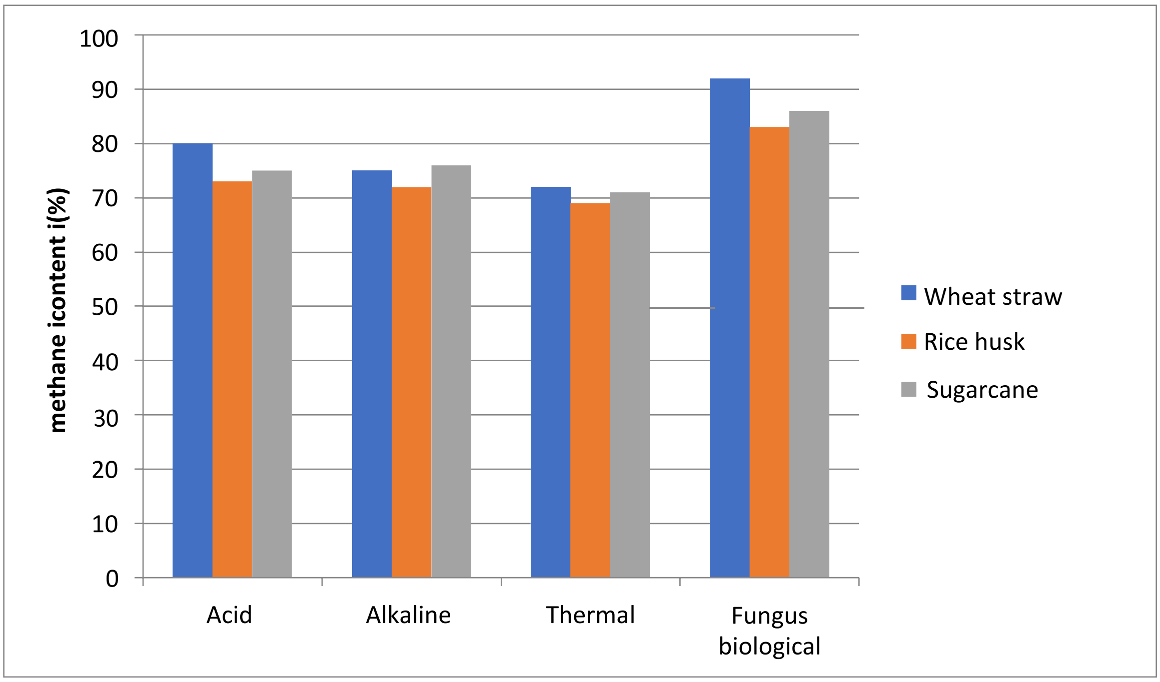


Fig. 4.12 Comparative biomethane content of Biomass (SB,RH &WS)

