Effect of Chemical Pretreatment on Characteristics and Biogas Production of Corn Cob through Mono and Co-digestion

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(2024)

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A thesis submitted in partial fulfillment of requirements for the degree of Master of Science in Environmental Engineering

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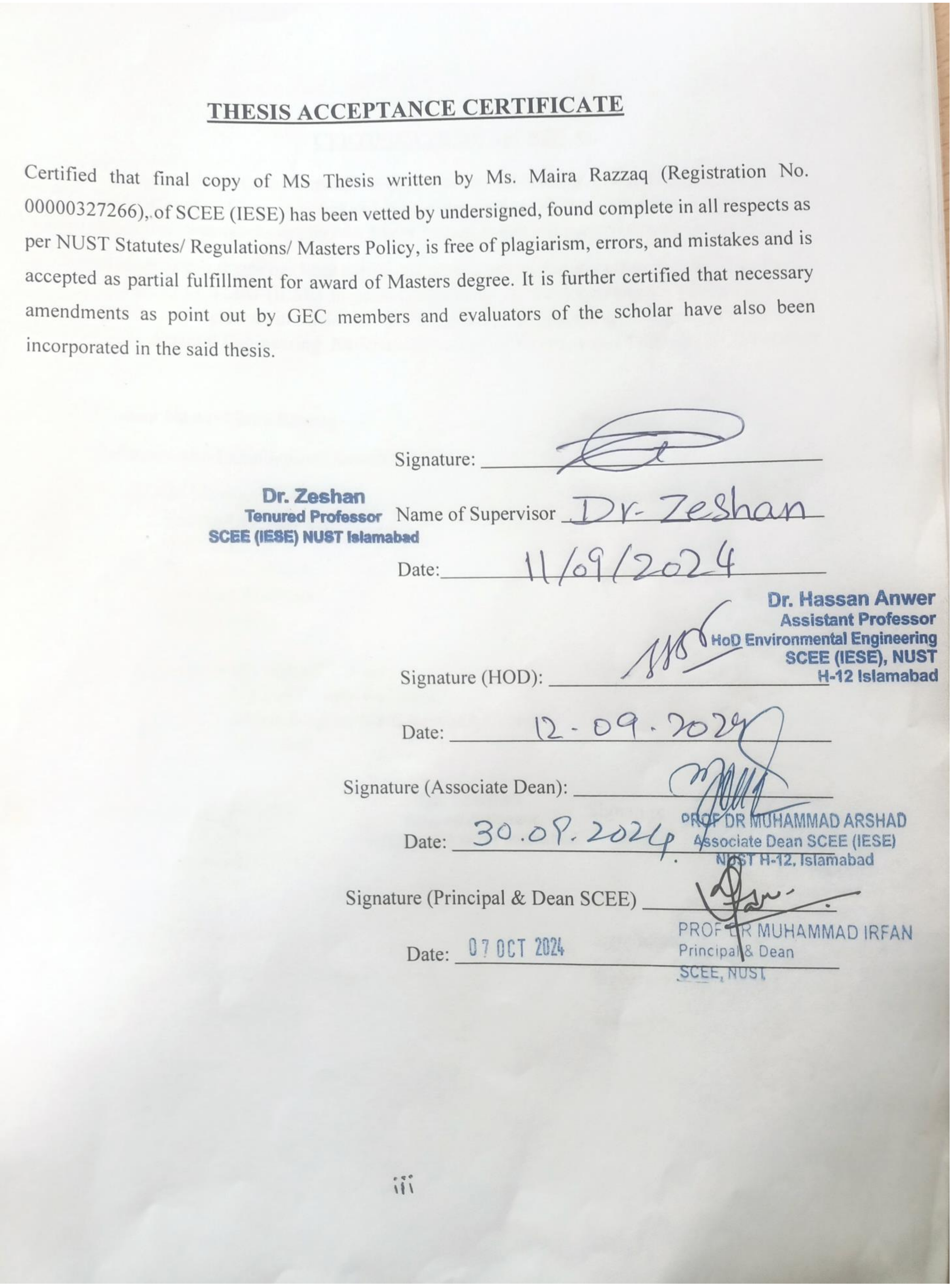

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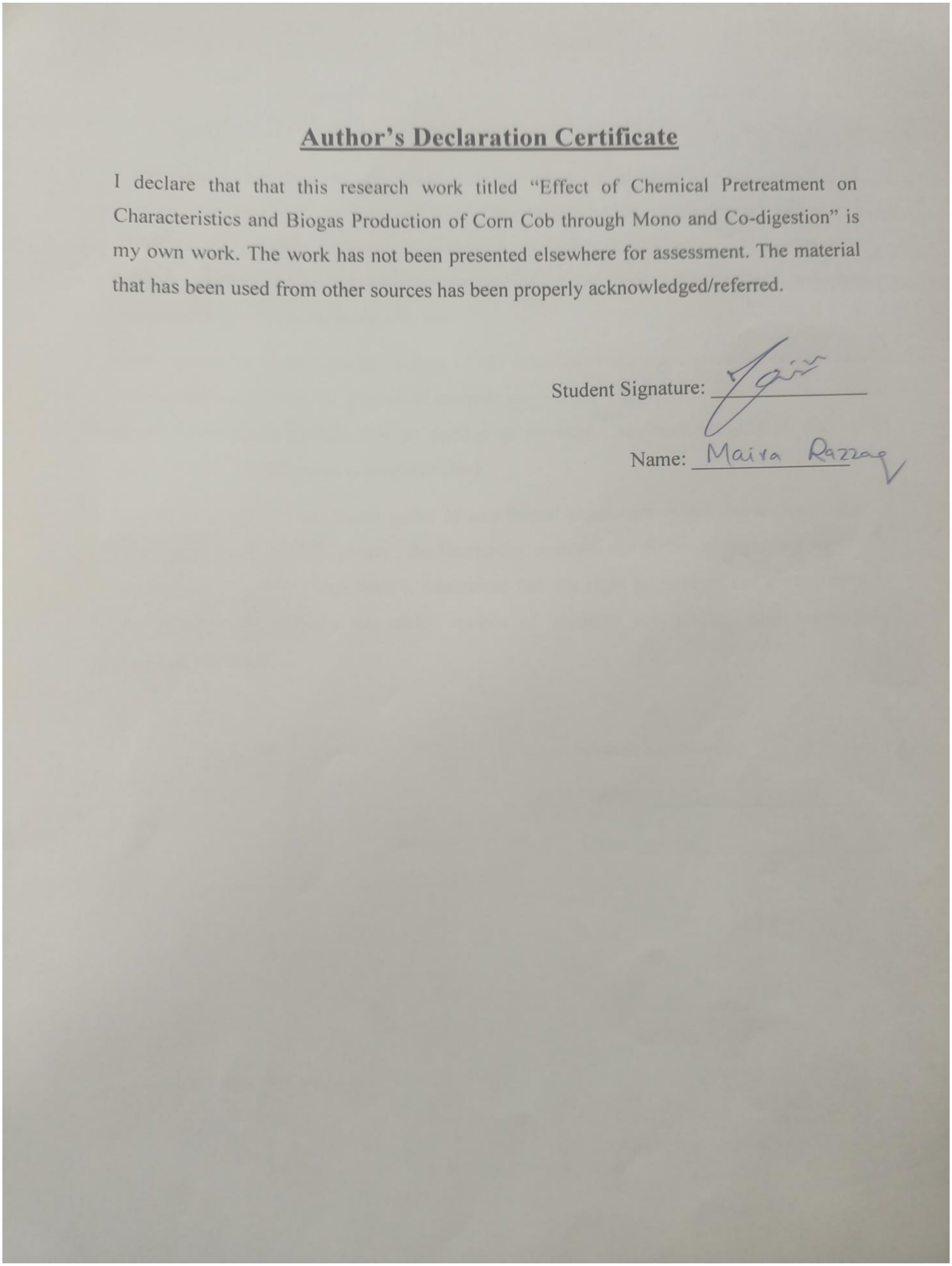

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Dedication

This research is dedicated to my loving parents whose efforts and sacrifice have made my dream of having this degree a reality. Words cannot adequately express my deep gratitude to them.

"O My Sustainer, bestow on my parents your mercy even as they cherished me in my childhood"

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LIST OF ABBREVIATIONS

ACD	Anaerobic Codigestion
AD	Anaerobic Digestion
CC	Corn Cob
CD	Cow Dung
FW	Food Waste
MC	Moisture Content
NaOH	Sodium Hydroxide
OL	Organic Loading
TA	Total Alkalinity
TS	Total Solids

VFA	Volatile Fatty Acids
VS	Volatile Solids

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ABSTRACT

Anaerobic digestion (AD) process has the capability to sustainably meet the growing global energy needs and thus energy crisis. It helps in converting a variety of biomass into bioenergy and digestate. Agricultural waste especially corn cob (CC) is a very suitable option for AD process. However, the recalcitrant structure of corn cob and high carbon to nitrogen ratio (C/N) makes it less susceptible to AD. Therefore, the focus of the study was to perform alkaline pretreatment of the CC and to perform its mono and co-digestion with food waste (FW) i.e. cooked rice as co-substrate to optimize the C/N for effective AD. The corn cob was subjected to pretreatment with varying NaOH concentrations of 1%, 1.5%, 2%, 2.5% and 3%. The raw and pretreated corn cob was subjected to AD i.e. both batch mode mono and co-digestion in mesophilic condition for 40 d. The ultimate analysis of food waste was done which assured its nutrient richness thus making it a good option to be used as a co-substrate with CC. The results revealed that maximum cellulose recovery was obtained in 1.5% and 1% NaOH pretreated samples showing cellulose recovery of 128% and 101.4% respectively. While the lignin removal for 1.5% NaOH pretreated CC was 63.38% and for 1% NaOH pretreated CC, it was 68.75%. The cumulative biogas yield of 1%, 1.5% and 2% increased by 64.76%, 24.36% and 10.32%, respectively in comparison to control group. In short, lower dosages of NaOH i.e. 1%, 1.5%, 2% were more effective than the higher dosages of 2.5% and 3% which showed decline in biogas yield due to excessive delignification. Furthermore, the more biogas production was indicated in co-digestion as compared to the mono-digestion process. The order of cumulative biogas produced in anaerobic co-digestion (ACD) of CC with FW is: 1% >1.5%>2.5%>2%> 3%. The research concluded that alkaline pretreatment was successful in increasing cellulose recovery and reducing lignin content, and that co-digestion of pretreated lignocellulosic substrate with other organic waste generated more biogas than its mono-digestion.

Chapter 1

Introduction

1.1. Background

Fossil fuels have been the primary source of energy across the globe. The fossil fuels come with the cost of emitting greenhouse gases in the environment which contributes to global warming and climate change. The global energy demands have further amplified owing to the increasing world population and rampant industrialization. This has further resulted in the overconsumption of fossil fuels and thus elevated greenhouse gasses emissions. Fossil fuels are now diminishing globally which is leading to energy crisis across the globe (Nosratpour et al., 2018). The skyrocketing costs and dearth of fossil fuels have resulted in the world turning towards the use of more clean and sustainable options of energy such as biofuels (Adeniyi et al., 2018; Bateni et al 2018; Hosseini et al., 2016). Biofuels prove to be a very suitable option of clean, affordable and sustainable energy source (Sekoai et al., 2019; Oh et al., 2018). Pakistan is one of the countries that are suffering the brunt of energy crisis. The country being an agrarian economy has the option to switch to biofuels to suffice its energy needs as it produces 121 million metric tons of agricultural residues (Khan et al., 2022). The lignocellulosic biomass is the most promising and sustainable renewable energy source as it may be converted to biogas (50-75% CH_4 and 25-50% CO_2) at a relatively lower costs (Isikgor and Becer, 2015; Zheng et al., 2014).

The expanding demand for alternate energy sources has resulted in research into biomass as a possible alternative. Corn cob being an agricultural residue presents as a viable potential to produce biogas through AD. The lignocellulosic composition of corn cob includes 44.4±5.2% of hemicellulose, 11.9±2.3% of lignin and 38.8±2.5% of Cellulose (Thangavelu et al., 2018). Pakistan being an agrarian country has a huge potential to produce bioenergy from lignocellulosic biomass which includes maize straw, corn cob, wheat straw, rice straw, cotton straw, sugarcane bagasse, animal dung, wood and poultry litter (Saeed et al., 2015; Asif, 2009). The country produces waste from 8,000-10,000 tonnes of maize crop annually which includes 720-900 tonnes of corn cobs (Sustainable

Agribusiness Forum). In Pakistan, the residue from maize crop is either indiscriminately discarded, burnt in mud stoves, made into handicrafts or some portion of it is included in animal feed (Miranda et al., 2021). The open burning of this waste results in the emission of greenhouses gases and air pollution in the form of smog (Rajput & Sheikh, 2019). The bioconversion of corn cob to bio products has been done in the past and it qualifies to be a great potential source of biomass for the low-carbon substitute to fossil fuels (Elegbede et al., 2021).

There are various conversion technologies of biomass especially corn cob, often regarded as agricultural waste. Its conversion can be done for generating bioenergy through various biomass conversion methods. These methods include thermal conversion, biochemical processes, and anaerobic digestion. Thermal conversion involves pyrolysis or gasification, both of these are energy intensive processes and may result in incomplete combustion. On the other hand, the biochemical conversion processes include microbial or enzymatic breakdown which are slow in nature and may require costly enzymes or conditions for process effectiveness (Gupta et al., 2022). Anaerobic digestion (AD) is a feasible option in comparison to the previously mentioned biomass conversion methods. AD is cost effective and requires low energy demands while generating biogas and lowering greenhouse gas emissions. AD helps recycle nutrients and controls odour (Zhang et al., 2019). In AD, the biodegradable organic matter results in methane (CH_4) and carbon dioxide (CO_2) production by the microbial activity in anaerobic environment (Zhou et al., 2016). The process of AD includes four phases which are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Najafpour et al., 2015). In the first phase i.e. hydrolysis, the large organic matter is disintegrated into smaller water soluble compounds such as fatty acids, glucose and amino acids. The next phase of AD is the Acidogenesis, which results in converting those smaller water soluble compounds into volatile fatty acids (VFAs) and some biproducts such as NH_3, CO_2, and H_2S. Then during acetogenesis, the already produced VFAs are converted to CO_2, acetate, H_2 and other compounds. During the last step, methanogenesis, the methanogens convert the acetate into biomethane. Among all the steps of AD, hydrolysis is the slowest and rate determining phase of the entire process (Hassan et al., 2017; Rodriguez et al., 2017). By dint of AD, organic waste can be managed and a cleaner environment may be fostered by harnessing energy from it. The process also helps in reducing harmful emissions. The produced bioenergy acts as a renewable energy source, while the byproducts are rich in nutrients and can be used as fertilizers thus promoting sustainability.

