EFFECT OF ALKALI PRETREATMENT OF RICE STRAW CATALYZED BY $BiFeO_3$ ON BIOGAS PRODUCTION

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

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Dedication

This research is dedicated to my loving, caring, and exceptional grandparents and my parents whose tremendous support and cooperation led me to this wonderful accomplishment. 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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Muneeb Hassan

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List of Abbreviations

Abbreviations	Description
AD	Anaerobic Digestion
Ag	Silver
AOP	Advance Oxidation Process
°C	Degree Centigrade
$Ca(OH)_2$	Calcium Hydroxide
CH_4	Methane
C/N	Carbon to Nitrogen Ratio
CO_2	Carbon Dioxide
CSTRs	Continuous Stirred-Tank Reactors
Cu	Copper
d	Day
EDS	Energy Dispersive X-ray Spectroscopy
FT-IR	Fourier Transform Infrared Spectroscopy
g	Gram
GC	Gas Chromatograph
g/kg VS	Gram Per Kilogram of Volatile Solids

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Abstract

Rice straw can be used as a potential source of bioenergy but its recalcitrant structure hinders its degradation. To breakdown the recalcitrant structure of rice straw, its pretreatment needs to be done. In this study, rice straw was subjected to photocatalytic, alkaline and combined pretreatment using bismuth ferrite ($BiFeO_3$) nanoparticles, sodium hydroxide (NaOH) and $BiFeO_3$ + NaOH, respectively. The effect of pretreatments on rice straw characteristics and biogas production was investigated. In photocatalytic pretreatment, highest lignin removal of 64.17% was observed at 0.25 g/L dose of $BiFeO_3$ with cellulose increase of 68.32% as compared to control. Alkaline pretreatment showed maximum cellulose increase of 135.23% with lignin removal of 25.62% at 0.6% concentration of NaOH. The combined pretreatment of 0.125 g/L $BiFeO_3$ and 0.6% NaOH showed highest cellulose increase than all pretreatments, which was 144.4% more than the control. The photocatalysis, alkali and combinely pretreated rice straw showed maximum methane yield enhancement at 0.25 g/L $BiFeO_3$, 0.6% NaOH and 0.125 g/L $BiFeO_3$ + 0.6% NaOH, which was 39.9%, 40.3% and 54.3% more than the control group, respectively. The highest methane yield enhancement was observed with combined pretreatment due to more lignin removal and cellulose increase. Furthermore, modified Gompertz kinetic model was applied to the experimental results for data validation. The results showed that the predicted values matched well with the experimental ones.

CHAPTER 1

1 INTRODUCTION

1.1 Background

The social, economic and technological development of a country is majorly dependent on energy supply. Over the past century, the increasing world's population and economic activities have redundantly consumed fossil fuels to meet the energy demands. Like many developing countries, Pakistan is facing energy crises and is majorly dependent on conventional non-renewable energy resources. In Pakistan, the consumption of natural gas was drastically increased that it shared almost 50% of the total energy consumption by 2005 (Kardon et al., 2020). Also, Pakistan imports a massive amount of crude oil to fulfill the energy demand (Kamran, 2018). The excessive consumption of non-renewable energy resources not only caused diminution of fossil fuel reserves but also resulted in environmental damage (Agyekum et al., 2021; Ansari et al., 2020). These concerns have made the world to consider natural renewable resources as a primary energy source which are environmentally friendly and vastly present (Shah et al., 2019; Xu et al., 2019).

Due to its geographical region, Pakistan has a lot of potential of renewable energy resources including solar, marine, wind and biomass. Biomass has gain so much attention in the recent years because of its low carbon energy and huge production. The annual lignocellulosic yield is estimated to be 200 billion metric tons (Ahmad et al., 2020). The lignocellulosic biomass mainly consists of agricultural waste, economic crops, horticulture residues and forestry waste (Ufodike et al., 2020). According to Pakistan economic survey 2017-18, agriculture is considered to be the largest sector of Pakistan contributing approximately 18.9% to the gross domestic product (GDP) of the country. Along with that, it provides employment opportunities to 42.3% of its population (Mahmood et al., 2020). In a country with such a rich agricultural area, all the major crops including wheat, rice, sugarcane, maize and cotton are harvested. Pakistan is the tenth largest producer of rice worldwide. Besides that, it is also ranked as the fourth largest exporter of rice in the world (RMM., 2018). Such huge production of rice leads to massive production of rice straw. It was estimated that about 9.24 million metric tons of rice straw was produced in 2017 in Pakistan (Swain et al., 2019). To deal with such bulk of waste, farmers find it easy to burn away the straw in order to get rid of it. This practice is one the major sources of smoke, harmful greenhouse gasses and soot which leads to many respiratory problems and global warming (Khalid et al., 2019). The bioconversion of rice straw in to clean and green energy in the form of bioethanol, biogas, bio-hydrogen etc. is the only way to manage the waste and control environmental damage (Li et al., 2020).

Among different conversion techniques of biomass such as thermochemical (combustion, pyrolysis gasification, liquefaction) biochemical (fermentation, anaerobic digestion) and physicochemical (mechanical extraction, esterification), anaerobic digestion from biochemical process is considered more safe and economically feasible that converts carbohydrates of lignocellulosic biomass into methane rich gas and nutrient rich digestate (Zhang et al., 2019; Cao et al., 2018; Neshat et al., 2017). Anaerobic digestion (AD) is a series of processes (Hydrolysis, acidogenesis, acetogenesis and methanogenesis) performed by consortia of microorganisms in the absence of oxygen. AD breaks the complex carbohydrates of biomass in to carbon dioxide (CO_2) and methane (CH_4) leaving behind nutrient rich digestate which can be used as a fertilizer (Maharaj et al., 2019; Lim et al., 2018; Zhou et al, 2016).

Lignocellulosic biomass consists of three major components including cellulose, hemicellulose and lignin (Kassaye et al., 2016). Cellulose is a polymer of glucose combined with β-1-4 glycosidic bonds and is mainly responsible for the biogas production during anaerobic digestion (Koupaie et al., 2019). One of the major problems associated with the conversion of lignocellulosic material into biogas is the shielding of cellulose by lignin and hemicellulose content. Both components show rigidness against anaerobic digestion and reduce the biogas yield (Hassan et al., 2017). Lignin is composed of phenolic compounds and forms complex polymers with other components of cell wall including cellulose and hemicellulose forming a three dimensional (3D) recalcitrant structure throughout the cell wall. The 3D structure provides protection to cellulose and prevents it from degradation through microbial activity leaving a large of portion of cellulose undigested (Neshat et al., 2017; Sun et al., 2016).

To break the lignocellulosic bonds and release the soluble reducing sugars, pretreatment of the biomass is required done in order to enhance the biogas production by utilizing more biomass in the process of anaerobic digestion (Zheng et al., 2014). In previous studies, different physical, chemical and biological pretreatment methods have been introduced (Zheng et al., 2014; Nges et al., 2016). Physical methods include physical destruction of the biomass through grinding, extrusion, milling and steam explosion in order to breakdown the recalcitrant structure (Liu et al., 2015). While, biological (fungal, bacterial, enzymatic) and chemical methods (alkaline, acidic, advanced oxidation process) work by exposing cellulose and hemicellulose content to anaerobic digestion by degrading lignin. Among all these methods, the best option for anaerobic digestion should be one with minimal size reduction, maximum lignin degradation while, preserving cellulose and hemicellulose, less generation of inhibitors, and energy & cost effectiveness (Cybulska et al., 2019; Rabemanolontsoa and Saka, 2016).

Alkaline pretreatment is considered practically effective as high pH dissolves more lignin and hemicellulose and reduces crystallinity of cellulose (Bolado et al., 2016). Along with that, alkaline pretreatment has other advantages including low operation cost, mild operative conditions and less formation of inihibitory compounds (Sharma et al., 2019). Most commonly used alkaline reagents are $Ca(OH)_2$, NaOH, KOH and $NH_3.H_2O$ (Veluchamy et al., 2018). Among all the reagents, sodium hydroxide has been studied the most, as it shows more effective results in response to lignin solubilization and preservation of cellulose by reducing the degree of polymerization of lignin. Moreover, it swells the cell wall structure providing more surface area for microbial attack (Sharma et al., 2019; Paudel et al., 2017).

Besides, advanced oxidation processes have been introduced by the scientists keeping in mind the energy crises, climate change and sustainability issues (Villaseñor & Ríos, 2018). In recent times, the use of AOPs are increasing in bioenergy sector, including the pretreatment of lignocellulose for lignin degradation through photo-catalysis (Tamilarasan, 2019). The problem associated with photocatalysis is that, most of the highly active semiconductor photo-catalysts are only active under UV light due to wide band gap. UV light accounts less than 5% of the solar spectrum, this not only limits the utilization of the solar energy but also reduced the activity of photo-catalysts under sunlight (Satar et al., 2019). Recently, bismuth ferrite ($BiFeO_3$, BFO) has gained attention owing to its high efficiency in visible light because of its smaller energy band gap and good chemical stability (Soltani et al., 2016). Different studies showed the efficiency of $BiFeO_3$ in the degradation of organic pollutants like phenol, malachite green dye (Jaffari et al., 2020), benzene (Soltani et al., 2016), antibiotics (Tang et al., 2018) and ammonia (Zou et al., 2017). Bismuth ferrite ($BiFeO_3$) has been used for the degradation of lignocellulosic biomass in combination with fenton-like pretreatment (Zhang et al., 2019)

According to the literature, no study has been done on photocatalytic degradation of lignocellulosic biomass using $BiFeO_3$. In the current study, rice straw has been treated with different concentrations of $BiFeO_3$ and NaOH. The combined pretreatment using the same concentrations of $BiFeO_3$ with the optimum value of NaOH was also performed. Moreover, the effect of pretreatment on biogas yield and methane content was also analyzed, including different parameters like pH, alkalinity and volatile fatty acids.

1.2 Objectives

1. Evaluate the effect of $BiFeO_3$ nanoparticles photocatalytic pretreatment on characteristics and biogas production of rice straw.

2. Evaluate the effect of alkaline (NaOH) pretreatment on characteristics and biogas production of rice straw.

3. Evaluate the effect of alkaline (NaOH) pretreatment catalyzed by $BiFeO_3$ nanoparticles on characteristics and biogas production of rice straw.

CHAPTER 2

2 LITERATURE REVIEW

This chapter will provide the information of the past work done regarding the pretreatment of rice straw and its biogas production.

2.1 Lignocellulosic biomass

Lignocellulosic material is the dry matter of a plant, which is abundantly present and considered as the potential source for the production of biogas, biofuels etc. in order to replace the conventional methods for energy production using fossil fuels. It is estimated that approximately 1.3 billion tons of lignocellulosic biomass is generated worldwide annually out of which only 3% is utilized in the form of value added products. Lignocellulosic biomass mainly includes forestry waste, horticulture residues and agricultural waste (Areepak et al., 2022; Bhatia et al., 2019). Agriculture is considered as the largest sector of Pakistan as it contributes almost 18.9% to the GDP of the country and employs approximately 42.3% of the total labor force. The main agricultural crops include wheat, sugarcane, maize, cotton, and rice. Pakistan is ranked as the tenth-largest producer and 4th largest exporter of rice in the world, leaving a huge amount of straw behind. Despite the fact, they are being burned causing more damage to the atmosphere by releasing greenhouse gasses and playing a major role in global warming (Ufodike et al., 2020; Mahmood et al., 2020).

Lignocellulose consists of two carbohydrate polymers i.e. cellulose (35–50%), and hemicellulose (20–35%) and an aromatic polymer i.e. lignin (10–25%). However, the amount of these polymers vary according to their species, age, nutrients availability, environment etc. (Ghaemi et al., 2019).

Cellulose in lignocellulose is comprised of D-glucose which is linked by β-1, 4 glycosidic bonds. Cellulose consists of crystalline and amorphous structure. The material in cellulose combine together to form microfibrils. These microfibrils are bound together through hydrogen bonding and are considered as the richest biopolymer present on earth surface (Liu et al., 2020; Wang et al., 2020; Zheng et al., 2014).