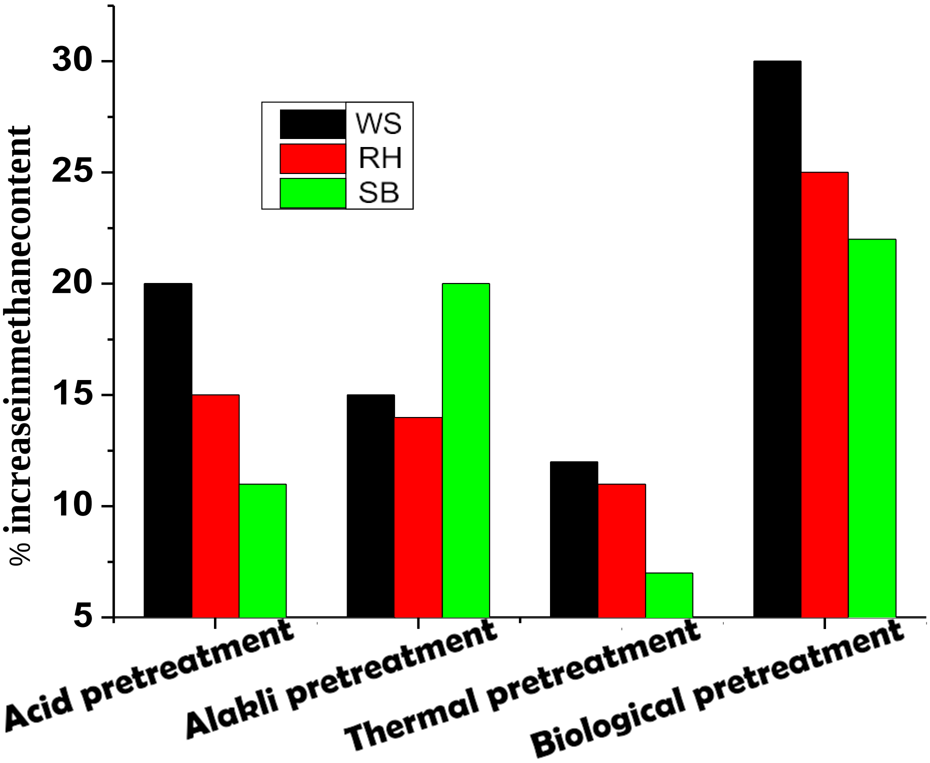


Fig 4.13 Effect of Pretreatment on percentage increase in methanecontent of biomass(WS,RH &SB)

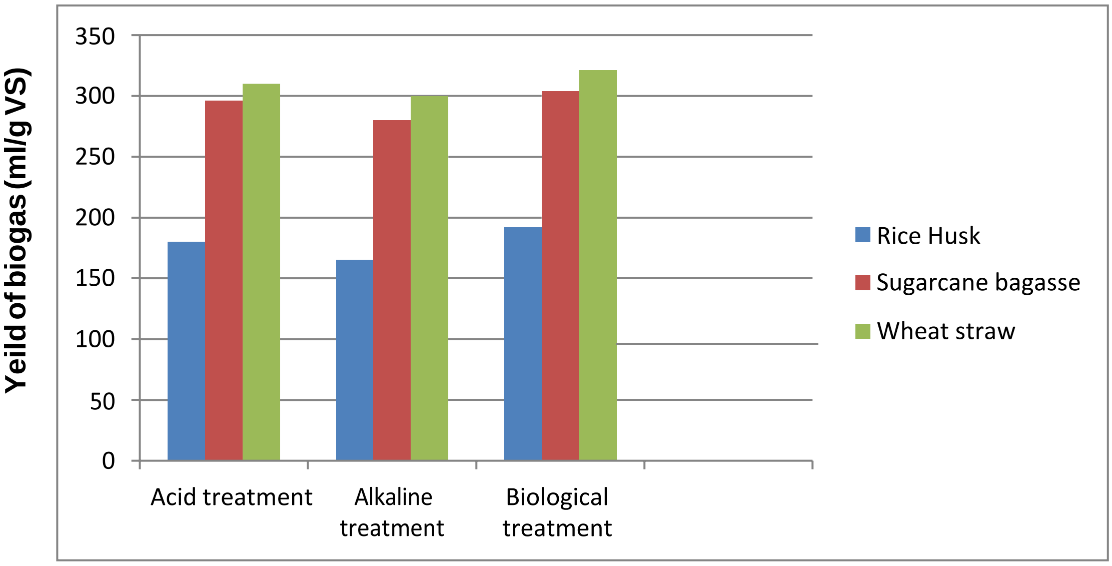


Fig 4.14 Effect of Pretreatment on yeild of biogas(ml/gVs) content of biomass(WS,RH &SB)

CHAPTER – 5

CONCLUSIONS AND SCOPE FOR FUTURE WORK

The consumption level of fossil fuels has far surpassed its formation- level that encouraged scientists to search for an alternative energy source that is sympathetic to the atmosphere and cost- efficient. Nevertheless, their re-calcitrant structure and composition is their major task when using lignocellulose substrates like agricultural residues and energy crops. Different techniques of pre-treatment were thus recommended and evaluated to change the composition of the lignocellulosic substrates, facilitate their enzyme hydrolysis and therefore improve their methane output. It can be helpful to compare the finest kind of treatment with pretreatment used wheat straw, rice husk, and sugarcane bagasse. Renewable power sources such as lignocellulose biomass have appeared as a great option as their unlimited and unused energy supply has been identified. In most emerging nations, such as India, China, etc., a big amount of lignocellulosic biomass is also generated from the agriculture, the plant factories, etc. This study aims to investigate the efficacy of various pretreatment techniques and the result features of lignocellulosic digestion (WS, RH, SB) for biogas production. Pretreatment technologies for lignocellulosic biomass include biological, mechanical, chemical methods, and various combinations thereof. Pretreatments must improve the digestibility of lignocellulosic biomaterials, and each pretreatment has its effect on the cellulose-, hemicellulose- and lignin fractions.

Table 5.1 A summary of techniques investigated for enhancing biogas production from lignocellulosic materials.