Pakistan, being an agrarian economy, produces agricultural residue in the form of corn stover, corn cob, maize straw, wheat straw, rice straw, cotton straw, sugarcane bagasse, animal dung, poultry litter, and wood (Saeed et al., 2015; Asif, 2009). The aforementioned sources of biomass have cellulose, hemicellulose, and lignin in abundance, which makes them ideal for bioenergy production through various processed. Corn cob specifically is often wasted across many regions of the world. It is an abundantly produced agricultural byproduct which is a low cost option for bioenergy production. By using corn cob results in the reduction of maize crop residue thus making it an economically and environmentally viable option. As mentioned, corn cob can be a low cost and readily available resource for sustainable biofuel production. The contents in corn cob include lignocellulosic biomass which is not readily accessible for reactions to occur for the production of biogas. Characteristics such as availability of surface area for reactions, crystallinity of lignin and cellulose presence limit the breakdown of lignocellulosic biomass. For addressing the aforementioned factors, pretreatment is needed before anaerobic digestion (Mancini et al., 2018). Research has revealed that in order to enhance the production of biogas, pretreatment of the biomass is essential. There are various methods, physical, chemical, or biological, that may be employed for the enhances yield. The physical pretreatment methods include steam explosion, comminution and irradiation breakdown the biomass through mechanical force thus making this process more energy intensive and give less biodegradability of biomass (Fernandes et al., 2009). The biological pretreatment processes have limitations such as slow hydrolysis (Gupta et al., 2022). The drawbacks of the aforementioned methods make chemical pretreatment methods to be more preferable. Chemical pretreatment methods have garnered attention due to lesser cost, fast reaction rates and efficiency in decomposing the complex organic matter. Alkali pretreatment has emerged to be a good option to enhance the carbohydrates accessibility to microbes thus leading to enhanced methane yield (Mancini et al., 2018).

As mentioned above, the biogas potential of corn cob in Pakistan is promising on various fronts. Its use can aid in addressing the country's looming energy crisis. Additionally, it can result in reducing the fossil-fuel dependence and mitigating greenhouse gasses emissions. The conversion of corn cob into bioenergy would also translate into economic opportunities by proving to be as a source of income to the farmer community and job creation. The process can also help in reducing agricultural waste and uncontrolled burning, which is frequently practiced in the rural localities (Miranda et al., 2021). However, despite pretreatment of corn cob, there exists a limitation in its sole AD. The limiting factor in AD of corn cob is its high carbon-to-nitrogen ratio (C/N) in comparison to the optimum value of 20-30. Although the corn cob is rich in hemicellulose and cellulose but its low nitrogen content leads to an imbalance in C/N of AD process which halts the microbial activity. The high C/N results in slow biodegradability of the biomass (corn cob) and suboptimal biogas production. For the optimization of AD process, a co-substrate with low C/N ratio is needed. In this research, boiled rice sourced from a local restaurant's leftover food was employed as a co-substrate. The C/N of cooked rice makes the AD optimal by the balancing the C/N of corn cob in the reactor. Boiled rice are also readily biodegradable and a sustainable option for food waste valorization (Gao et al., 2021). Co-digestion helps in enhancing biogas yield by the improvement of balance in nutrients and microbial activity. It helps in stabilizing the AD process through the dilution of inhibitory compounds from the substrate. Research suggests that if lignocellulosic biomass is co-digested with food waste, energy recovery is enhanced and chance of AD process failure is reduced (Mata-Alvarez et al., 2014).

The lignocellulosic biomass includes three primary contents i.e. lignin, cellulose and hemicellulose. Cellulose comprises of D-glucose chains and has crystalline as well as amorphous regions with indefinite structure. These cellulose fibres are in the form of bundles. On the other hand, hemicellulose comprises of networks xylose of, galactose, mannose, glucose, arabinose, rhamnose and uranic acids. They do not have a definite structure and are readily available to be broken down in comparison to cellulose. Lignin comprises of hydrophobic heteropolymers. These polymers are derived from three phenylpropane alcohols: p-coumaryl (H), sinapyl (S), and coniferyl (G). Lignin has an amorphous i.e. indefinite structure that helps in providing resistance to the plants against microbial activity. Within the cell walls of plants, lignin molecules are bonded to polysaccharides through lignin-carbohydrate network, resulting in halting the cellulose and hemicellulose biodegradation (Carrere et al., 2016). The utilization of alkaline pretreatment, specifically through sodium hydroxide (NaOH), is being utilized in the pretreatment of various lignocellulosic compounds. The alkaline pretreatment helps in enhancing biodegradability of biomass through lignin removal and enhancement of its porosity. These variations in the structure result in improving hydrolysis and higher yield of biogas production (Mancini et al., 2018).

1.2. Pretreatment of lignocellulosic biomass

A number of pretreatment options, biological, physical and chemical, are employed to increase biogas yield (Zheng et al., 2014). Physical methods involve the extrusion, comminution, steam explosion, irradiation and hot water. The drawback of using physical methods of pretreatment is that they give low biodegradability and yield less bioenergy (Fernandes et al., 2009). Whereas biological methods employ the utilization of of fungi, enzymes and microbial consortium. It is less preferred at industrial scale owing to the lower hydrolysis rates of lignocellulosic biomass (Chandra et al., 2012a). The chemical methods involve the usage of acids, organic solvents, bases, and ionic liquids for the pre-treatment of lignocellulosic substrate. The acidic treatment is not generally preferred due to the corrosive, toxic and hazardous nature of acids. This treatment has lesser biogas production in comparison to the alkaline pretreatment (Gao et al., 2013). Furthermore, comprehensive research is required to explore the usage of ionic liquids for the pretreatment. Chemical pretreatment methods are less utilized due to the cost intensiveness and possible intoxication of the microbes by the chemicals (Muthangya et al., 2009). The chemical pretreatment methods are utilized to expose the compact lignocellulosic organic matter through harsh conditions i.e. high temperature and pressure and high chemicals concentrations. These chemicals can potentially induce toxicity to the AD reactor and halt the biogas production (Puskelova et al., 2014).

1.3. Advance oxidation process (AOPs) for biogas enhancement

In the recent past, the use of Advance Oxidation Process (AOPs) to convert organic substrate into biofuel has been explored by the researchers. AOPs are used to treat recalcitrant lignocellulosic structure by using strong radicals to oxidize it. Most of the AOPs methods make use of the hydroxyl radical for the purpose of oxidation (M'Arimi et al., 2020; Luo et al., 2018).

AOPs have potential to breakdown the complex lignocellulosic biomass into simpler, hydrolysable and soluble forms thus resulting in enhanced biodegradability. This in turn increases the biofuel production. On the contrary, the AOPs methods have drawbacks as well which include high operational costs due to energy intensiveness of the process. These are also complex processes and also have the potential to form by-products, limited pollutant efficiency, equipment corrosion, and potential environmental impact.

1.4. Benefits of biogas technology

Biogas, being a very promising fuel source, can replace fossil fuels while providing environmental and socio-economic benefits. A few benefits of biogas are mentioned below:

Cost effectiveness

- Inexpensive feedstock
- Lower market price than petrol and diesel (Mao et al., 2015)
- Low cost of waste treatment (Holm-Nielsen et al., 2009)
- Low operational cost
- Availability of local materials for build and repair (Raja et al.,2017)

Green energy production (Rehl and Muller, 2011)

- Generation of heat and electricity
- Used as a vehicular fuel

GHG emission reduction (Cuellar and Webber, 2008)

- Limiting the greenhouse gases release into the atmosphere

Environmental protection (Cuellar and Webber, 2008)

- Digestate as a nutrient content bio-fertilizer
- Avert air and water pollution
- Minimize pathogenic nuisance caused by flies and insects
- Reduce acidification and eutrophication phenomena
- Conservation of woods and conserve forest vegetation

Organic waste disposal (Cuellar and Webber, 2008)

- Efficient management of organic waste such as house hold waste, municipal waste, industrial waste and agricultural crop residues etc.

1.5. Objectives of the study

1. To investigate the effect of chemical pretreatment on characteristics of corn cob
2. To evaluate effect of Chemical Pretreatment on Biogas Production of corn cob
3. To assess biogas production of chemically pretreated Corn Cob and Food Waste through codigestion

1.6. Scope of study

The defined scope of subject study is as below:

1. The impact of alkaline pretreatment on the biogas production of corn cob was assessed.
2. The corn cob was collected from a corn hawker at NUST while the boiled rice was acquired from a local restaurant in Islamabad.
3. Cow dung, acquired from a farm in sector H-13, near NUST, was used as a source of inoculum.
4. The characteristics of alkali pretreated corn cob were assessed by the changes in its lignocellulosic composition.
5. Mono and codigestion reactors set-up at laboratory scale.

Chapter 2

Literature Review

This chapter entails literature review in details on the topics covered in the ambit of this study.

2.1 Lignocellulosic biomass

Lignocellulosic biomass, including corn cobs, corn straw, corn stover, rice straw, cotton stalks, wheat straw, sugar cane bagasse and rice husks etc., is an abundant organic matter source suitable for bioenergy production such as biogas (50-75% CH_4 and 25-50% CO_2) (Isikgor and Becer, 2015; Zheng et al., 2014). The lignocellulosic biomass contents include lignin (10–25%), cellulose (35–50%), hemicelluloses (20–35%) (Liu et al., 2008) and very small amounts of extractives as illustrated in Figure 2.1. Cellulose and hemicellulose are fermented by enzymatic hydrolysis resulting in making lignocellulosic biomass to be an appropriate substrate for bioenergy yield. The ranges for these lignocellulosic contents varies with the maturation, growth conditions and specie type.

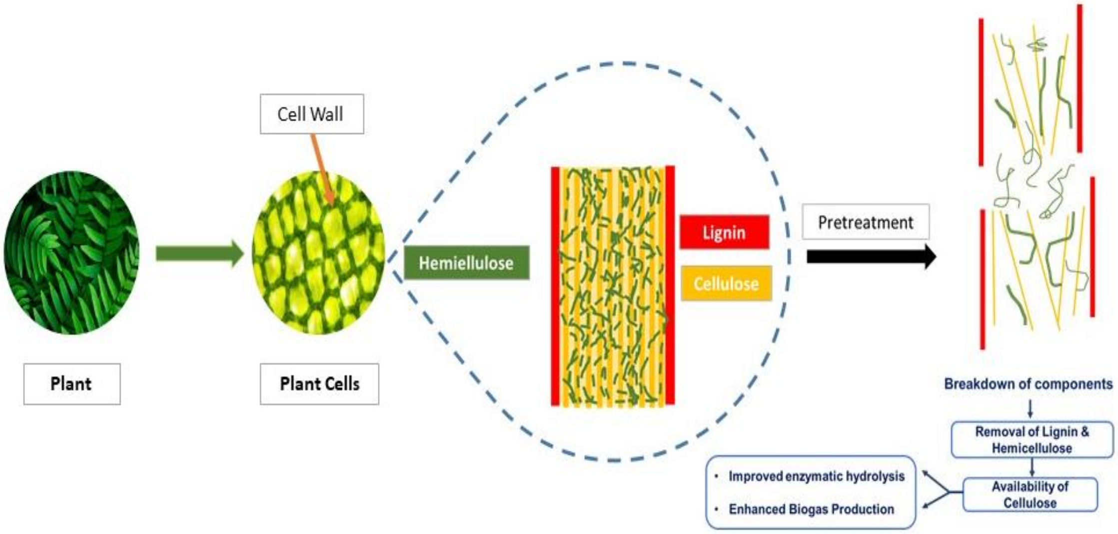

Figure 2.1 Lignocellulosic biomass structure in plant cell walls

2.2 Properties of lignocellulosic biomass

Plant cell wall is primarily made up of cellulose. Cellulose has a linear homopolysaccharide structure of b-1 chain which is strongly attached to 10,000–15,000 units of D-glucose. They are interconnected through vander Waals forces and hydrogen bonds forming microfibrils. The cellulose molecule has two different crystallinity levels i.e. amorphous having low and high crystallinity regions. Hemicellulose attaches the microfibrils to each other which are then covered by lignin. This compact and tough framework resists any biological and chemical attacks (Zheng et al., 2014). Nevertheless, the nature of hemicellulose is very amorphous and is branched carbohydrate having a variety of sugars (C5 and C6). The type of hemicellulose sugars varies with each plant species. However, the breakdown of hemicellulose always gives rise to pentose (xylose and arbinose), hexoses (mannose, galactose, glucose and and/or rhamnose) and acids (galacturonic acid, methyl glucuronic acid and glucuronic acid). The branches of hemicellulose form a nexus with the lignin and cellulose fibres making a matrix of cellulose-hemicellulos-lignin which is very rigid and compact. This amorphous and branched structure makes the monomer of hemicellulose to be very vulnerable to biological, chemical and thermal hydrolysis (Zheng et al., 2014; Singh et al., 2014).

Lignin being the most tough constituent of lignocellulosic biomass, is very non-reactive and insoluble in water. It has a complicated structure comprising of aromatic and hydrophobic heteropolymer made up of three cross linked phenylpropane (C6–C3) units of sinapyl alcohol, p-cuomaryl alcohol and coniferyl alcohol. The lignin connects cellulose and hemicellulose resulting in a 3-D unit of the plant cell wall. These resisrive properties make lignin a highly recalcitrant component, making it impossible for the lignocellulosic biomass to be bioconverted (Zheng et al., 2014).

2.3 Pretreatment of lignocellulosic biomass

The biomass biodegradation is halted due to its recalcitrance nature which results in reducing the biogas yield. The fractionation of the biomass is shown in Figure 2.2 (Singh et al., 2014).

