Effect of Alkaline Pretreatment on Characteristics and Biogas Production of Peanut (*Arachis hypogaea*) Shells

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2024

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science in

Environmental Science

Supervisor: Dr. Zeshan

Institute of Environmental Sciences and Engineering (IESE)

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Islamabad, Pakistan

2024

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No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to SCEE (IESE) in partial fulfillment of the requirements for the degree of Master of Science in Field of Environmental Science.

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I would like to dedicate my thesis project to my parents and supervisor who guided and assisted me throughout the journey.

ACKNOWLEDGEMENTS

All praise to Allah Almighty, the most Merciful and Beneficent, on whom ultimately, we depend for sustenance and guidance. Allah blessed me with wisdom and courage to accomplish this research and who fetch me to this stage and helped me to come up with this effort.

I would like to express special gratitude to my supervisor **Dr. Zeshan**. This thesis is truly an outcome of his dedication, continuous support, guidance, valuable ideas and suggestions. I am grateful to my Guidance and Examination Committee comprising, **Dr. Muhammad Arshad** and **Dr. Musharib Khan** for their valuable guidance.

I would like to thank all the laboratory staff of IESE and technicians for their help and support. Special Thanks to **Mr. Muhammad Basharat** (Lab technician, Environmental Chemistry and Teaching Lab) for his kind behavior and invaluable assistance during experimental work.

I pay gratitude to my **parents and siblings**, who supported me throughout my research journey. Also special thanks to all my seniors and lab mates, Mr. Junaid, Mr. Zia Ullah, MS. Sahar Saleem, Ms. Sadia Fida, Ms. Bushra Kulsoom, Ms. Safina Ali, Mr. Shoaib Gillani for their continuous help and guidance. My deepest gratitude to my group of friends especially Ms. Shanza Shabbir, Ms. Maira Razzaq, Ms. Mariam Ali, Ms. Hajra Bibi, Mr. Ebadat-ur-Rehman for being my constant support throughout my research journey.

Momina Hanif Malik

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LIST OF ABBREVIATIONS

Abbreviations	Description
ACD	Anaerobic co-digestion
AD	Anaerobic digestion
AMD	Anaerobic mono-digestion
APHA	American Public Health Association
C=C	Carbon to Carbon double bonding
CH_4	Methane
C-H	Carbon to Hydrogen bond
CM	Cow manure
C/N	Carbon to Nitrogen ratio
CO_2	Carbon dioxide
C-O-C	Carbon-Oxygen-Carbon or Ether bond
°C	Degree centigrade
d	Days
DM	Dry matter
3D	Three dimensional
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Gas chromatograph
GDP	Gross Domestic Product
g/L	Gram per liter
gVS/L	Gram of volatile solids per liter
h	Hours
HRT	Hydraulic retention time
H_2	Hydrogen
H_2O	Water vapors
H_2SO_4	Sulphuric acid
KBr	Potassium bromide
KOH	Potassium hydroxide
LCH	Lignin, cellulose and hemicellulose
mm	Millimeter

M	Molar solution
MC	Moisture content
mg/L	Milligram per liter
Mt	Million tonnes
mL	Milliliters
N	Normal solution
N_2	Nitrogen
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NH_3	Ammonia
NmL	Normal milliliter (Volume at gas at standard temperature pressure and without moisture content)
NmL/gVS	Normal milliliter per gram of volatile solids
OLR	Organic loading rate
PS	Peanut shells
rpm	Revolutions per minute
S/I	Substrate to inoculum ratio
TA	Total alkalinity
TKN	Total Kjeldal nitrogen
TOC	Total organic carbon
TS	Total solids
USEIA	US Energy Information Administration
VFAs	Volatile fatty acids
VS	Volatile solids
VW	Vegetable waste
w/v	Weight per volume
%	Percentage
%T	Transmittance in percentage
2% PS	2% NaOH pretreated peanut shells
4% PS	4% NaOH pretreated peanut shells
6% PS	6% NaOH pretreated peanut shells
8% PS	8% NaOH pretreated peanut shells

ABSTRACT

Anaerobic digestion (AD) mitigates high energy demand by converting lignocellulosic waste into biogas and digestate. This process supports the economy, reduces energy crises, and enhances environmental sustainability. Agricultural residues serve as promising energy resource in carrying out AD process. Recalcitrant lignocellulosic structure of *Arachis hypogea* shells and high carbon to nitrogen (C/N) ratio of peanut shells (PS) usually impedes efficiency of AD process. Therefore, this study was conducted with two aims i.e. application of alkaline pretreatment on PS before initiating AD and use of vegetable waste (VW) as a co-substrate to balance the C/N for effective AD performance. Exposure of PS was given to varying NaOH concentrations of 2%, 4%, 6% and 8%, which was followed by batch-mode AD including mono- and co-digestion under mesophilic temperature range for 45d. The characterization of untreated and pretreated PS was done using FT-IR (Fourier Transform Infrared) spectroscopy. FT-IR results confirmed the change in structural peaks after pretreatment. Additional ultimate analysis depicted the enrichment of essential nutrients in VW, making it suitable for using as co-substrate with PS. Results showed that 4% NaOH provided maximum cellulose recovery (96.04%) and 75% lignin removal. Further investigation revealed an improvement in biogas production during co-digestion setup rather than mono-digestion. Anaerobic co-digestion (ACD) of NaOH pretreated PS with VW has recorded cumulative biogas production in following order: 4% > 2% > 6% > 8% which produced 65% , 58.75%, 42.2% and 37.5% respectively more cumulative biogas as compared with control group. Overall, low NaOH dosage i.e. 2% to 4% was found more effective than high dosage 6% to 8% in delignification. This study concluded that alkaline pretreatment has effectively improved cellulose recovery and lignin degradation, while ACD resulted in greater biogas production than mono-digestion.

Key words: Anaerobic co-digestion, Biogas enhancement, Lignocellulosic waste, Peanut shells, Vegetable waste.

CHAPTER 1: INTRODUCTION

1.1 Background

Rapid growth of global population, better lifestyles and increased industrialization has triggered a notable upsurge in energy demand, forecasted to escalate by 56% by year 2040 according to the US Energy Information Administration (2020). To improve country's economic growth and its development, energy serves as a key element. At global level, the exploitation of fossil fuels as a conventional option for producing energy has become a serious concern regarding environmental problems like climate change and global warming (Agyekum et al., 2021; Ansari et al., 2021; Peter, 2018).

Tragically, like other developing countries, Pakistan is also facing various issues owing to energy crisis and mainly reliant on non-renewable fossil fuels. By year 2005, the use of natural gas in Pakistan had significantly ascended, comprising nearly 50% of the total energy consumption (Kardon et al., 2020). Likewise, this country relied heavily on importing large quantities of crude oil to meet its energy requirements (Kamran, 2018). The overuse of non-renewable energy resources has caused environmental degradation along with depletion of fossil fuel reserves (Agyekum et al., 2021; Ansari et al., 2021). To battle these concerns, country is considering plenty of sustainable and renewable energy options (Xu et al., 2019; Zhang et al., 2017). At present, bioenergy stands out as a key option in alleviating energy shortage, being recognized as the fourth-largest energy resource worldwide (Dutta et al., 2021; Chen & Lee, 2014). In this scenario, bio-waste serves as a potential renewable source for a green and clean energy production which also fulfilling the 3Rs (reduce, reuse, recycle) principle (Aklilu et al., 2021). Ahmed et al. (2020) reported that roughly 120 billion tons of lignocellulose waste gets discharge into environment every year, equivalent to approximately 2.2 x $10^{\wedge 21}$ joules. This amount surpasses the current worldwide energy requirement by more than 300 times (Guo et al., 2015).

Pakistan's geographic location provides ample opportunities for harnessing renewable energy resources such as marine, solar, wind and biomass. Among renewable options, biomass has received significant attention being a promising, suitable and sustainable alternate for fossil fuels to produce substantial amounts of low-carbon energy (Ahmad et al., 2020; Dahunsi et al., 2019)

and other valuable energy by-products i.e. biogas and bioenergy (Isikgor and Becer, 2015; Zheng et al., 2018). Pakistan's status as an agrarian country underscores its vast potential for producing bioenergy from a diverse array of lignocellulosic biomasses such as agricultural residues, crop residues, food waste, sugar bagasse, wheat straw, rice straw, animal and other poultry litter etc. (Maryam et al., 2021; Abraham et al., 2020; Ufodike et al., 2020; Saeed et al., 2015; Asif, 2009).

Peanut is a prominent leguminous crop that is extensively distributed across various zones like warm temperate areas in Asia, Africa, South America, North America, Europe and other tropical countries (Freeman et al., 1999). Based on Agricultural Statistics of Pakistan (2022), approximately, 84% of the total peanut-producing area is situated in Punjab, with 13% in KPK and the remaining 3% in Sindh illustrated in Figure 1.1.

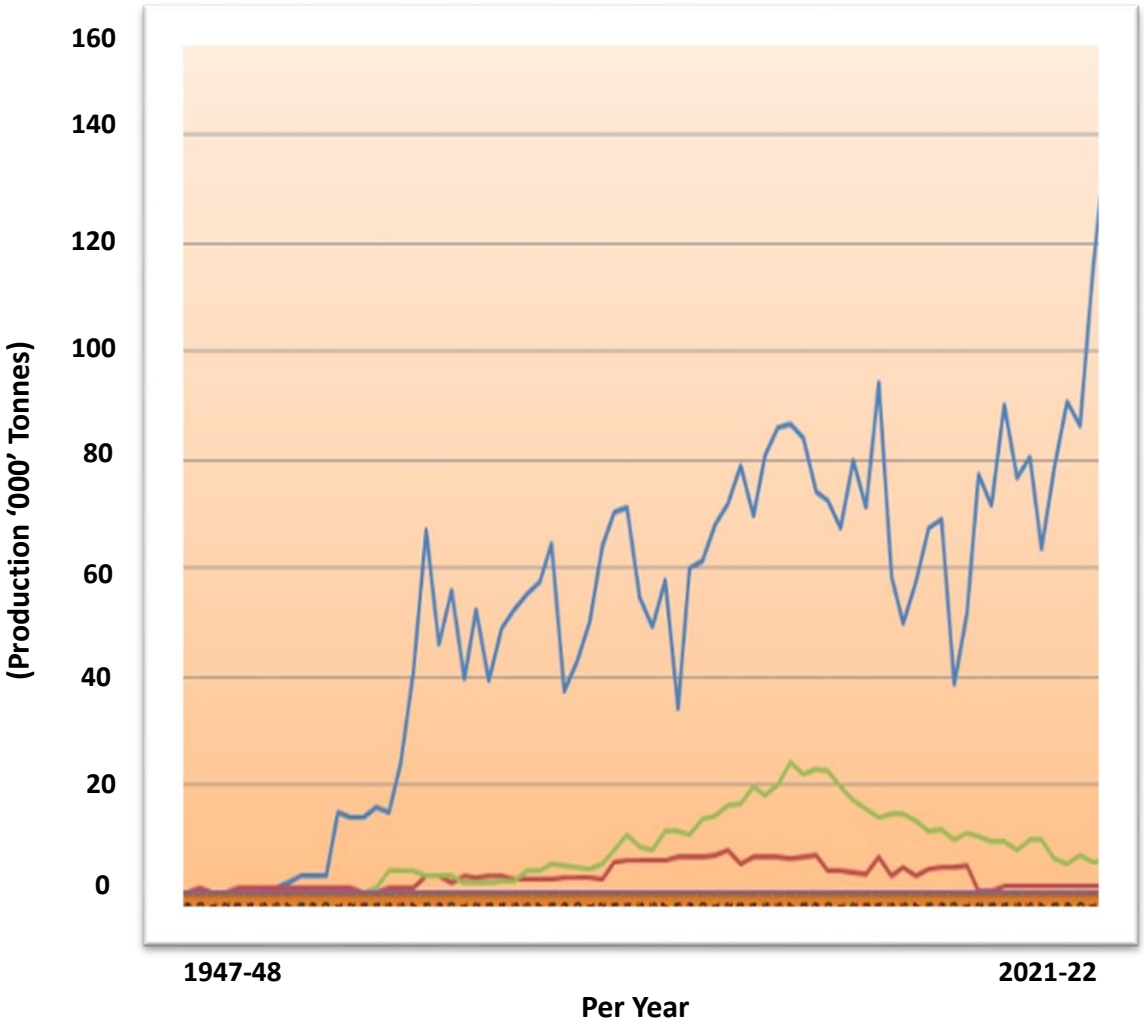

Figure 1.1 Province-wise production of *Arachis hypogaea* in Pakistan (1947-2022)

In time span of 1998-1999, peanut cultivation extended over 97,500 hectares, resulting in a production of 104,000 tons, with an average yield of 1067 kilograms per hectare (Abbas, 2021). Based on USDA (2016) report, the worldwide production of peanuts, including shells, was noted to be 40 million tons during 2015. China accounted for 40%, while other Asian countries contributed 19%, Americas 11% and Africa 18% (Fletcher et al., 2016). Another study (FAOSTAT, 2022) presented nearly 53,638,932 tonnes of peanut were produced globally in the year 2020. The peanut pod typically comprises of 65-75% seed, while the remaining 25–35% constitutes the protective shell layer (Nigam, 2014), resulting in 8 Mt of residual biomass in Asia alone (Perea et al., 2018; Kristoferson et al., 1986). These shells contain high percentages of cellulose, hemicellulose, and lignin content (Almeida et al., 2024; Jekayinfa et al., 2020). During harvesting and processing, bulky amount of peanut residues are mostly left on fields that can harbor the pathogens growth and if burned, produce harmful gases (GHGs) directly into atmosphere and squander their potential with losing inherent energy (Singh et al., 2021; Jekayinfa et al., 2020; Heuze et al., 2017; Kerr et al., 1986). This is seemed as a deadly practice for waste management as well as environment sustainability (Khalid et al., 2019).

From recent years, peanut shells as a lignocellulosic material came under the limelight since it's been produced extensively. Approximately, 28 million tonnes (Mt) of peanuts are yearly produced. Past researches highlight the opportunity to consume peanut shells for producing biogas which in turns uphold low carbon energy and zero-waste production (Olatunji et al., 2024). Such residual waste entails high energy content that is worth-exploring. For positive environmental impact, these organic shells must be processed into valuable green by-products i.e., biogas, biofuels, biochar and bioenergy prior to their disposing and burning (Lokasundaram, 2023; Lin et al., 2023; Qi et al., 2020; Dharanipriya et al., 2019).

1.2 Prosperities of biogas technology

Biogas emerges as a promising substitute for fossil fuels, offering plenty of environmental and socioeconomic benefits enlisted below:

- Cost effective (Holm-Nielsen et al., 2009, Mao et al., 2015)
- Clean and Eco-friendly (Ngan et al., 2020).
- Safeguards the environment (Cuellar and Webber, 2008)
- Limits the atmospheric emissions of GHGs (Cuellar and Webber, 2008)

- Green energy production (Rehl and Muller, 2011)
- Manages waste from direct disposal or landfilling (Cuellar and Webber, 2008)

Anaerobic digestion is basically a series of processes i.e. Hydrolysis, acidogenesis, acetogenesis and methanogenesis) performed by consortia of microbes in absence of O_2 that helps in degrading the complex carbohydrates of bio-waste into carbon dioxide (CO_2) and methane (CH_4). During AD process, hydrolysis is often envisaged as a rate-limiting step that often prolongs the AD process with low biogas yield (Dutta et al., 2021; Maharaj et al., 2019). To tackle this, pretreatment step is highly needed prior to AD process as it facilitates the de-lignification more effectively with high cellulose provision to enzymatic hydrolysis as well as bacterial attack which subsequently produce more biogas (Dutta et al., 2021).

Several methods are readily available for converting biomass into valuable by-products for instance combustion, fermentation, pyrolysis and anaerobic digestion (AD) etc. Combustion and pyrolysis are usually energy-intensive processes to treat humid-rich waste with high operational cost (Grande et al., 2021). Thus, AD technique has been a common practice for biogas generation at domestic and commercial level as well (Ngan et al., 2020) due to its cost effectiveness and nutrient rich bio-digestate and more CH_4 as by-products for past decades (Zhang et al., 2019; Hawash et al., 2017; Neshat et al., 2017; Zhou et al., 2017).

1.3 Need for pretreatment of lignocellulosic biomass

Any lignocellulosic substrate contains three key components including cellulose, hemicellulose and lignin in their composition (Almeida et al., 2024; Kassaye et al., 2016). In the structure, cellulose polymer makes β-1-4 glycosidic bonds and is highly accountable for biogas generation during AD (Koupaie et al., 2019). Due to shielding of cellulose by other polymers (lignin and hemicellulose), conversion of biomass into biogas is not very easy, often delayed and results into low biogas generation (Hassan et al., 2017; Rodriguez et al., 2017). Similarly, lignin makes a complex recalcitrant 3D structure throughout cell wall and inhibits the microbial degradation leaving behind the more cellulose undigested (Almeida et al., 2024; Neshat et al., 2017; Sun et al., 2016). Therefore, to break such rigid bonds, pretreatment of lignocellulose material is an essential step to achieve high yield of biogas after digesting major cellulose portion and to overcome structural impediments of feedstock during AD (Zheng et al., 2018). The impact of pretreatments significantly fluctuates from feedstock to feedstock, showing no universal

consistency (Olatunji et al., 2024; Olatunji et al., 2023; Olatunji et al., 2022; Siddhu et al., 2016; Menardo et al., 2012).

Previous studies have mentioned countless appropriate pretreatment techniques such as mechanical (grinding, extrusion and milling), chemical (alkaline, acidic, advanced oxidation process), biological (fungal, bacterial and enzymatic) and thermal pretreatments to improve the rate-limiting step during AD process for maximum biogas production (Awoyale et al., 2021; Nges et al., 2016; Liu et al., 2015). Thermal and biological pretreatments have few limitations because of its high cost, more energy consumption, time-consuming method; incomplete hydrolysis, formation of inhibitory compounds as well as bacterial or fungal strains is not easily cultivated upon lab conditions (Osorio-Gonzalez et al., 2018).

Previous work declared alkaline pretreatment to be more effective in use due to its low operational cost with mild conditions; easily decline the cellulose crystallinity as well as high pH can greatly dissolve more lignin and hemicellulose. Moreover, alkaline pretreatment have resulted into more lignin degradation (Khalid et al., 2019). More frequently used alkaline chemicals are NaOH and KOH (Maryam et al., 2021; Veluchamy et al., 2018).

It is highly challenging to optimize the anaerobic digestion (AD) process exclusively with utilizing mono-substrates due to various factors including inadequate microbial communities, nutrient imbalances and influence of operational parameters (Ngan et al., 2019). Despite of securing energy, solely-used substrate can create hindrance in the performance of microbes during digestion due to excessive nitrogen and ammonia (Moestedt et al., 2016). Low value of C/N ratio can cause carbon deficiency and ammonia accumulation similarly more value of C/N can produce nitrogen deficiency thus produce less methane yield (Li et al., 2011). Hence, solely use of peanut shells in AD is seemed barely effective as it limits the biogas generation due to its high carbon to nitrogen (C/N) ratio which means nitrogen content is very low in PS thus impedes efficiency of AD process owing to nutrient imbalancing. Regarding this, co-digestion factor could assist in improving and optimizing AD after C/N adjustment (Olatunji et al., 2023, Aklilu et al., 2021; Rajput et al., 2021). For that reason, co-digestion with nitrogen rich waste such as vegetable waste might be helpful in supplementing the nitrogen (N_2) deficiency. The nitrogen content in vegetable waste is relatively high which could help in adjusting C/N ratio to ideal range i.e. 20-30 (Rajput et al., 2021).

1.4 Significance of the study

Globally, to manage our ecosystem and fulfilling energy demand, the right use of existing energy resources has gained alot of attention for past decades. Like other crop residues, managing peanut shells has become another emerging challenge in developing countries. In a world where sustainability is no longer but a necessity, every bit of resource efficiency matters. Therefore, the benefits of these shells shouldn't be limited to gardens only. These casings hold a wealth of value because of its lignocellulosic nature which makes them far mightier to generate renewable green energy through AD process. Past studies revealed that suitable pretreatment is desired to overcome this compositional hindrance in order to produce more biogas. Besides this, ensure the nutrient balancing is another main element during AD process.

Hence, this study was conducted with two aims i.e. using alkaline pretreatment of peanut shells before initiating anaerobic digestion and use of selective vegetable waste as a co-substrate to balance the nutrients for a greater microbial performance. Peanut shells are carbon enriched that act as a potent energy source for microbes while vegetable waste holds nitrogen enrichment to balance the C/N ratio. Yet, to best of our knowledge, no past studies are available on anaerobic co-digestion of pretreated peanut casings with mixture of selective vegetable waste as co-substrate.