Technique	Subdivision
Mechanical	Milling
Thermal	Steam explosion Thermal hydrolysis
Chemical	Acid hydrolysis Alkaline pretreatment
Biological	Fungi

CONTRIBUTION OF THE PRESENT WORK

Three lignocellulose biomasses were selected and studied for its potentiality for biogas production. They are found to have a fairly good amount of cellulose content in it although they contain lignin too. In this research, therefore, an optimal achievement of the lignocelluloses plant has been evaluated in the pretreatment impact (physical, chemical, and biological) and multiple biogas manufacturing parameters. The pretreatment method focused on the removal of lignin content by applying different alkaline and acid condition and then anaerobic digestion of pretreated biomass (WS, RH, and SB). The parameters considered for the analysis TS of biomass, temperature of the substrate, C:N ratio, and pH. Methane manufacturing from lignocellulose (i.e. crop residue and power plants) seems to be one of the most successful solutions to food vs fuel from fossil fuels.

CONCLUSION OF THE STUDY

5.1 Improved characteristics ofbiomass

In chapter 3, rice husk and sugarcane bagasse biomass are described to achieve the goal and target of the current working on wheat straw along with livestock dung. Proximate analyzes reveals that rice husk, bagasse from sugarcane, grain straw, and livestock dung have solid oxygen ranges from 12% to 21.5%. The ash content of biomass, after subjection to an elevated degradation temperature, is the non-volatile inorganic material. It is found to vary from 3.6-12.35 % for all the above-mentioned biomasses. Volatile matter content of cattle dung, rice husk and wheat straw are found to be in the range of 66% to 68% whereas for the rest of the sugarcane bagasse biomass it is in the range of 82.17%. The result was that the biomass could readily be dissolved by volatile and fixed carbon. The final analysis of lignocellulose biomass revealed that the characteristics studied were wheat straws, rice husks, and sugarcane bagasse, while the biomass nitrogen content varies between 0.9 and 1.7 percent. High C: N is not beneficial for the manufacturing of optimum biogas. That is why the biomasses are mixed with cattle dung in 1:3 ratios to adjust the C: N of the substrate for better biogas production. Fiber analysis ofthe biomass’s lignin content of all the biomass feedstocks is found to be in the range of3% to 20% which is quite high.

5.2 Comparative pretreatment of different biomass (WS, RH, SB)

By contrast, the solution of cellulose, hemicellulose, and lignin for biomass (WS), RH, SB, alkaline, thermal, alkaline, and alkaline-biotherapy (HMB), is solventised by acid, alkaline,thermal, and biological

pretreatment (WH, HR, SB, etc.). **Lignin degradation is found to be the maximum acid and biological method used, and alkaline, and thermal methods.** Increased methane yields three for sugarcane bagasse, wheat straw, and rice husk, but led to increased anaerobic digestion mainly because of the solubilization of cellulose and hemicellulose fractions are beneficial thermal pretreatment carried out at 121°C. **Among thermal treatments, best results in the increase of methane manufacturing were observed with the treatment of wheat straw followed by sugar cane bagasse and rice husk at 121°C & 120 minutes (19,8%,18%, and 13%, respectively).** Pretreating alcohol has been commonly used in the biogas production method. Sulfuric acid (H_2SO_4) is the most commonly used acid in acid pretreatment. **Acid pretreatment at optimized condition (30%, (60 minutes) and % increase in methane content is found maximum with anaerobic digestion of wheat straw (25%), sugarcane bagasse (20%) followed by rice husk (17%). Acid pretreatment has a maximum impact on bio methanation of wheat straw biomass at optimized conditions.** Biological pretreatments, performed with fungus led to an increase of methane production of biomass (WS, RH, SB). **Biological pretreatments performed with a fungal strain, improve methane production. The percentage increase in methane content after pretreatment with fungal strain is found maximum for wheat straw (34%), followed by sugarcane bagasse (30.2%) and rice husk (27.7%) respectively.The maximum biogas yeild 321ml/gVS is found with fungal treated wheat staw.** Results from all classifications of pretreatment have been acquired. **There are more results in acid and biological pre-treatment in methane content, but for the energy as well as the economy, the only alkaline pre-treatment is also preferred.** Also , wheat straw was the largest output of methane among the three substrates.