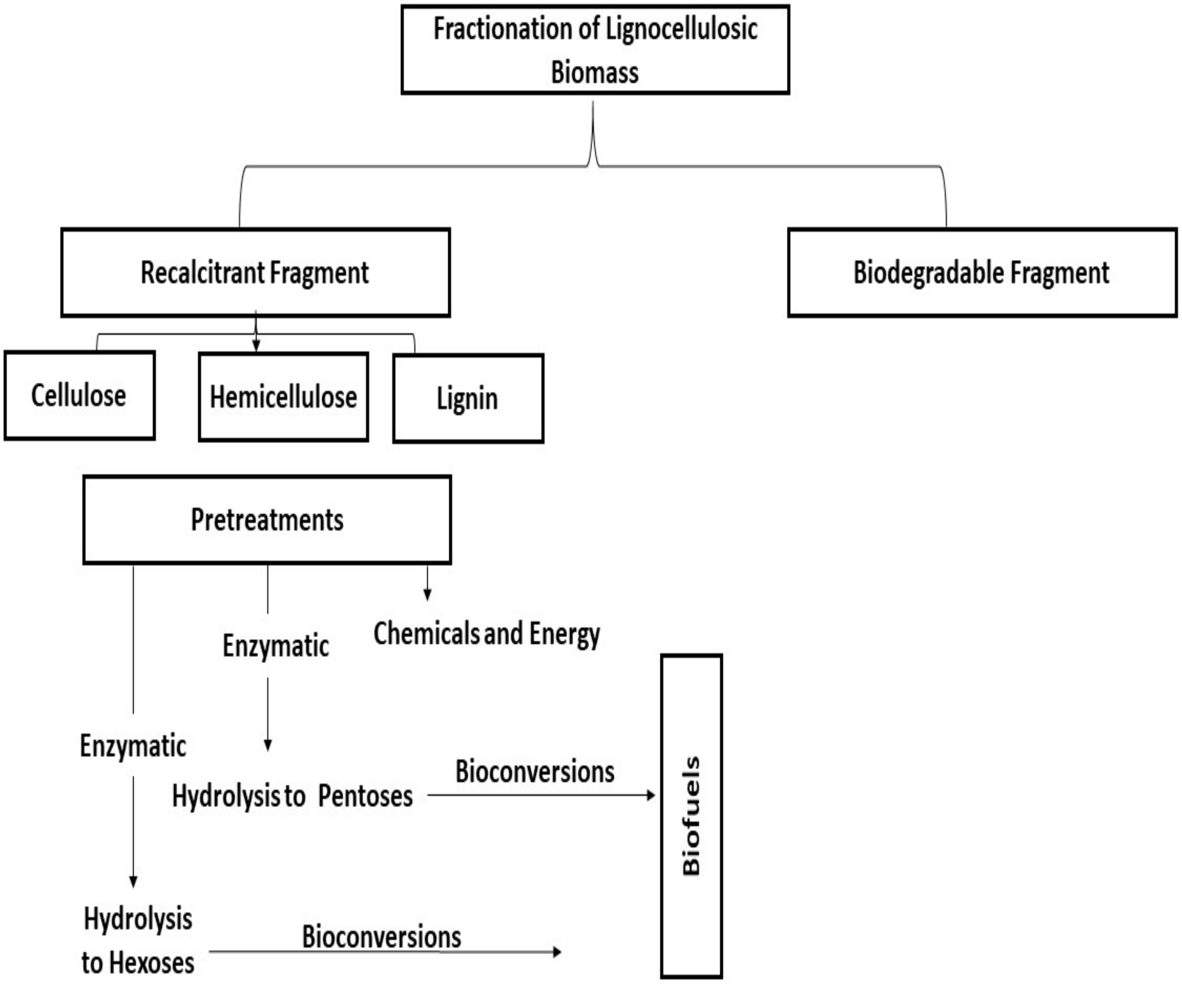

Figure 2.2 Fractionation of lignocellulosic biomass

The biomass fractionation occurs on two main parts of it one being biodegradable and other is recalcitrant. Breakdown of the recalcitrant part results in enhancing the biodegradability of lignocellulosic biomass. Research has shown that certain compositional and structural characteristics affect biodegradation of lignocellulosic substrate e.g. exposed surface area, cellulose polymerization and crystallinity, hemicellulose acetylation degree and lignin and hemicellulose presence. Thus pretreatment is used to enhance the properties of biomass so that biodegradation may be increased. Pretreatment converts lignocellulosic components i.e. hemicellulose and cellulose into smaller organic molecules which are easily biodegradable by microbes during anaerobic digestion (Yang et al., 2015).

There are three main categories of pretreatment which include physical (liquid hot water, pyrolysis, microwave, ultrasound, irradiation, extrusion and comminution), biological (enzymes, bacterial consortium and fungi) and chemical (acid, alkaline, ionic liquids ozonolysis, wet oxidation and catalyzed steam-explosion) (Zheng et al., 2014). The below mentioned factors are to be considered for breaking the crystalline matrix of lignocellulosic material (Behera et al.,2014)

1. The kind of lignocellulosic feedstock and downstream performance of the processes
2. No or limited amount of sugar production
3. Enhance delignification
4. High cellulose digestibility
5. Less water and energy requirements
6. Effluent volume recovered for pretreatment
7. Final products yield
8. Costs for the inclusion of catalyst or solvent recovery process, detoxification, use of anticorrosive materials and washing
9. Energy efficient and economical
10. High carbohydrate recovery
11. Emissions of Carbondioxide
12. Formation of by-products

2.4 Anaerobic digestion (AD) process

AD process is widely employed to covert organic matter into bioenergy in its useful form by the use of a variety of microbes in oxygen deficient environment. The final product of AD is biogas (60-70 % CH_4), organic byproducts, CO_2 (30-40%) and minor quantities of water vapors (H_2O), Nitrogen gas (N_2), hydrogen sulfide (H_2S) and ammonia (NH_3). The biogas composition differs with the substrate type and conditions of digestion. The biogas is produced through the synergistic effect of microbial consortia involves four phases-hydrolysis, acidogenesis, acetogenesis and methanogenesis (Najafpour, 2015; Vavilin et al. 2008; Madigan, 2005; Metcalf, 2003). All the four phases involved in anaerobic digestion are represented in the Figure 2.3 (Li et al., 2011).

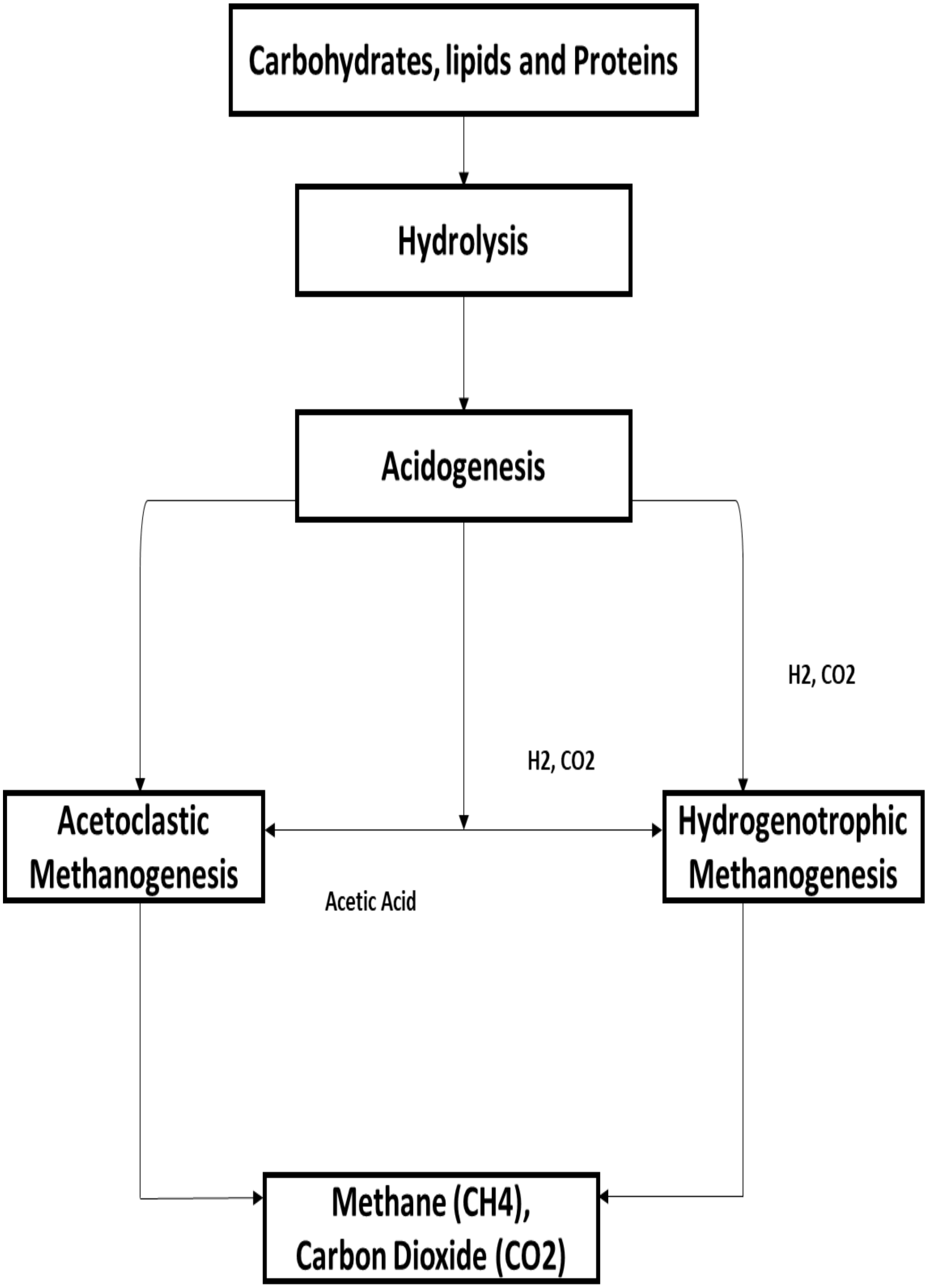

Figure 2.3 Mechanism of anaerobic digestion process

2.4.1 Hydrolysis

During the initial stage of hydrolysis, the well integrated organic matter including carbohydrates, proteins, nucleic acid and lipids are disintegrated into simpler and smaller constituents such as soluble compounds amino acids, sugars, purines, pyrimidines and fatty acids through extracellular enzymes.

2.4.2 Acidogenesis

The next phase after hydrolysis is the acidogenesis where the reduced compounds are converted into carbon dioxide, hydrogen, propionate, acetate, formate, butyrate, mehtylamines and methanol by the fermentative activity of bacteria.

2.4.3 Acetogenesis

During this phase, the products produced during acidogenesis are further broken down into acetate, carbon dioxide and hydrogen which are afterwards utilized for methane generation.

2.4.4 Methanogenesis

The final phase in AD is the methanogenesis. As name indicates, during this process, the consortium of microbes, Acetoclastic methanogens (acetate utilizer) and Hydrogenotrophic methanogens (H_2/CO_2 utilizer), synthesize methane. Acetotrophic methanogens convert acetate into carbondioxide and methane using hydrogen as an electron donor. While Hydrogenotrophic methanogens are responsible for converting carbondioxide into methane using hydrogen as an electron donor. Methane production from various precursors is explained in the equations 2.1-2.6 (Madigan, 2005; Metcalf, 2003).

$$4\ H_2 + CO_2 \longrightarrow CH_4 + 2\ O_2 \tag{2.1}$$

$$4\ HCOO^- + 4\ H^+ \longrightarrow CH_4 + 3\ CO_2 + 2\ H_2O \tag{2.2}$$

$$4\ CO + 2\ H_2O \longrightarrow CH_4 + 3\ CO_2 \tag{2.3}$$

$$4\ CH_3OH \longrightarrow 3\ CH_4 + CO_2 + 2\ H_2O \tag{2.4}$$

$$4\ (CH_3)_3N + 6\ H_2O \longrightarrow 9\ CH_4 + 3\ CO_2 + 4\ NH_3 \tag{2.5}$$

$$CH_3COOH \longrightarrow CH_4 + CO_2 \tag{2.6}$$

2.5 Factors affecting AD process for biogas production

The bioenergy production from biomass using AD is a complex process. In order for achieving the maximum yield of bioenergy, the optimization of AD stability parameters is necessary. The stability parameters include C/N ratio, Alkalinity, pH, VFA, retention time, OLR and toxicity. Optimization of these parameters is crucial as any anomaly in these parameters can result in AD failure. Some of the crucial AD parameters are discussed in the upcoming paragraphs.

2.5.1 C/N ratio

AD process is very sensitive to the C/N as the microbial growth depends on it. The optimal range of C/N lies between 20-30 for proper running of AD process (Risberg et al., 2013). The decrease in C/N ratio leads to the shortage of carbon and an increased risk of the accumulation of VFAs and NH_3 within the reactor thus halting the microbial growth (Li et al., 2011). A higher C/N ratio results in the insufficient presence of N_2 for microbial growth. The low concentration results in reduced methane yield owing to the AD process failure (Kayhanian, 1999). C/N of lignocellulosic biomass is very high which makes it unfit to be used as the only substrate for AD method. While the low C/N of cow dung is also an impediment in effective AD process. The issue is addressed by the co-digestion of biomass with a co-substrate and cow dung for a viable mixture for the enhancement of bioenergy yield (Risberg et al., 2013).

2.5.2 pH

The stability of AD digester is highly dependent upon its pH. The optimal value of pH lies in the range 6.8-7.4. For the proper development of methanogenic microbes in a stable AD process, the maintenance of appropriate pH is necessary. Methanogenic activity ceases if the pH falls below 6.8 while increase of alkalinity results in lower methane production (Hagos et al., 2017).

2.5.3 Alkalinity

Another fundamental parameter necessary for the stability of the AD reactor is the alkalinity. Methane production in an AD process ideally occurs at a pH of 7. This pH can only be upheld by substrate that has higher levels of alkalinity against CO_2 and VFAs produced during the process. The digestion process ceases due to excessive VFAs if the pH of the system is not maintained properly (Neshat et al., 2017). pH is adjusted by maintaining the buffering capacity and adding reagents such as sodium carbonate (Na_2CO_3), sodium bicarbonate (NaHCO3) and lime (CaO) (Li et al., 2009).

2.5.4 VFAs

The intermediate byproducts of an AD reactor include VFAs (acetic acid, butyric acid, propionic acid). The accumulation of these VFAs results in the reduction of pH below 6. This leads to the process acidification which stops the methanogenic activity. This results in the production of toxic chemicals leading to the AD process failure (Bah et al., 2014). VFAs in the range of 1500-2000 mg/L prevents the AD process. The intermediate byproducts of an AD reactor include VFAs. The accumulation of these VFAs results in the reduction of pH below 6. This leads to the process acidification which ceases the methanogenic activity. This results in the production of toxic chemicals that result in the AD process failure (Bah et al., 2014). VFAs in the range of 1500-2000 mg/L prevents the AD process.

2.5.5 Temperature

The most crucial role among all AD parameters is played by temperature. It affects the process performance and stability. AD may be carried out at all temperature ranges i.e. ambient, psychrophilic (<25 °C), mesophilic (25-42 °C) and thermophilic (50-70 °C) (Lettinga et al., 1999).

Table 1 Evaluation of mesophilic and thermophilic AD process

Process Operation	Thermophilic (55 °C)	Mesophilic (35 °C)
Methane yield	Higher	Lower
Degradation rate	Higher	Lower
Hydraulic retention time	Shorter/ same	Longer/ same
Process stability	Lower	Higher
Temperature sensitivity	High	Low
Energy demand	High	Low
Sanitation	Possible	No

The AD bacteria are very sensitive to the variations in the temperature which affects the degradation of organic substances, methane and hydrogen generation. Temperature decrease results in reduced substrate utilization rate, decreased ammonia and VFA concentrations (Bowen et al., 2014). Higher digester temperature suggests the increase in hydrolysis, pH and methane yield (Wang et al., 2014). There is higher biogas production in thermophilic AD owing to higher load bearing capacity, quicker biochemical and chemical reaction rates, lower gas solubility in the liquid, more pathogen deactivation and lesser odor emission. Nevertheless, it also results in higher acidification, poor methanogenesis, toxicity, reduced stability of reactor, increased energy inputs and greater investments and also inhibited biogas production. In addition, thermophilic AD is more environment dependent than mesophilic process. The latter AD process depicts better performance, enhanced stability and is less prone to inhibition (Bowen et al., 2014). However, thermophilic process is said to supersede the mesophilic AD in light of the aforementioned benefits but they are not very viable economically owing to higher energy utilization. Mesophilic process represents process strength, stability, inactivation of pathogens and lesser consumption of energy (Neshat et al., 2017). A comparison is drawn between thermophilic and mesophilic AD processes in Table 1 (Forgacs et al., 2012).

2.5.6 Organic loading rate (OLR)

OLR is the dry substrate added per volume of anaerobic digester per unit time. It is an important factor for the optimal microbial activity and increasing biogas yield. Very high OLR has adverse effects to the reactor's environment giving rise to hindring bacterial activity. Inhibition of bacterial activity leads to increased acidogenesis in comparison to methanogenesis microbial activity and as a result, VFAs are accumulated thus leading to a sudden decrease in the pH of AD. The bacteria are incapable of converting VFAs into CH_4 in low pH, this leads to the process failure (Rincon et al., 2008).