1.5 Objectives of the study

Anaerobic digestion of peanut shells is highly challenging due to its intricate lignocellulosic composition and high C/N value. To address this issue, current study has focused on following main objectives:

1. To study the effect of alkaline pretreatment on lignocellulosic composition of peanut shells.
2. To study the effect of pretreated peanut shells and untreated peanut shells on biogas production.
3. To study the effect of co-digestion with pretreated and untreated peanut shells on biogas production.

1.6 Scope of the study

The defined scope of present study is as follows:

1. The effect of alkaline pretreatment on the biogas production of peanut shells was assayed.
2. Peanut shells were collected from field at Attock district and vegetable waste were collected from local market (Itwar-bazar) in Islamabad.
3. Fresh cow manure was used as an inoculum which was collected from local animal farm at H-13 Islamabad, near NUST.
4. Effectiveness of alkaline pretreatment was monitored through changes in lignocellulosic composition of peanut shells.
5. Batch-mode experiments of mono-digestion and co-digestion were carried out at lab-scale.

CHAPTER 2: LITERATURE REVIEW

This chapter mainly focused on availability of lignocellulosic waste with detailed review on the pretreatment of waste with synergistic effect of using more suitable substrates and how it influence the biogas production , the mechanism of an anaerobic digestion and factors affecting it.

2.1 Lignocellulosic biomass

Lignocellulose biomass is basically an organic dry matter of a plant which is ultimately available in huge amount and pondered as optimal source for energy production (biogas, biofuels etc.) instead of consuming conventional fossil fuels. This waste categorizes many different kind of bio-materials including agricultural residues, crops residues, forestry residues, shells (Santos et al., 2021).

Globally, lignocellulosic waste has been spawned about 1.3 billion tons on yearly basis however merely 3% gets consume for producing valuable energy by-products (Areepak et al., 2022). The lignocellulosic material is typically made up of cellulose, hemicellulose, and lignin as 35-50%, 20-35% and 10-25% respectively (Almeida et al., 2024; Liu et al., 2008) in addition to small amount of extractives (Ghaemi et al., 2019). Though, these percentages vary from substrate to substrate on the basis of their origin, type and growing conditions etc. Figure 2.1 is displaying the common lignocellulosic structure of plant cell wall (Sankaran et al., 2021).

2.1.1 Components of lignocellulosic biomass

The major component of lignocellulosic biomass is cellulose which makes a linear structure of β-1, 4 glycosidic bonds strongly linked with D-glucose units (10,000-15,000) forming a long chains. The strong hydrogen bonding as well as Vander Waals forces between these cellulose chains forms micro fibrils. This biopolymer structure consists of two parts, one is highly crystalline and other is amorphous (less crystalline). The pectin, hemicellulose and lignin also assist these micro fibrils of cellulose to make a highly compact structure with more protection against any microbial or chemical attack (Wang et al., 2020; Zheng et al., 2018).

Unlike cellulose, hemicellulose is a biopolymer builds a nonlinear (branched) structure with highly amorphous nature. Such kind of arrangement makes it extremely vulnerable to heat and

other biological and chemical attack (Singh et al., 2014). This carbohydrate polymer is mainly composed of several types of sugar polymers (C5, C6) such as hexose, pentose as well as sugar acids through glycosidic bonds (β-1, 4 and β-1, 3) present between them. Hemicellulose provides high rigidity to the lignocellulosic structure by connecting lignin and cellulose together to itself (behaving as a connecter) by forming a matrix (cellulose-hemicellulose-lignin). Moreover, its solubility rises with temperature (Liu et al., 2019).

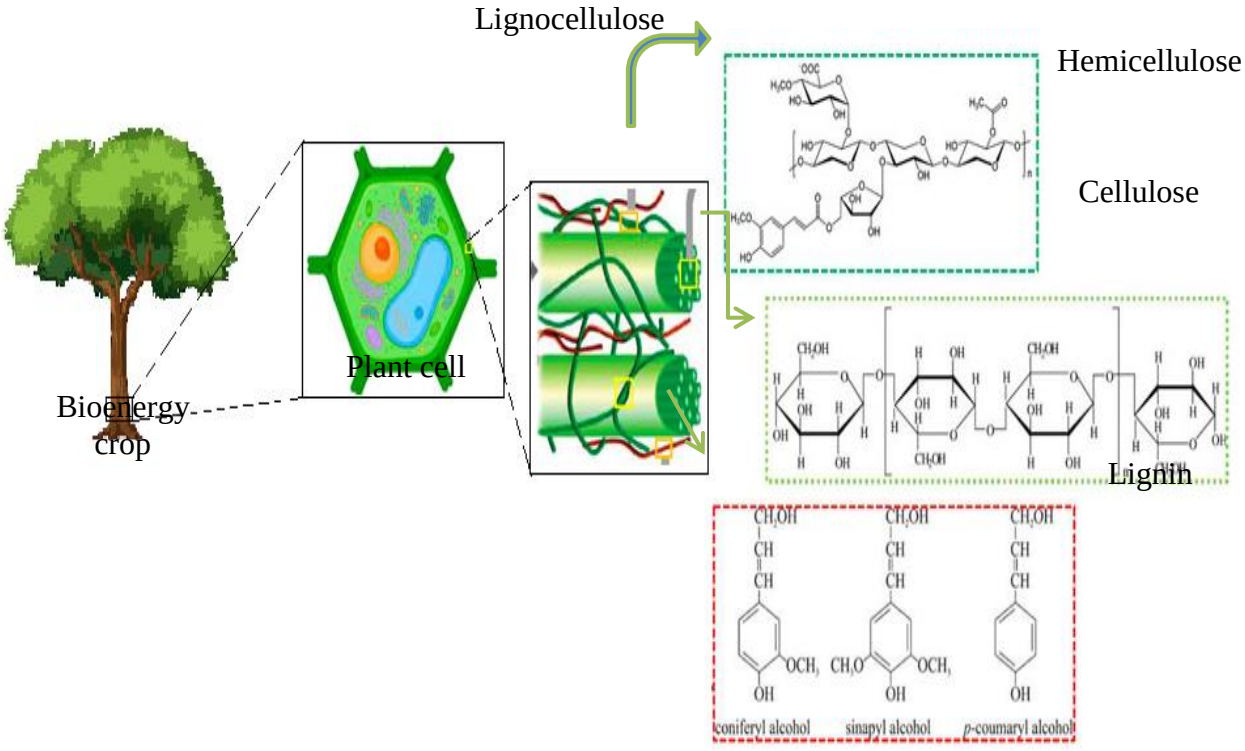

Figure 2.1 Lignocellulosic structure of plant cell wall

The third principle component of lignocellulose is lignin. This aromatic biopolymer is hydrophobic in nature (water repellent) which makes it insoluble in water as well as forms an amorphous structure. It is mostly present in cell wall and protects other polymers (cellulose and hemicellulose) against biological attack as well as strengthens the structure of lignocellulose. It is formed by three alcohol units (C6-C3) i.e., coniferyl alcohol, sinapyl alcohol and p-cuomaryl alcohol in cross-linkage manner. The lignin provides the 3D structure to lignocellulose by interlinking hemicellulose as well as cellulose. Lignin acts as a recalcitrant component which prohibit the bioconversion that effortlessly (Kucharska et al., 2018; Zheng et al., 2018).

2.2 Pretreatment of lignocellulosic biomass

Lignocellulosic materials are considered as a key energy resource due to its cellulose and hemicellulose portions that are highly responsible for biogas generation during AD process. On the other hand, another portion (lignin) of lignocellulose, being a recalcitrant constituent, impedes the biomass conversion through degradation anaerobically which ultimately reduces the biogas yield. Lignin basically shields the cellulose and hemicellulose which in turns lessen the cellulose digestion. Thus, to enrich the yield of biogas, the application of pretreatment techniques is obligatory prior to anaerobic digestion process for lignin degradation and ensures the high availability of cellulose towards biological attack (Bhatia et al., 2019; Sindhu et al., 2017)

Lignocellulosic substrates are primarily classified into two portions, one is biodegradable and other is recalcitrant which is shown in Figure 2.2. Therefore, it is highly needed to degrade the recalcitrant portion in order to increase the lignocellulose feedstock biodegradability. Past literature has observed that several factors disturb the biodegradability of feedstock based on structural nature for instance, degree of crystallinity of cellulose and available surface area etc. Therefore, pretreatment helps to modify the structure for enhanced biodegradation (Yang et al., 2015).

Zheng et al. (2018) reported several pretreatment techniques which are categorized into: physical (including pyrolysis, ultrasound, microwave, grinding), biological (such as microbes, enzymes and fungi) and chemical (alkali, wet oxidation, acid, steam explosion) etc. Another study (Behera et al., 2014) mentioned various significant factors that need to be considered while selecting the pretreatment method such as cost effective, more economical (energy efficient), more yield, enhance digestibility of cellulose, high sugar (carbohydrate) recovery, nutrient rich digestate and increased lignin degradation etc. (Kumar and Sharma, 2017).

2.3 Mechanism of anaerobic digestion (AD) process

The mechanism of anaerobic digestion process is illustrated in Figure 2.3. Anaerobic digestion (AD) is considered as a highly versatile method that is carried out by group of microbial activity to breakdown the complex structure of organic waste into valuable energy by-products (nutrient rich digestate, bioenergy and biofuels) in the absence of oxygen (Di Maria et al.,2017). The final

products of AD process includes biogas (60-75% methane, 30-40% carbon dioxide and some other trace gases (H_2S, N_2, H_2, and water vapors), nutrient enriched digestate that can further be used as a fertilizer. The generation of biogas takes place through a collaborative process involving a consortium of microbes, progressing through four separate phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Each phase results into different by-products (Wei et al., 2018; Li et al., 2012).

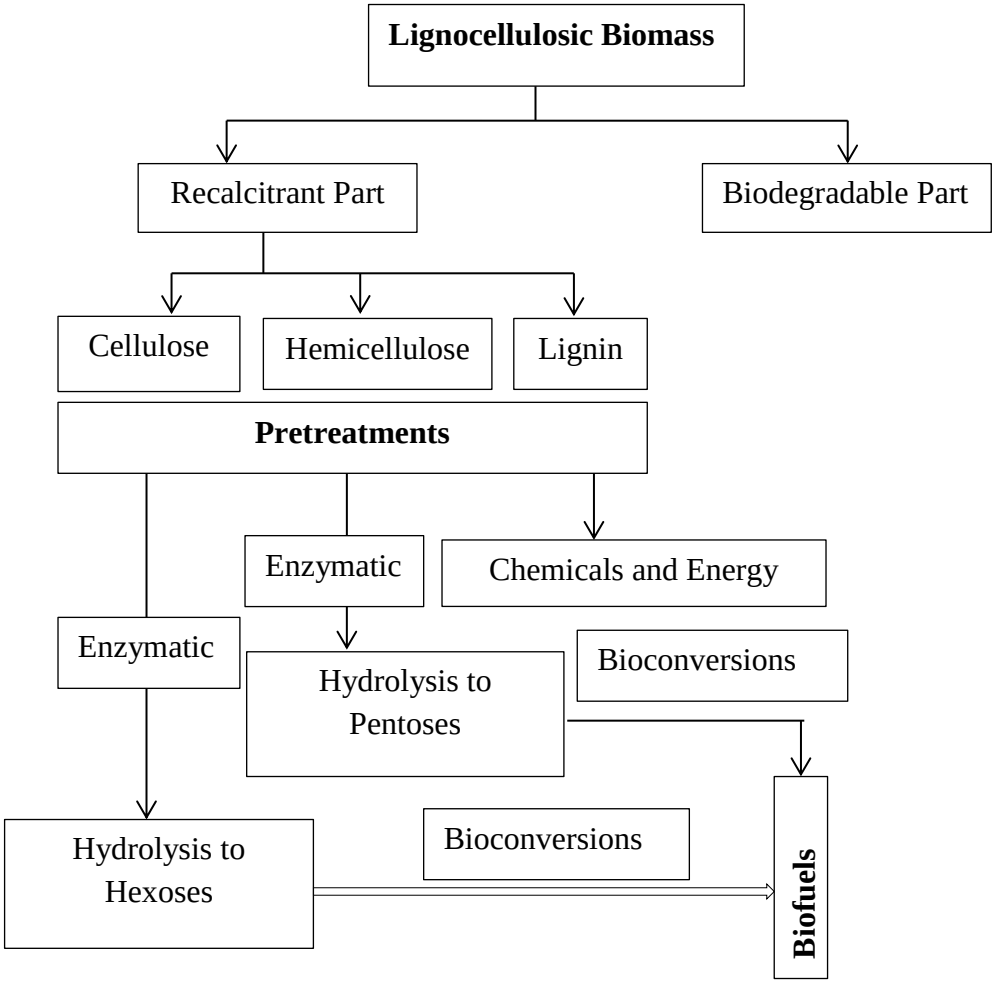

Figure 2.2 Classification of lignocellulosic biomass

2.3.1 Hydrolysis

Firstly, hydrolysis reaction takes place when complex insoluble organic material is broken down into simple soluble compounds. For instance, carbohydrates, proteins, lipids convert into sugars, amino acids and fatty acids using extracellular enzymes. This extracellular step is necessary

before acidification process occurs which helps fermentative microbes to integrate complex compound directly into their body. This whole step ensues in the presence of various enzymes including xylanase, cellulose, amylase and cellobiase responsible for degrading carbohydrates, lipase enzyme is accountable for breaking down of lipids while protease upholds the degradation of proteins (Schon, 2010). Due to recalcitrant nature of organic matter, this hydrolysis step is often termed as rate-limiting step. Because of which, anticipation of pretreatment is considered for breaking down of crystallinity in biomass structure (Bajpai, 2017; Hendriks & Zeeman, 2009).

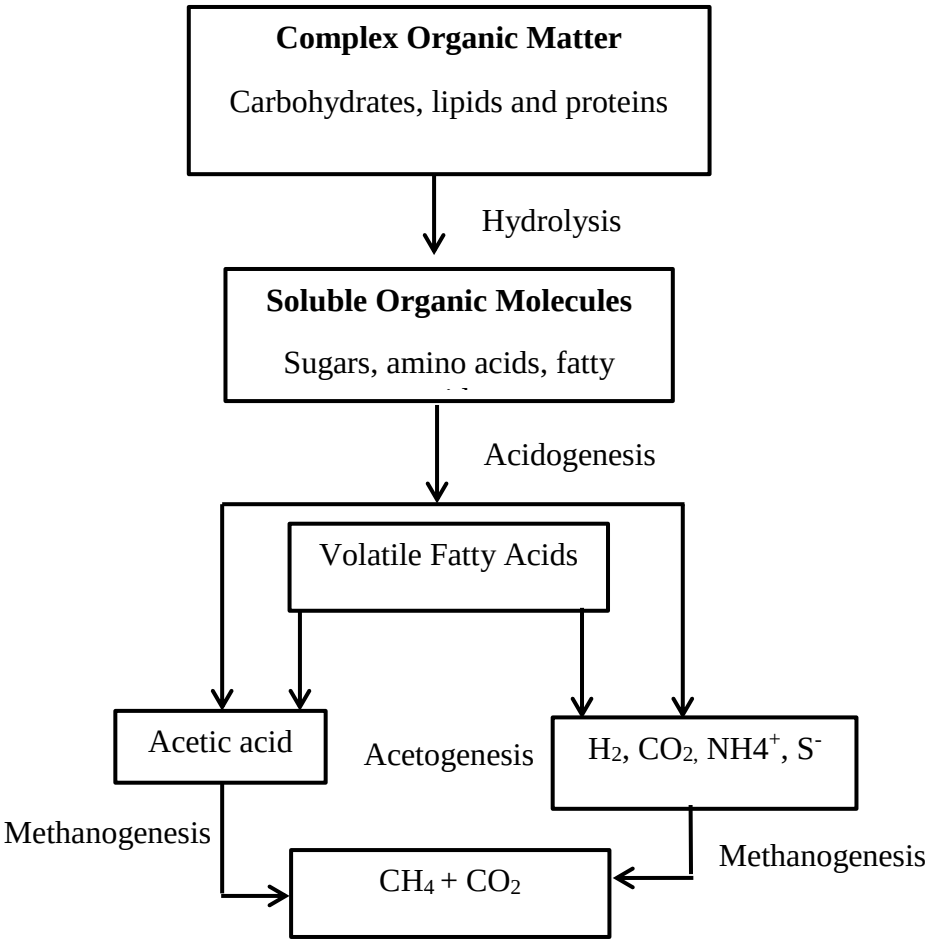

Figure 2.3 Mechanism of anaerobic digestion process

2.3.2 Acidogenesis

Fermentative microbe is responsible to carry out this step to further breakdown the compounds into carbon dioxide, formate, propionate, methanol, hydrogen, butyrate and acetate etc. During

this phase, acidogenic microbes (known as acid-formers) also facilitate the conversion of hydrolysis by-products into volatile fatty acids. The organic matter degrades spontaneously and liberates additional energy for bacterial community (Bajpai, 2017; Vavilin et al., 2008).

2.3.3 Acetogenesis

Acetogenic microbes become very active during this phase and convert the previous step by-products (organic acids) into acetic acid, acetate, H_2 and CO_2 through oxidizing which ultimately can be utilized for producing methane (Liu et al., 2018).

2.3.4 Methanogenesis

Two distinct microbial consortia named as Acetoclastic (acetate consumer) and hydrogenotrophic (H_2 and CO_2 consumer) microbes (methanogens) are responsible in producing methane at the end of this last phase.

These methanogenic microbes are also termed as strict anaerobes. The conversion of acetate into CH_4 and CO_2 is facilitated by acetotrophic methanogenic bacteria while direct conversion of carbon dioxide into CH_4 is ensued by hydrogenotrophic methanogenic bacteria that consume H_2 as electron donor (Bajpai, 2017; Angelidaki et al., 2011).

2.4 Factors affecting anaerobic digestion process

A variety of microbes are accountable to govern entire anaerobic digestion process. Therefore, favorable environment is their necessity to perform effectively and efficiently. Weiland (2010) highlights some significant factors that can disturb the favorable environment of microbes and ultimately devastate the AD process if not properly considered. The detailed discussion of these stability parameters which play a key role in making reactor more stable is provided here.

2.4.1 Particle size

Kratky and Jirout, (2011) reports the size of particles used in AD process truly matters in case of successful digestion i.e. small size of substrate particles can be easily digested by microbes due to low crystallinity of cellulose with availability of more surface area then enhanced methane generation. Yadvika et al. (2004) demonstrated that hydrolysis phase of AD process can get more effective and efficient by utilizing substrate particles of small size (Eryildiz et al., 2020).

2.4.2 Moisture content

All chemical and bio-chemical reactions necessitate the moisture content which assists microbial community to do the AD process through increased material transfer as well as more nutrient absorption hence increased production of biogas (Eryildiz et al., 2020; Heiske et al., 2015).

2.4.3 pH

This is considered as the most critical parameter in AD process. Since the whole process of anaerobic digestion is greatly depending upon pH which needs to lie within optimal range to benefit the microbial community otherwise this can directly disturb the stability of a reactor as well as the microbial performance. Literature shows that methanogenic microbes work actively in the pH around 6.8-7.5 (optimal range) which means if pH drops below 6.8 or goes above 7.5 can create disturbance in both conditions.

For instance, any sudden deviation from this optimal range can considerably distress the microbial growth, their activity and restrain the overall digestion system due to the occurrence of many toxic intermediates and more alkalinity levels can produce less methane yield. This all can happened due to the rapid increase in VFAs, acetic acid and ammonia which execute negative impact on AD process (Eryildiz et al., 2020; Hagos et al., 2017).

2.4.4 Temperature

Temperature is another most significant parameter of AD process that can tremendously upset the overall performance and stability of anaerobic digester. Generally, AD process can be operated under multiple temperature ranges for instance hyper-thermophilic range (above 55°C) thermophilic range (50^{o}C to 55^{o}C), mesophilic range (35^{o}C to 40^{o}C) and psychrophilic range (11^{o}C to 25^{o}C) based on study (Uddin et al., 2016).

The microbial community requires temperature maintenance to perform actively because they are highly sensitive towards temperature fluxes which ultimately influence the substrate degrading rate and less methane formation. Yadvika et al. (2004) observed that biogas generating anaerobes perform well under mesophilic and thermophilic regimes than other ranges.