5.3 Optimized anaerobic condition forbiogas production

Optimization of the anaerobic absorption procedure brought about the improvement of biogas amount and quality. A few changes demonstrated to be fundamental while others demonstrated to be insufficient. Cluster testing upon the absorption procedure demonstrated that pH was a viable pointer about the phase of processing. Through consistent pH checking, each phase of assimilation could be distinguished, giving an approach to treatment plants to screen digester activity. It was resolved through parametric examinations that every single tried parameter were coupled. Accordingly, one parameter can't be adjusted without influencing others Biogas creation was most elevated close to an unbiased pH. The ideal range was characterized to be 7.0, as the examples inside this range demonstrated the most noteworthy mole division of methane all through the testing period. The ideal temperature for assimilation fell inside the mesophilic run. Most noteworthy creation was accomplished at 35°C, yet vitality adjusts demonstrated that keeping up assimilation inside the lower part of the mesophilic range would be more vitality effective. The impact of C: N proportion was discovered greatest at an ideal proportion of 30:1. **The all- out strong concentration of 8% gave maximum biogas amount.**

5.4 End product of the pre-treatment and digester: Manures

Alongside the biogas delivered, AD additionally changes the additional feedstock into digestate that can be utilized as a compost which is high in nitrogen, potassium, and phosphorus substance. The digestate can be put away at that point utilized in farmlands for yield creation at a proper time moving along without any more treatment.Also , it very well may be isolated to create fiber and alcohol. The fiber can be sold or utilized as a decent compost or a dirt conditioner, while the alcohol contains different supplements and could be utilized as a fluid manure that could be sold or utilized nearby. The digestate nearly stays all the non-degradable substances from the percent, which kind of feedstock (WS,RH,SB) is processed.Besides , AD procedure of excrement or other natural biomass could change some portion of natural bound supplements to a mineral structure. This impact is significant for nitrogen. It likewise lessens the requirement for utilizing extra mineral nitrogen composts. **So the digestate from anaerobic aging is considered as an improved and profitable compost that could substitute mineral manure because of the expanded accessibility of nitrogen to crops**. Likewise, anaerobic treatment limits the survival of pathogens from the feedstock, which is significant for the digestate utilized as a manure. **The N (%) from spent slurry from anaerobic assimilation of biomass (WS, RH, SB) is in the scope of 0.93 to 0.98, most noteworthy P(%) and K(%) found from the slurry of anaerobic processing of rice husk.**

FUTURE STUDIES/RECOMMENDATIONS

Lignocellulose residuals are currently being manufactured from biogas in its preparatory phase and far more research needs to be undertaken to enable the economically feasible, large-scale use of lignocellulose in the future to producebiogas.

Interesting and difficult topics and fields for potential research:

- The reasonable pretreatment strategies, which are not just financially achievable on a full- scale process, yet additionally viable, vitality effective and use as meager synthetic compounds as would beprudent.
- So far, the majority of the pretreatment techniques and conditions examined have been assessed by anaerobic clump assimilation tests. This has been done to decide the methane potential accomplished after the pretreatment. Almost no test work has been done to research the long haul impacts in a ceaseless co-assimilation process where lignocelluloses are one of

the substrates used. This progression is essential to have the option to explore the possible restraining just as synergistic impacts. Besides, pilot- scale thinks about, including the pretreatment and anaerobic absorption steps, are vital toward the commercialization of these sorts of procedures.

- Specific structural modifications affecting the microbial degradation of lignocelluloses are still very unknown. Substrate features which are essential for elevated degradation levels and how they can be evaluated have been limitedly investigated. It will be easier in the future to build a pretreatment technique that operates most effectively when we are prepared to discover responses to these fundamentalissues.
- On the relationship between pretreated feedstock lignocelluloses and a microbial composition has only beenstudied for a few years; therefore, more information is required.
- The cellulosomes, or the cellulose corrupting compound- complex, is another intriguing territory, where there is still a lot to learn. Do the microorganisms embrace the plan of the cellulosomes of the substrate they meet? Is it conceivable to hereditarily alter these microorganisms to create cellulosomes, with an ideal structure and arrangement of chemicals to accomplish the bestdebasement?

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Appendix – I

Characterization of Biomass

I.1 Sample calculations forproximate analysis for sugarcane baggase

Total solids in biomass

$$\text{Total solid (\%) for Sugarcane baggase} = \frac{1.8794 - 0.156}{2} \times 100$$

$$= 86.17\%$$

where

Weight of dry pan + dry sample is 1.8794 gm

Weight of dry pan is 0.156 gm

Weight of sample as received is 2 gm

Moisture content of sugarcane baggase

$$\text{Moisture content in sugarcane baggase(\%)} = 100 \; \square \; \frac{1.8794-0.156}{2} \times 100$$

Moisture content in sugarcane baggase (%) = 13.83 %

$$\text{Volatile content in husksugarcane baggase} = \frac{w_2 - w_4}{w_2 - w_1} \times 100$$

$$\text{Volatile content insugarcane baggase} = \frac{57.56439 - 58.3860}{57.56439 - 5655819} \times 100$$

Volatile content insugarcane baggase = 82.17%

where

w_1 = mass of the empty crucible with the lid is 56.55819 gm.

w_2=mass ofthe crucible with lid and the sample before heating is 57.56439 gm. W_4 = mass ofthe crucible with lid and the sample after heating is 58.3860 gm.