2.5.7 Hydraulic retention time (HRT)

HRT is also very crucial in AD process as the microorganisms require time to decompose the organic matter and synthesize the final products. The optimum HRT is required to be maintained as a high HRT causes microbial death owing to the nutrient deficiency while a short HRT results in VFA accumulation, toxicity and reduced methane production. Thus optimal HRT is needed in order to avert undesirable products and unfavorable metabolic activity of microbes leading to process collapse (Metcalf, 2003).

2.5.8 Stirring

The mixing of slurry within the digester is essential to ensure that the microbes are well in contact with the organic feedstock, this results in enhancing the digestion and biogas yield. Studies have revealed that the microbes have limited access to the substrate (food) thus slight mixing has been found to enhance the digestion while violent stirring halts the AD process (Rai, 2011).

2.5.9 Particle size

Research has shown that smaller particle size of lignocellulosic substrate has improved degradation and methane production. Size reduction of lignocellulosic biomass results in modifying its structure, increasing surface area and reducing crystallinity and polymerization of cellulose (Kratky and Jirout, 2011). Studies have revealed that substrate's size reduction enhances the hydrolysis efficiency and subsequently enhance biogas production (Deublein and Steinhauser, 2011; Yadvika et al., 2004).

2.5.10 Toxicity

The stability and performance of AD are strongly affected by the excessive presence of VFAs and Total ammonium nitrogen (TAN). VFAs and TAN are formed from carbohydrate and protein based feedstock respectively. The codigesting of substrate with protein rich waste reduces the inhibition of ammonia and VFAs. The presence of macro and micro nutrients in feedstock results in enhancing the resilience and growth of bacteria (Yang et al., 2015).

2.6 Types of anaerobic digesters based on mode of operation

The bacteria are provided with a closed, oxygen deficient and heated digester in anaerobic digestion process. These conditions are appropriate for the anaerobic bacterial growth and conversion of organic waste into biogas. Commonly, the AD digesters are rectangular or rounded concrete tanks having covers made up of insulated material. The feeding and removal of organic matter is done daily from the reactor for 20 d and then transferred to storage system. The AD microbes convert organic matter into biogas and other products during the operational mode of AD process (Leggett et al., 2006).

The organic feedstock may be introduced into the AD digester in batch or continuous mode.

2.6.1 Batch process

Batch anaerobic digester mode involves the feedstock feeding into a single AD digester (fill-and-draw unit) which is emptied upon the completion of AD process. After this, a fresh batch is fed into the reactor and the process is continued without any interruption. In short, all the steps of AD occur within a single unit. This type of batch reactors is easy to build and offer an economically viable option to convert waste to bioenergy (Igoni et al., 2008).

The key benefits of the batch reactor are its operational simplicity, ability to treat wide range of influent volume, low input process, better process control, high efficiency and cost effectiveness.

The limitations of batch process include clogging, channeling and instability in bacterial population, poor self-immobilization and uneven gas production (Singh and Srivastava, 2011).

2.6.2 Continuous process

The feedstock is mechanically or through pressure fed into the reactor in a continuous AD process. This feeding process drives out the digestate continuously. This type of process ensures the predictable and continuous biogas yield. Continuous reactors are either horizontal, vertical or multiple tank systems. In order to maintain continuous stirring, the digesters may be completely mixed type or plug flow reactor. The former is mostly vertical while the latter are horizontal in alignment (Al Seadi et al., 2008).

2.7 Feed stocks traditionally used for biogas production

A large quantity of waste, from livestock manure, municipal solid waste (MSW), food waste, waste activated sludge and lignocellulosic biomass, may be used as a source of substrate for bioenergy generation. However, the biogas generation from each source varies and depends upon its biodegradability rate and composition (Mao et al., 2015). In the recent past, the lignocellulosic biomass has drew immense attention to be a sustainable source of generating bioenergy and biofuel. The abundant availability of lignocellulosic biomass and the fact that it does not compete with food makes it a viable option for bioenergy generation. In the past, lignocellulosic biomass has been explored in research including maize straw, corn cob, cotton stalks, corn stover, wheat straw, rice straw, sugarcane bagasse, sunflower stalks, switch grass and yard waste etc. The reseach has revealed that owing to the tough structure of lignocellulosic biomass, it cannot be readily digested (Jerger et al., 1982).

2.8 Corn cob as a substrate for biogas production

Maize is known to be the Queen of Cereals owing to its high yield adaptability, versatility, and significant economic importance. It is the second most significant cereal crop globally with regards to the acreage. Global maize yield crossed 1,144.63 million metric tons (Mt) (United States Department of Agriculture, 2019). These figures hold significance because of the valorization value of its residues including stover and cobs, former being the primary biomass. Corncob comes as a secondary residue as it is also generated during corn processing. In Pakistan, cobs being the secondary residue are mostly discarded by field dumping, open burning or utilized in place of firewood in furnaces and boilers. Direct disposal of CC is a recurring issue globally thus it needs to be bio-converted. The bioconversion of CC to bio hydrogen, bio methanol, bioethanol and biofuel has been explored in the past making CC an appropriate biomass for energy production (Gandam et al., 2022). CC is a good low carbon replacement to fossil fuels raw material.

The major components of corn cob include lignin, hemicellulos and cellulose forming a complex network. The typical chemical constituents of CC are shown in the Table 2 (Thangavelu et al., 2018). The structure of cellulose comprises of β−1, 4-glycosidic bonds of D-glucose which is insoluble and non-biodegradable. The crystallinity of cellulose is the main hindrance to its bioconversion. Thus pretreatment is required in order to expose its amorphous form and carry out its bioconversion into fermentable sugars (Van Wyk, 2001).

Table 2 Typical composition of corn cob

Sr. No.	Parameter(s)	Percentage
1	Hemicellulose (%)	44.4±5.2
2	Lignin (%)	11.9±2.3
3	Cellulose (%)	38.8±2.5

CC is comprised of 44.4±5.2% hemicelluloses. These are heterogeneous polymers of hexoses, pentoses and sugar acids in hemicelluloses. Hemicellulose connects with cellulose by hydrogen bonds and with lignin by chemical bond in the cell wall of agricultural biomass. Since hemicellulose is the most abundantly available polysaccharide, it is difficult to detach it from the cell wall. Thus, a number of pretreatment methods have been researched in the past to completely detach hemicellulose with intact cell wall structure (Garcia-Cubero et al., 2009).

In CC, lignin is interconnected to cellulose and hemicellulose creating a physical barrier which is highly impermeable in nature. The presence of lignin in CC creates resistance to enzymatic or microbial attack. Lignin is a complex polymer with a three-dimensional structure; however, it is degradable in nature (Shirkavand et al., 2016).

2.9 Pretreatment methods for lignocellulosic biomass

Hemicellulose and lignin create hindrances in CC bioconversion of cellulose (not broken down by any pretreatment method) to fermentable sugars. Thus, fragmentation of the crystalline tough structure prior to any bioconversion process is needed. The main motive of pretreatment is the enhancement of the recalcitrant structure to amorphous form in order to increase fragmentation of sugars in biomass (Tian et al.,2018).

The purpose to pretreat lignocellulosic biomass are to (Bharathiraja, 2017):

- Decompose the lignin
- Reduce the resistant nature of cellulose and its polymerization
- Enhance substrate porosity
- Ensure substrate enzymatic hydrolysis
- Avert the formation of inhibitory toxic products

Many methods of pretreatment such as chemical, physical, biological and physicochemical have been explored in past to improve the susceptibility of cellulose to being exposed to microbial or enzymatic activity. Physical pretreatments include extrusion, chipping, shredding, milling and grinding, and irradiation. Chemical pretreatment includes the usage of acids, alkalis, Ionic liquids and organic solvents etc. Biological pretreatment methods involve the usage of microbial consortium, fungi (soft rot, brown and white fungi) and enzymatic degradation of lignocellulosic feedstock. Physicochemical pretreatment includes using hot water treatment, ammonia fiber explosion (AFEX), steam explosion and CO_2 explosion. (Zheng et al., 2014). Table 3 below shows a few studies on various chemical pretreatments of lignocellulosic biomass.

Table 3 Studies on different chemical pretreatments of lignocellulosic biomass

Pretreatment Type	Substrate	Experimental Conditions and Findings	Reference
NaOH	Corn cob	The biogas yield was enhanced up to 28.57% by the NaOH pretreatment of corn cob at 6% (w/w) and 28 °C. While the methane yield was increased up to 29%	Omiyale et al. (2023)
NaOH	Corn cob	The alkali pretreatment of corn cob using NaOH at 2 wt. %, for 1.5 h at 90 °C and solid/liquid ratio (w/w) of 1/30 reduced lignin up to 50.98%	Araújo et al. (2019)
NaOH	Pennisetum Hybrid	The optimum results were obtained at 35°C for 24 hours using 2% NaOH. Methane yield was enhanced upto 21%.	Kang et al. (2018)
NaOH	Asparagus stover	6% NaOH pretreatment enhanced the biogas production 716.8% higher than that of the untreated asparagus stover	Sun et al. (2017)
HCl	Rice straw	12.5% hemicellulose dissolution of rice straw was observed while 74.9% biogas yield was enhanced by its pretreatment with 8% HCl at 35 °C	Dai et al. (2017)
KOH	Corn stalk	KOH solution at 60 °C reduces the recalcitrant nature of corn stalks, and obtained cumulative specific methane yield (CSMY) of 243.1 mL/g·VS, which was 56.4% higher than unpretreated corn stalks.	Siddhu et al. (2016)
$Ca(OH)_2$	Rice Straw	The pretreatment of rice straw with	Gu et al.

		$Ca(OH)_2$ at 8%, for 72 h at 25 ºC with S/L ratio of 1/20 resulted in the hydrolysis and methane production of 330.9 mL/g VS	(2015)
NaOH	Corn cob	1 % NaOH pretreatment of corn cob for 4 h at 50 °C resulted in the decrease of the Crystallinity Index (CrI) by approximately 61.54% while the Specific Surface Area (SSA) improved by around 538.46%	Sahare et al. (2012)
NaOH	Corn Stover	For an ORL of 65 g/L, corn stover was treated for a period of 3 days' time at 20 °C with 88% moisture content. The biogas yield was enhanced approximately 72.9%	Zheng et al. (2009)

2.10 Impact of NaOH pretreatment on lignocellulosic composition and biogas production

NaOH, $NH_3.H_2O$, $Ca(OH)_2$ and KOH are used for alkali pretreatment. Alkaline Pretreatment is used for the removal of lignin, hemicellulose and cellulose thus making lignocellulosic biomass more exposed and readily available for microbial and enzymatic activity (Garcia et al., 2013).

For the biomass valorization especially corn cob, NaOH pretreatment is the most reported chemical pretreatment method. It results in splitting the linkages of α, β-aryl ether that are responsible for connecting hemicellulose and lignin. This split detaches the lignin and the substitutions of uronic acid in hemicellulose and causes the cellulose to swell. This ultimately enhances the porosity of the otherwise recalcitrant lignocellulosic structure. Pretreatment using 2% wt. concentration NaOH, 90 °C for 1.5 h at 1:30 solid to liquid ration (w/w) degraded up to 50.98% of lignin (Araújo et al., 2019).

A number of studies involving the corncob valorization reported that the alkali pretreatment using NaOH is very effective. Dilute NaOH pretreatment of corn cob resulted in 85% enhanced enzymatic saccharification while the Crystallinity Index (CrI) reduced up to 61.54%. Furthermore, the Specific Surface Area (SSA) increase of CC was reported to be 538.46% (Sahare et al., 2012).

Corn cob has a very high carbon to nitrogen ratio (C/N). AD is C/N sensitive process as the growth of microbes depends on it. For the optimal running of AD reactor, an optimum

C/N in the range 20-30 must be maintained (Risberg et al., 2013). Due to this reason, the codigestion of corn cob is necessary to optimize the reactor's C/N.

A study by Gao et al., (2021) explored the co-digestion of food waste and hydrothermal pretreated corn cob. CC was used as lignocellulosic biomass source for its co-digestion with FW at different VS ratios of 1:3 (S1–S4) and 1:6 (S5–S8), to assess the effect of HTP of CC at varying temperature gradients (125, 150 and 175 °C) on the co-digestion performance. At the VS ratio of FW/CC of 1:3, the S3 group (CC was pretreated at 150 °C) reached the highest cumulative biogas yield (CBY) of 4660 mL.

Cow slurry and corn cob were codigested to study the effect on biogas yield. The codigestion was done at cow dung slurry to corn cob ratios of 1:1, 1:3 and 3:1 on the basis of percent VS of each substrate. The biogas production of aforementioned ratios came out to be 0.417, 0.429 and 0.453 m3/kgoDM respectively. In the same sequence, the methane yield was 0.323, 0.323 and 0.334 CH_4/kgoDM respectively under mesophilic temperature of 37ºC. Overall the codigestion of cow dung slurry and corn cobs showed more than 70% methane production in each case. This infers that the codigestion of both the substrates depicts positive impact on biogas yield (Ademola O Adebayo et al., 2012).

A study on the mono and co-digestion at mesophilic conditions (37 °C) for batch mode was conducted using corn stover (CS), kitchen waste (KW) and chicken manure (CM). The study was conducted to assess the biodegradability, biomethane potential, methane production and process stability. Initially, the substrate-to-inoculum ratio (S/I) was taken to be 0.5 and tested over VS concentration of 3 g VS L^{-1}. Later on, two other S/I ratios were evaluated i.e. 1.5 and 3.0. The results were interpreted by making use of the Modified Gompertz Model. The results indicated that the BMP and specific methane yields were 725 and 683 mL g–1 VS added for KW, 470 and 214 mL g–1 VS added for CS, and 617 and 291 mL g–1 VS added for CM, respectively. KW had the maximum degradability i.e. 94% while the CS was degraded up to 45% and CM was degraded up to 47%. For the mono and co-digestion of KW with CM and CS, the S/I ratio of 1.5 performed better than 3.0. While for CM, CS and their mixture, both the S/I ratios of 1.5 and 3.0 proved to be suitable (Li et al., 2013).

Table 4 represents a few co-digestion studies of biomass.