Hemicellulose is also a carbohydrate polymer, like cellulose. The structure of hemicellulose contains a mixture of different types of sugar polymers like pentoses (such as xylose), hexoses (like mannose and galactose) and sugar acids. These polymers are branched together with the help of glycosidic bonds like β-1,4 and β-1,3-glycosidic bonds. The composition of hemicellulose varies according to the type of plant species like hardwood contains xylans whereas, softwood is the mixture of glucomannans, xylans, and glucans. The solubility of these compounds are dependent on the temperature i.e. more the temperature more will be the solubility. Hemicellulose increases the rigidity of the plant lignocellulosic structure by binding cellulose microfibrils and lignin and acts as a connector between them. (Liu et al., 2019; Yang et al., 2015; Zheng et al., 2014).

Lignin is an amorphous heteropolymer which means it is derived from different kinds of monomers. After cellulose and hemicellulose, it is also counted as one of the abundant polymers we found in Earth. Lignin is hydrophobic and inactive in nature and is found in cell wall of almost every plant. Its presence gives strength, support, impermeability and protection against microbial attacks. All these factors have made the degradation of biomass a big concern. As, lignin blocks the microbes to attack cellulose and hemicellulose, which are highly soluble and degradable (López-Mondéjar et al., 2019; Kucharska et al., 2018; Zheng et al., 2014).

2.2 Pretreatment of lignocellulosic biomass

Cellulose and hemicellulose, both are fermentable sugars and produce biogas under anaerobic digestion. But the sheathing of lignin around cellulose and protection of cellulose by hemicellulose and lignin affects the digestion of cellulose. This results in the very less production of biogas leaving behind a large amount of cellulose undigested. In order to enhance the production of biogas, pretreatment of the lignicellulosic material is done to dissolve the lignin and make cellulose available for the microbial attack (Bhatia et al., 2019; Sindhu et al., 2017).

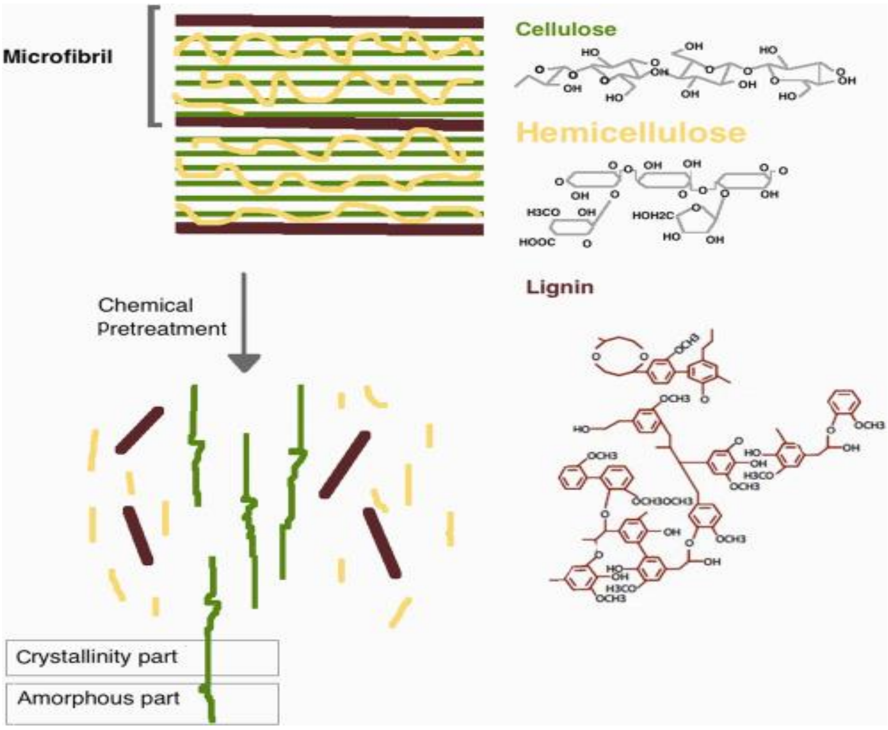

Figure 2:1 Effect of pretreatment on lignocellulosic structure

2.3 Types of pretreatment for lignocellulosic biomass

Different pretreatment methods have been reported in the previous studies to break the lignocellulosic structure. These different pretreatment methods include chemical, physical, biological and physicochemical techniques. Each method works in a different way by producing different kinds of products and by products. Also, each method effects the biogas yield, differently. Besides the advantages, there are also disadvantages related to each method (Sun et al., 2016). So, while selecting a pretreatment technique, many points have to be considered like; cost effectiveness, availale resources, energy efficiency etc. (Ravindran et al., 2018).

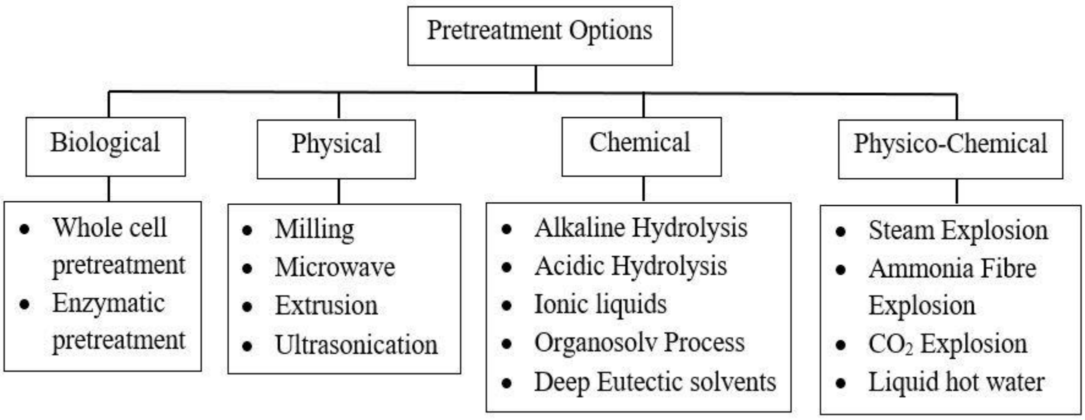

Figure 2:2 Different pre-treatment categories for lignocellulosic biomass

2.3.1 Physical pretreatment

In physical pretreatment, the particle size of the material is reduced causing the increase in surface area. As a result, the crystallinity of cellulose is reduced and lignin is depolymerized. The advantage of physical pretreatment is that no harmful by product is released during and after pretreatment. But the main disadvantage of physical pretreatment methods is the requirement of high energy which makes their use less suitable (Moset et al., 2018; Naimi et al., 2018). Milling, microwave, extrusion and ultrasonication are examples of physical pretreatment methods. Different studies have been reported with physical pretreatment (Gu et al., 2018; El Achkar et al., 2018; Savoo and Mudhoo, 2018). Savoo and Mudhoo, (2018) reported the increase of 64.7% in biogas production after pretreating cauliflower and cabbage leaves (1:1 on wet mass basis) with microwave power of 350 W for 25 min.

2.3.2 Biological pretreatment

In biological pretreatment, the consortia of microorganisms degrade the ignin and hemicellulose by releasing certain enzymes. The biological pretreatment is considered more safe, eco-friendly and cost efficient than other pretreatment methods. The major drawback of this pretreatment is that microorganism required large surface area and around 10-14 days for acclimatization and functioning (Shirkavand et al., 2017; Agbor et al., 2011). The use of biological pretreatment has been reported in various studies (Bari et al., 2016; Hua et al., 2016; Wang et al., 2016; Adebayo et al., 2015). Wang et al. (2016) reported 110% increase in methane yield after enzymatic pretreatment of corn straw using amylase. In the pretreatment using cellulase enzymes, the increase in methane yield was reported to be 103%.

2.3.3 Chemical pretreatment

In chemical pretreatment, the recalcitrant structure of lignocellulosic material is degraded through different chemical reactions. As a result of chemical reactions, the cell wall gets swell, increasing the surface area of cellulose and solubilizing lignin. The efficiency of chemical pretreatment has been reported in previous studies (Buratti et al., 2018; CaO et al., 2018; Zheng et al., 2018; Solé-Bundó et al., 2017). It was reported that, the pretreatment of wheat straw with 4% NaOH at 121°C increases the cellulose content upto 87% improving the enzymatic hydrolysis (Zheng et al., 2018).

2.3.4 Physico-chemical pretreatment

In physico-chemical pretreatment the effect of physical and chemical pretreatments are combined. The physical changes and chemical reactions enhanced destruction of recalcitrant structure of lignocellulose. Physio-chemical pretreatments have been reported in various studies (Tang et al., 2018; Feng et al., 2018; Sundaram et al., 2017; Liu et al., 2017). Sundaram et al. (2017) reported the increase of bio-char and bio-oil from 22%-25% and 46%-48% from the pyrolysis of corn stover pretreated from ammonia fiber explosion method at 100°C for 15 min, respectively.

2.4 Anaerobic Digestion

In anaerobic digestion, a group of microorganisms degrade organic matter in the absence of oxygen producing biogas and digestate as the end product (Di Maria et al.,2014). The process occurs in four stages i.e. hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each step is carried out with different groups of bacteria producing different end products. The steps in anaerobic digestion is shown in the figure 4 (Li et al., 2011).

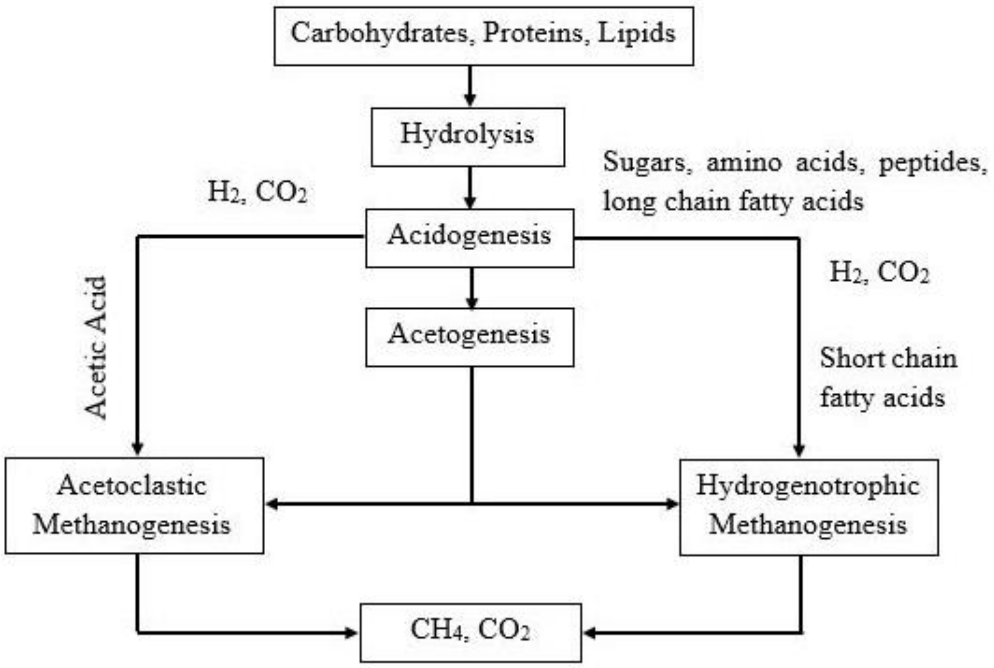

Figure 2:3 Steps in anaerobic digestion

2.4.1 Hydrolysis

In anaerobic digestion, hydrolysis is the first step in which a group of bacteria converts organic matter (which are in the form of polymers) into simple and soluble form (oligomers and monomers). It is an essential step prior to acidogenesis as the consortia of bacteria in acidification cannot intake complex form of organic matter. In other words, hydrolysis provides substrate to the group of bacteria involves in acidogenesis. Enzymes like cellulase, amylase and xylanase converts carbohydrate into monosaccharides i.e. simple sugars, protease degrades proteins into amino acids and lipase converts lipids into fatty acids and glycerol. The optimum temperature for hydrolysis is reported to be 30ºC-50ºC with the optimum pH range of 5-7 (Azman, 2016).