Both temperature regimes demonstrate some pros and cons as well. For instance, low temperature (mesophilic) demands more retention time (nearly 45d or more) as compared to

thermophilic (15d to 25d) (Eryildiz et al., 2020; Jain et al., 2015). Mesophilic regime is more cost effective than thermophilic as it consumes less energy (Neshat et al., 2017).

Moreover, thermophilic is vulnerable to the surrounding conditions as compared to mesophilic. Despite of high biogas production through thermophilic operation, it is not commonly preferred in AD process due to more heat consumption rather mesophilic operation upholds better performance, high stability (Bowen et al., 2014). The summarized evaluation of mesophilic and thermophilic operations during AD procedure is illustrated in Table 2.1.

Table 2.1 Summary of mesophilic and thermophilic anaerobic digestion (AD) process

Operations	**Mesophilic** (35 ^{o}C - 40 ^{o}C)	**Thermophilic** (50 ^{o}C - 55 ^{o}C)
Energy demand	Less	More
AD system stability	More	Less
Methane yield	Less	More
Retention time	More (45 d)	Less (15 d – 25 d)
Degradation rate	Less	More
Temperature sensitivity	Less	More

2.4.5 Organic loading rate (OLR)

This is another significant parameter in AD process which defines the quantity of substrate (dry) that is used per digester volume per time. This seems a key parameter in attaining the active performance of microbes which helps in improving biogas yield as well. Undeniably, microbes need extremely favorable loading conditions thus maintaining the OLR is very significant else

obstruction to system can occur eventually i.e. increased feeding in the digesters can unfavorably influence the system by microbial (methanogens) inactivation which further dominates the acid formation through acidogenesis and overall pH of reactor falls. Subsequently, methanogenic microbes become impotent to convert large amount of acids into methane during acidic condition and poorly cease the process (Eryildiz et al., 2020; Rincon et al., 2008).

2.4.6 Carbon to Nitrogen ratio (C/N)

For an effective and faster growth of microbes and their active performance has based on nutrients availability within a system. Adding the appropriate amount of nutrients either micro or macro seems very essential for continuing the AD process more effectively. Micro-nutrients are highly accountable for methane generation (Nges et al., 2012). Risberg et al. (2013) stated that 20-30 range is found to be the optimum (ideal) for C/N ratio of anaerobic digester.

The C/N is basically a carbon to nitrogen ratio and any deviation from this ideal range either more or less can influence the system stability. For instance, less value of carbon to nitrogen ratio can cause carbon deficiency and ammonia accumulation similarly more value of C/N can produce nitrogen deficiency and produce less methane yield. Therefore, any change from optimal C/N value can lead failure to AD process (Li et al., 2011).

Risberg et al. (2013) discussed that lignocellulosic materials contain more C/N value while manure contains less C/N value. In this way, application of co-digestion can achieve the nutrient balancing more efficiently rather than sole substrate.

2.4.7 Hydraulic retention time (HRT)

Likewise OLR, hydraulic retention time is also an obligatory parameter during AD process. Microorganisms necessitate a proper feeding time to provide by-products. The maintenance of optimal HRTs in system must be satisfied for effective AD process. For example, increased HRTs can cause nutrient shortage and ultimately microbial death occurs while decreased HRTs give rise to more acid accumulation in system and eventually less methane production (Eryildiz et al., 2020; Metcalf, 2003).

2.4.8 Volatile fatty acids (VFAs) and alkalinity

During AD process, several different by-products are obtained at the end of each phase. So, formation of volatile fatty acids is one of them such as butyric acid, acetic acid and propionic

acid. VFAs are seemed very useful in evaluating the stability and performance of the whole anaerobic digestion process i.e. pH value drops if more VFA's accumulation occur and cause acidification as well which hinders the microbial activity and overall AD system gets ceased (Bah et al., 2014). Cirne et al. (2007) reported that the VFA value of nearly 1500-2000 (mg/L) can lead to AD failure.

Similarly, Alkalinity is another important factor to evaluate the working and stability of anaerobic reactor. At neutral pH, the AD process remains stable and produce relatively high methane yield. This level of pH can only be attained if substrate contains high value of alkalinity against VFAs. In order to maintain the system pH, several buffering reagents can be utilized mainly sodium bicarbonate and lime etc. (Khalid et al., 2019; Neshat et al., 2017; Li et al., 2009).

2.4.9 Inoculation

Inoculation is highly needed in the process of anaerobic digestion to avoid the lagging period during biogas yield as several communities of bacteria are present in inoculum which actually digest the material or else whole process will get prolonged to initiate.

Conventionally, animal manure (Dhamodharan et al., 2015) is used as inoculum which is easily available or digestate (Ye et al., 2013) from treatment plants can also be utilized. The past study examined that methane yield from using cow manure was relatively higher.

2.4.10 Stirring

For degrading more substrate and increased biogas yield, firstly build the interaction between feedstock and anaerobes is really promising. It can be possible through proper mixing of materials (slurry) present in a reactor. Otherwise limited substrate will be available to microbes for digestion and fewer biogas yields will be obtained at the end.

Past study (Rai, 2011) has discovered that a slight manual stirring for 2-3 min on each day can increase degradation, avoid layering and high yield. Moreover, a proper mixing can favors the AD system by evenly distributing the feed throughout system without clogging, liberating more gas trapped between material layers in bubble form and transferring heat easily. Another study has witnessed that layering in reactor can reduce the daily yield of biogas which was noted to be 88% (Tian et al., 2018).

2.4.11 Toxicity

Yang et al. (2015) studied that excessive production of volatile fatty acids from using carbohydrate enriched substrate have negative impact on stable AD process. Similarly, protein enriched substrate can produce large quantities of total ammonium nitrogen. Both these intermediates if produce in excessive amount can create more toxicity to system so highly needed to be under control. The use of co-digestion can better resolve this issue of toxicity such as using protein based substrate can effectively lessen the impact of VFA's inhibition.

2.5 Types of anaerobic digesters

Anaerobic digesters are mainly used to carry out AD process for biogas generation. It is usually a closed bio-digester with insulated lid to ensure the anaerobic conditions (no oxygen) for anaerobes to nurture and work properly under suitable environment. Anaerobes digest the complex organic feedstock and produces biogas as well as other valuable by-products (Wei et al., 2018; Leggett et al., 2006). There are two distinct types of anaerobic digesters depending upon the mode of operation.

2.5.1 Batch process

Anaerobic digestion reaction can be carried out in batch mode where feed is introduced once at the beginning and emptied on finishing the AD process. It is also known as fill and draw reactor. The batch mode reactors are simply designed, easy to operate, more substrate digestion and more economical in carrying out AD process within a single unit (Wei et al., 2018; Carrere et al., 2016; Igoni et al., 2008). During initial days, production of biogas is found minimal and slowly exceeded to high level with time (d) and becomes constant at the end. Singh and Srivastava, (2011) reported some shortcomings of using such reactors including clog formation, uneven distribution of microbes, layering of materials, trapping of gas in bubble form etc.

2.5.2 Continuous process

Anaerobic digestion process can be operated in continuous mode where continuous feeding in a system is done. Feeding into reactor and removal of degraded matter from system can be done through manually or automatically. It usually provides continuous generation of biogas. This type of reactors is designed either in horizontal position (plug-flow), vertically (completely mixed) or with multiple feeding inlets (Wei et al., 2018; Al-Seadi et al., 2008). In such reactors,

there is relatively short time for substrate to digest as the previously fed material gets removed with the introduction of newly fed material in order to adjust volume in a system.

2.6 Studies on availability of different type of lignocellulosic feedstock

Variety of different kind of organic materials as feedstocks have been utilized in energy purposes so far i.e. animal dung, food (kitchen) waste, agro-industrial residues (shells), agricultural waste, crop residues, anaerobic sludge, municipal solid waste etc. Though, these different feedstocks contain distinct potential for biogas based on their nature, origin as well as lignocellulosic arrangements etc. Lignocellulosic materials containing high biogas potential are basic resource for fulfilling energy demands that's why have been used greatly in bioenergy applications because such biomasses are readily accessible and produce in huge amount globally. In past literature, most commonly used lignocellulosic waste for energy generation purpose includes agricultural waste (wheat straw, garden waste, rice straw), corn cobs, crop residues and sugarcane bagasse etc. (Mao et al., 2015).

Food waste including fruits and vegetables is also considered as a good energy resource that is nutrient sufficient. The remarkable huge production of food waste is estimated among different countries like Asia, South America and other under-developing countries. Amongst all, Asia contributes towards 61% of worldwide production of vegetables. Mainly food-processing industries are highly responsible in generating such type of waste. The final destination for this waste is either landfilling or burnt off instead this waste can be a remarkable alternative for energy resource (Wadhwa and Bakhshi, 2013).

Since decades, the use of animal dung (manure) has been a common practice for biogas production. Such waste if not properly managed can pollute the environment by liberating GHGs. Therefore, its effective use in biogas production is mandatory that lessen the environmental pollution as well as give valuable by-product. Amongst all, cow dung is the most commonly used manure so far with greater microbial community. Animal manure contains high C/N ratio in comparison with food waste (Wadhwa and Bakhshi, 2013).

2.6.1 Peanut shells as lignocellulosic feedstock

Zhou et al. (2017) reported that peanut hulls came under the limelight due to its extensive yearly production. This peanut waste (shells) can be effectively used to give valuable energy products

(bioenergy and biofuels). Globally, peanut has been considered a vital crop with inherent energy properties and broadly cultivated throughout different zones with over-all production of nearly 46 million tons/yr. while China contributes 37% to overall peanut generation worldwide (FAOSTAT, 2022).

Amongst agro-industrial waste, *Arachis hypogaea* shells, also well-known as peanut shells, are imperative lignocellulosic substrates which abundantly produce at global level because of extensive worldwide peanut production. For instance, during 2011-2012, approximately fourty-five percent increase was observed solely in US that ultimately upsurges the peanut production (3.04 million metric tons) based on analysis (Agricultural Marketing and Resource Centre, 2015). Polachini et al. (2016) reported that 90,000 tons of shells were produced in Brazil during 2014. Nearly, 3.64 million tons of peanut shells were recorded as yearly production in Asia (Wang et al., 2016). At global level, the overall peanut production per year is noted to be 45.6 million metric tons which give rise to shells generation of about 13.7 million metric tons (FAOSTAT, 2013). Another study (Araujo et al., 2014) reported that peanut waste (shells) contributes approximately 30% towards overall peanut production.

In Pakistan, agriculture has been a very common practice since decades. So, agricultural residues can serve as an abundant energy resource for energy yield. Generally, agricultural practices may lead to high production of agricultural residues. Such residues are basically energy inherent including shells, barks, seeds, vegetables, straws etc. (Santos et al., 2021; Bilotta and Ross, 2016). Moreover, the increased production of peanuts is majorly contributing in accumulating large quantities of peanut shells as well. These shells are usually burnt off or maybe used for animal feed or as firewood. But these shells can be a major resource of energy to benefit our environment through accompanies waste-to-energy purpose (Zhao et al., 2012; Kerr et al., 1986). Past work shows mainly these hulls (shells) used for fertilizer purpose (Fang et al., 2014). In previous work, peanut shells have been used to generate ethanol and other biofuels through fermentation (Polachini et al., 2016) or as an adsorbent for removing trichloroethylene (Ahmad et al., 2012).

Bolognesi et al. (2021) revealed that peanut shells can be more helpful in influencing the agricultural economy. A lot of work has been done concerning the utilization of peanut hulls as a potential resource of biofuel generation and its use in industrial and residential heating processes

so far (Perea et al., 2018). Bhaduri et al. (2016) reported the impact of using peanut shell based biochar for increasing crop production as well as soil heath (Nazir et al., 2021). The shells being a lignocellulosic material consist of three major constituents such as cellulose, hemicellulose and lignin.

Previous data has revealed the presence of high lignin (36-43%) in shells (Almeida et al., 2024; Fang et al., 2014) as compared to other agro-biomasses (wheat straw, rice straw etc) based on (Iqbal et al., 2013). Regardless of high peanut generation, very limited studies are available on utilizing peanut shells as productive resource for biogas production. Mostly, the peanut shells were consumed for biofuels (ethanol, isoprene etc.) production (Herring and Narayanan, 2016; Polachini et al., 2016; Wang et al., 2016)

2.7 Studies on impact of pretreatment methods on feedstock

The recalitrant nature and compostion of lignocelluose based materials just remained a bigger problem in AD process. Moreover, several techniques for pretreatment are available and readily used to overcome this problem succesfully by altering the tighlty bound rigid structure through bond cleavage. The studies conducted on these pretreatment methods have been reviewed in detail in below section. After pretreatment, the mode of action on structure of biomass has been shown in Figure 2.4. Effect of various pretreatments on different lignocellulosic feedstock is given in Table 2.2.

Tian et al. (2018) also discussed that the closely-packed (compact) structure and recalcitrant arrangement of organic materials serves as a barrier which makes it difficult to get degraded easily. Consequently, pretreatment step is a major prerequisite which mainly focuses on increasing lignin degradation, to reduce degree of polymerization, produce less crystalline arrangement and raise material's porosity level with high surface area (Bharathiraja, 2017). Pretreatment step is highly preferable to adopt before initiating the biomass digestion process otherwise it will not be very effective in producing more biogas.

Zheng et al. (2018) reported several pretreatment techniques that have been employed in the past work to increase cellulose availability. For instance biological based pretreatment (fungi, bacteria, enzymes), chemical based pretreatment (acids, bases, other organic reagents) and mechanical or physical (grinding) etc (Dahunsi et al., 2019).

Mechanical treatment is one of the common pretreatment methods, also referred as physical treatment. It usually alters the structure of biomass when undergone shredding and results in providing large surface area. According to Carrere et al. (2010), biomass with more surface area create better interaction with microbial community hence improve the AD procedure There are numerous physical processes including sonication, liquefaction, grinding which mainly focuses on size reduction of biomass particles. A past study proved that using small sizes of particle can produce biogas in greater quantity through AD process (Esposito et al., 2011).

Olivia et al. (2023) has investigated the biogas potential of peanut hulls (shells) in AD process using two distinct pretreatments for instance, ultrasound (physical) as well as organosolv (chemical) methods. This study has used three (1:5, 1:10, 1:20) different substrate-to-liquid (solvent) ratios during organosolv method. Results showed that ultrasound pretreatment method remained more effective as compared to organosolv pretreatment in this study as it solubilized more sugars about 37.90 mg per grams total solids hence increased methane yield was obtained i.e. nearly 64%. While organosolv method has only maximized the solubilisation of polyphenols which noted to be 4.90 mg per grams TS irrespective of any impact seen on methane yield.

Table 2.2 Studies on various pretreatments on different lignocellulosic feedstock

Pretreatment Type	Substrate	Experimental conditions	Findings	Reference
Thermal pretreatment	Wheat straw	Temperature range: 120 ^{o}C, 140 ^{o}C, 160 ^{o}C and 180 ^{o}C Time: 1 h	Thermal pretreatment (180 ^{o}C) increased biogas production by 53% more than control.	Rajput et al., 2021
Mechanical pretreatment	Peanut shells	Size reduction: 2 mm, 4 mm, 6 mm at 37 ^{o}C	Increased biogas production of 732.5 mL was observed from 6 mm PS.	Jekayinfa et al., 2020
NaOH pretreatment	Rice straw	NaOH dosage: 0.5%, 1% 1.5%, and 2% (w/v),	Maximum biogas was achieved at 1.5% NaOH pretreatment	Sabeeh et al., 2020

		Duration (h): 3 at 1:20 (S/L ratio)	recorded as 57% and methane content upto 70%.	
Mechanical pretreatment	Peanut shells	Size reduction: 3 mm at 37 ^{o}C	The results showed that PS have potency to produce 22.3 m^3 biogas equivalent to 31.84 Kwh/month energy	Santos et al., 2020
NaOH pretreatment	Rice straw	NaOH dosage: 2%, 4%,6%,8% and 10% (w/v). Time (d) duration: five, 1:15 (S/L ratio)	More delignification (88%) was observed at pretreatment of 2% with more biogas (57%) production.	Khalid et al., 2019
Fungal pretreatment	Rice straw	Size reduction: ≤ 2 mm Incubation time (d): 30	Delignification of 33% was observed after pretreatment of rice straw with 160% methane production.	Mustafa et al., 2017

2.7.1 Studies on impact of NaOH pretreatment on peanut shells

Due to high lignin presence in these peanut shells can overcome greater use of this type of waste in energy production purpose. Therefore, use of suitable pretreatment can overcome this problem (Zhou et al., 2017). Costa et al. (2014) has discovered that alkali based treatment is most effective in facilitating high degradation of lignin, with increased surface area as well as more porosity.

Furthermore, alkali based treatment also improved the enzymatic hydrolysis thus enhanced biogas yield even under individual treatment (Jang et al., 2013). Besides this, alkaline based pretreatment method has been considered more economical. For this treatment, frequently

consumed bases (alkali) are sodium hydroxide and potassium hydroxide etc. It certainly helps in delignification in an easy way through breaking of glycosidic bonds and makes the substrate highly available to microbes as well as towards reaction of enzymes (Garcia et al., 2013). The greater part of feedstock comes under degradation and introduces the amendments in the lignocellulosic arrangement.

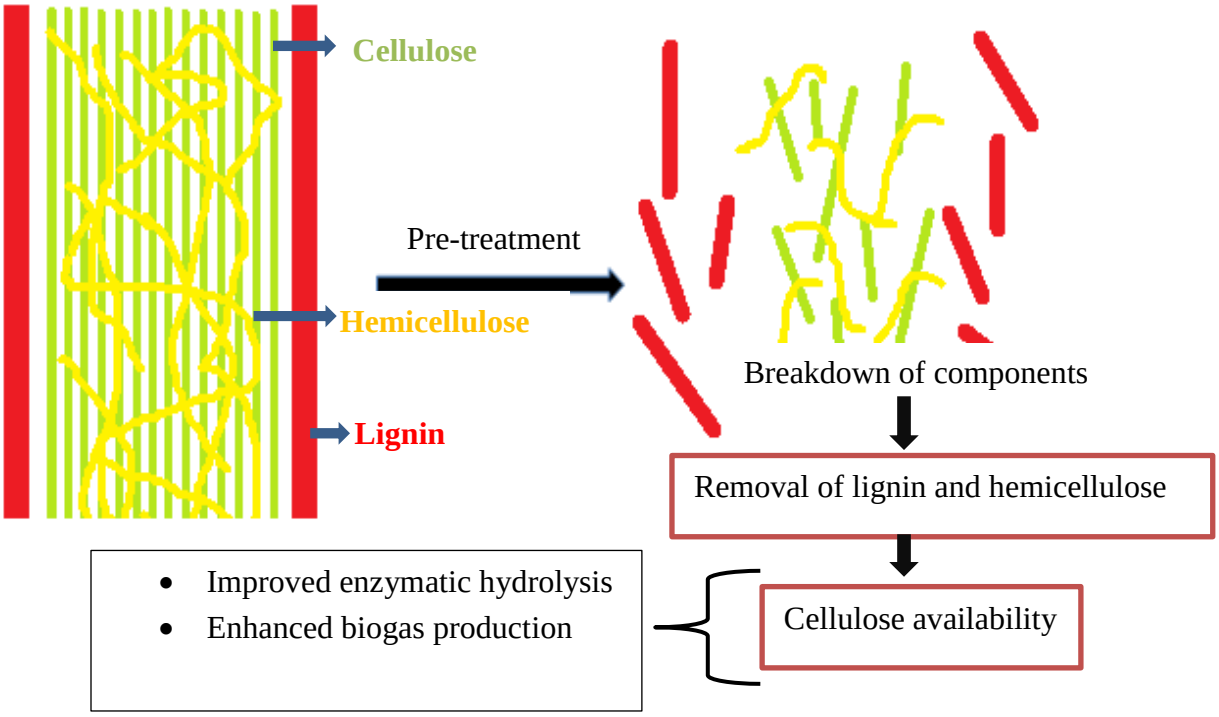

Figure 2.4 Pretreatment: Mode of Action

The few shortcomings of this alkali treatment have also been observed including time-consuming etc. But this treatment also results in high porosity to substrate and more surface area to degrade effectively as well as alter the crystallinity structure etc. (Li et al., 2010). Generally, alkalis target the lignin part of biomass and somehow hemicellulose part as well to increase the availability of cellulose of organic material. Literature shows that such pretreatment has been utilized more commonly in paper industry. The breaking of glycosidic linkage helps in increased delignification reduces the tightly bound crystallinity structure and gives rise to highly porous surface with increased area for bacterial and enzymatic reaction (Paudel et al., 2017).