Table 4 Studies on codigestion of biomass

Substrate(s)	Findings	Reference
Durian shell Pig manure	Durian shell to pig manure ratio of 1:1 resulted in the maximum biogas enhancement 224.8 mL/gVS	Shen et al. (2019)
Sludge Rice straw	Sludge and rice straw co-digestion resulted in the optimization of C/N ratio being a crucial parameter in AD process.	Suraj et al. (2018)
Banana Pseudo-Stem Fiber	NaOH concentration of 7.8%, a fiber length of 0.2 cm, and a temperature of 48°C for 3 d were used for pretreatment. This NaOH pretreatment significantly enhanced the biogas yield from banana pseudo-stem fiber. The maximum biogas yield achieved was 463.0 $mL \cdot g^{-1}$ VS added, representing an 89.2% increase compared to the control, which produced 244.7 mLg^{-1} VS added.	Pei et al. (2014)
Wood waste Pig manure	The NaOH pretreatment of wood waste resulted in the enhancement of methane production by 38.5% in comparison to untreated wood waste. While when it was co-digested with pig manure, the methane production was increased up to 75.8%	Li et al. (2019)
Rose stalk Pig Manure	Codigestion of rose stalks and pig manure enhanced the methane yield from 41% to 52%. This also improved the kinetics of biogas production in comparison to the rose stalk monodigestion.	Chen et al. (2019)
Food waste Corn cob	FW/CC co-digestion at the VS ratio of 1:3, (CC was pretreated at 150 °C) gave the highest cumulative biogas yield (CBY) of 4660 mL	Gao et al. (2021)

2.11 Summary

The utilization of lignocellulosic biomass such as corn cob has the capability to meet our growing energy demands sustainably. However, there arises a need to treat the corn cob prior to subjecting it to anaerobic digestion. This is because lignocellulosic biomass has a recalcitrant structure which makes it less susceptible to be digested by microorganisms. The pretreatment helps in enhancing decomposition and biodegradability of lignocellulosic biomass by disintegrating the linkages that exist among lignin and polysaccharide, thus exposing cellulose and hemicellulose to microbial action. A diverse range of pretreatment options, exist including physical, chemical, physico-chemical and biological, have been explored in the past. Moreover, use of alkaline pretreatment has reported to be an efficient method in terms of enhancing the structure of biomass and bioenergy yield. Since the C/N value of corn cob is very high thus co-digestate such as food waste to balance out its C/N up to optimum range is necessary to enhance the bioenergy production. On the basis of above literature review, this research aims 1) To investigate the effect of Chemical Pretreatment on Characteristics of Corn Cob; 2) To evaluate effect of Chemical Pretreatment on Biogas Production of Corn Cob; and 3) To assess biogas production of chemically pretreated Corn Cob and Food Waste through codigestion.

Chapter 3

Materials and Methods

This chapter is dedicated for the provision of details on the materials utilized, experimental setup and the methodologies employed in this analysis. The objectives of this research are: To investigate the effect of Chemical Pretreatment on Characteristics of Corn Cob; To evaluate effect of Chemical Pretreatment on Biogas Production of Corn Cob**;** and to assess biogas production of chemically pretreated Corn Cob and Food Waste through codigestion.

The methodological approach is shown in Figure 3.1 below.

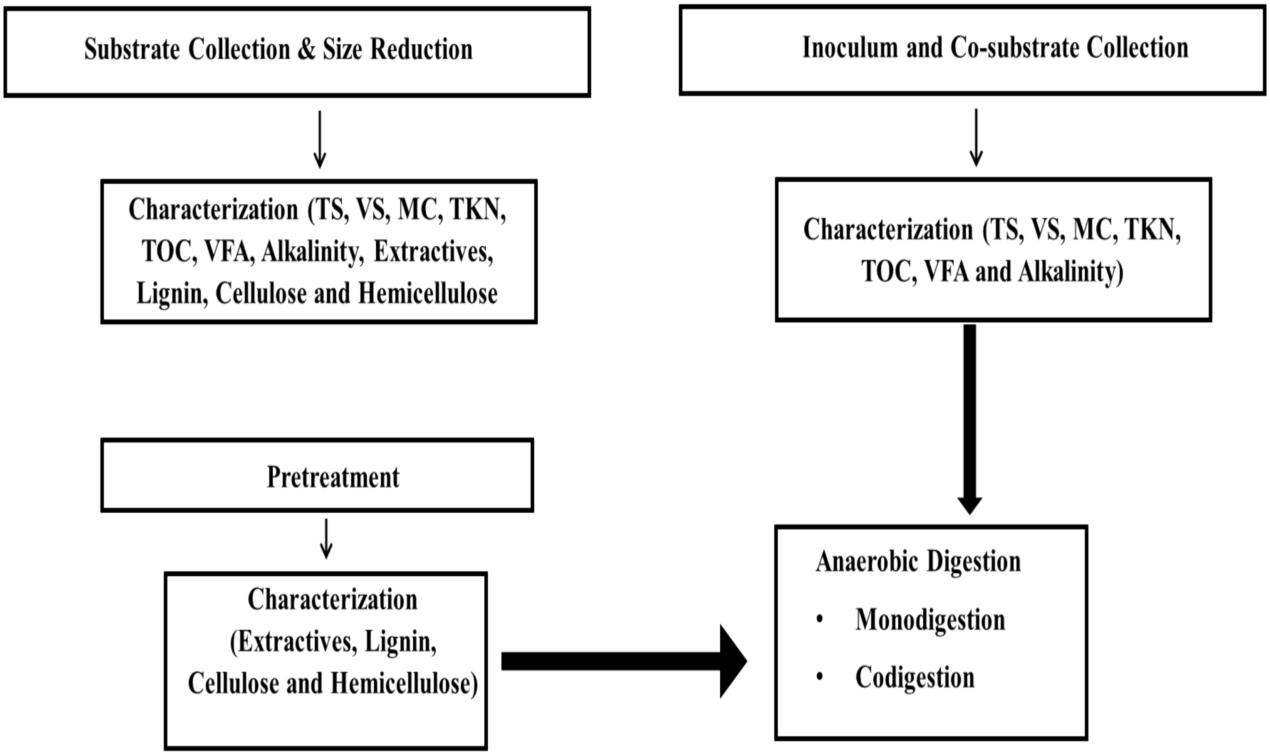

Figure 3.1 Methodological approach

3.1 Substrate and Inoculum Preparation

Corn cob was utilized as a substrate. Corn cobs were acquired from a corn hawker at NUST, H-12, Islamabad. These were then sun dried for a few days for the moisture to evaporate. The cobs were carefully commuted to the laboratory and oven dried at 80 °C for a few hours to a constant weight in order to avoid fungal growth (oven model-UNB 400). For achieving the desired biomass, the cobs were shredded well using a small-scale shredder and then sieved through 20-mesh size, resulting in particle size up to 1mm. The sieved biomass was stored at room temperature in airtight zip-lock bags for further analysis (Figure 3.2).

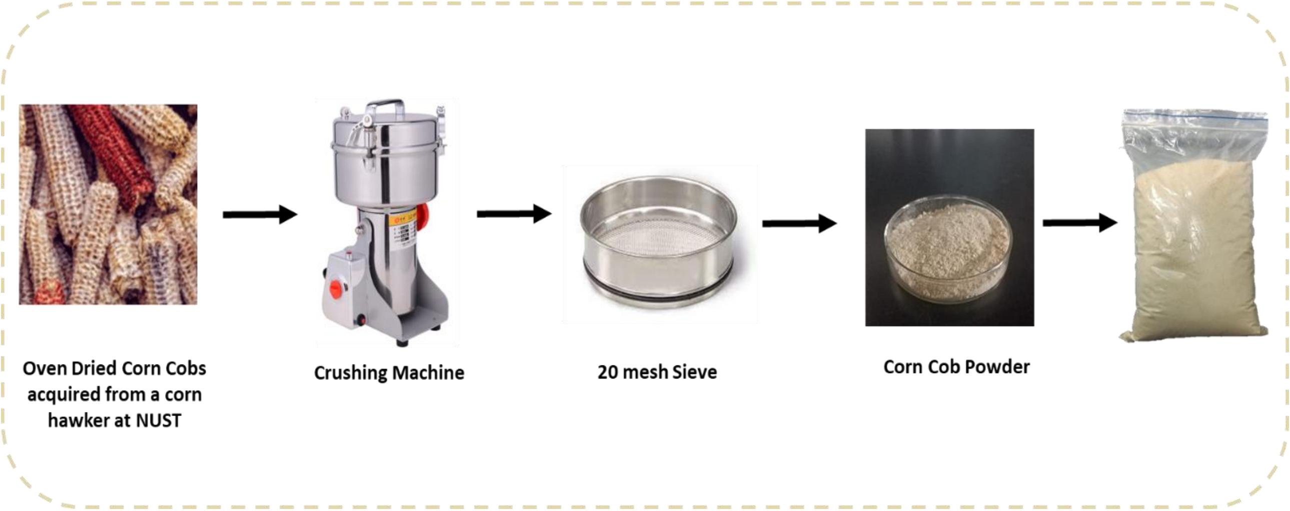

Figure 3.2 Substrate preparation

In this research, boiled rice was used as a co-substrate with corn cob. The boiled rice was sourced from leftover food at a local restaurant in Islamabad. The use of readily available leftover food waste aligned well with the sustainability principle of waste valorization.

Fresh cow dung (CD) was used as an inoculum during the AD process. It was acquired from a nearby cow farm located in Sector H-13, Islamabad (Figure 3.3).

Figure 3.3 Cow Dung as a source of inoculum

3.2 Primary characterization and fiber analysis

After the collection of cow manure and shredding of corn cob, both were subjected to preliminary analysis including proximate analysis (total solids (TS), volatile solids (VS), moisture content (MC), total organic carbon (TOC)), total Kjeldahl nitrogen (TKN), volatile fatty acids (VFAs). The procedure followed for these analysis was performed as stipulated in Standard Methods for the Examination of Water and Wastewater, 22nd edition (2012)" (APHA, AWWA, and WEF, USA). Additionally, fiber analysis was also performed of the corn cob which helped in assessing its structural contents such as lignin, hemicellulose and cellulose as per the method stated by Li et al. (2004). This analysis also helped in understanding of the chemical composition of corn cob.

3.3 Chemical Pretreatment of Substrate

Corn cob was subjected to chemical pretreatment. The chemical treatment used was Alkaline Pretreatment by using varying concentrations of NaOH. 1%, 1.5%, 2%, 2.5% and 3% w/v NaOH solutions were made by dissolving NaOH pallets of 3 g, 4.5 g, 6 g, 7.5 g and 9 g respectively in distilled water. The solutions were made in heavy-walled glass reagent bottles. Substrate i.e. corn cob was then added to the solutions at 1:10 w/v (30 g corncob in 300 mL distilled water). The bottles were then covered with aluminum foil and these were subjected to autoclave for 30 min, 121 °C and 15 psi. After pretreatment, the treated solution was then filtered using a ketti cloth filter followed by continuous washing using deionized water until the pH was neutralized. It was then oven-dried (oven model-UNB 400) at 105 °C until constant weight was achieved and stored in air tight bags for further characterization (Angulo-Padilla et al., 2018). The complete process of pretreatment is illustrated in Figure 3.4 below.

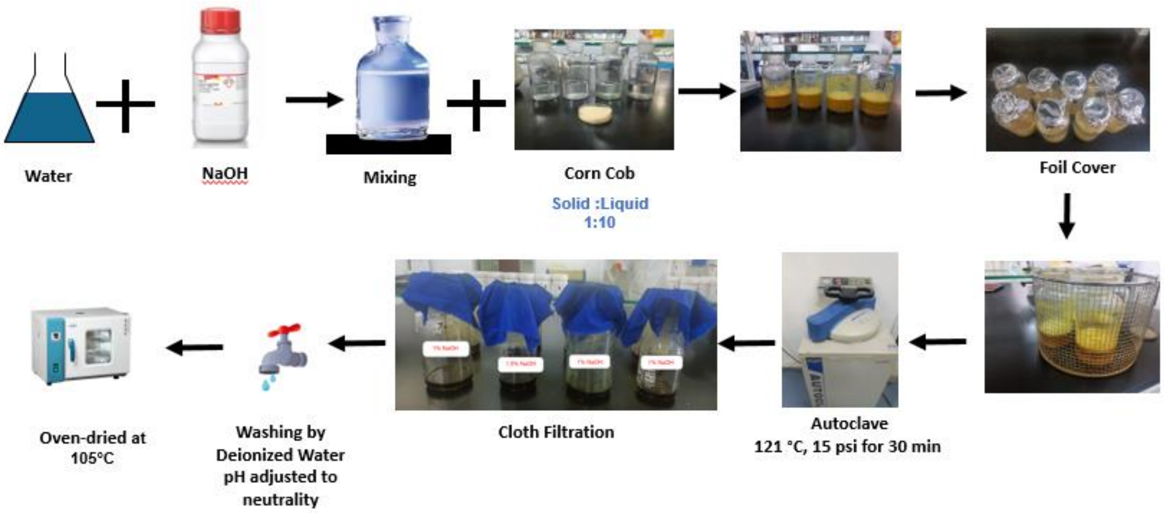

Figure 3.4 Alkali pretreatment of substrate

The pretreated samples were the subjected to further analysis prior to being used in anaerobic digestion. Anaerobic digestion was done in monodigestion of solely corn cob and its codigestion with food waste i.e. boiled rice. Below Figure 3.5 represents the raw and alkaline pretreated corn cob.

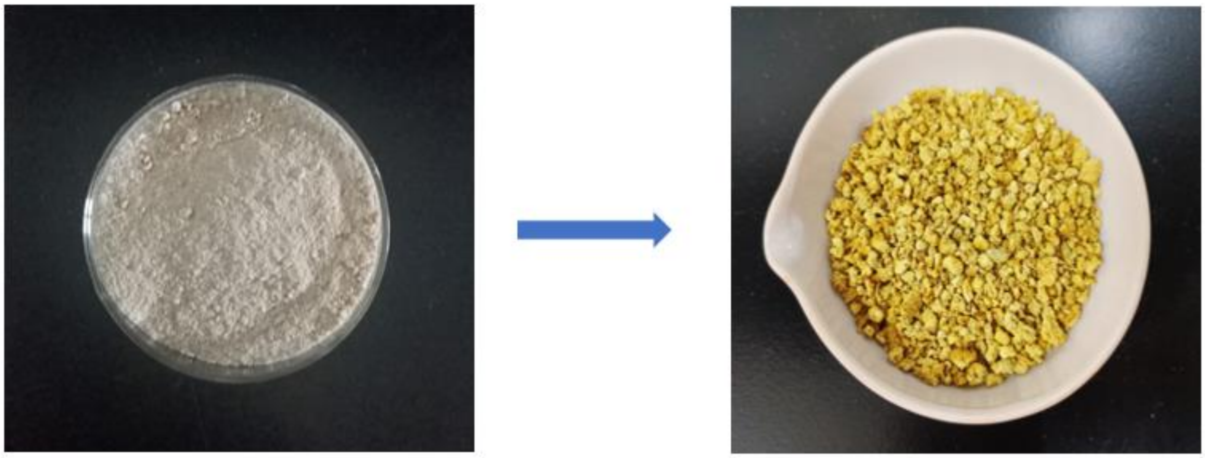

Figure 3.5 Raw and alkaline treated corn cob

3.4 Characterization of Substrate

TS, VS and Moisture content in untreated as well as pretreated corn cob and boiled rice were analyzed following standard method 2540 G (APHA, 2012). Extractives, lignin, hemicellulose and cellulose of untreated and pretreated corn cob samples were analyzed as per procedures stipulated by Li et al. (2004). All analyses for untreated and pretreated substrate and co-substrate samples were performed in triplicates.