2.4.2 Acidogenesis

In acidogenesis, bacteria convert the products of hydrolysis into volatile fatty acids (acetate, butyrate etc.), aldehydes, carbon dioxide, hydrogen, nitrogen and sulfur compounds etc. This process releases a high amount of energy for microorganisms. Products of this phase is utilized in further steps of anaerobic digestion especially acetic acid which is majorly utilized in methanogenesis. In acidogenesis, pH falls because of the acid production (Bajpai, 2017).

2.4.3 Acetogenesis

Acetogens convert organic acids (which are produced in acidogenesis) into acetic acid, carbon dioxide, hydrogen etc. The main product of acetogenesis is acetic acid, which will be utilized by methanogens. Acetogenetic bacteria are slow growing and produce less energy because of which a small change in the environment could affect them. (Moraes et al., 2015).

2.4.4 Methanogenesis

In the last stage of anaerobic digestion, methanogens convert acetic acid, carbon dioxide and hydrogen into methane. In this stage, the production of methane occurs in two paths. In first, acetoclastic methanogens convert acetic acid in to methane. While, on the other hand, carbon dioxide and hydrogen is converted in to methane by hydrogenotrophic methanogens. A large amount of methane is produced by acetic acid. Whereas, very few amount of methane is produced by hydrogenotrophic methanogens using carbon dioxide and hydrogen (Richards et al., 2016; Angelidaki et al., 2011).

2.5 Parameters for anaerobic digestion

As anaerobic digestion is performed by different groups of bacteria, they require favorable conditions to work at optimum level. Many parameters affect their growth and working which ultimately affects the biogas yield. These parameters are described below:

2.5.1 Temperature

Temperature is one of the most important factors in anaerobic digestion as it directly effects the microbial growth and plays a vital in separation of gases like methane and carbon dioxide from liquid phase (Ogbonna et al., 2015). Anaerobic digestion could take place in different ranges of temperature including psychrophilic (11-25 ^{O}C), mesophilic (35-40 ^{O}C) thermophilic (50-55 ^{O}C) and hyperthermophilic (> 55 °C) (Moset et al., 2015). In the previous studies it has been seen that mesophilic and thermophilic digestion gives better results as compared to others. Thermophilic digestion requires high energy and maintenance cost that is why mesophilic digestion is preferred over it. Although, more retention time is required for mesophilic digestion as compared to thermophilic (Labatut et al., 2014).

2.5.2 pH

pH is also one of the most important parameter whose slight change can highly effect the microbial growth and performance. The optimum pH for methanogenesis is reported to be 6.8-7.2. Latif et al. (2017) observed maximum methane production at pH 7 during the continuous anaerobic digestion of sludge while, 88% reduction in methane yield was observed at pH 5.5. During acidogenesis and acetogenesis the pH falls due to the acid formation but if the pH drops from 6 it will cause inhibition in the biogas production. Same is the case with methanogenesis, as methanogens cannot work in the environment with low pH. As a result of which hydrogen and acetic acid starts to accumulate, inhibiting the biogas production (Eryildiz et al., 2020).

2.5.3 VFA and alkalinity

Volatile fatty acids (VFA) leads to the production of methane during anaerobic digestion. But their accumulation lowers the pH of mixture effecting the performance and growth of methanogens (Eryildiz et al., 2020). Whereas, formation of ammonia increases the pH and also acts as the buffer (alkalinity) in response to VFA. The optimum VFA/TA ratio that shows stable anaerobic digestion is <4 (Wang et al, 2016).

2.5.4 Water content

Water is very important in AD as it provides medium for biochemical reactions, microbial activities, nutrient absorbance and locomotion (Heiske et al., 2015). Different moisture content is required for different substrates. Usually, 75-80 % of moisture content is maintained in the reactors (Khalid et al., 2011).

2.5.5 Inoculum

Inoculum is very essential to start the anaerobic digestion as it contains rich consortia of microbes. In the absence of inoculum, the process takes too much time to start. It reduces the lag phase and increases the biogas and methane yield. Animal manure, wetland sludge and digestate of already running biogas plant are mostly used as inoculum (Gu et al., 2014; Raposo et al., 2011).

2.6 Types of anaerobic digestion

On the basis of operational mode, anaerobic digestion can carry out in two different ways i.e. batch mode digestion and continuous mode digestion. Both modes are described below:

2.6.1 Batch mode digestion

In batch mode digestion, the reactor is fed with inoculum, substrate and water at the start with the buffer solution to control the pH change during the anaerobic digestion. After that the bottles are sealed for retention time. In batch mode the digestion of substrate is higher than continuous as it spends more time in the digester. The production of biogas is low in the start but slowly increases to the maximum and then decreases at the end (Carrere et al., 2016).

2.6.2 Continuous mode digestion

In continuous mode digestion, the reactor is fed with substrate on daily basis around 1-8 times. The old one is replaced by the freshly added substrate. As, the substrate has less retention time in the digester hence, most of the substrate remained undigested. The biogas production in continuous mode is constant as compared to batch mode (Wei et al., 2018).

2.7 Effect of different pretreatments on biomass

2.7.1 Alkaline pretreatment

In the previous studies, alkaline pretreatment of the lignocellulosic biomass has been performed in order to solubilize the lignin content to make the holocellulose readily available for the microbial attack. Alkaline pretreatment works in a way that it undergoes saponification and break lignin-carbohydrates bond as a result of which the crystalline structure swells and the crystallinity of the biomass decreases (Paudel et al., 2017). Mainly used alkalis are NaOH, $Ca(OH)_2$, KOH, and NH_4OH. Some of the studies are given in table 2.1.

The reduction in efficiency of alkaline pretreatment at higher concentration has been reported in various studies (Khalid et al., 2019; Kang et al., 2018; Shetty et al., 2017; Gu et al., 2015). Khalid et al. (2019) reported the decrease in biogas and methane yield when the NaOH concentration was increased from 2-10% (w/v). Gu et al. (2015) also reported the reduction in methane yield with increasing concentration of $Ca(OH)_2$ from 8-15% (w/w). The effect of temperature during alkaline pretreatment has also been reported. Kang et al. (2018) observed optimum results at 35ºC during alkaline pretreatment and reported the decline in methane yield at higher temperatures of 55ºC and 121ºC.

Table 2.1 Effect of alkaline pretreatment on structural degradation and biofuel production of lignocellulosic wastes.

Alkali used	**Substrate**	**Conditions**	**Findings**	**Reference**
NaOH	Rice Straw	Temperature: 37°C, NaOH concentration: 2,4,6,8 and 10% w/v, Duration: 5 days, Solid/Liquid ratio: 1:15	The pretreatment using 2% NaOH degrades the lignin by 88% and recover cellulose by 102%. Biogas and methane yields from the pretreated rice straw was increased by 57 and 60% respectively.	Khalid et al., 2019
NaOH	Silver Grass	Temperature: 35°C, NaOH concentration: 6% w/v, Duration:3 hours, S/L ratio: 1:10	Degradation of Lignin and hemicellulose was observed by 12 and 13% respectively.	Fu et al., 2018
NaOH	Wheat Straw	Temperature:30°C, NaOH concentration: 1.6% w/w. Duration:24 hours, S/L ratio: 1:6	Lignin degradation of 37% was observed while, methane yield was enhanced by 15%	Mancini et al., 2018
NaOH	Pennisetum Hybrid	Temperature:35, 55 and 121°C, NaOH concentration: 2-8% w/w , Duration:24, 24 and 1 hours, S/L ratio: 1:10	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	Rice Straw	Room temperature, NaOH Concentration: 0.5-5% w/v, Duration: 3 hours, S/L ratio: 1:13	Highest biogas yield of 514 mL/g observed at 1% NaOH.	Shetty et al., 2017
$Ca(OH)_2$	Rice Straw	Temperature:25°C, $Ca(OH)_2$ concentrations: 5, 8,10,12 and 15% w/w, Duration:72 hours S/L ratio: 1:20	8% $Ca(OH)_2$ yielded best results in terms of hydrolysis and methane production i.e. 330.9 mL/g VS	Gu et al., 2015

Besides, it can be seen that alkaline pretreatment has shown effective increase in biogas and methane yield because of the efficient removal of lignin under mild conditions i.e. low temperature and lesser time duration. Also, NaOH is the most used base for alkaline pretreatment of biomass like corn strover, rice straw, wheat straw, sugar baggase etc. (Sun et al., 2016).

2.7.2 Photocatalytic pretreatment of biomass using TiO_2

Many studies have been carried out in photocatalysis of organic compounds especially lignocellulosic material in order to enhance the biogas production. As, in advance oxidation process, photocatalysis is considered most efficient technology. In this technology highly active radicals are generated which degrades unwanted organic pollutants. Few studies are mentioned in table 2.2.

It can be seen that photocatalysis plays a significant role in solubilization of lignin and biogas enhancement. Although, high doses of catalyst show less lignin and hemicellulose removal. The optimum irradiation time in many studies have been reported to be 3 h. Less irradiation time showed poor lignin solubilization. Moreover, the combine pretreatment using alkali shows more promising as compared to simple photocatalysis. In most of the studies, TiO_2 has been used due to its high photocatalytic activity, low toxicity commercial availability and low cost (Stucchi et al., 2015). Besides these benefits, TiO_2 has a wide band gap of 3.23 eV that enables it to performs under UV light, which is only 4-5% of the sunlight. The ability to perform under UV light only, restricts the use sunlight (Guo et al., 2017).

In order to utilize maximum solar light and make the system energy efficient, it is necessary to use the catalyst with low band gap so that it can be active in visible light. Therefore, in the current study, $BiFeO_3$ has been used as a catalyst as the band gap of this catalyst is 2.1 eV which makes it highly active under visible light (Soltani et al., 2016).

Table 2.2 *Effect of photocatalysis on biogas production and methane yield.*

Pretreatment	**Conditions**	**Substrate**	**Findings**	**Reference**
TiO_2/UV/ NaOH	TiO_2 Concentrations: 0.1-1 g/L, NaOH concentrations %w/v: 0.5-2, Irradiation time: 3 hours, S/L: 1:20	Wheat Straw	Biogas and methane yield was enhanced by 74 and 122 % respectively in combined pretreatment of 1.5 % NaOH and 0.25 g/L TiO_2..	Sabeeh et al., 2020
TiO_2/UV/ H_2O_2	TiO_2 Concentrations %w/v: 0-2 H_2O_2 concentration: 1-16 mM Irradiation time: 1-8 hours, S/L: 1:40	Rice Straw	Optimum results were observed at conditions having 0.5% TiO_2 with 13 mM H_2O_2 for 3 hours. Released sugar was increased by 58%	Chang et al., 2018
TiO_2/UV	TiO_2 Concentrations %w/w: 1-2, Irradiation time: 0-3 hours, S/L: 1:260	Wheat Straw	1.5% (w/w) TiO_2 and 3 h increased methane production by 37%	Alvarado-Morales et al., 2017
Cu-TiO_2/ Sunlight	TiO_2 Concentration: 100g Irradiation time: 7 h (9 am-4 pm) for 30 days, S/L: 1:5	Coffee Pulp	Degradation of lignin was observed upto 41.13% and cellulose was increased upto 32.39%	Corro et al., 2014
NaOH/TiO_2/ UV	TiO_2 concentration: 2 g/L, NaOH concentration: 3% w/v, Irradiation time: 1 hour, S/L: 1:40	Rice Straw	Lignin degradation of 68.75% was achieved	Niu et al., 2009

2.7.3 Photocatalytic pretreatment using $BiFeO_3$

In the recent times $BiFeO_3$ has gained so much attention because of its narrow band gap and high photovoltaic effect. Many studies have been made to degrade biomass and also organic pollutants in wastewater using bismuth ferrite. Some of the studies have been given in the table 2.3.

Table 2.3 Effect of photocatalysis of BiFeO3 on organic compounds.