Ash content in sugarcane baggase

% of Ash in sugarcane baggase = (33.229 – 33.193) × 100 = 3.6%

where

Weight of crucible + sugarcane baggase is 33.229 gm

Weight of crucible is 33.193 gm

Weight of sample as received is 1 gm

CV analysis of sugarcane baggase

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

W_c = Water equivalent ofthe bombcalorimeter, MJ/°C =10.74×10^{-3} MJ/°C Initial water temperature =26.04°C

Final water temperature = 26.89°C

ΔT = Rise in temperature, °C = 0.85 °C

M_s = Mass of the biomass sample burnt, kg = 0.503 gm

$$C = \frac{W_c \times \Delta T}{M_s}$$

$$= \frac{10.73 \times 10^{-3} \times (26.89 - 26.04)}{0.503 \times 10^{-3}}$$

= 18.266 MJ

I.2 Sample calculations forproximate analysis for Rice husk

Total solids in biomass

$$\text{Total solid (\%) for Rice husk} = \frac{1.9442 - 0.156}{2} \times 100$$

= 89.41%

where

Weight of dry pan + dry sample is 1.9442 gm

Weight of dry pan is 0.156 gm

Weight of sample as received is 2 gm

Moisture content of Rice husk

Moisture content in Rice husk (%) =100 □ $\frac{1.9442-0.156}{2} \times 100$

Moisture content in Rice husk (%) = 10.59 %

Volatile content in Rice husk = $\frac{w_2 - w_4}{w_2 - w_1} \times 100$

Volatile content in Rice husk = $\frac{57.56439 – 58.2539}{57.56439 – 56.55830} \times 100$

Volatile content in Ricehusk = 68.96%

where

w_1 = mass of the empty crucible with the lid is 56.55830 gm.

w_2=mass ofthe crucible with lid and the sample before heating is 57.56439 gm.

w_4 = mass of the crucible with lid and the sample after heating is 58.2539gm.

Ash content in Rice husk

% of Ash in Rice husk = (33.334 – 33.193) × 100

= 14.1%

where

Weight of crucible + Rice husk is 33.334 gm

Weight of crucible is 33.193 gm

Weight of sample as received is 1 gm

CV analysis of Rice husk

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

W_c = Water equivalent of the bomb calorimeter, MJ/°C =10.74×10^{-3} MJ/°C

Initial water temperature = 26.04 °C

Final water temperature = 26.70 °C

ΔT = Rise in temperature, °C = 0.66 °C

M_s = Mass of the biomass sample burnt, kg = 0.508 gm

$$C = \frac{W_C \times \Delta T}{M_s}$$

$$= \frac{10.73 \times 10^{-3} \times (26.70 - 26.04)}{0.508 \times 10^{-3}}$$

$$= 14.10 \text{ MJ}$$

I.3 Sample calculations forproximate analysis for Wheat straw

Total solids in biomass

$$\text{Total solid (\%) for Wheat straw} = \frac{1.9026 - 0.156}{2} \times 100$$

$$= 87.33\%$$

where

Weight of dry pan + dry sample is 1.9026 gm

Weight of dry pan is 0.156 gm

Weight of sample as received is 2 gm

Moisture content of wheat straw

$$\text{Moisture content in wheat straw (\%)} = 100 \ \square \ \frac{1.9026-0.156}{2} \times 100$$

Moisture content in wheat straw (%) = 12.67%

$$\text{Volatile content in wheat straw} = \frac{w_2 - w_4}{w_2 - w_1} \times 100$$

$$\text{Volatile content in wheat straw} = \frac{57.56439 - 58.1958}{57.56439 - 56.55840} \times 100$$

Volatile content in wheat straw = 63.15 %

where

w_1 = mass of the empty crucible with the lid is 56.55840 gm.

w_2=mass ofthe crucible with lid and the sample before heating is 57.56439 gm. W_4 = mass ofthe crucible with lid and the sample after heating is 58.1958gm.