3.4.1 Total solids

In order to determine total solids, first of all an empty china dish was thoroughly washed with deionized water and it was kept in a furnace for 550 ºC for 1 h. It was then removed and placed in the desiccator for cooling. The china dish was subsequently weighed and 50 g material was put into it and it was weighed again. The china dish with material was then left in an oven at 105 ºC overnight. The dish was then taken out and weighed using an analytical balance, it was again put in the oven for 1 h and reweighed and cooled until a constant weight is achieved and results do not vary more than 4% or 50 mg. The quantity of the materials in the china dish was the total solids and can be calculated by using the below mentioned equation 3.1.

$$\text{TS (\%)} = \frac{(\text{w1}-\text{w2})\text{x}100}{\text{w3}-\text{w2}} \qquad (3.1)$$

Whereby, w1 = weight of dried sample residue + china dish after evaporation,
w2= weight of empty china dish, w3 = weight of moist/unheated sample + china dish

w4= weight of residue + dish after ignition

3.4.2 Moisture content

The mass lost due to 105 ºC oven heating refers to the moisture content which can easily be determined by using equation 3.2.

$$\text{MC (\%)} = 100\text{-TS (\%)} \qquad (3.2)$$

3.4.3 Volatile solids

The volatile solids may be calculated by putting the oven dried material from TS to muffle furnace (model JSMF-270 H) and ignite it for 1 h at 550 °C in a crucible. The crucible is then removed and placed in a desiccator for cooling. After cooling, it is weighed and out in the oven for another 1 h until the weight does not differ more than 4% or 50 mg. The VS are calculated by using equation 3.3 below.

$$\text{VS (\%)} = \frac{(\text{w1}-\text{w4})\text{x}100}{\text{w1}-\text{w2}} \qquad (3.3)$$

w4 = weight of residue + crucible after ignition

3.4.4 Total Organic Carbon (TOC) and Total Kjeldahl Nitrogen (TKN)

The TOC was determined by using the equation 3.4 (Adams et al., 1951).

$$\text{TOC (\%)} = \frac{\text{VS(\% of TS)x}100}{1.724} \qquad (3.4)$$

TKN of treated and untreated substrate (corn cob) as well as co-substrate (boiled rice) was performed by using standard method 2540 G (APHA-2012). All analyses were performed in triplicates.

3.4.5 C/N ratio

C/N ratio was determined by simply diving the already calculated TOC by TKN as per the equation 3.5 (Wang et al., 2014).

$$\text{C/N} = \frac{\text{w1xC1}+\text{w2xC2}}{\text{w1xN1}+\text{w2xN2}} \qquad (3.5)$$

w1= weight of corn cob (g)
w2= weight cow dung (g)
C1= Total organic carbon of corn cob (g Kg^{-1} VS)
C2= Total organic carbon of cow dung (g Kg^{-1} VS)
N1= Nitrogen content of corn cob (g Kg^{-1} VS)
N2= Nitrogen content of cow dung (g Kg^{-1} VS).

3.4.6 Extractives

The already dried and stored corn cob sample (wo, 7 g) was leached using 300 mL Acetone for 3 h at 90°C in a Soxhlet Extractives apparatus. After this, the corn cob sample was removed and oven dried at 103-105 °C until constant weight was achieved. Then it was cooled down in a desiccator at room temperature and weighed (w1, g). Extractives weight was calculated using the below equation 3.6

$$\text{Extractives (\%)} = \frac{(\text{wo}-\text{w1})\text{x}100}{\text{wo}} \qquad (3.6)$$

3.4.7 Hemicellulose

Hemicellulose was calculated by using the extractive-free corn cob sample (w1, 1g), which was added to 150 mL NaOH solution. The solution was boiled at 80 °C for 3.5 h on hot plate. The mixture was then filtered and washed using deionized water until pH was neutralized. It was then shifted in an empty china dish and dried at 103 to 105 °C until constant weight was achieved. Cooling was done in the desiccator and the sample was then weighed (w2, g). The calculations made to determine hemicellose is as under (3.7).

$$\text{Hemicelulose (\%)} = \frac{(\mathrm{w1}-\mathrm{w2})\mathrm{x}100}{\mathrm{w1}} \qquad (3.7)$$

3.4.8 Lignin

Lignin was determined by adding extractive-free corn cob sample (w3, 1 g) to 30 mL of 98% sulphuric acid (H_2SO_4). It was placed at 15 ºC for 24 h. After 24 h, 300 mL distilled water is added to it and it was boiled at 100 °C for for 1 h. The residue was then filtered using ketti cloth filter and washing was done with DI water so that no more sulfate ion in the filtrate was detected (detected by 10% barium chloride solution). The corn cob sample was then dried in the oven, cooled in a desiccator and weighed (w4, g). The lignin content was subsequently calculated using the following equation (3.8)

$$\text{Lignin (\%)} = \frac{\mathrm{w4}\ \mathrm{x}(1-\left(\frac{Extractives}{100}\right))\mathrm{x}100}{\mathrm{w3}} \qquad (3.8)$$

3.4.9 Cellulose

Cellulose is calculated by making an assumption that extractives, hemicellulose and lignin are the only components of the entire biomass, the cellulose weight is calculated according to following equation 3.9.

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

3.5 Anaerobic digestion experiment

The anaerobic digestion was done in 300 mL biomethane potential (BMP) glass reactors which functioned as anaerobic digesters working in batch mode. The reactor working volume was 225 mL. The substrates and inoculum were taken based on their VS content. OLR was adjusted at 10 g VS/L. Inoculum to substrate was taken as 1:1. Experiment was done in two modes i.e. monodigestion and co-digestion with boiled rice (food waste). Since the C/N ratio of corn cob is high, the food waste was added to adjust the C/N ratio to 25-26. The bottles were filled up to 225 mL mark with distilled water while the pH was upheld at 7.2-7.3 by using sodium bicarbonate buffer. After the pH adjustment (pH meter model-WTW 720), the bottles were sealed with airtight rubber septa and screw caps while the headspace was filled with nitrogen (N_2) gas through purging for 2 min each for fostering anaerobic conditions. The reactors were then place at mesophilic conditions 30±1 °C for 40 days (d) in the Land Resources Research Institute (LRRI) Laboratory incubator at the National Agricultural Research Centre (NARC). The reactors were mixed manually by shaking for 1 min twice each day. For the monodigestion reactors, cow dung was used as inoculum and corn cob (treated/untreated) was used as substrate source. For the co-digestion, corn cob was the primary substrate while boiled rice were used as co-substrate (substrate to co-substrate ratio at 1:1). For each of mono and co-digestion setups, raw corn cob was used as control. One blank reactor with solely cow dung in distilled water without any substrate was also put up in order to assess methane yield contributed by the cow dung. All the digesters were put up in triplicates. Figure 3.6 represents the different phases of setting up the AD reactors while Figure 3.7 represents complete experimental design.

Figure 3.6 Different phases during the setting up of anaerobic digestion process

3.5.1 Analytical method for anaerobic digestion

The daily biogas volume was assessed using the water displacement method. The measured biogas was then converted to normal milliliter (NmL) (dry gas, P = 100 kPa = 760 mm Hg, T = 0 ºC) using the equation 3.10.

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

Where;

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

V= biogas volume (mL)

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

T= ambient temperature (ºK)

Analysis such as pH, TA, VFAs, TS and VS were done at the initiation as well as termination of the digestion process. pH analysis was done by dissolving 10 g sample in 100 mL DI water. VFA and alkalinity samples were prepared by taking 10 g AD sample in 30 mL deionized water which was subsequently centrifuged at 8000 rpm for 5 min. Below Table 5 shows each analysis and its respective standard method.

Table 5 Analysis of various parameters

Parameters	Methods
pH	Digital pH meter
Total Solids	Method 2540B
VS	Method 2540E
TKN	Method 4500-Norg B
Volatile Fatty Acids (mg/L)	2310B Titration method
Alkalinity (mg/L)	2320B Titration method
Volume of Biogas	Water Displacement Method

The removal of TS and VS was calculated by using the below mentioned equations:

$$\text{TS Removal (\%)} = \frac{Initial\ gTS\ of\ Substrate - Final\ gTS\ of\ Substrate}{Initial\ gTS\ of\ Substrate} \qquad (3.11)$$

Final gTS of Substrate = Final gTS – Final gTS of Inoculum

$$\text{VS Removal (\%)} = \frac{Initial\ gVS\ of\ Substrate - Final\ gVS\ of\ Substrate}{Initial\ gVS\ of\ Substrate} \qquad (3.12)$$

Final gVS of Substrate = Final gVS – Final gVS of Inoculum

3.6 Biogas data validation by a kinetic model

In order to validate the biogas data, the Modified Gompertz Model was used in the study in order to predict the methane and cumulative methane production rates for anaerobic digestion (Hu et al., 2015). The analysis was carried out using the IBM SPSS 16.0 while rest of the analysis were done on excel 2013.

The Modified Gompertz Model equation is:

$$P_t = P_m \text{ x } \exp\{-\exp[\frac{Rmax\ .e}{Pm}(\lambda\text{-}t) + 1\ ]\} \qquad (3.13)$$

Where;

P_t = Cumulative methane yield (NmL/gVS) at a given time t

P_m = Predicted methane yield (NmL/gVS) at the termination of digestion

R_{max} = Maximum CH_4 production rate (NmL/gVS/day)

λ = Lag time (days)

e = base of logarithm i.e. 2.72

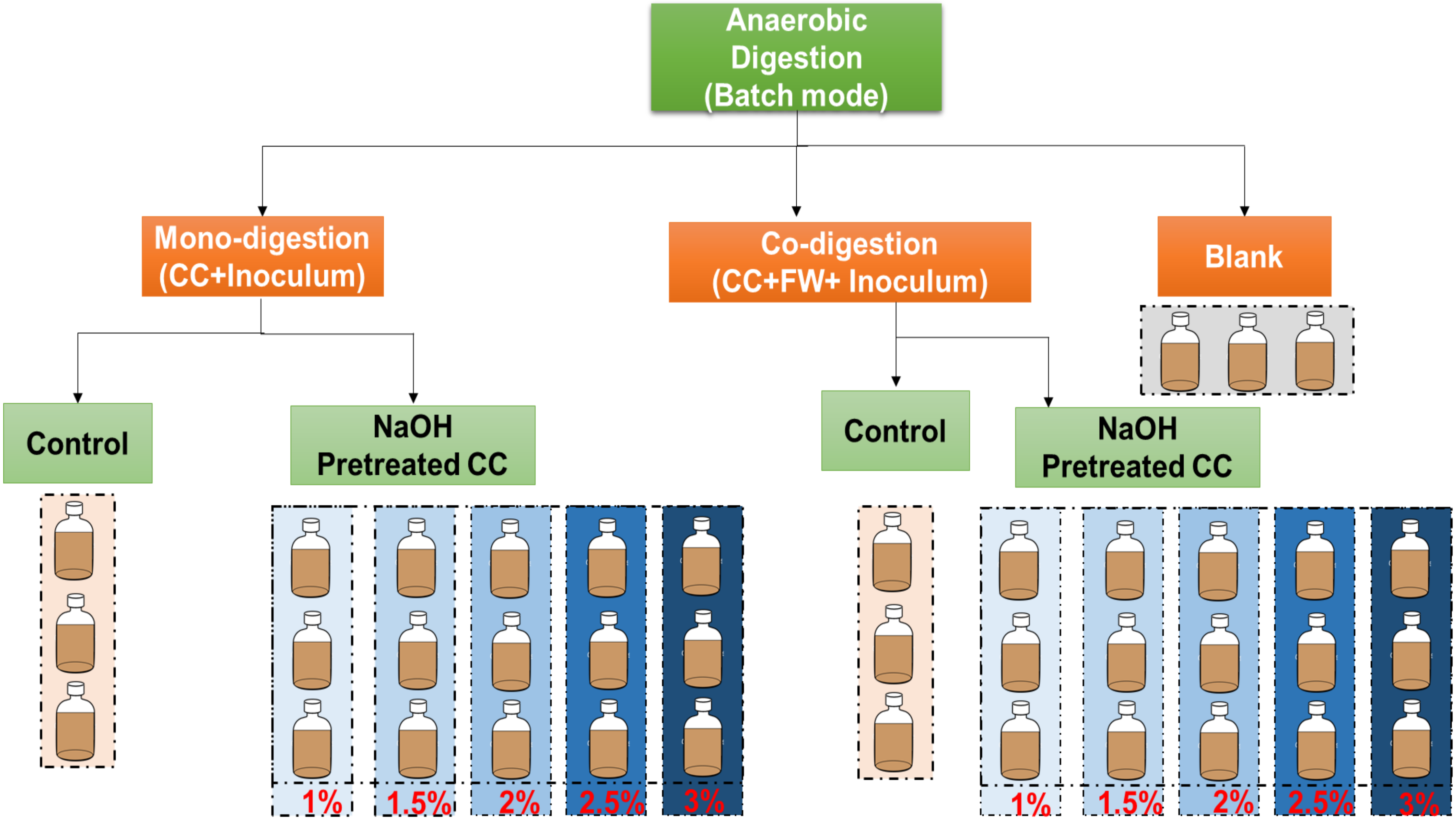

Figure 3.7 Experimental Design

Chapter 4

Results and Discussion

This chapter entails a detailed discussion of results achieved during the experimental phase. The discussions are based on characterization of substrate and inoculum, pretreatment of corncob and the effect of CC co-digestion with FW on biogas yield and reactor stability.

4.1 Characteristics of corncob, food waste and cow dung

Table 6 shows characteristics of corncob and cow dung. CC had higher values of both TS and VS than cow dung. This is due to the fact that dry biomass tends to have less moisture content in it while cow dung has higher moisture content thus it has low contents of TS and VS.

Table 6 Characteristics of substrate and inoculum

Parameters	Unit	Substrate Corn cob	Co-substrate Food waste (boiled rice)	Inoculum Cow dung
Total Solids (TS)	%	93.23±0.81	73.63±0.91	10.04±0.03
Volatile Solids (VS)	%TS	85.55±1.40	98.84±1.1	86±0.81
Total Organic Carbon (TOC)		47.52±0.57	40.3±1.2	48.52±0.46
Total Kjeldahl Nitrogen (TKN)		0.36 ± 0.05	2.65±0.08	0.42±0.01
Extractives	%	1.79 ± 0.03	---	---
Lignin		16 ± 1.3	---	---
Hemicellulose		49 ± 1.2	---	---
Cellulose		33.21 ± 2.1	---	---

4.2 Effect of NaOH pretreatment on lignocellulosic composition and physical structure of CC

The impact of NaOH pretreatment on lignocellulosic composition (MC, TS, VS, extractives, lignin, cellulose and hemicellulose) and physical structure are discussed in this section. The initial characterization results of untreated corncob are depicted in Figure 4.1. NaOH pretreatment of CC was carried out at varying doses i.e. 1%, 1.5%, 2%, 2.5% and 3%.