In past work (Sun et al., 2016), sodium hydroxide has been used more excessively for alkali pretreatment to increase biogas yield from different type of lignocellulosic feedstocks such as

agricultural waste (wheat straw, rice straw, corn straw), crop residues, agro-industrial waste (shells) and other wood based residues etc.

Olatunji et al. (2022) has studied the effect of basic pretreatment on peanut hulls (shells). This work preferred to use NaOH as a base with four different concentrations i.e. from 1% to 4% under specific temperature (90 ^{o}C) with different time exposures i.e. 45 to 10 mins. The surface area after pretreatment was studied using SEM technique (scanning electron microscope). Similarly, lignocellulosic changes (functional groups) occurred during pretreatment were also analyzed using different characterization techniques i.e. FTIR (Fourier transform infrared spectroscopy. The AD process of this study was designed at mesophilic temperature. Results showed that alkali treatment lowered the crystallinity and modified the functional groups present in peanut shells. Likewise, modification in the rigid structure of these shells was also seen and confirmed by SEM pictures. The enhanced production of methane (256.78 mL/gVS) was observed after alkali-pretreatment in comparison with untreated peanut shells (151.23 mL/gVS).

Olatunji et al. (2024) further studied the impact of pretreatments on biogas yield of peanut shells either individually or combined. This study mainly focused on using chemical pretreatment (acids and base), thermal pretreatment method and nano-particle additive method as well as combined. The whole study was designed under mesophilic conditions. Results showed that all pretreatment processes have positive impact on the structural composition of peanut hulls by modifying it. The highest CH_4 yield (cumulative) was observed at combined pretreatment i.e. 261.36 mL methane per grams VS which was 160% more in comparison with raw substrate. Numerous studies on the effect of alkaline (NaOH) pretreatment of peanut shells are given in Table 2.3.

2.8 Studies on impact of co-digestion on biogas production

The nature, origin and composition of feed (substrate) has found significant in carrying out successful anaerobic digestion throughout process. Generally, appropriate nutrient balancing (C/N) in AD process is really important otherwise can produce disturbance and failure of overall process. In this regard, co-digestion can be a useful alternative which helps in balancing the nutrients by using more than one substrate at a time in a reactor to digest anaerobically for greater yield. Inappropriate nutrient balancing can create process failure within less time span. The selection of suitable substrate is also very important depending upon nature and composition

of substrate as well as nutrient requirement. The optimum range of C/N is recorded to be 20-30 in previous work (Rajput et al., 2021).

Table 2.3 Studies on effect of NaOH pretreatment of peanut shells

Pretreatment Type	Substrate	Experimental conditions	Findings	Reference
NaOH pretreatment	Peanut shells	NaOH dosage: 1%, 2%, 3% and 4% (w/w), 90 ^{o}C, 1:10 (S/L ratio), time duration: 10 to 45 mins	In case of 3% NaOH pretreatment, higher methane production (256.78 mL/gVS) was achieved.	Olatunji et al., 2024
NaOH pretreatment	Peanut Shells	NaOH dosage: 4% (w/v), 80 ^{o}C, 200 rpm for 2 h duration, 1:10 (S/L ratio)	Peanut shells produced 55.45% carbohydrate percentage upon NaOH pretreatment rather than acid. High PS porosity and more surface area were also observed in case of alkali pretreatment.	Lin et al., 2023
NaOH pretreatment	Peanut shells	NaOH dosage: 4%, 6% and 8%, Mesophilic temperature range (37 ^{o}C)	Higher biogas production was recorded from PS pretreated at 4% which was 48.91% more than its control group and methane production was noted to be above 60%.	YanQiu et al., 2011

Anike and Yusuf, (2017) studied the co-digestion effect of peanut shells and cornstalks on biodegradation process. In this study, solid state AD process was designed using different co-digestion ratios (9:1, 1:1 and 3:1) with controls for 120 d. A white rot fungal strain named as Pleurotus Ostreatus was used throughout fermentation process. As a result, cornstalks with peanut shells degraded more lignin of about 41%. Best results were produced at 1:1 with highest (24.1%) organic matter degradation as compared to individual peanut shells. This means digestion of matter increased by 2.4 times than solely use peanut shells.

Dahunsi et al. (2019) also examined the effect of co-digestion on biogas generation in AD process. This work has consumed peanut shells with poultry manure as co-substrates during anaerobic digestion. Practically, co-substrates were undergone two types of pretreatments namely thermal-alkaline and physical. This literature mainly focused on studying five important parameters including pH, TS, VS, temperature and time with application of two models (RSM and ANNs). Results from this study revealed highest biogas production of about 3903.15 $10^{-3}m^3$ per kilogram TS under model RSM while 3338.3 $10^{-3}m^3$ per kilogram TS under ANNs model for thermal-alkali based pretreatment reaction than physical. About 65% methane yield was observed through GC (gas chromatography) technique while up to 71% production of biogas was obtained after alkali-pretreatment rather than raw substrate.

Li et al. (2020) has investigated the effect of mono-digestion, co-digestion and tri-digestion during AD process by using food based substrates (kitchen waste, food waste and vegetable plus fruit waste) on methane yield. Amongst all, tri-digestion (combination of these three waste) produced the best results with more yield of CH_4 nearly 355 mL per grams VS as well as enhanced biogas production due to improved synergism. Table 2.4 is presenting several studies on effect of anaerobic co-digestion for biogas production.

Table 2.4 Studies on anaerobic co-digestion for biogas production

Digestion Type	Substrate	Experimental conditions	Findings	Reference
Co-digestion	Peanut shells and food waste	1:1 at Mesophilic range (37^{o}C)	Methane yield was high for thermal pretreatment of about 222.92 mL CH_4/gVS.	Olatunji et al., 2023
Co-digestion	Peanut shells Sludge inoculum	1:5	More than 64% methane content was estimated	Oliva et al., 2023
Co-digestion	Food waste and pig sludge	1:1	Biogas generation was estimated as 253 mL/gVS.	Dutta et al., 2021
Tri-digestion	kitchen and	1:1	Results investigated	Li et al.,

	vegetable waste, food waste		that tri-digestion produced high yield of CH_4 (methane) nearly 355 mL/gVS.	2020
Co-digestion	Peanut shells and swine manure	NaOH dosage: 6% and temp. (25 ^oC), 1:1, time duration: 8 days	The increased production of biogas was noted as 1280.65 mL/gVS with 686.06 mL CH_4/gVS.	Yuanfang et al., 2019
Co-digestion	Kitchen waste and cow dung	1:1	Biogas yield was reached upto 179.8 mL/gVS.	Amin et al., 2017
Co-digestion	Peanut shells and cornstalks	Different mixing ratios: 9:1, 1:1 and 3:1 under solid-state AD, Time duration (d): 120	Biogas production was more at 1:1 mixing ratio with highest (24.1%) organic matter degradation as compared to individual peanut shells.	Anike & Yousaf (2017)
Co-digestion	Peanut shells and poultry manure	PS size: ≤ 20 nm, 1:1, 55 ^oC, 24 h	More biogas production was observed with methane production of 65.5% after thermal pretreatment.	Dahunsi et al., 2017
Co-digestion	Peanut shells and corn cobs	1:1 37 ^oC	Maximum biogas production (2810 cm^3) was estimated after co-digesting more than one substrate. Daily yield was measured as 93 cm^3.	Ibrahim et al., 2016

2.9 Summary

Literature review shows that using peanut shells for biogas generation via AD method can be more practicable to encounter energy demands as best alternative in more sustainable way because of its energy inherent property after suitable pretreatment with co-digestion. The presence of high lignin levels in peanut shells can create hindrance in AD process thus contributes towards less yield. Fortunately, using pretreatment step before initiating anaerobic

digestion can be more productive in terms of increased biogas yield as pretreatment facilitates the de-lignification more effectively with high cellulose provision to enzymatic hydrolysis as well as bacterial attack. Since past decades, a variety of pretreatment techniques such as mechanical, biological and chemical have been explored so far. Amongst all available pretreatments, alkaline is found more feasible, more economical with less quantity consumed under even low temperatures.

Furthermore, co-digesting of the peanut shells with additional suitable substrate can produce more biogas by balancing the nutrients and C/N ratio. For instance, there is high carbon to nitrogen ratio for peanut shells while vegetable waste contains less carbon to nitrogen ratio due to more nitrogen presence. Based on previous literature, this study has been conducted with two aims i.e. using alkaline pretreatment of peanut shells before initiating anaerobic digestion and use of selective vegetable waste as a co-substrate to balance the nutrients for a greater microbial performance. Peanut shells are carbon enriched that act as a potent energy source for microbes while vegetable waste holds nitrogen enrichment to balance the C/N ratio. Yet, to best of our knowledge, no past studies are available on anaerobic co-digestion of pretreated peanut casings with selective vegetable waste as co-substrate.

CHAPTER 3: MATERIALS AND METHODS

This chapter primarily enlightens the experimental part of the study i.e. materials and chemicals (reagents) used for pretreatment of feedstock and anaerobic digestion setup. The framework of a complete experimental setup is shown in Figure 3.1:

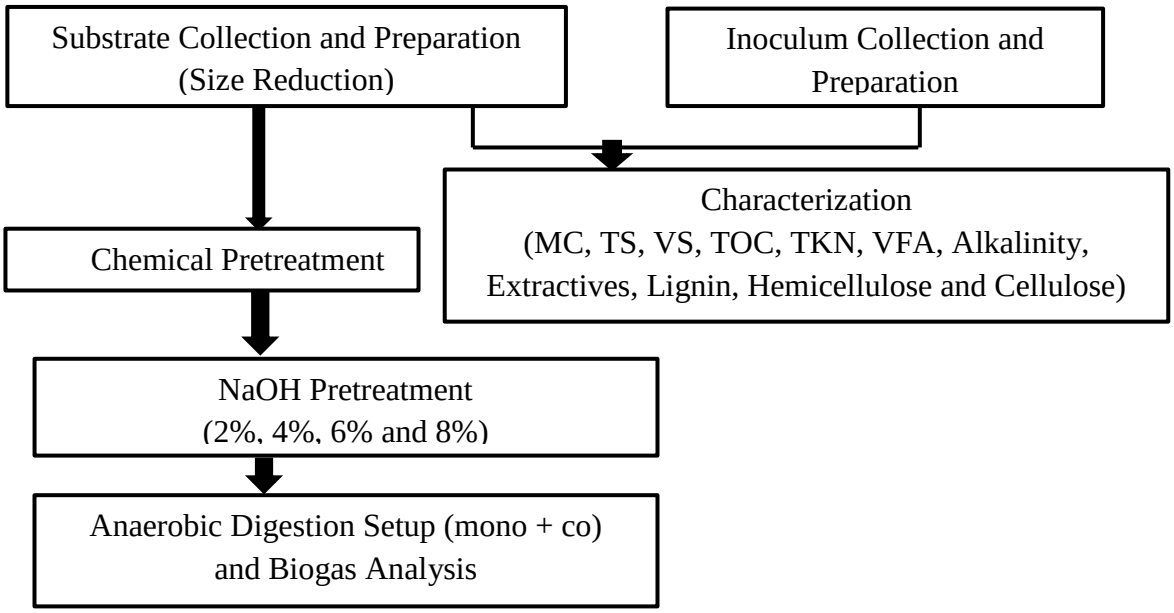

Figure 3.1 Framework of experimental setup

3.1 Feedstock and inoculum collection and preparation

The collection and preparation steps for both feedstocks are shown in Figure 3.2.

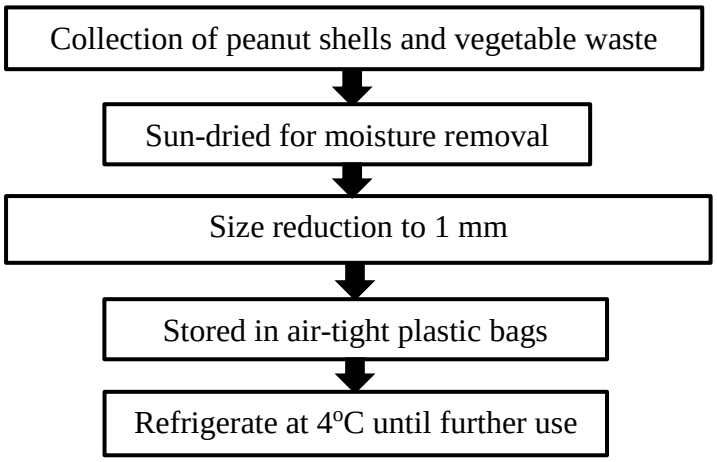

Figure 3.2 Steps used in feedstock collection and preparation

3.1.1 Feedstocks for anaerobic digestion (AD)

In this study, two different type of organic substrates i.e. peanut shells as a feedstock and vegetable waste as a co-substrate were utilized. Peanut shells (PS) were collected from a field located at Attock district in Punjab, Pakistan whereas vegetable waste (VW) was collected from local market (Itwar-bazar) in Islamabad. After collection, peanut shells were separated from seeds followed by shredding using shredder available in laboratory. The grinded form of peanut shells were then passed through 1 mm sieve to obtain standard particle size and stored in refrigerator using air-tight bags till further use. Likewise, five selective vegetable waste including mint, salad leaves, coriander, cucumber and bottle gourd were undergone same steps employed for PS preparation as shown in Figure 3.3.

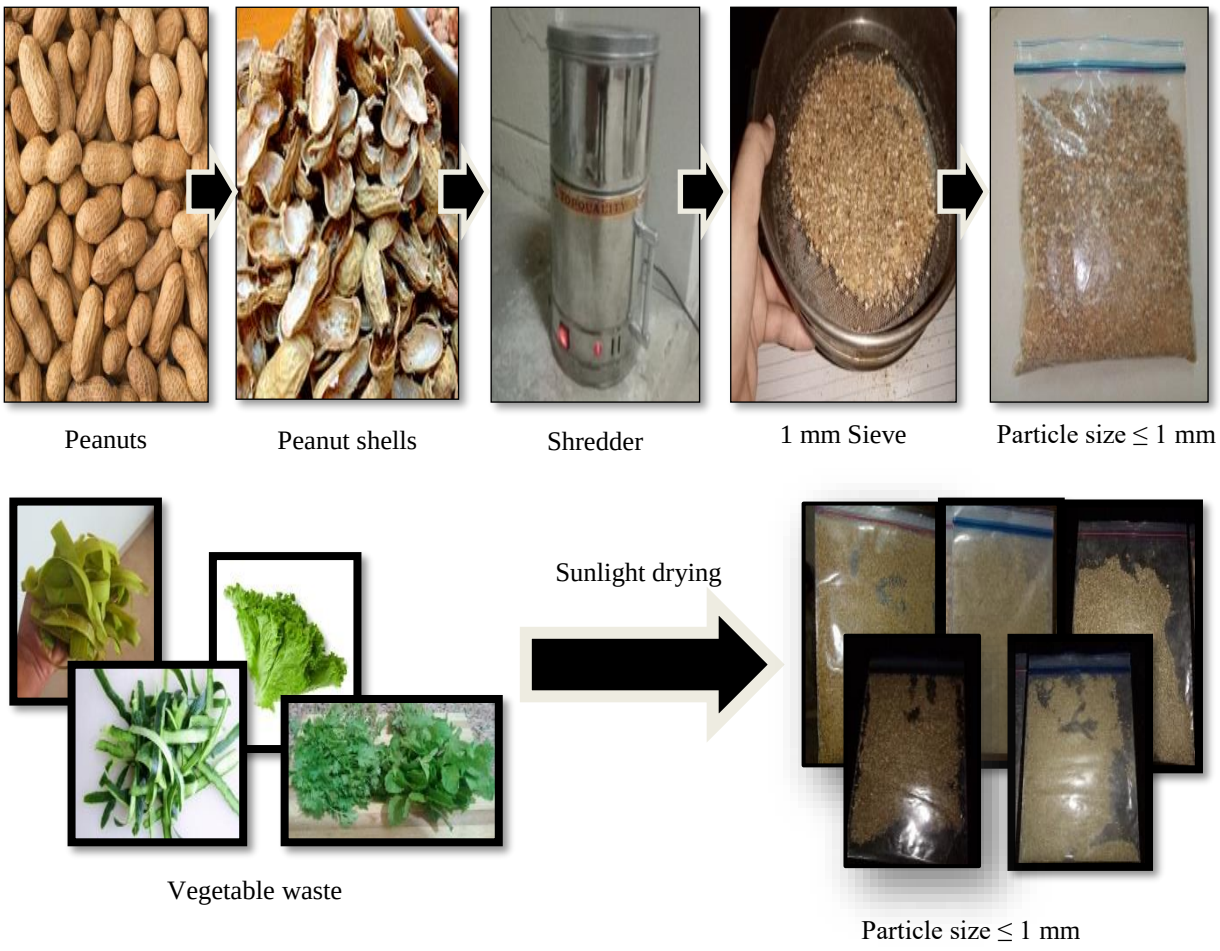

Figure 3.3 Feedstock collection and preparation

3.1.2 Inoculum for anaerobic digestion (AD)

Figure 3.4 is illustrating the preparation (degassing) steps for inoculum.

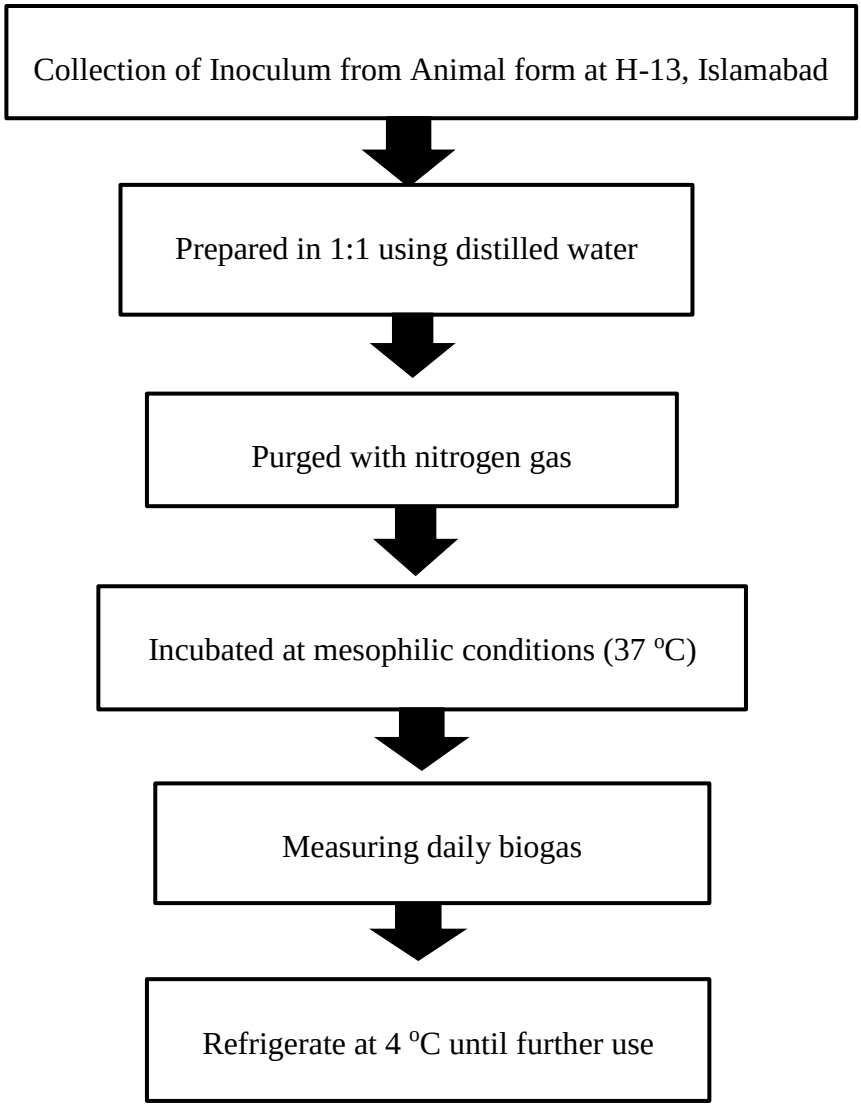

Figure 3.4 Steps used in inoculum preparation (degassing)

Fresh cow manure (CM) was used as an inoculum which was collected from an animal farm located at H-13, Islamabad. Previously, the inoculum was mixed with water to make slurry in 1:1 followed by nitrogen purging to ensure anaerobic conditions and then incubated under mesophilic conditions (37 ºC) for 20-25 d under regular monitoring and degassing. Later on, the degassed inoculum was refrigerated at 4 ºC till further used as shown in Figure 3.5.