Pretreatment	Conditions	Substrate	Findings	Reference
$BiFeO_3/H_2O_2$	$BiFeO_3$ Concentrations: 0.05 g/LH_2O_2 concentrations: 10 ml of 30% H_2O_2. S/L: 4:1	Corn stover	Increase in cellulose was seen upto 26% and lignin was degraded upto 75%.	Li et al, 2021
$BiFeO_3$/ H_2O_2	$BiFeO_3$ Concentrations: 0.15 g/L H_2O_2 concentrations: 20 ml of 40% H_2O_2. Time: 3 hours Temperature: 60°C. S/L: 1:20	Sugar baggase	Reduced sugar yield was increased upto 25.8% with 36.6% sugar conversion rate.	Zhang et al., 2019
$BiFeO_3$/ Sunlight	$BiFeO_3$ Concentrations: 0.1 g/L, Irradiation time: 2 and 3 hours	Methylene Blue	In 2 hours 87% MB was degraded while in 3 hours it was 96.6%.	Satar et al., 2019
rG-$BiFeO_3$/ Visible light	$BiFeO_3$ Concentrations: 0.1 - 0.25 g/L Irradiation time: 8 hour	Ammonia	0.2 g/L rG-$BiFeO_3$ has shown the optimum results by giving 92.7% degradation of ammonia.	Zou et al., 2017

It can be seen in the table that photocatalysis using $BiFeO_3$ have shown remarkable degradation of organic compounds. The combination of $BiFeO_3$ with fenton like pretreatment has shown positive impacts on lignocellulose degradation. The direct photocatalysis using $BiFeO_3$ on lignocellulosic compounds has not been studied yet.

2.8 Mechanism of $BiFeO_3$

Photocatalysis starts when BFO absorbs light energy (*hv*) which is equal or greater than its band gap and generates e^- and h^+ pairs (Eq. 1). These photo-generated electrons react with oxygen that is absorbed by the surface and produce superoxide radicals (Eq. 2). The generated holes (h^+) react with water molecules and produce hydroxyl radicals (Eq. 3). After generation, superoxide radicals, hydroxyl radicals and holes take part in the degradation of substrate and produce CO_2, H_2O and other by products. (Eq. 4,5,6). In the whole process, photo-generated holes simultaneously move to the surface to react with water molecules and generate maximum hydroxyl radicals (Haruna, A., et al., 2020).

$$BFO + hv \rightarrow e^- + h^+ \quad \text{Eq. 1}$$

$$e^- + O_2 \rightarrow O_2^{\cdot -} + H_2O^2 \quad \text{Eq. 2}$$

$$h^+ + H_2O \rightarrow \cdot OH \quad \text{Eq. 3}$$

$$O_2^{\cdot -} + Substrate \rightarrow degradation\ products \quad \text{Eq. 4}$$

$$\cdot OH + Substrate \rightarrow degradation\ products \quad \text{Eq. 5}$$

$$h^+ + Substrate \rightarrow degradation\ products \quad \text{Eq. 6}$$

2.8.1 Schematic diagram of the mechanism

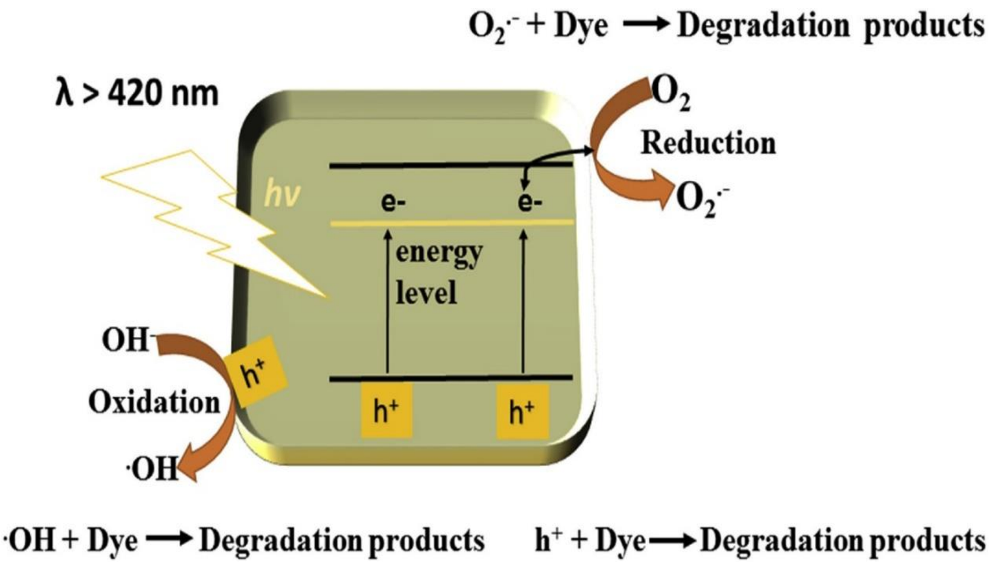

Figure 2:4 Schematic Diagram of BFO mechanism

2.9 Summary

The literature review has shown us the potential of rice straw and how the lignin cellulosic bonds are creating hindrance between its potential use. In order to fully utilize the cellulose for biogas production, pretreatment is necessary to solubilize lignin and hemicellulose. It has also seen that the alkaline pretreatment and photocatalysis using BFO is more environmental friendly because of their low toxicity, chemical stability, and high efficiency. Also, small band gap in case of catalyst as visible light is required for its activation. In current study, the efficiency of NaOH and BFO has witnessed using small quantities. Also, there combined effect was investigated.

CHAPTER 3

3 MATERIALS AND METHODS

In this chapter, the materials and methods will be described, which was used for catalyst preparation, rice straw pretreatment and characterization and biogas production.

3.1 Substrate and Inoculum

3.1.1 Collection and Preparation of Substrate

Rice straw was used as a substrate and it was collected from a rice field nearby Okara. It was air dried and shredded to get a particle size of $\leq$ 5 mm. To obtain a homogenized particle size, the shredded substrate was passed through a 5 mm sieve. After shredding, the substrate was air dried for 24 hours and stored in zip lock bags at ambient temperature.

3.1.2 Collection and Preparation of Inoculum

Fresh cow dung was used as an inoculum in the experiment. It was collected from the farm located in H-13, Islamabad, in front of NUST Gate-4. The degassing of the inoculum was done under anaerobic conditions for 21 days at 37^{o}C using water bath. After that, the digestate was stored at 4^{o}C.

3.2 Preparation of Bismuth Ferrite Oxide (BFO) Nanoparticles

$BiFeO_3$ nanoparticles were prepared using sol-gel method. In this, 7.7611 g of Bismuth (III) nitrate pentahydrate (reagent grade) and 6.4640 g of Iron (III) nitrate nanohydrate (reagent grade) were added in 36 ml ethylene glycol under continuous mixing using magnetic stirrer at room temperature. (Tang et al., 2019). After constant mixing for 1 hour, a colloidal solution of brownish red color was formed. The solution was kept in an oven for 48 hours at 80^{o}C. After drying, a xerogel powder was obtained which was calcinated at 400^{o}C for 30 minutes in the muffle furnace for the removal of organic compounds and nitrate ions. Before removing and bringing the sample to room temperature, it was kept at 500°C for 30 minutes. The formed particles were brought to room temperature and were washed multiple times using absolute alcohol and distilled water. Afterwards, the particles were again dried at 80°C (Wang et al., 2011). The obtained powder was cooled down in desiccator and then stored in glass bottles.

3.3 Characterization of Nanoparticles

3.3.1 X-ray Diffraction (XRD)

The XRD of nanoparticles was performed to find the crystal size of nanoparticles using X-ray diffractometer (Bruker, D8 Advance, Russia). The analysis was done at 0.15406 nm wavelength of Cu Kα radiation with 40 mA applied current and 40 kV voltage. The other parameters include 2θ range (10°-80°) and 0.02°/sec scan rate.

3.3.2 Scanning Electron Microscopy (SEM)

Morphology and particle size of the nanoparticles were determined through scanning electron microscope (JOEL, JSM-6490A, Japan) with 10 kV accelerating voltage. The SEM images were taken at 1 µm, 2 µm and 500 nm. Before the analysis, the particles were gold coated for good conduction.

3.3.3 Energy Dispersive X-ray Spectroscopy (EDX)

Energy Dispersive X-ray Spectroscopy (EDX) was performed to determine the purity of the compound by carrying out its elemental analysis through SEM-EDX (JOEL, JSM-6490A, Japan).

3.3.4 Energy band gap

The energy band gap of synthesized nanoparticles was determined using UV-vis spectroscopy (Total technology, T6OU, UK). The absorption spectrum of prepared nanoparticles was obtained under the range from 200 nm to 800 nm.

3.4 Pretreatment of substrate

3.4.1 Alkaline pretreatment of substrate

Different concentrations of NaOH were used to pretreat the rice straw. These concentrations were kept as 0.3, 0.6, 0.9 and 1.2 % w/v. The pretreatment was done at room temperature with a solid to liquid ratio of 1:50 for 3 hours. After pretreatment, the substrate was filtered using a conical flask. Washing of the filtrate with distilled water was done until the pH got normal. The substrate was further used for characterization and anaerobic digestion.

3.4.2 Photocatalytic pretreatment of substrate

Photocatalytic pretreatment was done with different concentrations of $BiFeO_3$ nanoparticles at room temperature. The concentrations of 0.125, 0.25, 0.5 and 1 g/L were used for the pretreatment of substrate under the constant stirring at 120 rpm. Air was also provided in the aqueous solution at a continuous flow rate of 2 ml/min to create natural conditions. For the mixture preparation, 30 g of rice straw was added in a 2 liter pyrex beaker, in which 1500 ml deionized water was added to attain a solid to liquid ratio at 1:50. At first, the mixture was kept in dark under constant stirring for 30 minutes to attain an adsorption-desorption equilibrium. Then the mixture was exposed to 60 Watt visible light for 3 hours. Afterwards, the nanoparticles were removed from the aqueous solution with the help of a magnet. The mixture was filtered and washed with distilled water to remove the remaining nanoparticles. The substrate was further used for characterization and anaerobic digestion.

3.4.3 Alkaline-Photocatalytic pretreatment of substrate

For the alkaline-photocatalytic pretreatment of substrate, all the concentrations of $BiFeO_3$ were combined with 0.6 % of NaoH (which showed the best results in alkaline pretreatment). To maintain a solid to liquid ratio at 1:50, 30 g of rice straw was added in 1500 ml of 0.6% NaOH solution. The mixture was kept in dark at 120 rpm and 2 mL min^{-1} air flow rate for 30 minutes at room temperature. Visible light of 60-watt was introduced for further 3 hours under the same conditions. The nanoparticles were removed, the mixture was filtered and washed until the pH get 7. The substrate was then used for anaerobic digestion and further characterization.