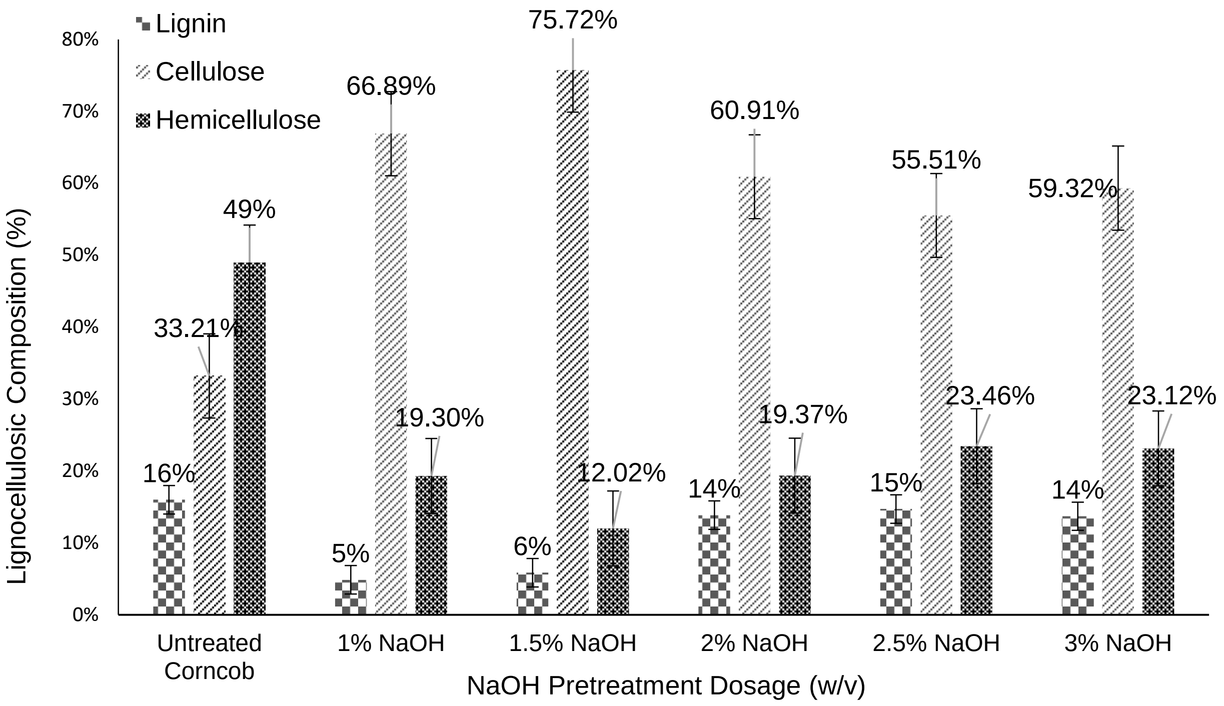

Figure 4.1 Impact of NaOH pretreatment on chemical composition of corn cob

The effect of NaOH pretreatment on the chemical composition of CC is represented in Figure 4.2. At the NaOH dosages of 1% and 1.5%, the lignin degradation was maximum and decreased from 16% to 5% and 6% respectively. Further increase in NaOH concentrations from 2% through 3% revealed not very significant delignification. Similarly, the hemicellulose degradation for 1% and 1.5% was also significant as it decreased from 49% to 19.30% and 12.02% respectively. This is due to the fact that delignification was optimal at 1% and 1.5% NaOH pretreatment over higher concentrations. Lower NaOH concentrations attribute to Optimal Delignification whereby the alkaline pretreatment disintegrates lignin and hemicellulose effectively thus enhancing the cellulose accessibility for microbial action. The higher NaOH concentrations result in excessive lignin removal which can potentially disrupt the structural coherence and cause swelling thus reducing its biogas production. In short, the alkaline pretreatment at lower concentrations indicated considerable degradation of lignin and hemicellulose. Furthermore, the alkaline pretreatment at 1% and 1.5% NaOH showed a sharp increase in cellulose i.e. from 33.21% to 66.89% and 75.72% respectively. The biodegradability of lignocellulosic biomass increased owing to alkaline pretreatment by breaking down the side chains of glycosides and esters thus causing cellulose to swell, hemicellulose solvation and structure change of lignin (Li et al., 2010).

4.3 Effect of NaOH pretreatment on cumulative biogas yield, solids removal and reactor stability of monodigestion and codigestion of CC

In this section the effect of mono and codigestion of corncob with FW on Biogas production, methane yield, solids reduction, AD reactor stability and Kinetic modeling are discussed.

4.3.1 Effect of NaOH pretreatment on cumulative biogas yield of monodigestion of CC

After the NaOH pretreatment of CC, the samples were thoroughly washed with DI water to achieve neutral pH. These samples were then oven dried (oven model-UNB 400) and later on fed into the AD digesters. The daily biogas yield of mono-digestion of untreated and treated CC with inoculum during 40 d of incubation is represented in Figure 4.2. The biogas production for the untreated CC. The cumulative biogas yield for untreated CC was recorded to be 249 NmL/gVS. The cumulative biogas yield of all the pretreated samples was more than the untreated CC. This attributes to the fact that the alkaline pretreatment biodegraded a portion of CC into smaller and simpler organic compounds, thereby enhancing biogas production. While biogas yield in monodigestion of 1% NaOH pretreated CC was very significant among all other pretreated samples. The cumulative biogas yield for 1% sample was 435 NmL/gVS. For 1% pretreated CC, the lignin degradation of 68.75% and cellulose recovery of 101.42%. While for 1.5% CC, the lignin degradation was 63.38% and cellulose recovery was 128%. The results imply that the degradation of lignin and cellulose recovery translate to increased biogas yield for NaOH pretreated CC. The biogas yield of increasing NaOH concentration pretreatment (2%, 2.5% and 3%) was relatively lower, this is because the lignin degradation was lower than 1% and 1.5% pretreated CC. The lignin degradation for 2%, 2.5% and 3% NaOH pretreated CC was 12.5%, 6.25% and 12.5% respectively. A number of research studies have revealed that alkaline pretreatment of lignocellulosic biomass by using NaOH leads to the increase in the lignin degradation and hydrolysis of cellulose and hemicellulose thus enhancing biogas yield (Sambusiti et al., 2012; Taherdanak and Zilouei, 2014;

Khalid et al., 2019; Sabeeh et al., 2020). A research by Sahare et al., (2012) studies the effect of alkali pretreatment on the structural properties and enzymatic hydrolysis of corn cob, and found out that the most effective pretreatment through hydrolysis of corn cob was achieved with 1 % alkali at 50 °C in 4 h. These findings are in agreement with aforementioned results.

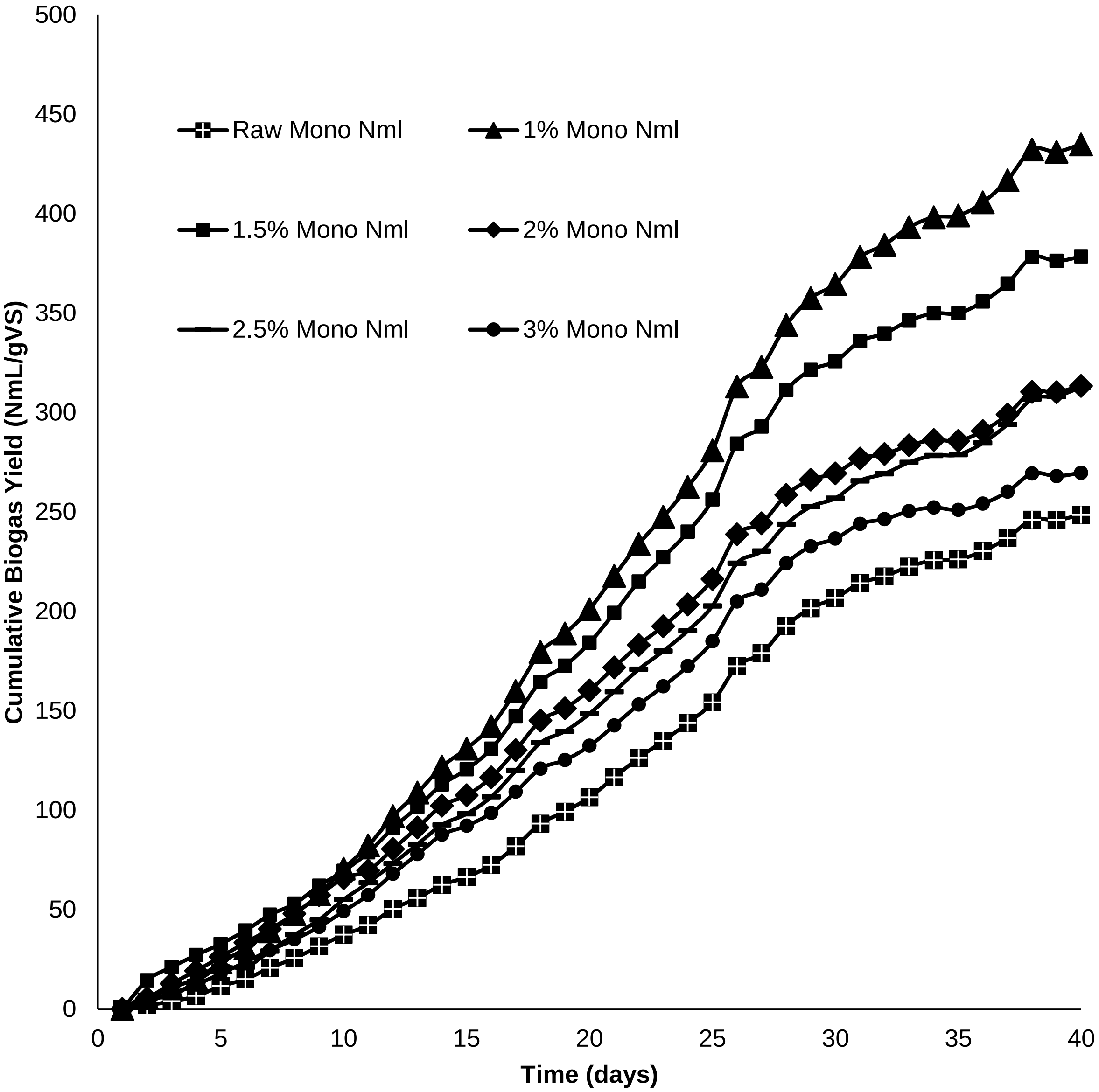

Figure 4.2 Cumulative biogas yield of mono-digestion of corn cob

4.3.2 Effect of NaOH pretreatment on cumulative biogas yield of co-digestion of CC with food waste (boiled rice)

During the study, it was noted that the cumulative biogas produced during co-digestion of CC with FW was significantly more than the mono-digestion of CC alone. The cumulative biogas produced during the co-digestion of CC with FW is represented in Figure 4.3.

This enhancement of the cumulative biogas can be attributed to the synergistic contribution of both the substrates. FW is rich in nutrients especially nitrogen which helps to optimize the C/N ratio of the digester which creates a more suitable environment for the microbial activity and biogas yield enhancement. As reported by Li et al., 2013, optimum C/N ratio is necessary for the efficient processing of AD digester as it results in the ample supply of carbon and nitrogen to the microbes for the production of biogas. The variety of microbial community existing in food waste may also contribute to the production of increased biogas, this may be due to the metabolic pathways and production of additional enzymes for the organic matter degradation (Zou et al., 2020). Dima, A.D. et al. (2020) studied the optimization of substrate composition in ACD of agricultural waste using central composite design, whereby C/N was adjusted to 13.515–30.485. The highest yield 347.48 mL/g VS was produced for the C/N ratio of 26.24. Thus C/N adjustment is necessary for the effective working of AD. Furthermore, another study, whereby co-digestion of cow slurry with maize cob is done. It in agreement with the notable positive effect of codigestion and C/N adjustment on biogas production. (Ademola O Adebayo et al., 2012).

The cumulative biogas produced by the co-digestion of untreated CC with FW produced 349 NmL of biogas which is an indication that prior to pretreatment, the lignocellulosic biomass of corn cob becomes a limitation to the access of microbes to the carbohydrates and thus halt anaerobic digestion resulting in lower methane yield. While during the codigestion of 1% NaOH pretreated CC, the methane production was the highest i.e. 575 NmL which attributes to the effectiveness of mild NaOH pretreatment. This suggests that at 1%, the pretreatment resulted in breaking down the matrix of lignocellulose thus making cellulose and hemicellulose to be more in access for microbial activity. The biogas produced by the co-digestion of 1.5% NaOH pretreated CC was 434 NmL which was lesser than that of 1% alkali pretreated CC. This attributes to the fact that 1% NaOH pretreatment had lower ratio of VFA to Alkalinity thus suggesting a better process stability and more biogas production in comparison to 1.5% NaOH pretreatment. Higher VFA/Alkalinity ratios are an indication of increased acid accumulation or reduced buffering capacity which inhibits microbial activity and biogas yield (Luo et al., 2018). The pretreatment at higher concentrations of NaOH (1.5% to 3%) have shown decreased methane production which pertains to excessive solubilization of lignin and other components thus forming inhibitory compounds and loss of substrate thereby decreasing the overall digestability and biogas yield (Cucina et al., 2021). This aligns with the findings of Sahare et al. (2012), which reports that the most efficient pretreatment of corn cob was achieved at 1 % alkali pretreatment, 50 °C temperature in 4 h which reduced the Crystallinity Index (CrI) by approximately 61.54%. The Specific Surface Area (SSA) was increased by approximately 538.46%. Which agrees that 1% NaOH pretreatment of corn cob effectively balanced lignin degradation and cellulose recovery, optimized digestibility and minimized inhibitory effects.

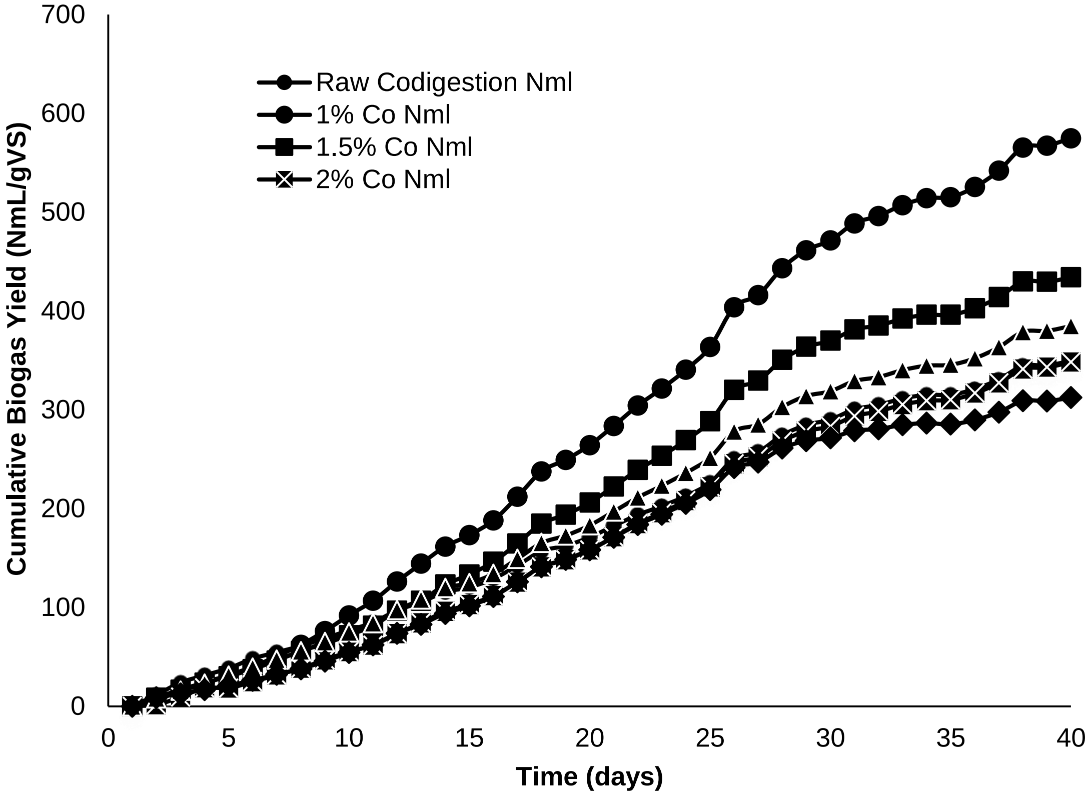

Figure 4.3 Cumulative biogas yield of co-digestion of corn cob with food waste

4.3.3 Effect of pretreatment on solids (TS and VS) removal

The effectiveness of AD is represented by the solids removal. The removal of the TS and VS of untreated and NaOH pretreated CC in mono and co-digestion is depicted in the Figure 4.4.