Ash content in wheat straw

% of Ash in wheat straw = (33.442 – 33.387) × 100

= 5.46%

where

Weight of crucible + wheat straw is 33.442 gm

Weight of crucible is 33.387 gm

Weight of sample as received is 1 gm

CV analysis of wheat straw

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

W_c = Water equivalent ofthe bombcalorimeter, MJ/°C =10.74×10^{-3} MJ/°C Initial water temperature =26.04°C

Final water temperature = 26.64°C

ΔT = Rise in temperature, °C = 0.66 °C

M_s = Mass of the biomass sample burnt, kg = 0.510 gm

$$C = \frac{W_c \times \Delta T}{M_s}$$

$$= \frac{10.73 \times 10^{-3} \times (26.64 - 26.04)}{0.510 \times 10^{-3}}$$

= 12.73 MJ

Table I.1 Characterzation results of lgnocellulosic biomass. Values correspond to mean ± standard deviation of measurement performed in triplicate

Sl. No	Sample	TS (%)	M (%)	Fixed Carbon	Ash (%)	VM (%)	PH	CV (MJ/Kg)	COD (mg/L)
1	Sugarcane Bagasse	86.17±0.40	13.83±0.15	12.10±0.25	3.60±0.03	82.17±0.20	7.85±0.01	18.26±0.16	4688.83±0.10
2	Rice Husk	89.41±0.1	10.59±0.20	14.13±0.2	14.10±0.20	68.96±0.15	8.95±0.02	14.01±0.02	37836.67±0.21
3	Wheat Straw	87.33±0.15	12.67±0.20	12.03±0.20	5.46±0.02	63.15±0.10	8.61±0.01	12.73±0.02	9292.12±0.014

Table-I.2 The proximate and ultimate analysis of various lignocellulose biomass (literature)

Biomass type	Ultimate analysis (db, % w/w)					Proximate analysis (% w/w)				LHV (MJ/kg)
	C	H	O	N	S	ASH	VM	FC	M	
Wheat straw	46.1	5.6	41.7	0.5	0.08	6.1	75.8	18.1		17.20
Sugar cane bagasse	48.58	5.97	38.94	0.2	0.05	2.26	70-84	10-25	DB	19.05
Rice husk	45.8	6.0	47.9	0.3	---	8	73.8	13.1	12.3	13.36

VM: Volatile matter, FC: Fixed Carbon, M: Moisture, DB:Dry basis

3.2 Fibre analysis results

Table I.3 Results obtained from the fibre analysis of the feed material

Sl. No.	Biomass	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Lignin to Cellulose ratio
1	Sugarcane Bagasse	24.5±0.10	41.5±0.20	20±0.14	0.481
2	Wheat straw	27±0.12	35±0.10	17±0.01	0.491
3	Rice husk	23.2±0.2	32±0.15	18±0.24	0.562

Appendix – II

Control of C:N ratio and TS of substrates

II.1 Sample calculation for TS% of biomass and cattle dung mixture with water

Total solid of Sugarcane bagasse = 86.17%

Total solid of cattle dung = 20.036%

Cattle dung is mixed with biomass in 1:2 ratio.

Total solid of sugarcane baggase and cattle dung mixture, TS% = $\frac{86.17 + 2 \times 20.036}{3}$

= 42.08 %

When water is added to the mixture in 1:2 ratio, TS% = $\frac{42.08}{3}$

= 14.02 %

When water is added to the mixture in 1:3 ratio, TS% = $\frac{42.08}{4}$

= 10.52 %

When water is added to the mixture in 1:5 ratio, TS% = $\frac{42.08}{6}$

= 7.01 %

II.2 Sample calculation for TS% of Rice husk and cattle dung mixture with water

Total solid of Rice husk = 89.41 %

Total solid of cattle dung = 20.036%

Cattle dung is mixed with biomass in 1:2 ratio.

Total solid of rice husk and cattle dung mixture, TS% = $\frac{89.41 + 2 \times 20.036}{3}$

= 43.16%

When water is added to the mixture in 1:2 ratio, TS% = $\frac{43.16}{3}$

= 14.38 %

When water is added to the mixture in 1:3 ratio, TS% = $\frac{43.16}{4}$

= 10.79 %

When water is added to the mixture in 1:5 ratio, $TS\% = \frac{43.16}{6}$

$= 7.19\ \%$

II.3 Sample calculation for TS% of wheat straw and cattle dung mixture with water

Total solid of Wheat straw = 87.33 %

Total solid of cattle dung = 20.036%

Cattle dung is mixed with biomass in 1:2 ratio.