3.5 Characterization of Substrate

3.5.1 Total solids, volatile solids and moisture content

The total solids, volatile solids and moisture content was obtained following ASTM standards (ASTM, 2015). At first, empty china dish was placed in an oven for 15 mins at 105^oC. Then it was put in a desiccator to cool down. The empty china dish was weighed. Later, 2g of sample was put in a pre-weighed china dish and placed in the oven for 1 hour at 105^oC. The china dish was put in the desiccator for 15 mins to cool down the temperature and then weighed. This procedure was repeated until the constant weight of china dish with sample was achieved. The percentage of moisture content and total solids were obtained using the equation 3.1 and 3.2, respectively.

$$\mathrm{MC}(\%) = \frac{\mathrm{w2-w3}}{\mathrm{w2-w1}} \times 100 \qquad (3.1)$$

$$\mathrm{TS}(\%) = \frac{\mathrm{w3-w1}}{\mathrm{w2-w1}} \times 100 \qquad (3.2)$$

where,

w_1 = weight of empty china dish

w_2= weight of china dish and sample

w_3 = weight of china dish and sample after 105^oC

to measure the volatile solids, the sample was placed in a muffle furnace at 550^oc for 30 minutes. The volatile solids were evaporated and the remaining solid was ash content. Volatile solids were calculated by using the equation 3.3.

$$\mathrm{VS}\ (\%\ \mathrm{of\ TS}) = \frac{\mathrm{w3-w4}}{\mathrm{w3-w1}} \times 100 \qquad (3.3)$$

where,

w_4 = weight of china dish and sample after igniting at 550^oC

3.5.2 Determination of cellulose, hemicellulose and lignin

Cellulose, hemicellulose and lignin were determined by using chemical method as mentioned by Li et al. (2004). Extractives were removed through solvent extraction method by using soxhlet apparatus. Acetone was used as a solvent with a substrate to solvent ratio of 1:60. (60 ml acetone for 1 g of rice straw). Pre-dried rice straw (a) was placed in a soxhlet apparatus for 2 hours at 90^{o}C. After that, the sample was dried in the oven until the constant weight of extractives free rice straw (b) was obtained. Percent extractives removal were determined by using the equation 3.4.

$$\text{Extractives (\%)} = \frac{\text{a}-\text{b}}{\text{a}} \times 100 \qquad (3.4)$$

For determination of hemicellulose content, 1 gram of pre-dried extractives free rice straw (c) was added in 150 ml of 0.5 mol/L NaOH solution. The mixture was kept at 80^{o}C for 3.5 hours. After the mixture got cool, it was filtered and washed with deionized water to bring the pH at 7. The sample was dried in the oven till the constant weight (d) was achieved. Percent hemicellulose content was determined by using the equation 3.5.

$$\text{Hemicellulose (\%)} = \frac{\text{c}-\text{d}}{\text{c}} \times 100 \qquad (3.5)$$

For lignin, 1 gram of extractives free rice straw (e) was soaked in 30 ml of 98% H_2SO_4 for 24 hours at 8-15^{o}C. 300 ml of distilled water was added. The solution was boiled at 100^{o}C for 1 hour. After the solution was brought to room temperature, filtration was done. The filtrate was washed with deionized water until no sulphate ion was detected (The detection was done by using 10% Barium chloride solution). The sample was dried till the constant weight (f). Percent lignin was determined with the equation 3.6.

$$\text{Lignin (\%)} = \frac{\text{f}}{\text{e}} \times 100 \qquad (3.6)$$

It is assumed that the extractives, lignin, hemicellulose and cellulose are the only components of the entire biomass. So, cellulose was determined by with equation 3.7.

$$\text{Cellulose (\%)} = 100 - (\text{Extractives} + \text{Hemicellulose} + \text{Lignin}) \qquad (3.7)$$

3.5.3 Total organic carbon (TOC) and total kjeldahl nitrogen (TKN)

Total Kjeldahl Nitrogen (TKN) was determined by following the APHA standard methods (APHA, 2017). For total organic carbon, the formula given in the equation 3.8 was used (Adams et al.,1951).

$$\text{Organic Carbon (\%)} = \frac{\text{VS(\% of TS)}}{1.8} \qquad (3.8)$$

3.6 Anaerobic digestion design and setup

To determine the effect of pretreatments on rice straw, batch mode anaerobic digestion was done at lab scale. Pretreated rice straw along with inoculum and untreated rice straw was used for anaerobic digestion. 300 ml serum bottles with a working volume of 75% were used. Organic loading rate (OLR) of 10 gVS/L was kept for both substrate and inoculum. Maintaining the OLR, substrate and inoculum were added at a ratio of 1:1 on gVS basis. Distilled water was added to fill the remaining volume upto 75% of the bottles. pH of the mixture was neutralized by adding 1M solution of sodium bicarbonate. Subsequently, the bottles were covered with rubber septa and sealed with aluminum caps by crimping to ensure air tight conditions. Two ports in the rubber septum were created using syringes from the headspace of the bottles. From one port, the nitrogen gas was introduced for 2 mins while, oxygen was released from the other port in order to provide anaerobic environment in the reactor. Afterwards, the bottles were placed in the incubator for 75 days at 37°C under mesophilic conditions.

Triplicate bottles were prepared for each pretreatment concentration and raw substrate, which was used as a control group. Along with that, triplicates of inoculum were also prepared as a blank group in order to remove the endogenous biogas production of inoculum to eliminate errors. Each bottle was manually mixed and their biogas volume was measured on daily basis by using water displacement method in order to find out daily and cumulative biogas production (Yuan et al., 2019).

3.6.1 Analytical methods for anaerobic digestion experiment

The measured biogas was converted into normalized volume (NmL) on daily basis to present it as dry gas by using equation 3.9 (Dinuccio et al., 2010).

$$V_{NmL} = (V \times 273 \times (760 - P_w))/((273 + T) \times 760) \qquad (3.9)$$

Where;

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

V= Daily measured biogas volume (mL)

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

T= ambient temperature (ºC)

Different analyses were performed including pH, total alkalinity (TA) and volatile fatty acids (VFA) according to APHA standards (APHA, 2017) to assess the stability of the reactor before and after the anaerobic digestion. To determine the effect of pretreatment on solid removal VS was also measured before and after anaerobic digestion. To determine the methane content, sample of biogas was taken at 10th, 20th and 30th day of digestion. Methane content was measured using gas Chromatograph (Shimadzu, GC 2010 plus, Japan).

3.7 Statistical Analysis

All the characterization of substrate and inoculum were performed in triplicates. Their average and standard deviation were measured using spss (version 22). Single factor ANOVA was applied using orign (version 2019b) to determine the effect of pretreatments on cellulose increase of rice straw. For data validation, Modified Gompertz kinetic model was run on the experimental results of biogas production using the equation 3.9 (Mu et al., 2006; Orozco et al., 2013).

$$H(t) = H_m . exp\left\{-exp\left[\frac{R_m . e}{H_m}(\lambda - t) + 1\right]\right\} \quad (3.9)$$

Where,

H(t) = measured biogas yield (NmL /g VS) with respect to time (t).

Hm = predicted biogas yield (NmL /g VS) with respect to time (t)

Rm = maximum biogas production rate (NmL /g VS /d)

e = Euler’s function (2.72)

λ = lag time in biogas production (d)

t = time of anaerobic digestion (d)

CHAPTER 4

4 RESULTS AND DISCUSSIONS

This chapter includes the results of rice straw characterization before and after pretreatment with its impacts on biogas and methane yield production.

4.1 Characterization of synthesized $BiFeO_3$ nanoparticles

4.1.1 Scanning electron microscopy

SEM was performed to determine the average size of the $BiFeO_3$ nanoparticles. The images of SEM are shown in figure 4.1. The average size of $BiFeO_3$ nanoparticles was found to be 56 nm at 44.5 kx magnification. SEM results confirm the nano size of the particles.

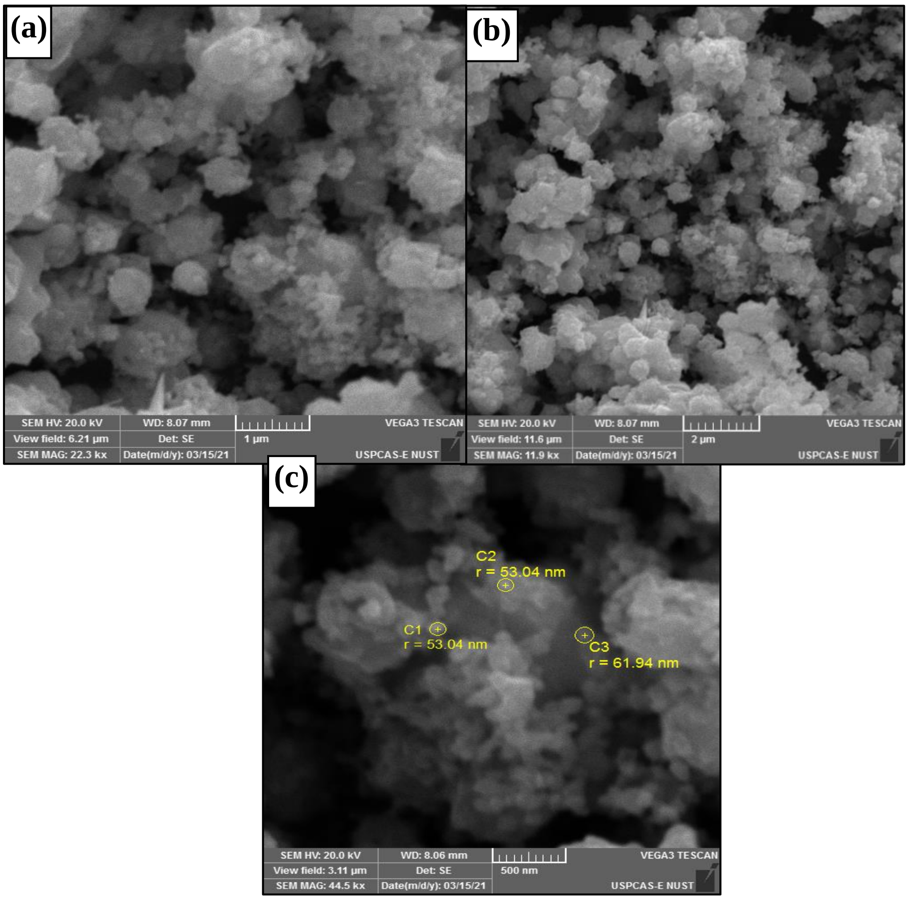

Figure 4:1 SEM images of synthesized $BiFeO_3$ nanoparticles at (a) 1 µm (b) 2 µm and (c) 500 nm

4.1.1 Energy dispersive X-ray spectroscopy

To determine the elemental composition and purity of $BiFeO_3$ nanoparticles energy dispersive x-ray spectroscopy was done. The image of elemental analysis through EDS is shown in figure 4.2. The result has confirmed the presence of bismuth, iron and oxygen with elemental composition of 47%, 17.3% and 29.67%, respectively. A trace amount of carbon was also evident in the EDS image which could be due to the carbon tape used to support the sample for the analysis.

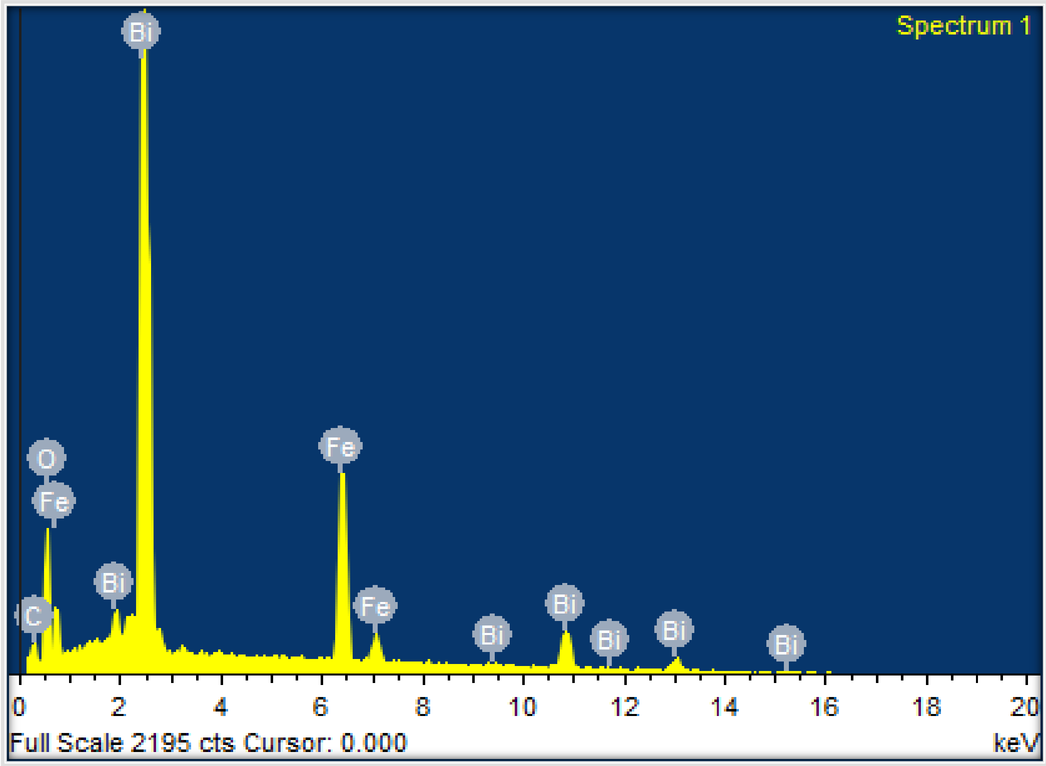

Figure 4:2 EDS analysis of synthesized $BiFeO_3$ nanoparticles

4.1.2 X-ray diffraction (XRD)

The structure and crystallite size of prepared $BiFeO_3$ nanoparticles was determined through XRD. The XRD spectra is shown in figure 4.3. All the diffraction peaks and intensity matched well with literature and can be indexed to perovskite rhombohedral structure (Jaffari et al., 2020; Jaffari et al., 2019; Sazali et al., 2019).