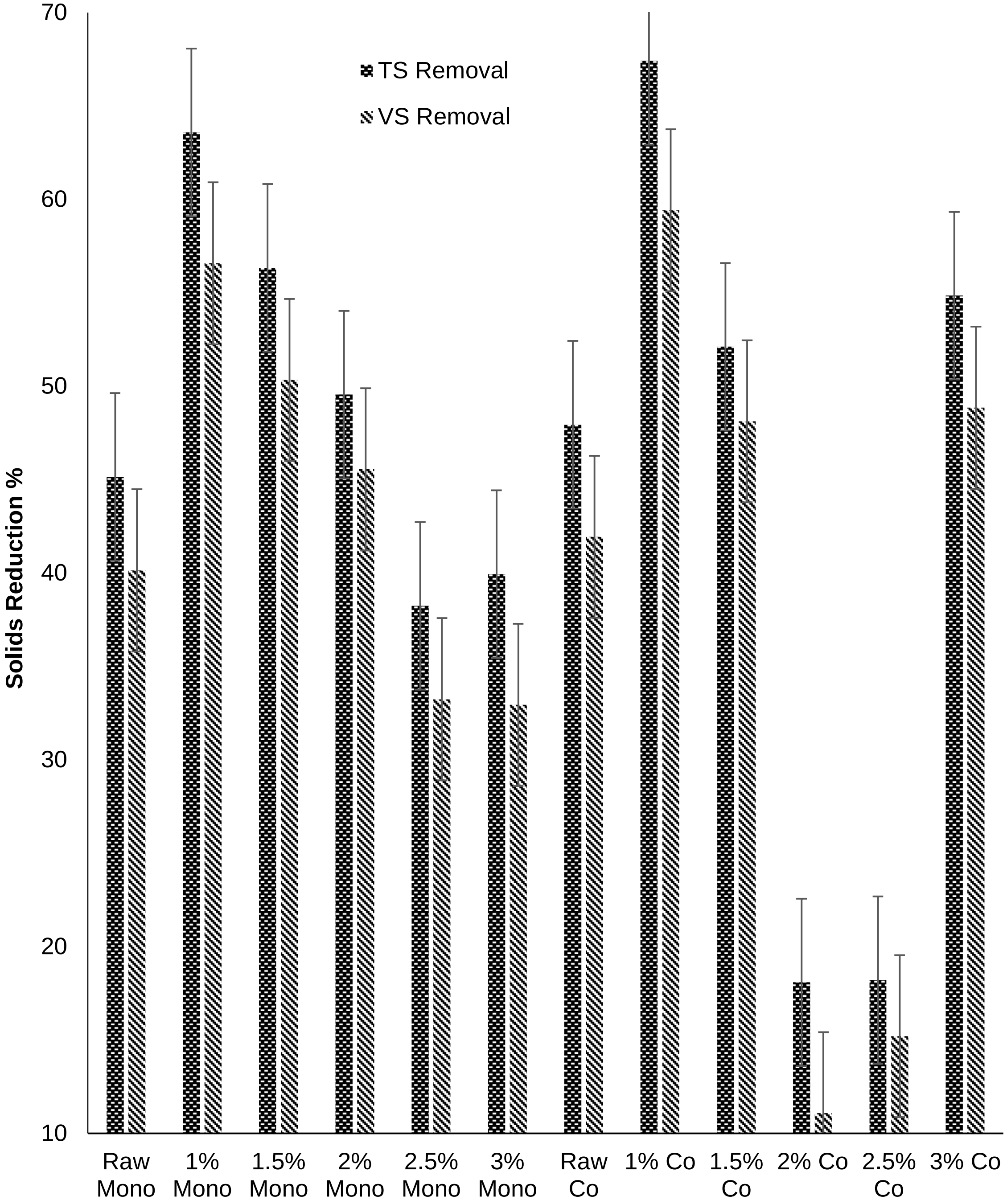

Figure 4.4 Effect of NaOH pretreatment on solids

Among all the pretreatments, the solids removal was the highest in 1% NaOH pretreated CC in both mono and codigestion. This aligns with the earlier observed increase in biogas production for 1% NaOH pretreated corn cob, supporting that moderate alkaline pretreatment optimizes the balance between lignin removal and the preservation of digestible cellulose and hemicellulose. TS removal in monodigestion of 1% pretreated CC was 63.58% as compared to raw CC, which was 47.94%. Similarly, the VS removal of former was 56.58% compared to 41.94% for raw corn cob. This is an indication that the alkaline pretreatment of CC has resulted in breaking down its lignocellulosic recalcitrant structure, enhancing its biodegradability and efficient microbial action on organic matter. On the same lines, the 1% NaOH pretreated CC codigestion had a higher TS and VS removal of 67.42% and 59.4% respectively, compared to that of untreated CC having 45.15% and 40.14% respectively. This improvement pertains to the understanding that the pretreatment helps in enhancing the degradation of biomass and efficient biogas production.

4.3.4 Effect of pretreatment on reactor stability

The process stability of an AD depends on the pH and VFAs of the reactor as methanogenic bacteria and process stability is sensitive to both of these parameters (Neshat et al., 2017). pH, Volatile Fatty Acids (VFAs), Total Alkalinity and VFA to alkalinity ratio are used to evaluate the AD performance. pH is a significant player in affecting the growth of bacteria, digestive process and products. The reactor efficiency was evaluated by monitoring the pH of the reactor before and after AD process. Figure 4.5 represents the initial and final pH of all the AD reactors. The initial pH was adjusted to around 7, within the optimum pH range of 6.8-7.2 of AD process (Hagos et al., 2017). The final pH of the reactors was recorded to be 5.18 to 6.65. The drop in the pH is an indication to the possible production of VFAs during the process (Neshat et al., 2017).

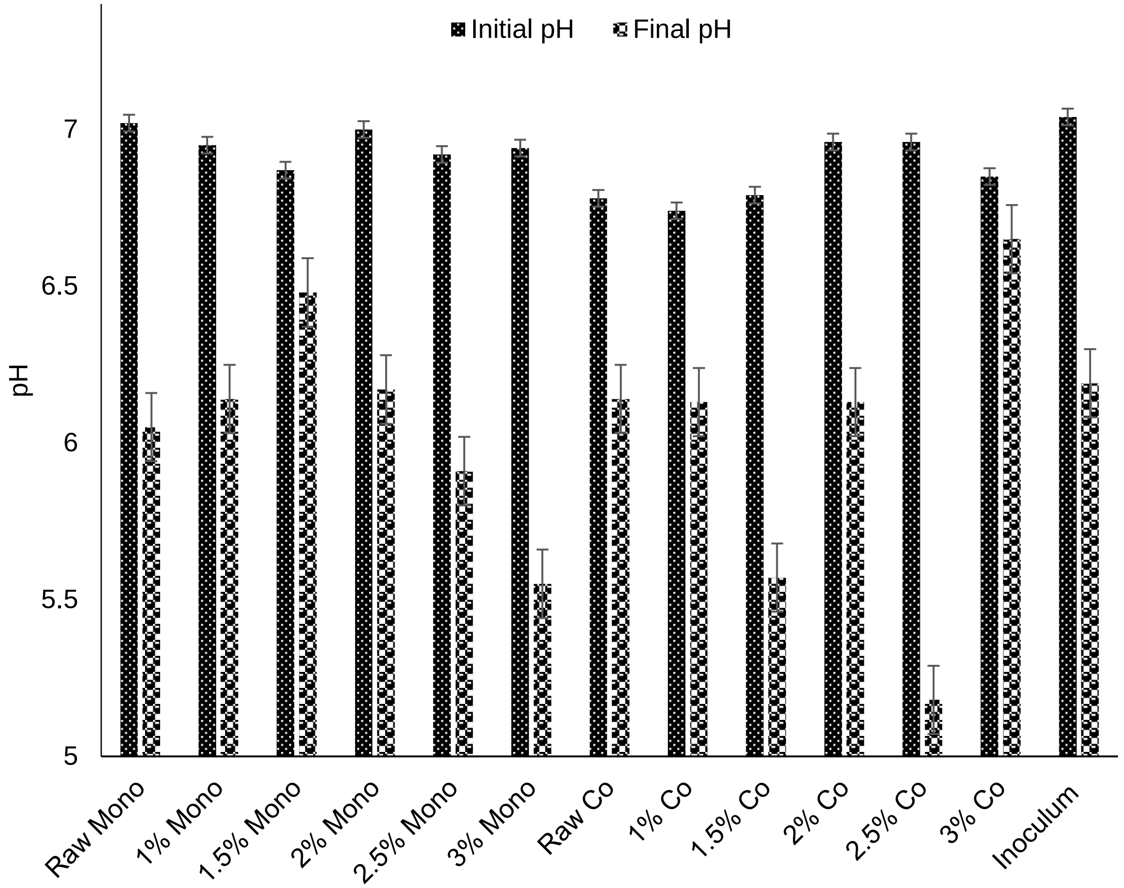

Figure 4.5 Effect of mono and codigestion of untreated and NaOH pretreated corn cob on pH

The VFA/Alkalinity ratio is one of the key parameters for the AD reactor's healthy performance and stability. The initial and final VFA/Alkalinity ratios of the reactors are represented in Figure 4.6. If the VFA/TA ratio of anaerobic digester is near to 1 but the VFA value is lower than 10,000 mg/L, it indicates the system stability (Kim et al., 2010).

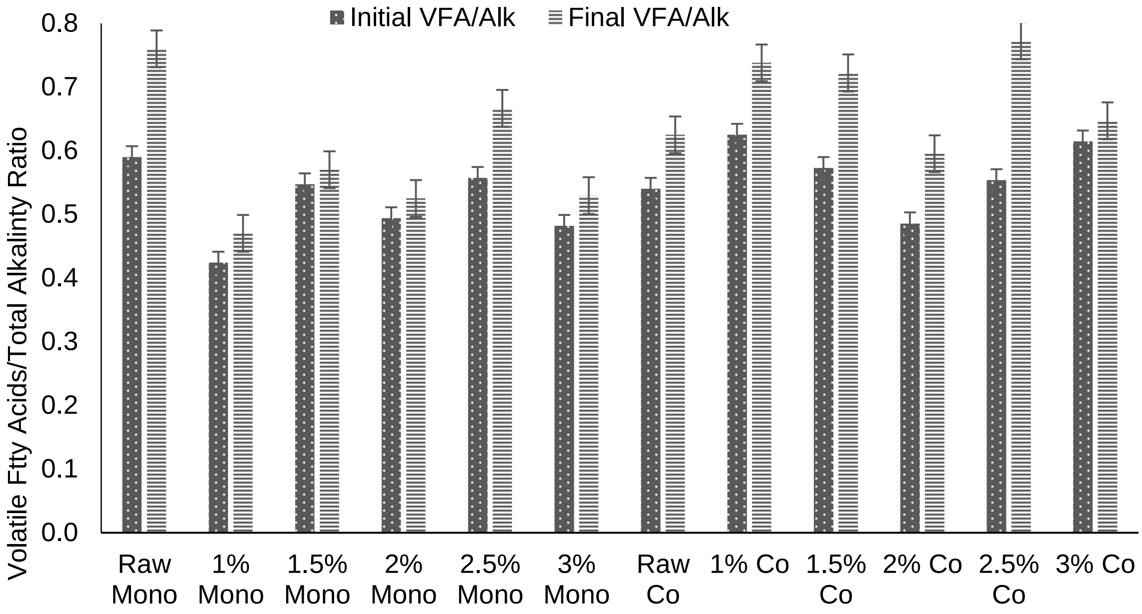

Figure 4.6 Effect of mono and codigestion of untreated and NaOH pretreated corn cob on total alkalinity

4.4 Biogas production data validation for mono and codigestion of CC

The cumulative methane yield was evaluated using the Gompertz model (modified). The results are represented in Table 7. The estimated parameters imply a positive effect of NaOH pretreatment on mono and codigestion of CC in AD process. The predicted yields (Hp) for monodigestion of untreated, 1%,1.5%, 2%, 2.5% and 3% pretreated CC were 310.75, 463.74, 366.88, 371.44 and 319.98 NmL/gVS respectively. While the predicted yields for the codigestion of untreated, 1%,1.5%, 2%, 2.5% and 3% pretreated CC with FW were 412.44, 696.71, 520.65, 419.56, 473.01 and 356.06 NmL/gVS respectively. The AD results under the model predicted the highest values for 1% NaOH pretreated sample for its codigestion with food waste. This indicates that codigestion of low NaOH pretreated CC significantly enhances biodegradability and methane enhancement. The predicted biogas yield and methane production rates values were consistent with aforementioned experimental results. It was observed that the lag phase (λ) for untreated CC sample was relatively short as compared to the pretreated samples in both mono and codigestion due to lack of inhibitors (Gao et al., 2021). For all the pretreated samples in both mono and codigestion, the lag phse was more than raw CC possibly because pretreatment would have resulted in the formation of inhibitors during the AD process which affected methnogenic activity. The R^2 values ranged 0.996-0.998 which was an implication of modified Gompertz model being a good fit to the experimental data.

Table 7 Cumulative methane yield as assessed using modified Gompertz model

Specimen	Hm (NmL/gVS)	Hp (NmL/gVS)	λ (days)	Rm (NmL/gVS/d)	R^2
Raw Monodigestion	249	310.75	7.7	9.26	0.997
1% Monodigestion	435	528	6.8	16.09	0.998
1.5% Monodigestion	379	463.74	5.7	13.67	0.996
2% Monodigestion	313	366.88	5.15	11.30	0.997
2.5% Monodigestion	313	371.44	6.11	11.12	0.998
3% Monodigestion	270	319.98	5.96	10.13	0.996
Raw Codigestion	349	412.44	5.90	10.20	0.996
1% Codigestion	575	696.71	6.7	20.60	0.998
1.5% Codigestion	434	520.65	6.5	16.04	0.997
2% Codigestion	348	419.56	6.78	12.51	0.998
2.5% Codigestion	385	473.01	5.34	13.14	0.997
3% Codigestion	312	356.06	6.29	12.13	0.997

Chapter 5

Conclusions and Recommendations

In this chapter conclusions drawn from present research are briefly discussed and also some future recommendations are proposed.

5.1 Conclusions

- For alkali pretreatment, all the concentrations have shown positive results in cellulose recovery and biogas enhancement. The concentration of 1.5% NaOH showed 227.86% cellulose recovery followed by 1% NaOH with 201.3% cellulose recovery.

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5.2 Recommendations

- More studies should be conducted with different pretreatment methods to enhance biogas yield
- Co-digestion of corn cob with other food waste may be studied.
- Studies on the comparison of different methods to find out lignocellulosic composition may be done

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