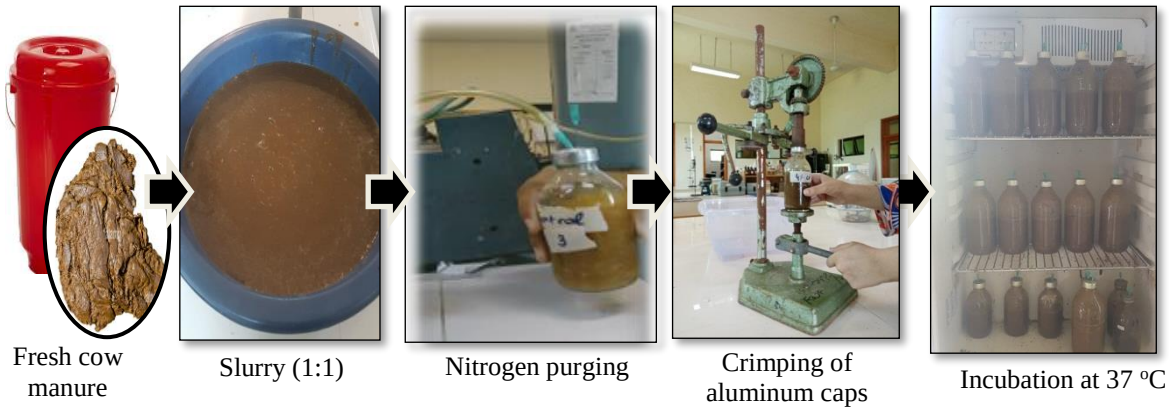

Figure 3.5 Inoculum collection and preparation (degassing)

3.2 Primary characterization of feedstock

Some basic parameters such as TS (total solids), VS (volatile solids), MC (moisture content), and TKN (total nitrogen content) of feedstocks i.e. PS and VW were analyzed on the basis of standard procedure given in report (APHA, 2017) whereas TOC (total organic carbon) was performed in accordance with procedure adopted by Adams et al. (1951). Moreover, peanut shells were also undergone fiber analysis to get better insight of its structure and composition. All the fiber analysis, extractives along with other structural constituents for instance cellulose, hemicellulose and lignin were done in accordance with method reported by Li et al. (2004).

3.3 Chemical pretreatment of feedstock

Prior to anaerobic digestion (AD) setup, peanut shells were pretreated chemically using base under alkali-pretreated method.

3.3.1 Alkaline pretreatment of peanut shells

For alkaline pretreatment of peanut shells, sodium hydroxide (NaOH) pellets were used as an alkali source. The whole experiment was performed at normal temperature for 24 h in 2 L Pyrex bottles for each percentage solution of NaOH i.e. 2%, 4%, 6% and 8% (w/v). For this pretreatment, the ratio of PS and sodium hydroxide solution were set to be 1:10 (w/v). To constant stirring, magnetic stirrer was put inside each 2 L Pyrex bottle at 200 rotations per minute (rpm). After completing the pretreatment step, the mixture was then filtered using a piece of cloth followed by continuous washing via distilled water until the pH was neutralized.

Later on, pretreated PS samples were subjected to oven-drying step providing 105 °C temperature for 24 h and were stored in refrigerator at 4 ^{o}C using air-tight bags until further use. All the pretreated and untreated (raw) samples of PS were undergone Fourier transform infrared spectroscopy (FT-IR) to characterize the functional group (structural) changes occur after pretreatment as illustrated in Figure 3.6.

Figure 3.6 Alkaline pretreatment of peanut shells: (a) Untreated PS (b) Pretreated PS

3.4 Experimental setup for anaerobic digestion (AD)

In current study, batch setups for both anaerobic digestions i.e. mono-digestion and co-digestion were conducted at lab scale for treated as well as untreated substrates. The serum bottles of 300 mL size were equipped as anaerobic digesters for both digestion setups. For entire anaerobic digestion setup including mono-digestion and co-digestion, total of 47 serum bottles were arranged. Out of 300 mL, only 75% volume of serum bottles i.e. 225 mL was utilized to fill the reactor bottles by keeping the headspace of remaining 25% for biogas.

3.4.1 Experimental conditions for anaerobic mono-digestion (AMD)

In case of mono-digestion setup, both untreated (control) and pretreated mono-digestion reactor bottles were prepared. Each set either for pretreated mono-digestion or control were prepared in triplicates. Total of 20 serum bottles of anaerobic mono-digestion setup along with its control were prepared i.e. four for control and sixteen bottles for pretreated mono-digestion. Based on organic loading rate of 10 gVS/L, a calculated amount of peanut shells either pretreated or untreated with inoculum were introduced into anaerobic digesters. For instance, 225 mL volume of serum bottles were occupied with only inoculum and peanut shells either pretreated or untreated in 1:1 (S/I) while remaining portion was filled with distilled water after pH adjustment as shown in Figure 3.7.

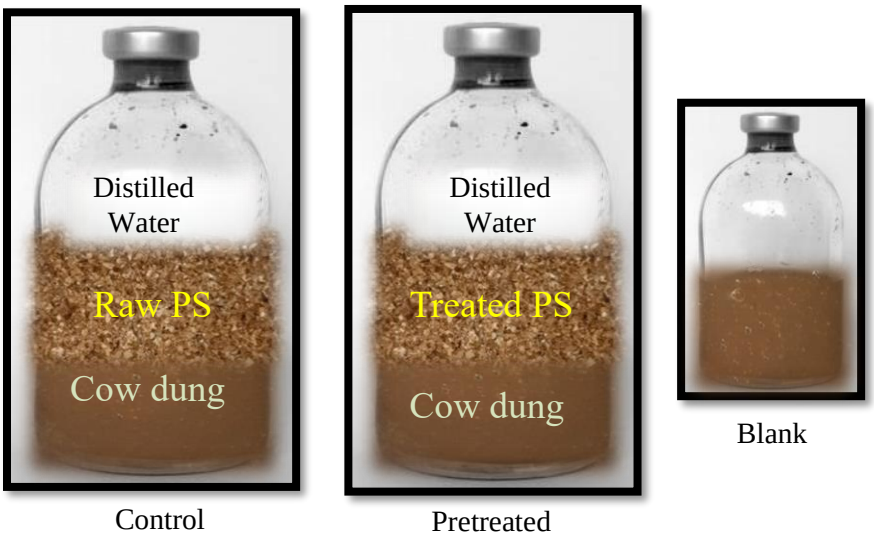

Figure 3.7 Anaerobic mono-digestion (AMD) setup

3.4.2 Experimental conditions for anaerobic co-digestion (ACD)

In case of co-digestion setup, known amount of peanut shells either pretreated or untreated, vegetable waste and inoculum were introduced for co-digestion setup by using mixing ratio of 1:1 (S/I) that achieved desired carbon to nitrogen ratio i.e. 25 as calculated using equation 3.5. A proper manual mixing was done using glass rod. To adjust the pH level of all reactors, few drops of 1 M sodium bicarbonate solution (act as buffer) were also added into all serum bottles. After neutralizing pH, distilled water was poured inside serum bottles up-to the mark to reach the working volume as shown in Figure 3.8.

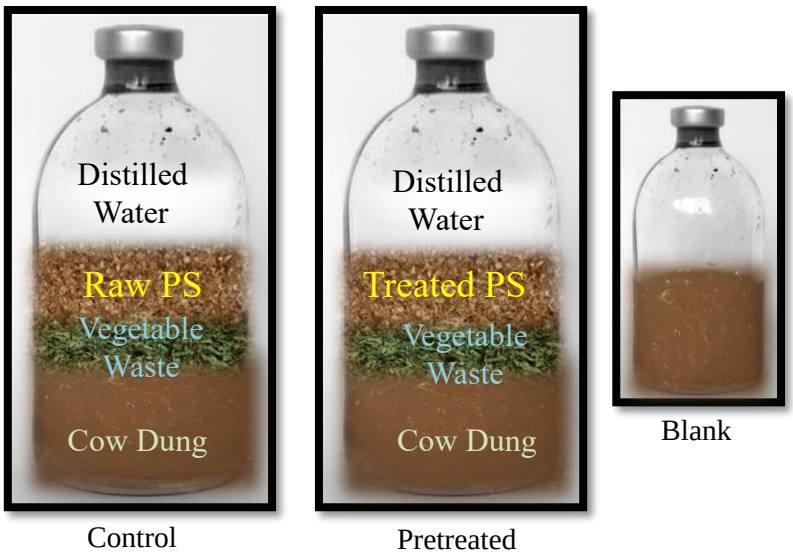

Figure 3.8 Anaerobic co-digestion (ACD) setup

At the end, these serum bottles were closed air-tightly by using rubber lids (septa). The aluminum caps were also used to proper sealed the bottles using crimping machine as shown in Figure 3.9 (a). Moreover, nitrogen purging was also done to ensure the anaerobic conditions inside digesters for at least 2 min where nitrogen gas is entered through rubber septa into reactor bottles using inlet and discharge by outlet. Then, all serum bottles were incubated at mesophilic temperature (37 ^{o}C) for duration of 45 d inside the incubator (Velp Scientifica-FOC 120E Cooled Incubator, Italy). Twice a day, about 2 to 3 min regular shaking of these reactor bottles were done manually before measuring the biogas volume using water displacement method.

To facilitate the biogas measurement, 500 mL size of water-filled graduated cylinder was placed in upside-down direction in water filled container to completely submerge the cylinder. A pipe was used to connect reactor bottle with cylinder in accordance with procedure (Yuan et al., 2019). Both initial and final water levels were noted to calculate biogas volume on regular basis to obtain daily as well as cumulative biogas production. All the reactor bottles were run in triplicates either for pretreated or untreated (control) along with blank (only inoculum) to overcome biogas error from inoculum as shown in Figure 3.9 (b).

(a) Crimping of Aluminum Caps (b) Incubation at 37ºC

Figure 3.9 Anaerobic digestion setup (a) Bottle crimping (b) Incubation

3.5 Characterization techniques for feedstock analysis

All the characterization techniques which have been equipped for analysis of feedstock and their procedures are discussed here in detail. The primary analysis of feedstock such as moisture content, total solids and volatile solids, TKN and TOC of peanut shells and vegetable waste was performed using standard method (Adams et al., 1951). While other fiber analysis (lignin, cellulose, hemicellulose and extractives) of peanut shells were done by following the method developed by (Li et al., 2004).

3.5.1 Total solids (TS) of feedstock

To determine TS of feedstock i.e. peanut shells and vegetable waste, china dish (CD) was rinsed using distilled water (DW) followed by oven-drying at 105 ^{o}C for 15 min and let it cooled in desiccator. Oven-dried empty china dish was weighed (W_2) using analytical balance. After putting substrate (50 g) in china dish (W_3), placed the CD in oven (Memmert, UNB-400, Germany) for 1-2 h at 105 ^{o}C followed by cooled down the CD in desiccator (W_1). After drying step, the amount of substrate left in CD was total solids content. The percentage amount of TS of substrate was determined using equation 3.1:

$$TS\% = \frac{w_1 - w_2}{w_3 - w_2} X\ 100 \tag{3.1}$$

Here, W_1 = weight of dried sample + china dish wt.

W_2 = weight of empty china dish

W_3 = weight of wet substrate sample + china dish wt.

3.5.2 Volatile solids (VS) of feedstock

In determining the VS of feedstock i.e. peanut shells and vegetable waste, placed the previously dried sample in CD obtained at the end of TS step in muffle furnace (JSR, JSMF-270H, Korea) for duration of 30 min by setting the temperature at 550 ^{o}C. Later, cooled the sample in desiccator and weighed (W_4). The percentage amount of VS of substrate was obtained using equation 3.2:

$$VS\% = \frac{w_1 - w_4}{w_1 - w_2} X\ 100 \tag{3.2}$$

Here,

W_4 = weight of sample residue + china dish wt. after ignition

3.5.3 Moisture content (MC) of feedstock

The moisture content is actually the amount loss while drying the sample at 105 ^{o}C and the percentage amount of MC was achieved using equation 3.3:

$$MC\% = \frac{w_3 - w_1}{w_3 - w_2} X\ 100 \quad (3.3)$$

3.5.4 Total organic carbon (TOC)

The percentage amount of total organic carbon (TOC) of feedstock i.e. peanut shells and vegetable waste was determined by using equation 3.4 (Abdelsalam et al., 2017; Adams et al., 1951):

$$TOC\ \% = \frac{VS\ (\%\ of\ TS)}{1.8} \quad (3.4)$$

3.5.5 Total Kjeldal nitrogen (TKN) of feedstock

To determine the amount of Total Kjeldal nitrogen (TKN) of feedstock i.e. peanut shells and vegetable waste, dry sample was put in TKN tubes with addition of copper sulfate, potassium sulfate, sulfuric acid and distilled water based on standard procedure (APHA, 2017).

3.5.6 Carbon to Nitrogen ratio (C/N) of feedstock

The amount of C/N of feedstock i.e. peanut shells and vegetable waste was determined by dividing TOC quantity for each substrate by TKN amount for each substrate as illustrated in equation 3.5 (Wang et al., 2014):

$$\frac{C}{N} = \frac{W_1 * C_1 + W_2 * C_2}{W_1 * N_1 + W_2 * N_2} \quad (3.5)$$

Here,

W_1= weight of peanut shells (PS)

W_2= weight of vegetable waste (VW)

C_1= total organic carbon content of PS

C_2= total organic carbon content of VW

N_1= nitrogen content of PS

N_2= nitrogen content of VW

3.5.7 Extractives of feedstock

For analysis of amount of extractives present in feedstock i.e. peanut shells, the application of solvent extraction process was equipped. In this method, acetone (60 mL) as a solvent was consumed for each gram of oven-dried substrate by setting 90 ^oC temperature for about 2 to 3 h. Thimbles were filled with dry PS wt. (W_0) and placed inside extraction tube with introducing condensation using water inlet and outlet to carry out process. Then, substrate sample (PS) was oven dried for 24 h at 105 ^oC followed by cooling in desiccator and sample was measured (W_1). The percentage amount of extractives of substrate was calculated using equation 3.6:

$$Extractives\ \% = \frac{w_0 - w_1}{w_o}\ X\ 100 \tag{3.6}$$

Here,

W_o = weight of dried PS before extraction

W_1 = weight of dried PS after extraction

3.5.8 Hemicellulose of feedstock

For analysis of hemicellulose content present in feedstock i.e. peanut shells, oven-dried PS sample (extractive-free) was put (W_1) in a beaker followed by addition of 150 mL of NaOH solution (0.5 mol/L). Then, boiling was done on hot plate (Velp Scientifica-ARE heating magnetic stirrer, Italy) at 80 ^oC for duration of 3.5 h using magnetic stirrers for complete constant mixing.

At the end, PS sample was undergone cooling, filtration and continuous washing with distilled water (DW) to neutralize pH. After pH adjustment, sample was subjected to oven dry at 105 ^oC followed by cooling in desiccator (W_2). The percentage amount of hemicellulose of substrate was determined using equation 3.7:

$$Hemicellulose\ \% = \frac{w_1 - w_2}{w_1} X\ 100 \tag{3.7}$$

Here,

W_1 = weight of extractive-free PS sample

W_2 = weight of PS sample after heating

3.5.9 Lignin of feedstock

To analyze the lignin content of feedstock i.e. peanut shells, oven-dried PS sample (extractive-free) was put (W_3) in a beaker followed by addition of 30 mL of sulfuric acid (98 % H_2SO_4) and kept for 24 h at ambient temperature. After this, dilution was done using 300 mL of distilled water (acid to water) followed by boiling at temperature of 100 ^oC for 1 h duration. Later, PS sample was cooled; filtered and continuous washing with DW was done to ensure complete removal of sulfate ions. At the end, PS sample was dried in oven at 105 ^oC followed by cooling in desiccator (W_4). The percentage amount of lignin of substrate was determined using equation 3.8:

$$Lignin\ \% = \frac{w_4 * (1 - \frac{extractives}{100})}{w_3}\ X\ 100 \tag{3.8}$$

3.5.10 Cellulose of feedstock

The cellulose content present in feedstock can be determined by difference from total (100) supposing that total biomass contains only extractives, lignin and hemicellulose content. The percentage amount of cellulose of substrate was determined using equation 3.9:

$$\text{Cellulose \%} = 100 - (\text{Extractives} + \text{Hemicellulose} + \text{Lignin}) \tag{3.9}$$

3.5.11 Fourier Transform Infrared spectroscopy (FT-IR) technique

In order to determine the structural changes of PS samples that may influenced by pretreatment, a very common characterization technique FT-IR termed as Fourier transform infrared spectroscopy was equipped. Both untreated PS sample and pretreated PS samples were undergone FTIR to compare the structural changes before and after treatment. The FTIR spectrophotometer (Perkin Elmer, Spectrum 100) helped to generate the required spectra of FTIR. Each tool was cleaned properly to avoid contamination. For this characterization, round-

shaped pellets were formed using the fined sample of substrate (PS) with addition of KBr in ratio (1:3) by applying 60 torr pressure. The range of 500 to 4000 cm^{-1} of wavenumber was set to obtain the required spectra of FTIR.

3.6 Analytical methods for anaerobic digestion (AD) setup

With the help of water displacement method, the volume of biogas was measured on daily basis i.e. twice a day. The daily measurement of biogas which was taken in mL unit was allowed to convert into NmL unit by using equation 3.10:

$$V_{NmL} = (V \times 273 \times (760 - P_w) / (273 + T) \times 760) \quad (3.10)$$

Here,

V_{NmL} = volume of dry biogas at standard temperature and pressure

V = volume of biogas measured in mL

P_w = water vapor pressure as a function of ambient temperature (mm Hg)

T= ambient temperature

For the analysis of biogas composition, the samples of biogas were collected in the first week of AD setup, in the middle and at the end of AD process. The biogas was carefully stored in air-tight bags to run on gas chromatography (GC) for quantifying the methane content in each biogas sample. The model (GC-2010 PLUS SHIMADZU) of gas chromatography equipped with TCD (thermal conductivity detector) was used containing 5A open tubular column with porous layer molecular sieve and employed helium (He) as a carrier gas.

Furthermore, to evaluate the digester stability; sample was taken from each set of reactors followed by analysis in accordance with method (APHA, 2017) at the beginning and ending of AD process, to carry out all the necessary parameters of AD process including pH, total alkalinity (TA), volatile fatty acids (VFAs), total solids (TS) and volatile solids (VS). Additional set of AD reactors was also prepared using the identical methodology employed for the experimental reactors for analysis purpose. The pH analysis for all the reactors was directly assessed on the day of AD setup using digital pH meter (HANNA-8521, USA) probe. For

conducting total alkalinity (TA) and VFAs analysis, 20 g sample was taken and subsequently subjected to centrifugation at 6000 rpm for 5 min. Later, the supernatant was further used for alkalinity and VFA analysis. Similarly, TS analysis was performed by initially weighing the reactors putting them in oven at 105 ^{o}C for 24 h duration. After drying, dried material from the reactors were shifted to china dishes and kept in muffle furnace for VS analysis at 550 ^{o}C for 30 min duration. All the analytical parameters responsible for stable AD process were analyzed using methods represented in Table 3.1:

Table 3.1 Various analytical parameters for AD stability

Parameters	**Methods**
pH	Digital pH meter
Total alkalinity (mg/L)	2320B Titration method (APHA, 2017)
VFA (mg/L)	2310B Titration method (APHA, 2017)
Total solids	APHA, 2017
Volatile solids	APHA, 2017
Daily biogas volume	Water Displacement Method
Biogas composition	GC Analyzer (GC-2010 Plus SHIMADZU)

The calculation of the percentage removal of total solids (TS) and volatile solids (VS) was done based on given equations 3.11 and 3.12 respectively:

$$TS\ removal\ \% = \frac{Initial\ gTS\ of\ substrate - Final\ gTS\ of\ substrate}{Initial\ gTS\ of\ substrate} \quad (3.11)$$

Final gTS of substrate = Final gTS – Final gTS of inoculum

$$VS\ removal\ \% = \frac{Initial\ gVS\ of\ substrate - Final\ gVS\ of\ substrate}{Initial\ gVS\ of\ substrate} \quad (3.12)$$

Final gVS of substrate = Final gVS – Final gVS of inoculum

CHAPTER 4: RESULTS AND DISCUSSION

In this chapter, detailed discussion of all the results obtained though experimentation has been presented including effect of NaOH (alkaline) pretreatment on lignocellulosic composition of peanut shells (PS) as well as all the structural changes occurred during pretreatment confirmed by Fourier transform infrared (FT-IR) spectroscopy and influence of alkaline pretreatment on biogas production from mono- and co-digestion, solids reduction and reactor stability in comparison with untreated peanut shells.