Total solid of wheat straw and cattle dung mixture, $TS\% = \frac{87.33 + 2 \times 20.036}{3}$

$= 42.46\%$

When water is added to the mixture in 1:2 ratio, $TS\% = \frac{42.46}{3}$

$= 14.15\ \%$

When water is added to the mixture in 1:3 ratio, $TS\% = \frac{42.46}{4}$

$= 10.61\ \%$

When water is added to the mixture in 1:5 ratio, $TS\% = \frac{42.46}{6}$

$= 7.07\ \%$

Table II.1 TS (%) of biomass before and after mixing with cattle dung and water

Biomass	**TS (%)**	**Biomass + Cattle dung in 1:2 ratio**	**TS (%)**	**Biomass + Cattle dung : Water**	**TS (%) for 1: 2 ratio**	**TS (%) for 1:3 ratio**	**TS (%) for 1:5 ratio**
Sugarcane Bagasse (SB)	83.17±0.20	SB+CD	41.08±0.05	SB + CD + Water	13.69±0.04	10.27±0.01	6.84±0.04
Rice Husk (RH)	89.41±0.14	RH+CD	43.16±0.02	RH + CD + Water	14.38±0.1	10.79±0.06	7.19±0.08
Wheat Straw (WS)	87.33 ±0.10	RS+CD	42.46±0.04	RS + CD + Water	14.150.05	10.61±0.08	7.07±0.10
Cattle Dung (CD)	20.03±0.10						

Appendix – III

List of Equipment/Instrument Used

List of instruments used

- Weighing balance: weighing scale panel, measuring range; max = 15 kg, min = 0.04 kg, error, error =2 gm, Model: SP/p1s-15-FLP, manufactured by Shyaam Switchgears Pvt.Ltd.
- Gas analyzer (Thermo)
- Muffle Furnance Bionics with model (BST/MF/900)
- Hot air oven (BST/HAO-1122)(300 x 300 x 300 mm)
- Indian standard sieve for particle size measurement (manufactured by Scientific Engineering Corporation)
- Rotameter for flow measurement: capacity 0 to 5lpm.
- Thermocouple sensor –type-K
- Thermocouple calibrator with constant temperaturebath.

Appendix – IV

Accessoried Used in the Experimental Set-Up

Fig. IV.1 Waterbathwhere smallscale digesters were kept for fermentation under constant temperaturecondition

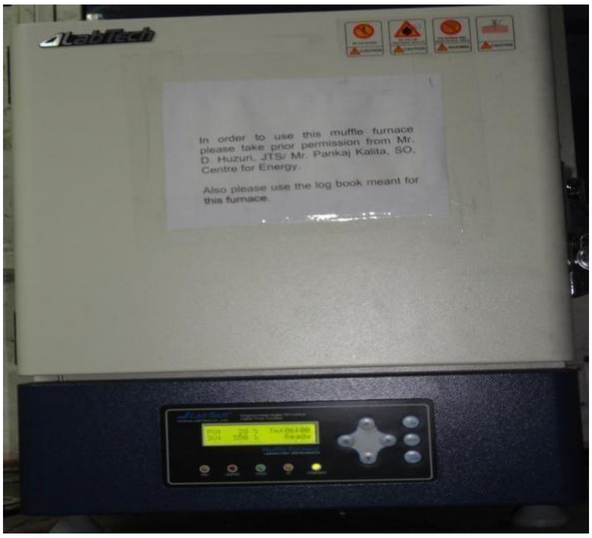
Fig. IV.2 Small scale digesters inside water bath kept for fermentation

	Accessories used: 1) water baths 2) digesters 3) connecting tube

	4) solution bottle 5) conical flask (beaker) 6) copper tube 7) Thermometer 8) measuring cylinder 9) beaker

Fig. IV.3 Various accessories used in the experimental set-up

Fig. IV.4 Convection Oven



In order to use this muffle furnace please take prior permission from Mr. D. Huzuri, JTS/ Mr. Pankaj Kalita, SO, Centre for Energy.

Also please use the log book meant for this furnace.