The crystalline size of nanoparticles was determined using Debye-Scherer equation. The average crystalline size was found to be 16.6 nm which was consistent with the particle size found by SEM.

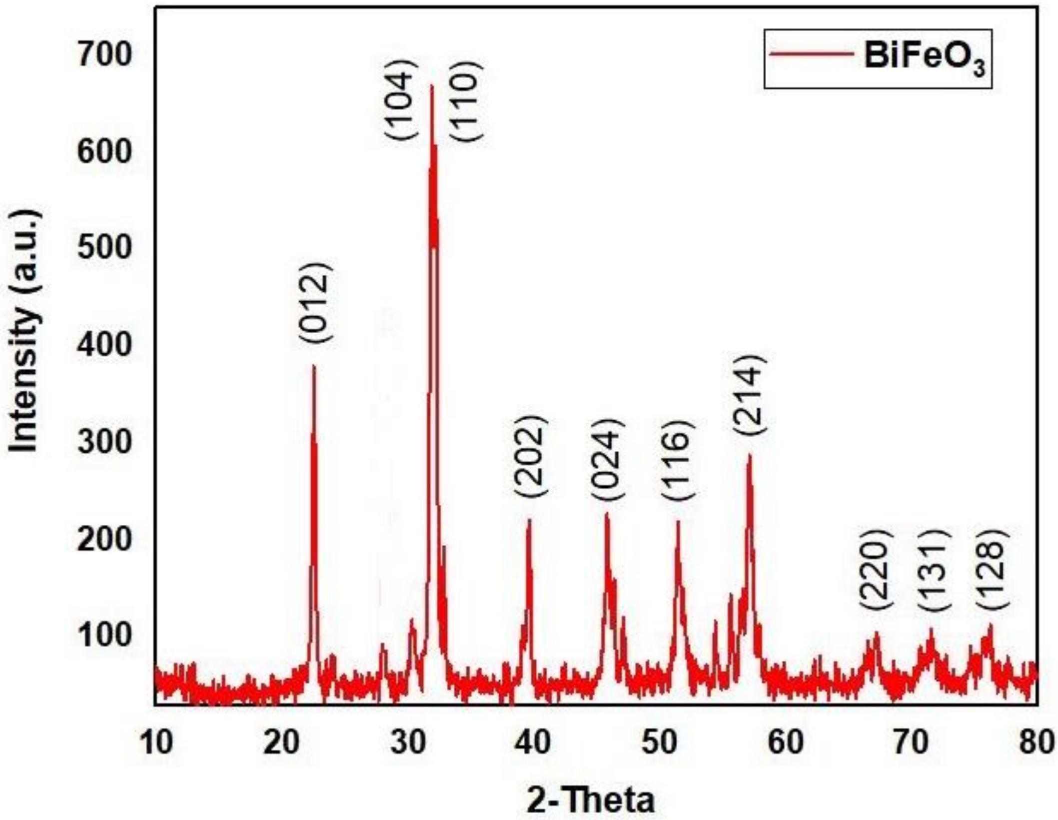

Figure 4:3 XRD spectra of synthesized BiFeO3 nanoparticles

4.1.2 Energy band gap

Energy band gap of synthesized $BiFeO_3$ nanoparticles was determined through linear extrapolation using data of UV-Vis spectroscopy. The energy band gap was found to be 2.07 eV as shown in figure 4.4. This small band gap shows that $BiFeO_3$ nanoparticles may perform promisingly under visible light range. Tang et al. (2019) reported the band gap of pure $BiFeO_3$ nanoparticles as 2.09 eV which is close to the band gap of nanoparticles prepared in the present study.

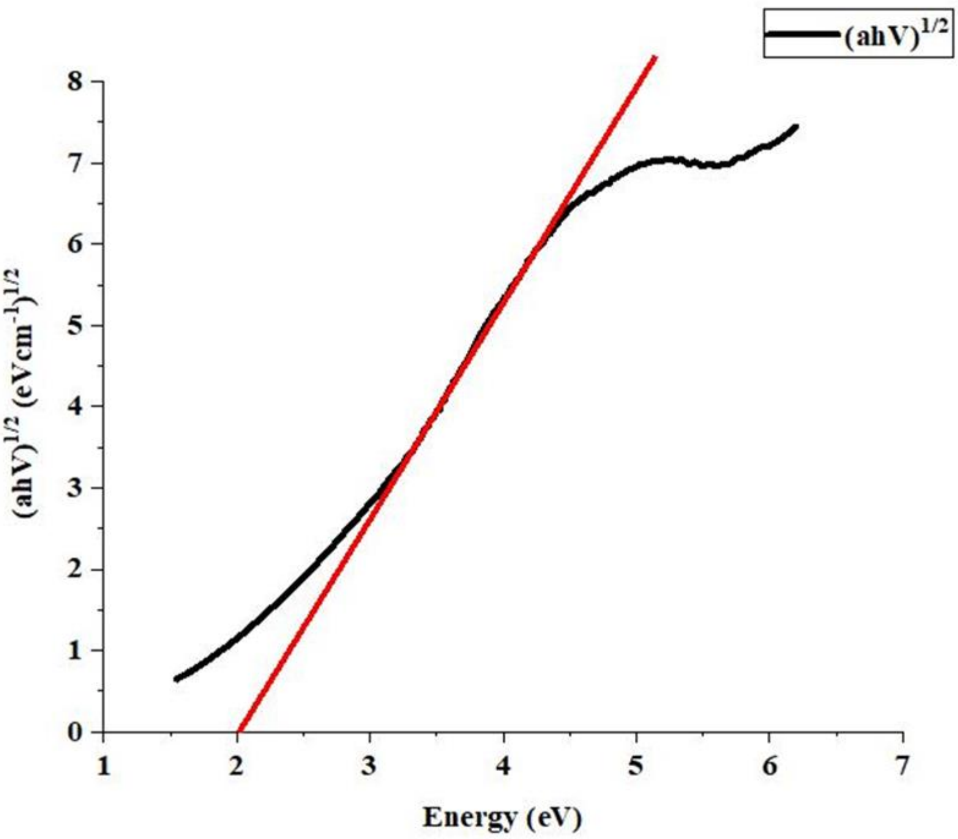

Figure 4:4 Energy band gap of synthesized BiFeO₃ nanoparticles

4.2 Characterization of rice straw and cow dung

Initial characterization of rice straw and cow dung are shown in table 4.1. Both substrate and inoculum have high volatile solids of 83.47% and 84.33%, respectively that favored the biogas production through anaerobic digestion. Haryanto et al. (2018) characterized rice straw and cow dung for anaerobic digestion and reported TS, VS, TOC and TKN for cow dung as 29%, 74.96%, 39.87% and 1.42% respectively. While, for rice straw TS, VS, TOC and TKN were 89%, 71.52%, 38.55% and 0.58% respectively. The lignocellulosic analysis of rice straw had shown that approximately 26% of cellulose in rice straw was present which served as a food for microbes during anaerobic digestion. Syaftika et al. (2018) reported the results of lignocellulosic composition of rice straw as 28% cellulose, 55% hemicellulose and 11% lignin which supports the results of current study.

Table 4.1 Characteristics of rice straw and cow dung

Parameters	Unit	Substrate	Inoculum
Total Solids (TS)	%	94.38 ± 0.03	14.03± 0.01
Volatile Solids (VS)	%TS	83.47 ± 0.003	84.33± 0.46
Total Kjeldahl Nitrogen (TKN)		0.22 ± 0.06	2.1 ± 1.07
Total Organic Carbon (TOC)		46.37 ± 0.002	46.85 ± 0.26
Extractives		3.31 ± 0.01	---
Lignin		21.35 ± 2.9	---
Hemicellulose		49.14 ± 0.08	---
Cellulose		26.2 ± 2.81	---

4.3 Effect of $BiFeO_3$, NaOH and combine ($BiFeO_3$ + NaOH) pretreatment on rice straw composition

4.3.1 Effect of $BiFeO_3$ photocatalytic pretreatment on rice straw composition

The effect of photocatalytic pretreatment using $BiFeO_3$ nanoparticles on rice straw is shown in figure 4.4. Pretreatment of rice straw with all the doses of $BiFeO_3$ nanoparticles (0.125, 0.25, 0.5 and 1 g/L) showed positive effect on cellulose increase through removal of lignin and hemicellulose. The optimum results were observed at dose 0.25 g/L of $BiFeO_3$ nanoparticles showing 68.32% cellulose increase with 7.06% and 64.17% removal of hemicellulose and lignin respectively. The higher dose of nanoparticles from 0.25 g/L to 1 g/L reduced the efficiency of photocatalysis showing less removal of lignin and hemicellulose. The cellulose increase was decreased from 68.3% to 45% and 31.8% when the doses of $BiFeO_3$ nanoparticles were increased to

0.5 g/L and 1 g/L respectively. This could be due to the high dose of nanoparticles might have blocked the light to pass through the solution and activate the other nanoparticles (Mahdavi et al., 2022; Chang et al., 2018). Sabeeh et al. (2020) pretreated rice straw with 0.25 g/L titania nanoparticles for 3 hours at room temperature with solid to liquid ratio of 1:20 and reported the cellulose increase of 14.17% with 16.62% and 9.79% removal of hemicellulose and lignin respectively.

Bismuth ferrite ($BiFeO_3$) has been proved as a proficient photocatalyst for degradation of organic compounds including antibiotics (Tang et al., 2018), ammonia (Zou et al., 2017), phenol, bacterial and fungal elimination (Jaffari et al., 2020). Jaffari et al. (2020) studied the photocatalytic degradation of phenol and malachite green dye with 1 g/L pladium (Pd) doped bismuth ferrite ($BiFeO_3$) nanocomposites for 4 hours at room temperature and reported 95.7% and 100% removal of malachite green dye and phenol respectively.

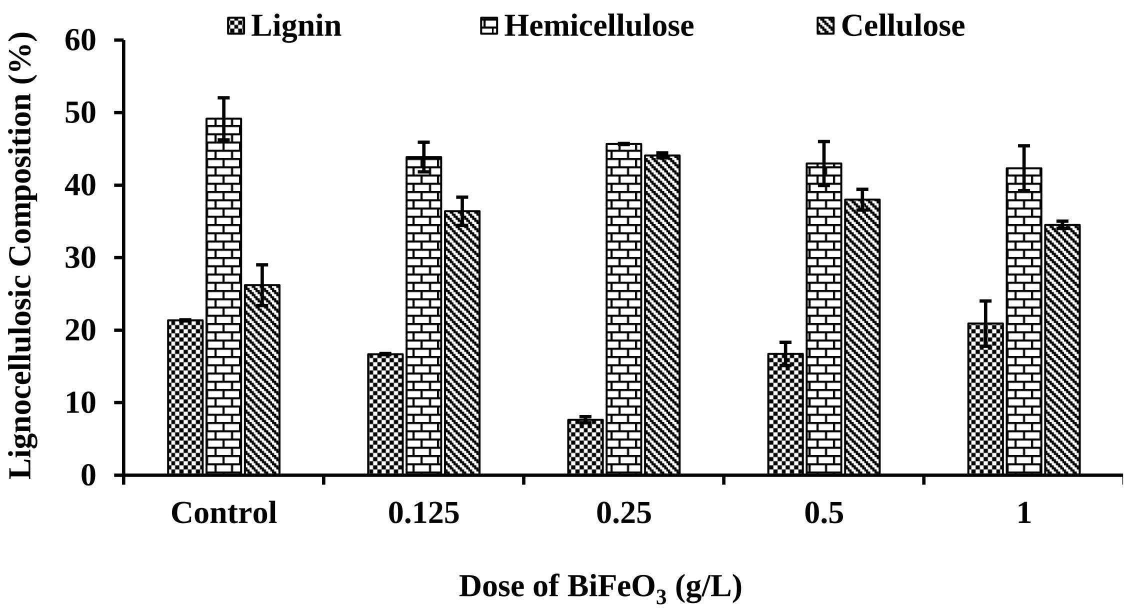

Figure 4:5 Effect of BiFeO3 nanoparticles photocatalytic pretreatment on rice straw

Photocatalysis works by generating free $OH^{\bullet}$ radicals exhibiting high oxidative properties which breaks the recalcitrant structure of hemicellulose and lignin (M'Arimi et al., 2020). Photocatalytic degradation of lignocellulosic material using only $BiFeO_3$ nanoparticles has not been studied yet. One-way ANOVA showed significance increase of cellulose after pretreatment with all doses of $BiFeO_3$ nanoparticles ($p < 0.05$).