4.1 Characteristic of substrates and inoculum

In order to design and operate a digester, it is significant to identify the initial characteristics of feedstocks used in it for obtaining better results. These characteristics are key parameters to startup any AD process as it greatly influence the startup, reactor stability as well as production of biogas during AD. All the initial characteristics of untreated peanut shells (PS), vegetable waste (VW) and inoculum were calculated and presented in Table 4.1.

Table 4.1 Characteristics of substrates and inoculum

Parameters	**Unit**	**Substrate** (Peanut shells)	**Co-substrate** (Vegetable waste)	**Inoculum**
Total Solids (TS)	%	93	90.3	19
Volatile Solids (VS)	%TS	96	87.6	67
Total Kjeldal Nitrogen (TKN)		0.2	3.54	1.9
Total Organic Carbon (TOC)	%	53	48.6	32.19
Extractives		2.00	-	-
Lignin		35	-	-
Hemicellulose		23	-	-
Cellulose		40	-	-

- Not Determined

Total solids (%) and volatile solids (%TS) of peanut shells and vegetable waste were noted to be high i.e. 93%, 96 (%TS) and 90.3%, 87.6 (%TS) respectively that favored the biogas production by AD process (Yuanfang et al., 2020).

Further TOC and TKN analysis of peanut shells were calculated as 53% and 0.2% respectively and 48.6% and 3.54% for vegetable waste (VW) respectively. The lignocellulosic composition (cellulose, hemicellulose and lignin) of peanut shells revealed the presence of high lignin content i.e. 35% in accordance with (Almeida et al., 2024; Wibowo et al., 2019; Sareena et al., 2015; Baskara et al., 2014). Table 4.1 is presenting the characteristics of feedstock (substrate + co-substrate) and inoculum before pretreatment.

4.2 Effect of NaOH pretreatment on lignocellulosic composition of peanut shells

All the results of lignocellulosic composition of peanut shells (PS) obtained before (untreated) and after pretreatment (alkali pretreated) are presented in Figure 4.1.

Peanut shells were pretreated with four varying concentration of NaOH i.e. 2%, 4%, 6% and 8%. From pretreated lignocellulosic results, it was observed that degradation of lignin and hemicellulose was increased with an increase in NaOH dosage up to 4% rather than other concentrations (Khalid et al., 2019). Relatively sharp increase in cellulose recovery with all the varying concentrations of NaOH was observed whereas lignin and hemicellulose content was degraded significantly upto 4% NaOH dosage.

The remarkable increase of 96.04% in cellulose recovery at 4% dose of NaOH indicated that highly complex 3D recalcitrant structure of lignin and hemicellulose were greatly broken down that helped in liberation of the crystalline cellulose portion after pretreatment. Whereas untreated PS exhibited lignocellulosic composition, comprising of 35% lignin, 40% cellulose and 23% hemicellulose (Almeida et al., 2024; Wibowo et al., 2019; Zahra et al., 2019; Zhou et al., 2017). In comparison with untreated PS, cellulose content was observed as 72.75%, 78.42%, 63.84% and 55.61% for NaOH concentration of 2%, 4%, 6% and 8% respectively. Likewise, the degradation in lignin content was high i.e. 75% at 4% dosage of NaOH followed by 2% dosage of NaOH (Remli et al., 2014). Similarly, the content of hemicellulose was gradually decrease with increase in dosage of NaOH upto 4% which showed that hemicellulose might be actively

solvated. Moreover, amount of extractives were gradually increased after pretreatment with increase in NaOH dosage from 2% to 8% i.e. 2.25% to 4.5% respectively (Almeida et al., 2024; Zhu et al., 2010).

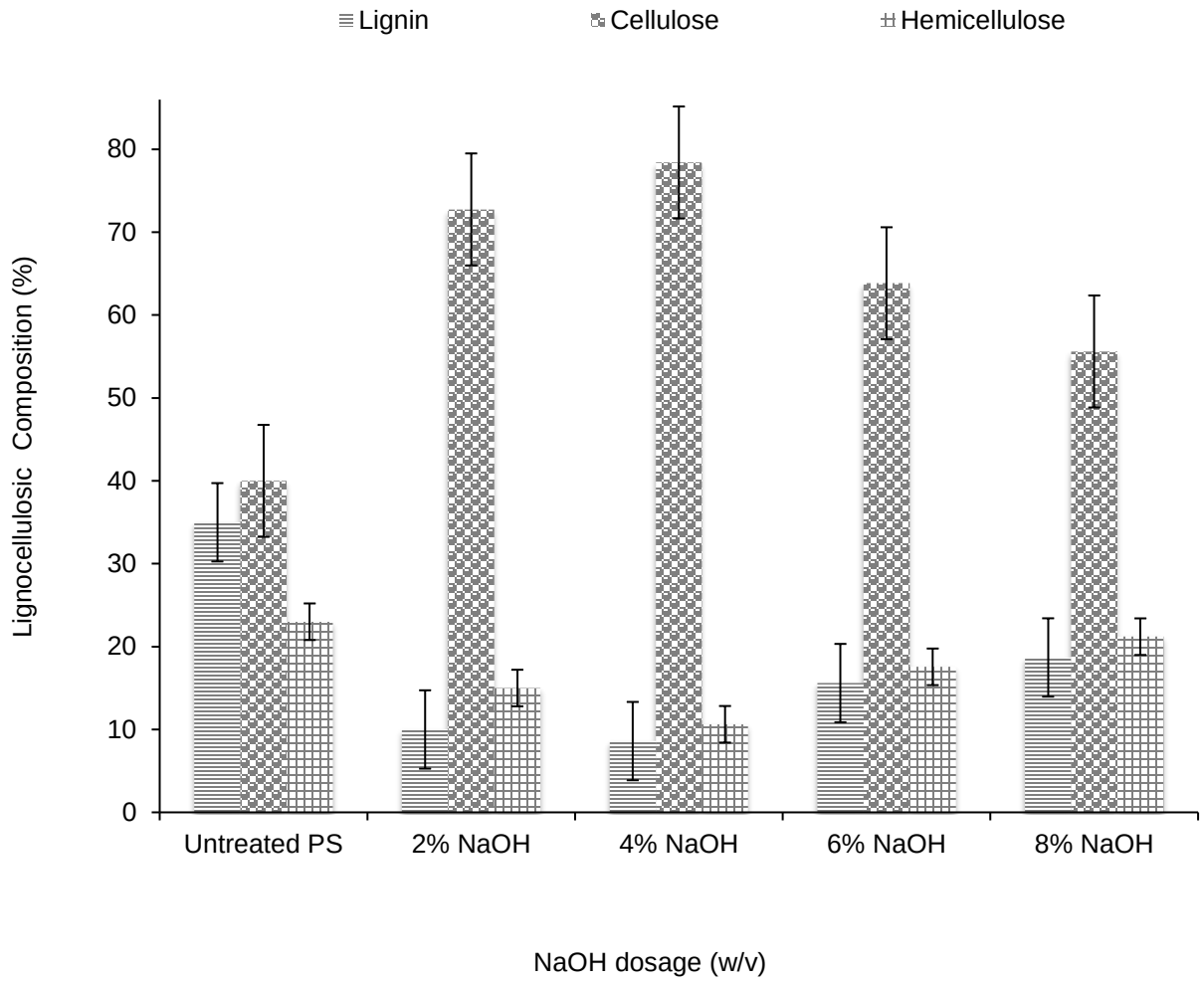

Figure 4.1 Effect of alkaline pretreatment on lignocellulosic composition of peanut shells

Among all NaOH dosage (w/v), alkaline pretreatment at 2% and 4% were remained effective than higher concentrations in terms of lignin degradation, cellulose recovery and conserving holo-cellulose content (Taherdanak and Zilouei et al., 2015). The high degradation of lignin and hemicellulose content suggests that significant portion of lignocellulosic biomass underwent more degradation and converted to various components. In addition, alkaline pretreatment was effective in destroying the intricate structure of lignocellulosic biomass that improved the

biodegradation after breaking up the linkage of ester and glycosidic bonding present in the side-chains and caused more hemicellulose solubilisation, swelling of cellulose as well as modifying the lignin (Samar et al., 2021; Li et al., 2010). It was clearly noticed that there was a slight decline in removing lignin as well as hemicellulose content with further increased in concentration of NaOH after certain point. This could be attributed towards extensive swelling of microfibers at higher NaOH dosage, potentially impeding the effective separation of hemicellulose from the cell's fibrous structure (Rambabu et al., 2016).

Ciftci et al. (2020) also revealed that increase in NaOH concentration upto 20% (w/v) during canola straw pretreatment at various temperatures reduced its degradation efficiency. No significant difference in lignin and hemicellulose removal between 15% and 20% NaOH (w/v) was seen. Another study also revealed more delignification and hemicellulose reduction upon alkaline pretreatment of wheat plant by giving specific conditions (0 ºC - 100 ºC) and resulted into more lignin degradation i.e. 10.8% and 5.4% (Taherdanak and Zilouei., 2015). The previous literature also stated the positive response of using alkaline pretreatment in degrading lignocellulosic components (Lin et al., 2023; Samar et al., 2021). Fu et al. (2018) and Paudel et al. (2017) also investigated the greater availability of cellulose content which served as food source for microbes that significantly improved the biogas production after alkaline pretreatment. All these findings were aligned with the results of current study.

4.2.1 Effect of NaOH pretreatment on chemical structure of peanut shells

The influence of alkaline (NaOH) pretreatment on the composition of peanut shells compared with untreated is previously illustrated in Figure 4.1. These findings verified the effective removal of lignin besides retaining more cellulose with hemicellulose (holocellulose) making them suitable for subsequent anaerobic digestion (AD). Also, these outcomes were validated through Fourier transform infrared (FT-IR) spectroscopy as well.

Fourier Transform Infrared Spectroscopy (FT-IR-8400, SHIMADZU) is basically a fascinating tool that is considered quite useful for assessing the structural modifications appear during pretreatment of lignocellulosic biomass. This spectroscopy also highlights notable alterations in the functional groups (chemical bonds) of cellulose, hemicellulose and lignin.

Figure 4.2 is displaying the FT-IR spectra of both untreated and alkali pretreated peanut shells. For a given FT-IR spectra of untreated and pretreated peanut shells (PS), wavenumber (cm^{-1}) were plotted in the range of 500-4000cm^{-1} along x-axis with percentage transmittance (%T) taken along y-axis. Prior to FT-IR analysis, both untreated and pretreated PS samples were oven dried by providing temperature at 105 °C for 2-4 h. All PS pellets (12 mm in diameter) were made by applying 60 torr pressure using moisture free Potassium Bromide (KBr) in 1:3 (w/w).

According to a given Figure 4.2, the broadband observed at 3423.56 cm^{-1} demonstrating the presence of hydroxyl group (O-H bond stretching) from cellulose. Following the pretreatment, a slight decline in this band suggests modifications in the partial H-bonding present in cellulose which was more prominent under 4% NaOH pretreatment. This outcome aligns with earlier research (Chen 2018; Mustafa et al., 2017; Awadhiya et al., 2017; Asghar et al., 2015; Prabhakar et al., 2015; Rahnama et al., 2013). Simultaneously, other spectral bands noticed at 2924.14 cm^{-1} and 2854.89 cm^{-1} are because of existing alkane group with C-H bond stretching. The sharpness of peak obtained at this band with untreated PS slightly reduced upon alkali pretreatment revealing the methylene group disruption present in cellulose (Zhao et al., 2016; Rahnama et al., 2013; He et al., 2009).

The visible peak at 1741.83 cm^{-1} and 1632.98 cm^{-1} recognizes the ester bond amongst lignin and hemicellulose, also credited to stretching vibrations of C-O bonding as well as stretching of C=C aromatic vibrations present in lignin and to acetyl stretching from hemicelluloses respectively (Gu et al., 2015; Mondal et al., 2015). This sharp peak was completely abated after alkali pretreatment confirming that lignin has been partially removed after ester group destruction via breaking up the carbohydrate-lignin connection supported by various studies (Zhang et al., 2018; Negrea 2018; Danial et al., 2015; Cherian et al., 2010).

Another band appeared at1429.98 cm^{-1} exhibiting the presence of lignin's methoxyl group was slightly modified upon alkaline pretreatment ensuring the partial lignin removal. Likewise, peak at 1382.24 cm^{-1} allotted towards phenolic rings from lignin got altered to some extent after pretreatment of NaOH ensures release of lignin's phenolic group. This phenolic group of lignin is presumed to form strong connection with cellulose and its release thereby liberates cellulose as discussed in previous literature (Zhao et al., 2018; Sun et al., 2016).

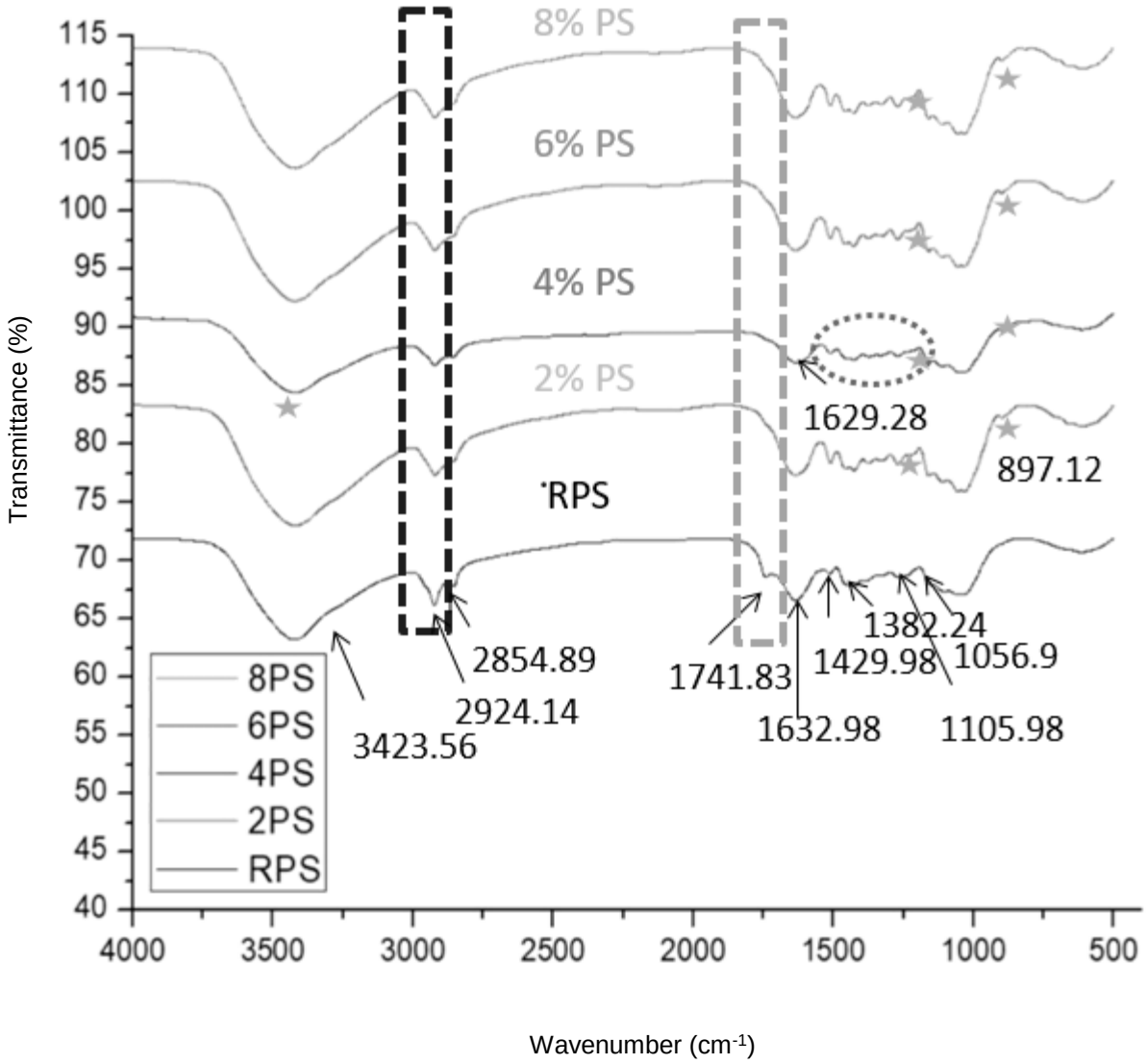

Figure 4.1 FT-IR spectra of untreated and alkali pretreated peanut shells

Furthermore, peak appearance at 1105.98 cm^{-1} and 1056.9 cm^{-1} depicts C-O-C stretching of cellulose and hemicellulose asymmetrically. After alkaline pretreatment, these peaks were subtly modified due to changes occurred within cellulose and hemicellulose. Additional band obtained at 897.12 cm^{-1} refers to linkages amongst sugar units through beta-glycosidic bonds that were clearly visible predicting the intermolecular disturbance in the arrangement of hemicellulose when undergone alkaline pretreatment (Zhang et al., 2019; Gierlinger et al., 2008).Overall representation of all the bands for both samples of untreated and pretreated peanut shells (PS) were relatively similar but the shift in band values of the pretreated PS endorse alterations in

their chemical bonding i.e. inter- and intra-molecular. The existence of all given peaks in untreated PS was highly defined whereas slight alteration or complete disappearance of all these peaks were also noticed upon alkaline pretreatment that confirms the positive response of using alkaline compound effectively. Complete disappearance of peak at 1741.83 cm^{-1} approves the effective lignin removal in all pretreatment samples of peanut shells. From the findings, it is declared that 4% NaOH concentration exhibits major alteration of these given bands guaranteed the greater lignin removal along with large portion of holocellulose rather than higher concentrations. This FT-IR data significantly aligned with the results presented in Figure 4.1. A brief summary of obtained structural peaks representing the functional groups obtained by FT-IR spectroscopy is discussed in Table 4.2:

Table 4.2 Summary of obtained structural peaks with functional groups

Sample	Obtained Peaks (cm^{-1})	Functional Groups	References
Peanut shells (PS)	3423.56	O-H bond stretch (s)	
	2924.14	C-H bond stretch (s)	Negrea et al., 2018
	2854.89	C-H bend (m)	Awadhiya et al., 2017
	1741.83	C=O bond (s)	Lopez et al., 2016
	1632.98	-C-O- stretch (s)	Mondal et al., 2015
	1429.98	C-H bending (m)	Zhao et al., 2016
	1382.24	C-H bond (m)	Rahnama et al., 2013
	1105.98	C-O-C bond (s)	Hsu et al., 2010
	1056.9	-C-O- bond (s)	Asghar et al., 2015
	897.12	O-H bend (m)	

4.3 Effect of NaOH pretreatment of peanut shells on biogas production, solids removal and reactor stability

In current study, the influence of NaOH pretreatment of PS at varying concentrations (%) on biogas yield was evaluated during anaerobic digestion either anaerobic mono-digestion (AMD) or anaerobic co-digestion (ACD) in comparison with their respective control groups (untreated). Additionally, the pretreatment effect on reactor stability i.e. pH levels, volatile fatty acids to total alkalinity ratio (VFA/TA) as well as solid removals i.e. total solids (TS) and volatile solids (VS) was investigated as well. In this study, 36 AD reactors were utilized for initiating the entire AD setup in triplicates including the control group and blank. For instance, control (untreated PS); NaOH pretreated PS (2%, 4%, 6%, and 8%) and only inoculum as a blank. The entire AD setup was continued for 45 d in a batch mode which remained progressive till the end.

4.3.1 Effect of NaOH Pretreatment of PS on Daily Biogas Production

The daily production of biogas from both AD setups (mono- and co-digestion) with untreated and alkali pretreated peanut shells (2%, 4%, 6% and 8%) was assessed that underwent successful performance with proper handling and mixing. Also, the analysis of results obtained from both AD setups was also discussed.

4.3.1.1 Effect of NaOH pretreatment of PS on daily biogas production from anaerobic mono-digestion (AMD)

The biogas production from anaerobic mono-digestion (AMD) setup with untreated and pretreated (NaOH) PS on daily basis is depicted in Figure 4.3.

The increase in biogas production was noticed during initial days of anaerobic mono-digestion (AMD) setup that significantly led to a short lag phase. In each AD reactor, the increase in biogas production was notably commenced from next day i.e. day 2. It could be possible due to well-prepared inoculum, which contained plenty of microbial population for substrate degradation. The optimum value of biogas production for untreated PS on daily basis was about 30 (NmL/gVS) that was recorded on day 14 and ultimately decline during last days i.e. 4 NmL/gVS was measured on day 45. Whereas, all the alkali pretreated peanut shells AMD

reactors i.e. 2%, 4%, 6% and 8% boosted the biogas production upto 53 NmL/gVS, 60 NmL/gVS, 48 NmL/gVS and 42 NmL/gVS respectively.