Fig. IV.5 Muffle Furnace

Fig. IV.6 Set-up for COD with heating mantle

Fig. IV.7 Gas Chromatography machine

Fig. IV.8 Laboratory scale digesters

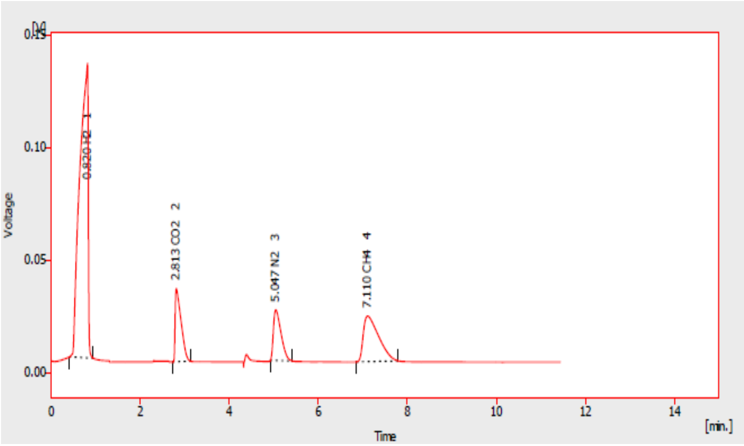


Fig. V.1 GC analysis of biogas from cattle dung

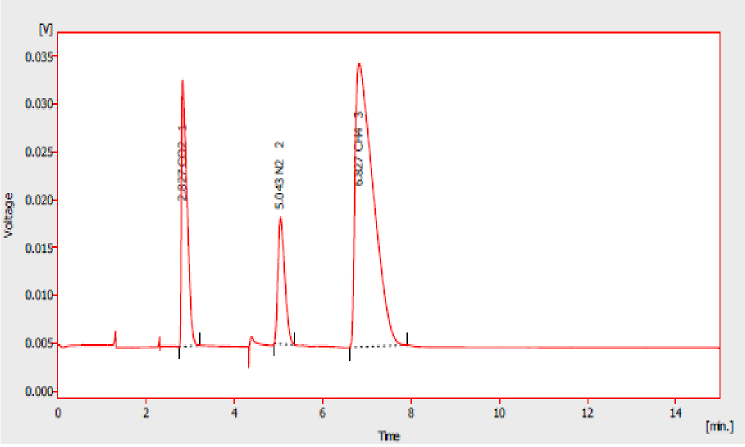


Fig. V.2 GC analysis of biogas from sugarcane bagasse and cattle dung mixture

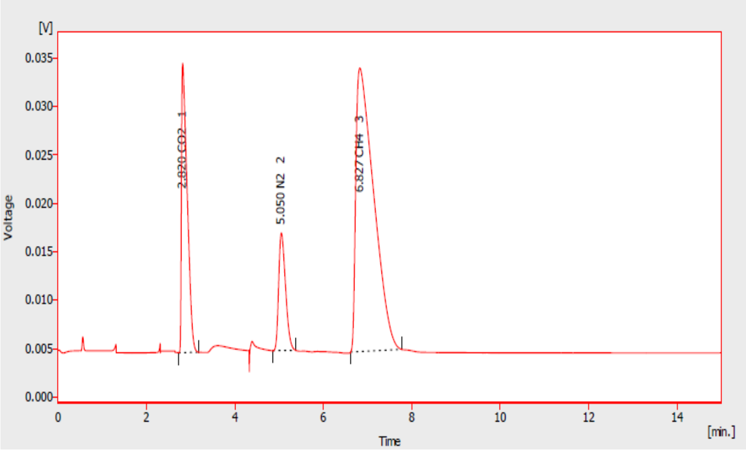


Fig. V.3 GC analysis of biogas from Rice husk and cattle dung mixture

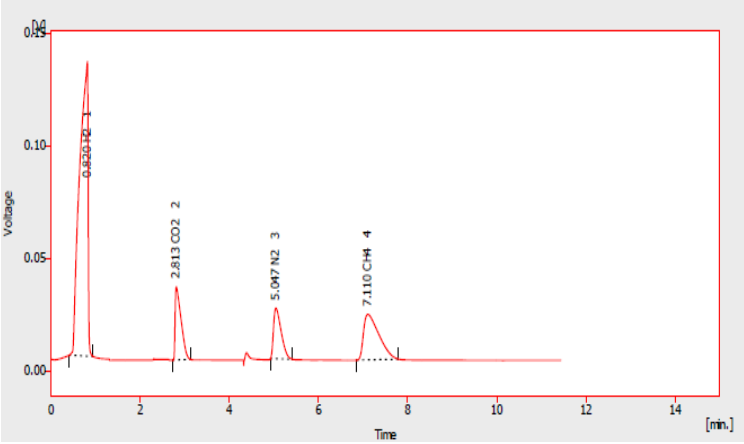


Fig. V.4 GC Analysis of biogas from Wheat straw and cattle dung mixture

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- **Rajan Sharma**, Shailey Singhal and Avanish K. Tiwari, "Pretreatment Impact on Biomethanation of Lignocellulosic Waste" Published in Single Cell Biology Journal 2016, 5:1, ISSN: 2168-9431, DOI10.4172/2168-9431.1000130.
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