4.3.2 Effect of NaOH pretreatment on rice straw composition

Effect of alkaline (NaOH) pretreatment on lignocellulosic composition of rice straw is shown in figure 4.5. Pretreatment with each concentration of NaOH i.e. 0.3, 0.6, 0.9 and 1.2% showed remarkable increase in cellulose content with high removal of lignin and hemicellulose. The optimum results with maximum cellulose increase of 135.2% were obtained at concentration of 0.6% showing lignin and hemicellulose removal of 25.62% and 55.55%, respectively. The efficiency of NaOH pretreatment in degradation of lignocellulosic compounds have been reported in various studies (Samar et al., 2021; Khalid et al., 2019). Samar et al. (2021) pretreated rice straw with 1% (w/v) NaOH at 121^{o}C for 30 minutes with 1:10 solid to liquid ratio and reported the cellulose increase of 61.19% with 37.51% lignin removal. Khalid et al. (2019) also reported significant increase of 102% in cellulose content with 88% lignin and 29% hemicellulose removal after pretreatment of rice straw with 2% (w/v) NaOH at 37^{o}C for 5 days with 1:15 solid to liquid ratio. The degradation of lignocellulose compounds through alkaline pretreatment works by swelling the cellulose and remodeling the lignin structure through breaking the ester bonds and glycosidic linkages present in the cell wall of the compound. This also breaks down the 3-D recalcitrant structure of lignin and hemicellulose (Fu et al., 2018; Paudel et al., 2017) exposing cellulose for microbial degradation which increases the biogas and methane yield. The analysis of variance (ANOVA) showed that the cellulose increase in rice straw pretreated with all concentrations of NaOH is significant ($p < 0.05$).

It was observed that with the increase of NaOH concentration to 0.9% and 1.2%, the lignin removal was reduced to 16.6% and 14.3%, while hemicellulose removal was reduced to 46% and 32.3%, respectively. As a result, cellulose increase was also reduced to 110% and 81.3%, respectively. The decrease in efficiency of NaOH pretreatment with increasing concentration after certain point has also been reported in previous studies (Saratale et al., 2020; Sabeeh et al., 2020; Shetty et al., 2017). Saratale et al. (2020) reported that no further decrease in lignin was observed with the increase of NaOH concentration from 2% to 3% (w/v) during the pretreatment of wheat straw at 100^{o}C with solid to liquid of 1:4 for 30 minutes. Ciftci et al. (2020) also reported the reduction of NaOH efficiency by increasing the concentration to 20% (w/v) during pretreatment of canola straw at different temperatures of 25^{o}C, 50^{o}C and 75^{o}C with solid to liquid ratio of 1:20 for 2 hours. It was reported that no significant difference was observed in lignin and hemicellulose removal when concentration of NaOH was increased from 15% to 20% (w/v). This could be due to excessive swelling of microfibers at high concentrations of NaOH that might prevent separation of hemicellulose from fibrous structure of the cell (Rambabu et al., 2016).

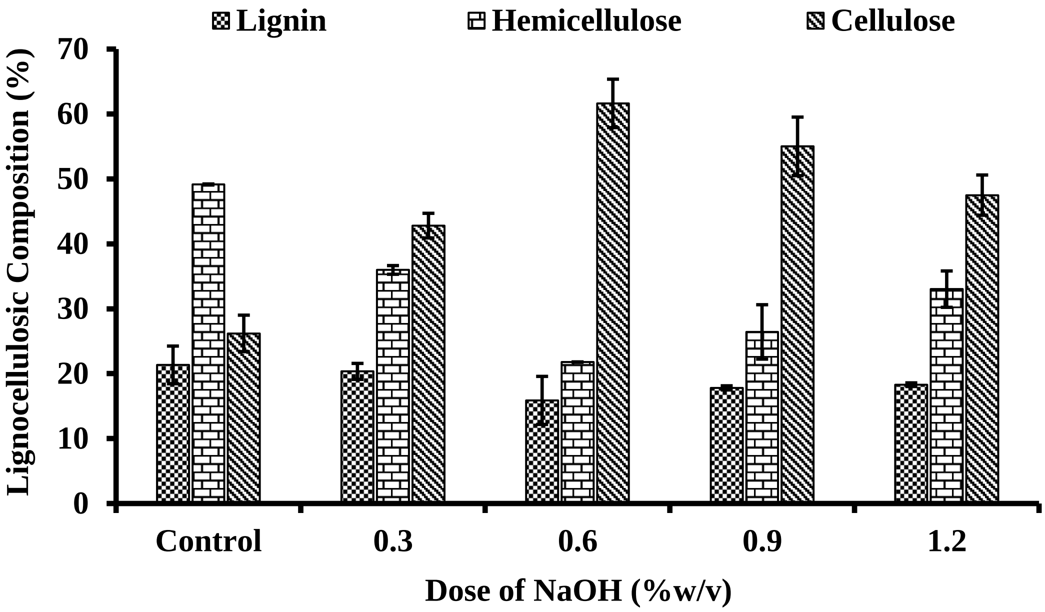

Figure 4:6 Effect of NaOH pretreatment on rice straw composition

4.3.3 Effect of combined ($BiFeO_3$ + NaOH) pretreatment on rice straw composition

Effect of combined pretreatment ($BiFeO_3$ + NaOH) on lignocellulosic composition of rice straw is shown in figure 4.6. It was observed that the combination of doses of $BiFeO_3$ nanoparticles (0.125. 0.25. 0.5 and 1 g/L) with 0.6% (w/v) NaOH showed better results of cellulose increase (from 91.4% to 144.4%) than the separate pretreatment of $BiFeO_3$ and NaOH. This might be because of the synergistic effect of two mechanisms of photocatalysis and NaOH pretreatment involved in the degradation of lignocellulosic compound. The decrease in cellulose crystallinity and alteration of lignin structure caused by alkaline pretreatment might aided the interaction of $OH^{\bullet}$ radicals, generated through photocatalysis of $BiFeO_3$ nanoparticles, enhancing the oxidization of lignin and hemicellulose (Fu et al., 2018; Paudel et al., 2017).

The optimum dose of combine pretreatment was observed to be 0.125 g/L $BiFeO_3$ nanoparticles and 0.6% NaOH that showed 144% cellulose increase with 50.2% and 51.59% removal of lignin and hemicellulose, respectively. When the amount of nanoparticles was increased from 0.1 to 1 g/L the lignin and hemicellulose removal started to reduce from 50.2% to 2% and 51.6% to 42.9%, respectively. Cellulose increase was also reduced from 144.4% to 91.4%. This could be as nanoparticles might have sticked together in the presence of NaOH and failed to attach to the substrate surface. Besides the fact, the cellulose increase of 144.4% with combine treatment is 3.9% and 45.2% more than the individual pretreatment with NaOH and $BiFeO_3$. ANOVA showed that the combine pretreatment showed significant ($p < 0.05$) increase in cellulose at all doses.

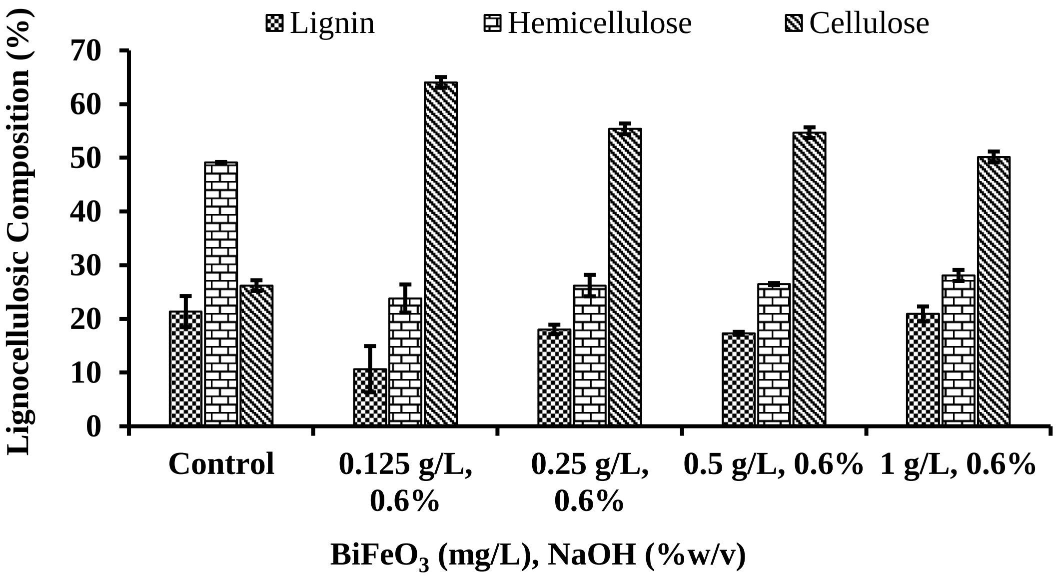

Figure 4:7 Effect of combine ($BiFeO_3$ + NaOH) pretreatment on rice straw composition

Sabeeh et al. (2020) studied the effect of combine pretreatment using NaOH and TiO_2 nanoparticles on rice straw and reported the cellulose increase of 94% with 66% and 75% removal of lignin and hemicellulose respectively, after the pretreatment of rice straw with 0.25 g/L TiO_2 and 1.5% NaOH at 37°C for 3 h.

$BiFeO_3$ has been reported to be used as fenton-catalyst with H_2O_2 to degrade phenol and lignocellulosic compounds like sugarcane baggase (Zhang et al., 2019; Soltani et al., 2014). Zhang et al. (2019) reported that bismuth ferrite ($BiFeO_3$) assisted fenton like pretreatment of sugar baggase showed better results with high lignin and hemicellulose removal than the fenton like pretreatment alone. The cellulose increase of bismuth ferrite ($BiFeO_3$) assisted fenton like pretreatment was reported to be 7.2% more than the simple fenton like pretreatment. The combination of $BiFeO_3$ with NaOH has not been reported yet.

4.4 Effect of $BiFeO_3$, NaOH and combine ($BiFeO_3$ + NaOH) pretreatment on cumulative biogas production

4.4.1 Effect of $BiFeO_3$ photocatalytic pretreatment on cumulative biogas production

Cumulative biogas production from untreated rice straw and rice straw pretreated with $BiFeO_3$ nanoparticles is shown in figure 4.7. All the doses showed increase in the biogas production. The cumulative biogas production from untreated rice straw was observed to be 409.9 NmL/g VS. In case of pretreated rice straw, the cumulative biogas production of 457.5, 499, 428.2 and 430.4 NmL/g VS was observed for the doses of 0.1, 0.25, 0.5 and 1 g/L. The highest biogas production shown by 0.25 g/L was observed to be 21.7% more than the untreated rice straw while the higher doses showed lesser biogas production. This was due to more lignin removal and high cellulose increase after the pretreatment with 0.25 g/L of $BiFeO_3$ nanoparticles.