From results, it is observed that 4% NaOH pretreatment remained more effective in improving biogas followed by 2% NaOH than untreated PS. Increase in biogas yield could occur due to successive removal of lignin and hemicellulose. The biogas production values for all the pretreated AMD reactors were surprisingly higher than control group (untreated). Additionally, untreated PS attains the optimum value sooner than all the pretreated PS. This might be possible due to delayed acclimatization of microbial population with alkaline (Sabeeh et al., 2020; Zhang et al., 2019; Dahunsi et al., 2017).

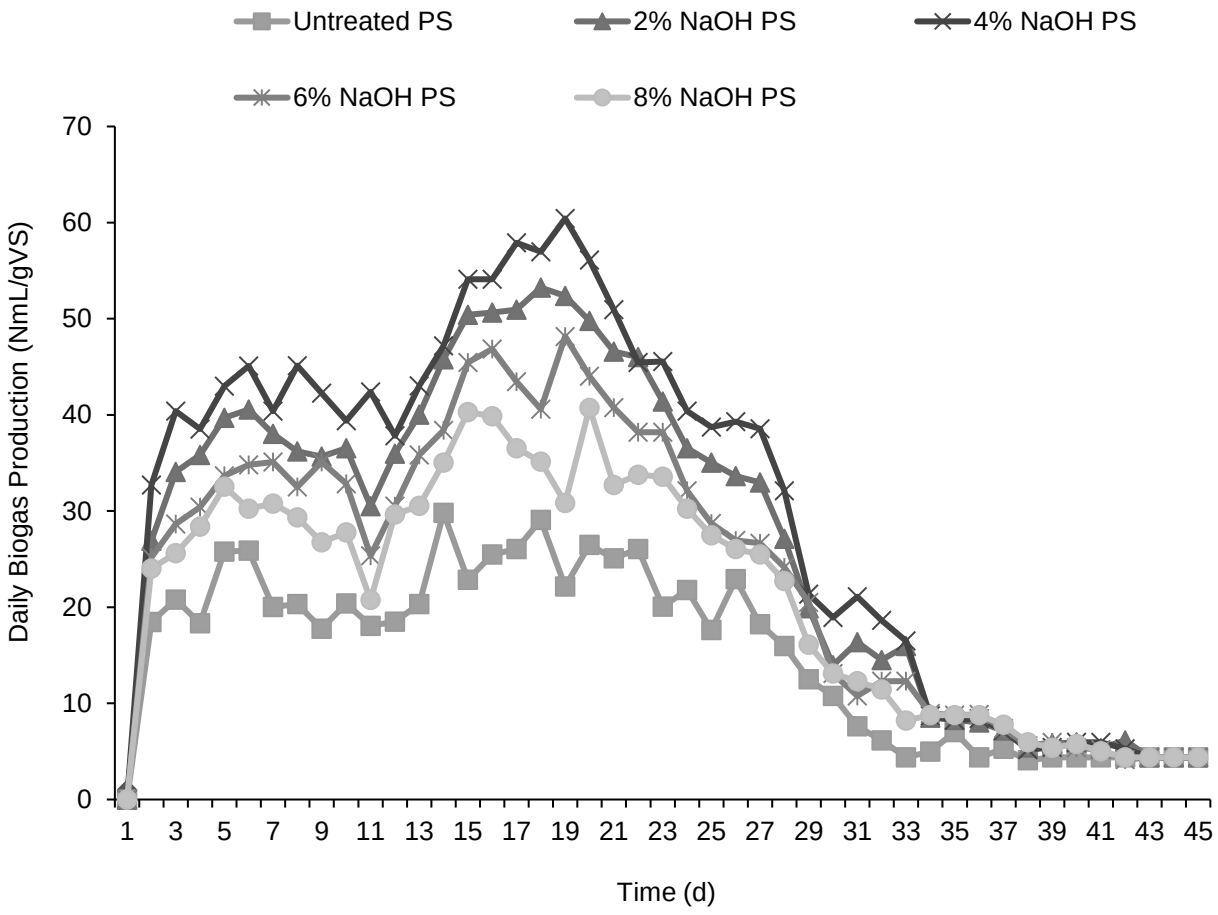

Figure 4.3 Daily biogas production from anaerobic mono-digestion (AMD) using alkali pretreated peanut shells

4.3.1.2 Effect of NaOH pretreatment of PS on daily biogas production from anaerobic co-digestion (ACD)

The daily production of biogas during anaerobic co-digestion (ACD) setup is displayed in Figure 4.4. In case of anaerobic co-digestion (ACD), alkali pretreated peanut shells were anaerobically co-digested with vegetable waste (VW) in 1:1 (S/I) with adjusted C/N ratio of 25 because this is considered optimal for successful AD process as supported by previous study (Rajput et al., 2021).

From given results, it is noticed that all the AD reactors initiate the biogas production from day 2. For untreated co-digestion AD reactor (PS+VW), maximum value for biogas production was recorded upto 50 NmL/gVS whereas considerable improvement in biogas yield was witnessed with all the other pretreated co-digestion AD reactors i.e. 69 NmL/gVS, 75 NmL/gVS, 60 NmL/gVS and 59 NmL/gVS for 2% PS+VW, 4% PS+VW, 6% PS+VW and 8% PS+VW respectively. Overall, all the reactors have amplified the biogas yield upon co-digestion rather than mono-digestion which could be owing to nutrient balancing in all the reactors after anaerobically co-digested with vegetable waste. During last days, biogas yield was gradually reduced upto 3.9 NmL/gVS on day 45 as methanogens might consume all the available substrate. The increased production of biogas confirms the complete degradation of substrates during AD process. From given analysis, 4% PS+VW reactor gave highest peak value of biogas yield followed by 2%, 6% and 8% which could be due to availability of more holocellulose with optimum pH that is considered suitable for AD process (Oliva et al., 2023; Khalid et al., 2019; Neshat et al., 2017; Meng et al., 2015).

4.3.2 Effect of NaOH pretreatment of PS on cumulative biogas production

This study further evaluated the cumulative biogas production from both AD setups i.e. mono- and co-anaerobic digestion. All the results were discussed in detail.

4.3.2.1 Effect of NaOH pretreatment of PS on cumulative piogas Production from anaerobic mono-digestion (AMD)

The cumulative biogas production from anaerobic mono-digestion (AMD) is given in Figure 4.5.

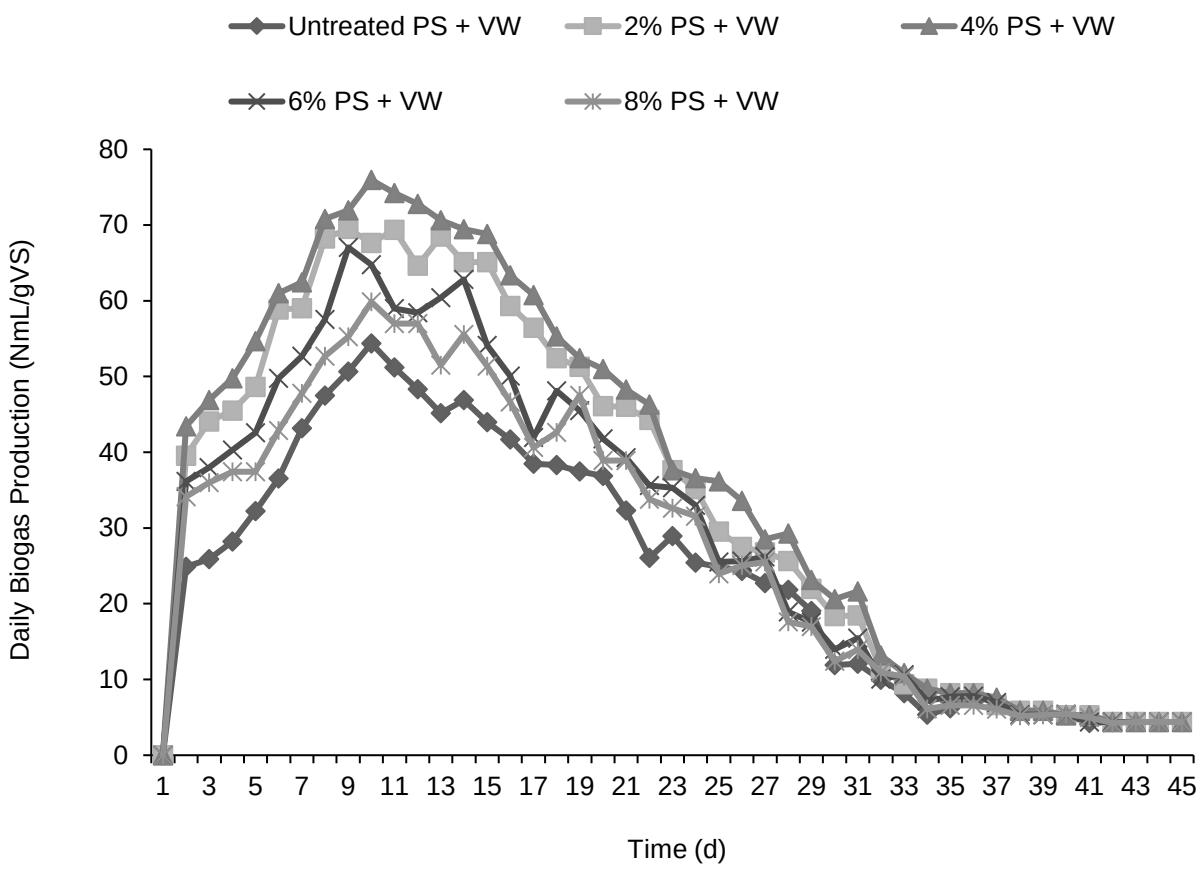

Figure 4.4 Daily biogas production from anaerobic co-digestion (ACD) using alkali pretreated peanut shells

The influence of varying alkali pretreatment of PS i.e. 2%, 4%, 6% and 8% on cumulative biogas production in comparison with untreated was evaluated as well.

From results, the optimum value for cumulative biogas production was obtained from 4% NaOH pretreated PS that was calculated 590 NmL/gVS followed by 2%, 6% and 8% which produced biogas recorded as 571 NmL/gVS, 505 NmL/gVS and 461 NmL/gVS respectively. Previous studies (Oliva et al., 2023; Santos et al., 2021; Wibowo et al., 2019; Shetty et al., 2017; Abudi et al., 2016) also experienced same increasing trend for biogas yield after undergone pretreatment. The biogas yield was more with 4% and 2% NaOH pretreatment due to providing more holocellulose exposure towards microbial population (Khatri et al., 2015). All the varying concentrations exhibited positive response towards improving biogas yield as compared to untreated. This could be directed towards high cellulose exposure for microbial community

during AD process after pretreatment. For instance, the alkaline pretreatment of peanut shells (PS) at 4% has provided maximum biogas which was 59.46% more followed by 2%, 6% and 8% which was recorded as 54%, 36.48% and 24.59% respectively higher than untreated peanut shells (Meng et al., 2015).

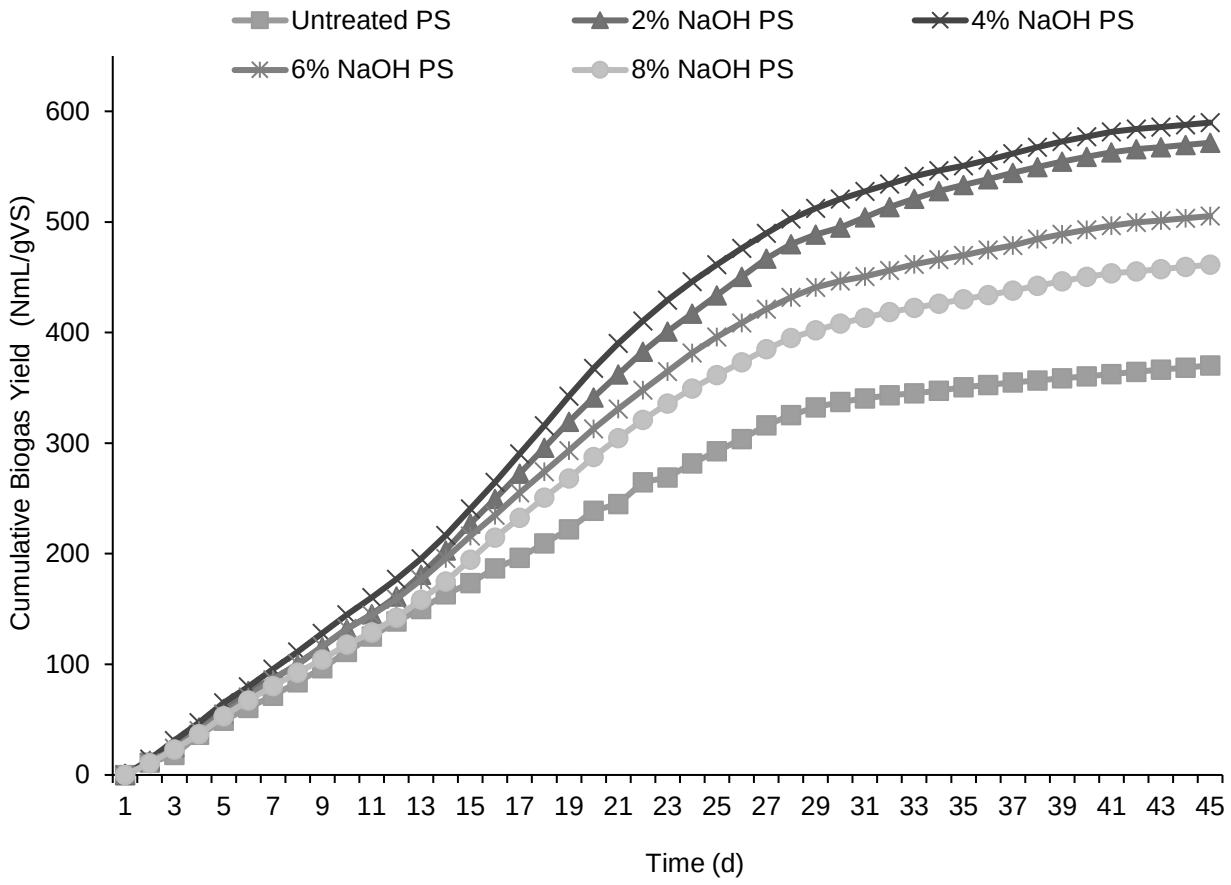

Figure 4.5 Cumulative biogas production from anaerobic mono-digestion (AMD) using alkali pretreated peanut shells

These results align with previous work (Khalid et al., 2019; He et al., 2009). The greater removal of lignin could be reason for higher holocellulose that was achieved at 4% and 2% NaOH pretreatments respectively. From overall results, it is noticed that high dosage of NaOH above 4% was not that much effective in further improving yield of biogas and gradually decline as compared to low concentrations (2% and 4%) due to fact of less delignification in addition to increased swelling of microfibers that impede the hemicellulose departure from complex structure. Such findings associated with results of past literature (Barman et al., 2014). The

portion of holocellulose for 4% NaOH PS was noted to be 89.04% in comparison with other pretreated PS samples as mentioned in Figure 4.1. This could be one of reasons for maximum biogas production (Khalid et al., 2019, Wibowo et al., 2019, Rambabu et al., 2016).

4.3.2.2 Effect of NaOH pretreatment of PS on cumulative biogas production from anaerobic co-digestion (ACD)

The cumulative biogas production from anaerobic co-digestion (ACD) is shown in Figure 4.6. The impact of using vegetable waste (VW) as a co-substrate in all the AD reactors along with untreated and pretreated PS at varying concentration of alkaline pretreatment i.e. 2%, 4%, 6% and 8% was also studied and compared with anaerobic mono-digestion (AMD) setup in detail.

According to Figure 4.6, all the reactors have successfully upgraded the production of cumulative biogas during anaerobic co-digestion (ACD) in comparison with anaerobic mono-digestion (AMD). The overall cumulative yield of biogas with co-digestion was remained effective throughout AD process rather than mono-digestion. This could be owing to imbalancing of nutrients accessible to microbial community in case of mono-digestion AD setup when PS was solely utilized that might not sufficient to satisfy the nutrient demand of microbes, led to incomplete biodegradation i.e. insufficient nitrogen levels as discussed in past literature (Rajput et al., 2021).

During anaerobic co-digestion (ACD), maximum cumulative biogas was achieved after varying concentration of alkaline pretreatment i.e. 709 NmL/gVS, 688 NmL/gVS, 635 NmL/gVS and 620 NmL/gVS for 4%, 2%, 6% and 8% pretreatment respectively. This could be attributed to increase substrate biodegradation upon co-digestion with adjusted C/N. The consumption of suitable co-substrate is measured as an additional key factor for investigating an effective anaerobic co-digestion (ACD) as recommended in Ugolini et al. (2015). PS with more carbon value (C/N = 265) might effectively co-digested with vegetable waste containing more nitrogen value (C/N = 13.72) to adjust the ratio successfully with improved biogas results. The increasing trend of biogas production under co-digestion was also supported by previous studies (Olatunji et al., 2023; Rajput et al., 2021; Li et al., 2020; Khalid et al., 2019; Meng et al., 2015).

High yield of biogas during co-digestion AD setup directed towards the fact of appropriate adjustment of carbon to nitrogen ratio upto 25 for all the reactors as numerous studies stated 20

to 30 ratio of C/N as an ideal range for effective AD process. Therefore, proper C/N adjustment for all the reactors is most-important factor for successive ACD setup. From findings, it is inspected that co-digestion of vegetable waste with pretreated PS at 4% NaOH produced 65% more biogas followed by 58.75%, 42.2% and 37.5% respectively in compared with control group (untreated PS +VW) could happen due to greater cellulose recovery (96.04%) after high delignification achieved at 4% followed by 2% as shown in Figure 4.1.

These findings were supported by previously published work (Olatunji et al., 2023; Oliva et al., 2023; Dutta et al., 2021; Li et al., 2020; Dahunsi et al., 2017; Ibrahim et al., 2016; Liu et al., 2014).

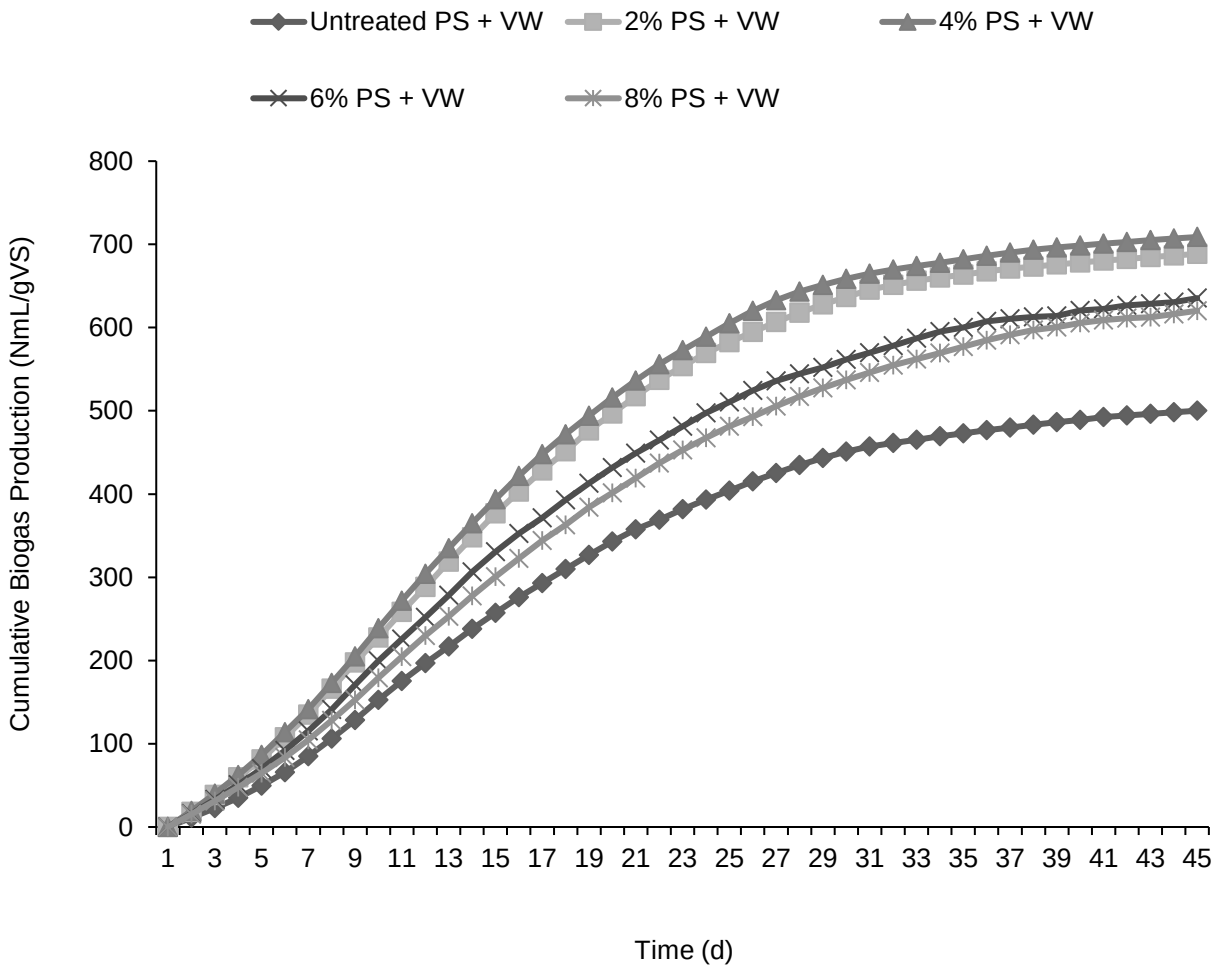

Figure 4.6 Cumulative biogas production from anaerobic co-digestion (ACD) using alkali pretreated peanut shells

4.3.3 Effect of NaOH pretreatment of PS on solids reduction

The efficient performance of anaerobic digestion (AD) setup is usually quantified through assessing its proficiency to reduce solid content. During time period of 45 d, the reduction of solid content i.e. total solids (TS) and volatile solids (VS) for all the reactors of untreated and alkali pretreated PS (2%, 4%, 6% and 8%) using both AD setups i.e. anaerobic mono-digestion (AMD) and anaerobic co-digestion (ACD) is presented in Figure 4.7.

From results, it is noticed that all the reactors of co-anaerobic digestion setup have successfully reduced greater portion of solids (TS and VS) as compared to all other reactors of mono-anaerobic digestion setup at relatively low concentration of alkali (NaOH). Amongst all the reactors, the highest reduction of total solids and volatile solids i.e. 70% and 68% respectively was identified at 4% NaOH PS + VW during co-digestion setup, that found to be 21% and 30.76% more in comparison with its control group (untreated PS+VW). The reactors with maximum VS reduction suggested that microbial community have consumed large portion of solids after successive lignin removal and high cellulose accessibility that greatly assisted in producing more biogas. Therefore, all the reactors that were undergone pretreatment resulted into greater solids reduction and biogas enhancement correspondingly as compared to untreated.

Overall, these notable outcomes are found consistent with results of biogas production. This might be related to active degradation of substrate that underwent co-digestion after pretreatment, which led to the confirmation that increased degradation of substrate is highly required for obtaining enhanced biogas yield. A strong correlation exists between VS removal and enhanced yield of biogas that was witnessed in current study as well. This type of connection was also seen in previous work (Elsayed et al., 2016; Haider et al., 2015). This increasing trend of solids removal per biogas production is found similar to previous work (Maryam et al., 2021; Sabeeh et al., 2020; Khalid et al., 2019; Abudi et al., 2016).

4.3.4 Effect of NaOH pretreatment of PS on reactor stability

Figure 4.8 is representing pH values of all the reactors before and after digestion process. Amongst all other essential parameters that are highly accountable for evaluating the effectiveness of anaerobic digestion (AD) process stability, pH is found one of them.

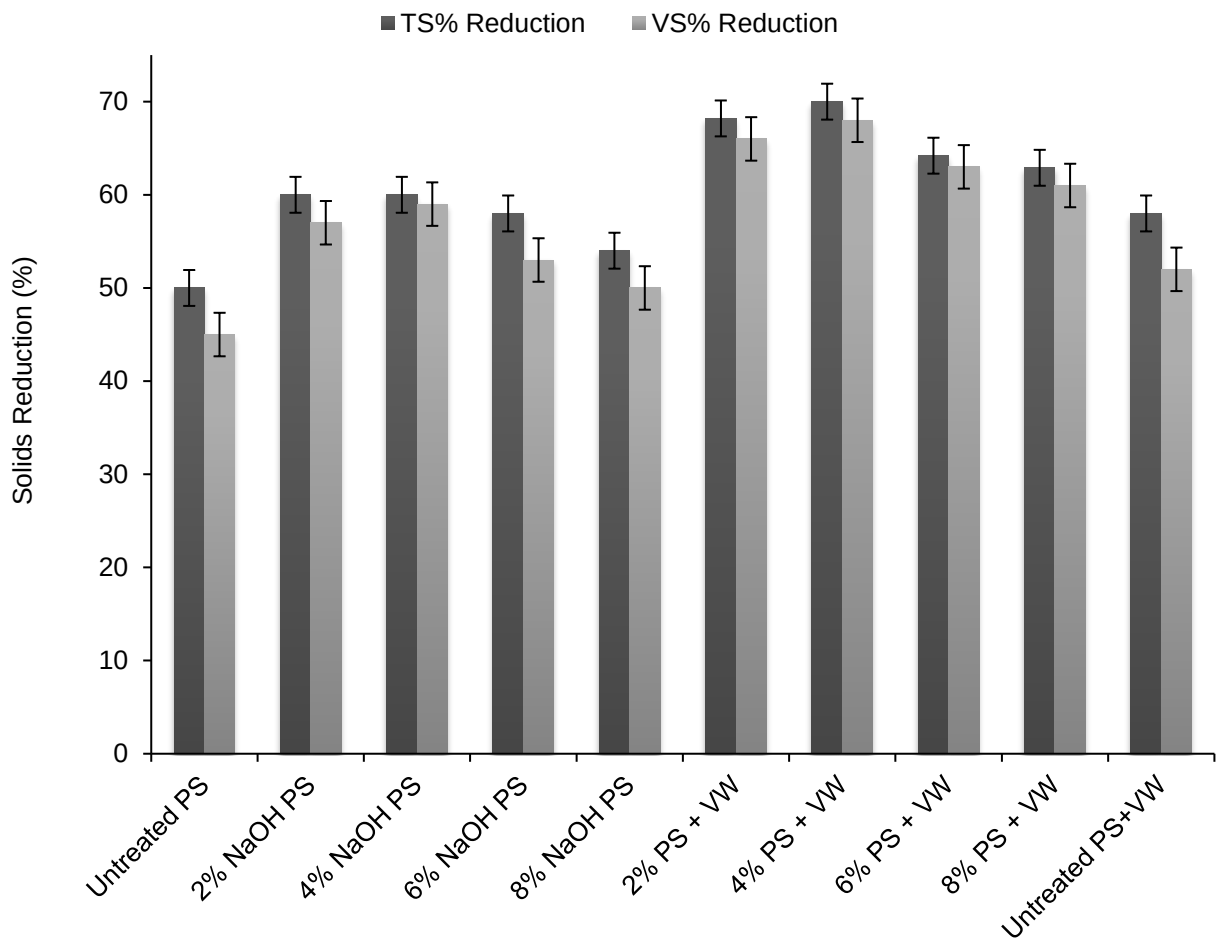

Figure 4.7 Effect of NaOH pretreatment of PS on solids reduction for both AD setups

In this study, initial value of pH (before digestion) and final value of pH (after digestion) was calculated for all reactors of both AD setups in order to gauge the AD efficiency. According to Figure 4.8, the starting pH of all the AD reactors was calibrated between ranges of 7.2 to 7.3 using sodium bicarbonate as a buffer solution to ensure its specific range that is highly operational. After 45 days, final pH levels for all reactors were measured as well i.e. 6.8 to 7.2. All the pH results demonstrated a very slight decline which could attribute towards accumulation of volatile fatty acids while digestion was being carried out for 45d period. Still, these pH values were identified within the range. The optimal range of pH for better functionality of methane producing microbes is stated to be 6.7 to 7.3 ranges (Gonde et al., 2023; Hagos et al., 2017; Neshat et al., 2017; Ye et al., 2013).

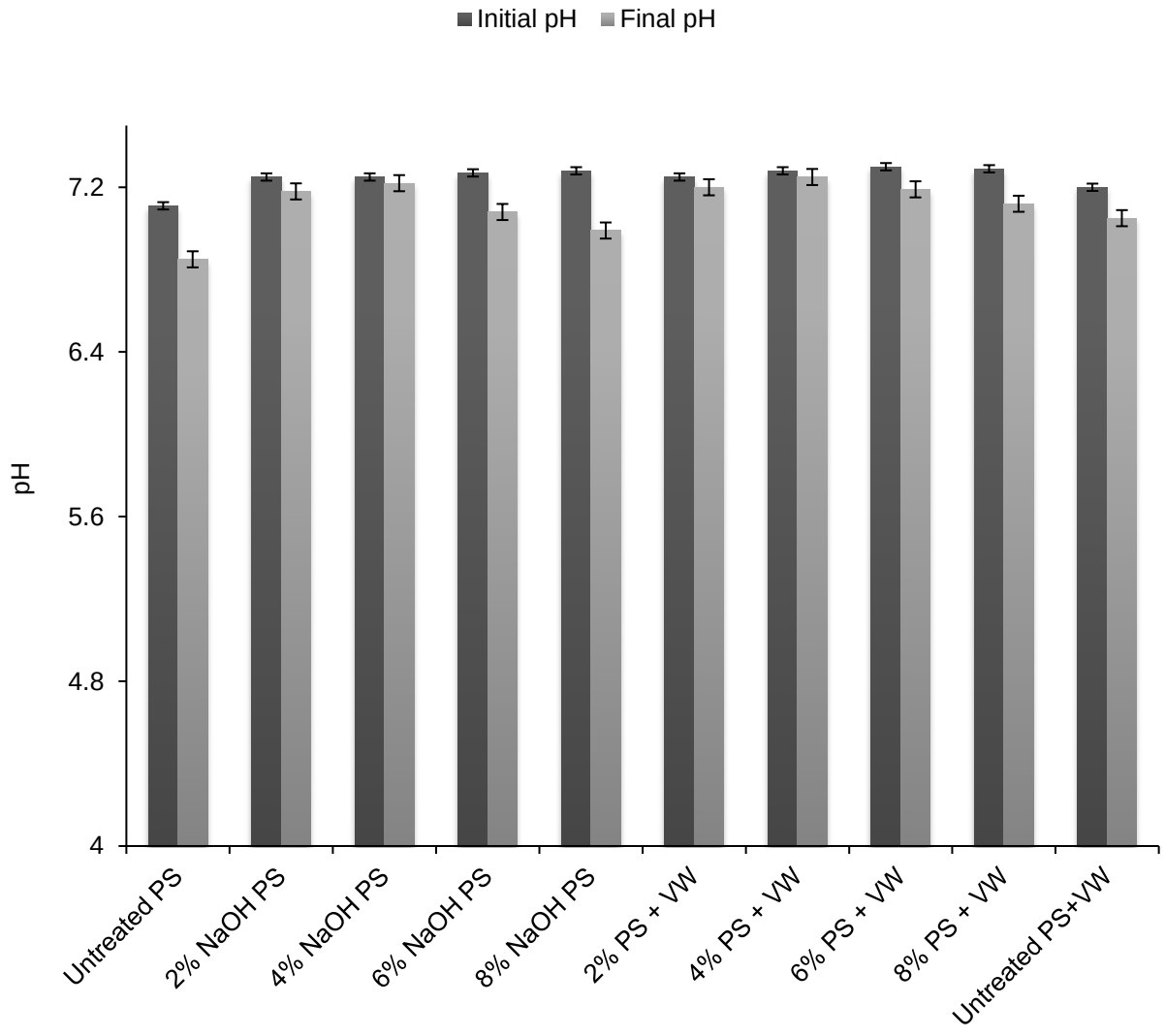

Figure 4.8 Effect of NaOH pretreatment of PS on reactor pH for both AD setups

After digestion process, no significant decline in pH levels were observed with reactors produced more biogas yield as shown in Figure 4.8. This could confirm the stability of all reactors and proper working of methanogens resulted in successful AD setup within neutral range. Because, any rapid change in dropping pH level can badly influence the AD reactor stability, and then led to failure of the entire AD setup. Besides this, survival of microbial community i.e. methane producing microbes (methanogens) under low pH levels (highly acidic) can become problematic as already acknowledged in previous study (Gonde et al., 2023; Neshat et al., 2017). Therefore, considering all the AD reactor's parameters i.e. pH as well as ratio of VFA to alkalinity (VFA/TA) became highly significant to analyze AD digester performance (Eryildiz et al., 2020).

Many researchers have stated that VFA to total alkalinity ratio (VFA/TA) is another important indicator while analyzing the stability and efficiency of anaerobic digestion (AD) setup. Figure 4.9 is depicting VFA/TA ratios for all reactors of both AD setups before and after digestion.

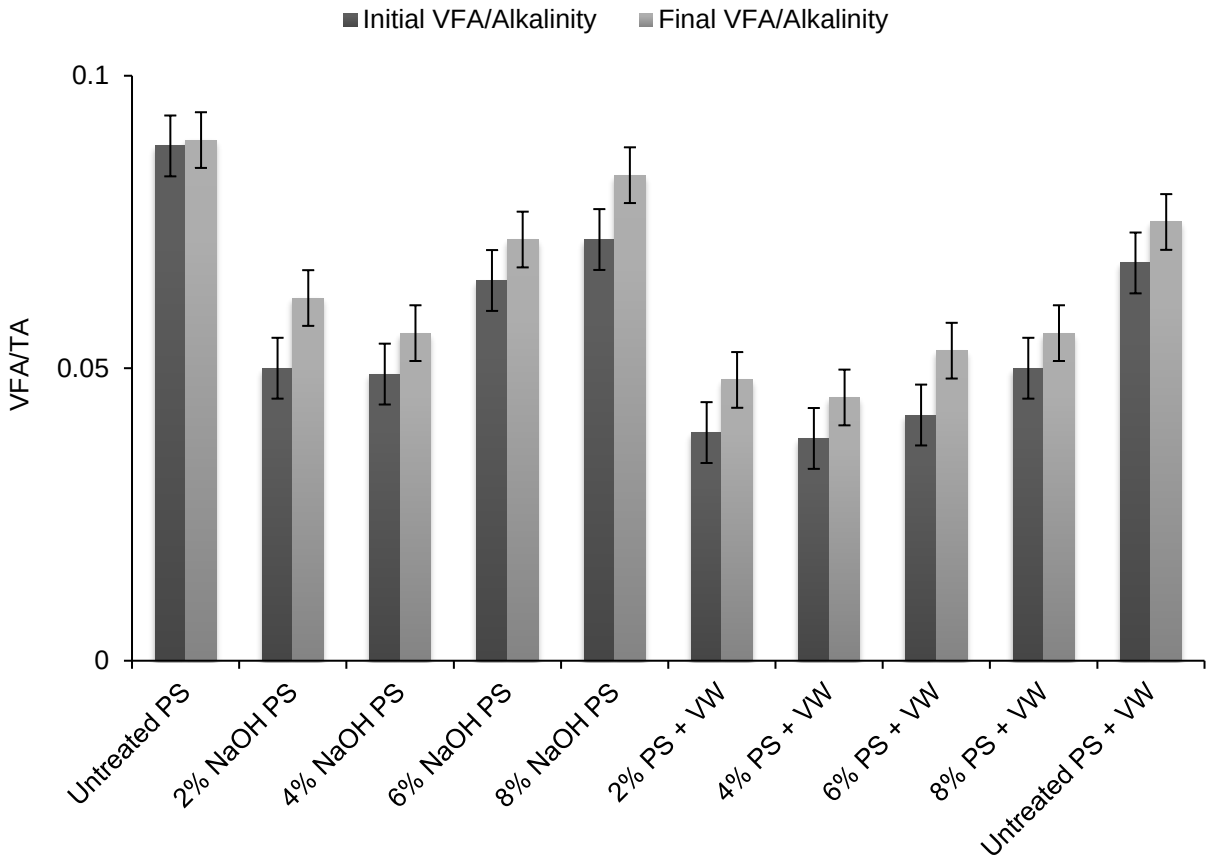

Figure 4.9 Effect of NaOH pretreatment of PS on reactor VFA/TA for both AD setups

Overall, the reactor stability was evaluated upon pH levels and volatile fatty acids to alkalinity ratio. The obtained results revealed effective buffering of all AD reactors. Previous reports (Eryildiz et al., 2020; Kafle and Kim, 2013) stated that VFA/TA ratio must be low i.e. < 0.4 for a well-functioning anaerobic digester (AD). From results, it is recognized that VFA/TA ratio for all reactors upto 0.1 pointing towards stability of AD process.

In current study, most of the reactors, with enhanced biogas production resulted into low VFA/TA ratio i.e. less than 0.4, showing the process stability of all reactors. These results were in agreement with past literature (Eryildiz et al., 2020; Khalid et al., 2019; Wang et al., 2018; Haider et al., 2015).

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

This chapter highlights the conclusions that were drawn from current research with brief discussion. Besides this, some future recommendations are also proposed here.

5.1 Conclusions

This study was conducted to evaluate the effect of alkaline pretreatment on lignocellulosic composition of peanut shells (PS) as objective-1. The obtained results from this objective have concluded that alkaline pretreatment for all varying concentrations have shown positive response towards cellulose recovery and lignin degradation. Moreover, the pretreated peanut shells (PS) at concentration of 4% NaOH have shown maximum cellulose recovery of about 96.04% and 75% decrease in Lignin followed by 2% NaOH. The lignin and hemicellulose degradation of PS was high at low concentrations of NaOH i.e. 2% and 4%. No significant hemicellulose degradation was observed with high dosage of NaOH i.e., 6% and 8% as compared to low dosage. But, all the pretreated PS samples underwent high cellulose recovery after pretreatment. These results were further supported by FT-IR characterization. The analysis of Fourier Transform Infrared (FT-IR) examined that lignocellulosic composition of peanut shells (PS) were undergone some positive changes in its structure and functional groups of cellulose, hemicellulose and lignin after alkaline pretreatment at varying concentrations.

This study also evaluated the effect of pretreated and untreated peanut shells (PS) on biogas production. During anaerobic mono-digestion (AMD) setup, the influence of 4% NaOH pretreatment on biogas yield was high due to more delignification which led to enhanced biogas production that boosted upto 59.46% more in comparison with untreated PS. Overall, biogas production from PS with varying concentrations of NaOH followed the increasing trend upto a certain point i.e. from 2% to 4% then slight decline was observed with further increase in NaOH dosage i.e. 6% to 8% that means low dosage remained effective in producing high biogas with availability of excessive hollo-cellulose content.

This study also investigated the effect of co-digestion with pretreated and untreated peanut shells (PS) on biogas production. During anaerobic co-digestion (ACD) setup, both substrate i.e. peanut shells and co-substrate i.e. vegetable waste were effective throughout biogas enhancement. It was clearly noticeable from results that biogas production using co-substrates was greatly

enhanced rather than solely used substrate. In comparison with control, 65% more biogas was achieved at 4% NaOH pretreated peanut shells followed by 2%, 6%, and 8% due to availability of sufficient nutrients to microbial community for complete degradation. The overall reactor stability were identified within the range i.e. pH and VFA/TA ratios. The volatile solids (VS) removal showed a strong correlation with biogas production i.e. 70% and 68% removal of TS and VS respectively was observed for 4% NaOH pretreated PS during co-anaerobic digestion which produced 65% more biogas as compared to control (untreated).

5.2 Recommendations

Following recommendations are noteworthy for further study:

- Conduct pilot-scale studies to evaluate the feasibility and scalability of alkaline pretreatment. Assess the economic viability and environmental impact at a larger scale to facilitate industrial application.
- Experiment with different alkaline agents beyond traditional ones like NaOH and KOH. Investigate the potential of using more environmentally friendly or cost-effective alternatives.
- Further studies should focus on comparative studies of how this alkaline pretreatment would be a better option than biological pretreatments. This will help in understanding the advantages and limitations of alkaline pretreatment.
- Study the potential utilization of by-products generated during alkaline pretreatment. For instance, explore the use of lignin-rich residues in value-added products such as bio-based materials or chemicals.
- Further investigation should focus on NaOH recovery after pretreatment to lessen the environmental influence.
- Exploring more suitable feedstocks for co-digestion to improve the AD process.

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