The similar trend of less biogas production at high catalyst doses has been reported in studies (Alvarado-Morales et al., 2017; Sabeeh et al., 2020). Alvarado-Morales et al. (2017) observed 37% increase in cumulative methane yield from the rice straw pretreated at 1.5% (w/w) TiO_2 nanoparticles for 3 h and reported no significant difference at the increase of dose to 2%(w/w). Sabeeh et al. (2020) reported 30% increase of cumulative biogas production from the rice straw pretreated with 0.25 g/L TiO_2 nanoparticles for 3 h and reported lesser biogas production at higher dose of nanoparticles. This could be because of lesser removal of lignin at higher doses as more amount of nanoparticles might have blocked the light to pass through the solution and most of the particles remained inactive (Mahdavi et al., 2022; Chang et al., 2018).

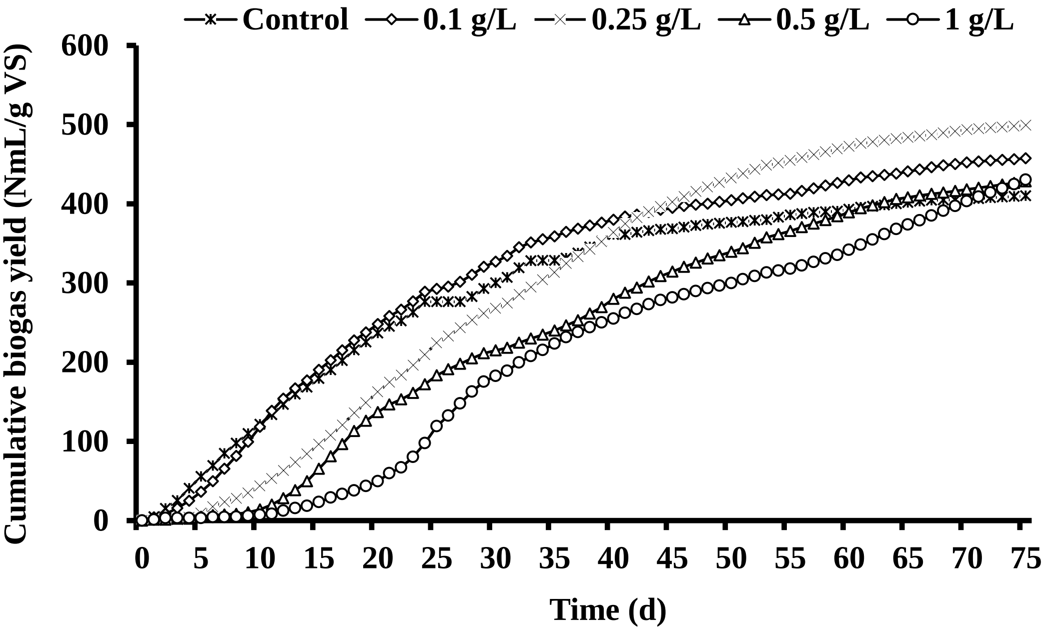

Figure 4:8 Effect of BiFeO3 photocatalytic pretreatment on cumulative biogas production

4.4.2 Effect of NaOH pretreatment on cumulative biogas production

The cumulative biogas yield of rice straw pretreated with different concentrations of NaOH is given in figure 4.8. All the concentrations showed positive effect on biogas production due to more cellulose availability to microorganisms during anaerobic digestion. The cumulative biogas yield of rice straw pretreated with 0.3, 0.6, 0.9 and 1.2% NaOH was observed to be 421.13, 489.43, 486.91 and 431.26 Nml/g VS.

The biogas production of 0.6% was observed to be optimum, followed by 0.9% showing biogas enhancement of 19.4% and 18.8% respectively. With the increase in concentration from 0.9% to 1.2% the biogas enhancement was also reduced from 18.8% to 5.2% due less lignin and hemicellulose removal after pretreatment.

Shetty et al. (2017) reported the optimum biogas production from the rice straw pretreated at 1% (w/v) NaOH at room temperature for 3 hours and reported the decrease in biogas yield when the NaOH concentration was increased to 2, 3, 4 and 5%. The current study has also showed the optimum results at the concentration of 0.6% and

0.9% which are closer to 1%. Khalid et al. (2019) observed the optimum biogas enhancement of 53.5% at 2% (w/v) NaOH concentration and reported the decreases in biogas yield with the increase in NaOH concentration (4, 6, 8, 10%). This could be due to excessive swelling of microfibers at high concentrations of NaOH that might prevent separation of hemicellulose from fibrous structure of the cell (Rambabu et al., 2016)

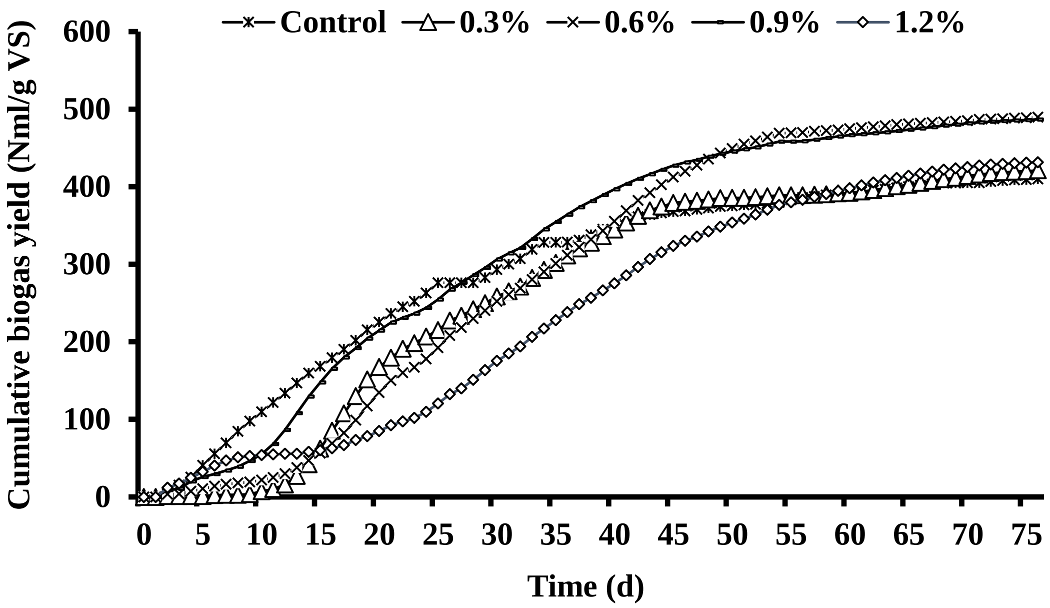

Figure 4:9 Effect of NaOH pretreatment on cumulative biogas production

4.4.3 Effect of combine ($BiFeO_3$ + NaOH) pretreatment on cumulative biogas production

The cumulative biogas yield of rice straw pretreated with $BiFeO_3$ (0.125, 0.25, 0.5 and 1 g/L) and 0.6% NaOH is shown in figure 4.9. It was observed that high doses of $BiFeO_3$ with NaOH showed negative impact on biogas production. Maximum biogas production of 511.97 Nml/g VS was observed at 0.125 g/L + 0.6%, which is the minimum dose of $BiFeO_3$ nanoparticles combined with 0.6% NaOH. While, the increased amount of $BiFeO_3$ nanoparticles (0.25, 0.5 and 1 g/L) showed cumulative biogas production 377.6, 338.4 and 272 Nml/g VS, which is 7.9, 17.4 and 33.7% less than the control group, respectively. This might be because $BiFeO_3$ nanoparticles got attached to the rice straw in the alkaline solution and remained stick after washing. As a result, particles might have transferred to the anaerobic digesters and caused toxicity, constraining the biogas production. Different studies have been reported on the inhibitory effect caused by metal ions in anaerobic digestion (Otero-González et al., 2014; Gonzalez-Estrella et al., 2013; Wang et al., 2012; Vodovnik et al., 2012). Gonzalez-Estrella et al. (2013) observed the inhibition effect of CuO and ZnO nanoparticles during the anaerobic digestion and reported the release of heavy metal ions (Cu^+, Zn^+) due to dissolution or corrosion of nanoparticles. The IC_{50} (half-maximal inhibitory concentration) for Cu^0, CuO and ZnO nanoparticles was reported to be 62-250 mg/L. The density of bismuth (9.79 g/cm^3) is reported to be more than copper (8.96 g/cm^3) and zinc (7.14 g/cm^3). Wang et al. (2012) reported the inactivation of bacteria due to bismuth vanadate nanotubes at concentration of 100 mg/L under anaerobic conditions by destructing the cell wall and cellular components. In the present study, the lowest biogas production with largest lag phase of 11 days was observed at the highest dose of 1 g/L bismuth ferrite with 0.6% NaOH because of the transfer of more residues of nanoparticles in the digester.

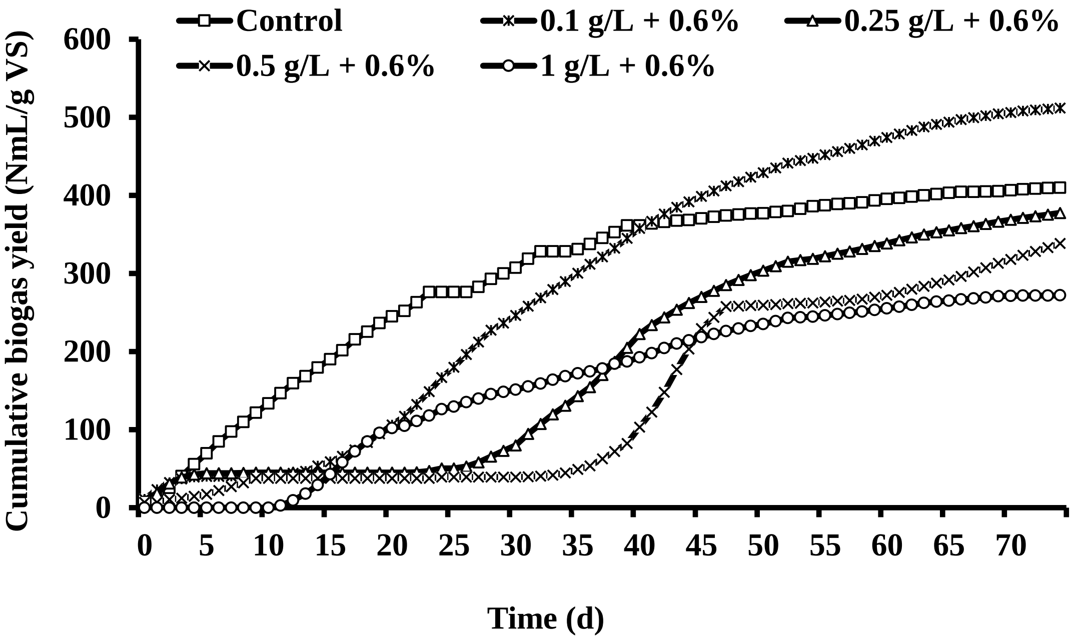

Figure 4:10 Effect of combine (BiFeO3 + NaOH) pretreatment on cumulative biogas production

Besides the fact, the cumulative biogas production from the combine pretreatment of 0.1 g/L + 0.6% was observed to be 25% more than the control group. In comparison to optimum doses of $BiFeO_3$ and NaOH pretreatment, the combine pretreatment ($BiFeO_3$ + NaOH) enhanced 2.5% and 4.7% more biogas respectively. The efficiency of $BiFeO_3$ in combination with fenton like pretreatment has been observed in the previous studies

(Zhang et al., 2019; Li et al., 2021). Zhang et al. (2019) reported that the efficiency of combined pretreatment of $BiFeO_3$ and fenton like pretreatment of sugarcane baggase at $60^{o}C$ for 72 h in reducing sugar yield was 2.4 fold more than the conventional fenton like pretreatment.

4.5 Effect of pretreatment on cumulative methane yield

Effect of $BiFeO_3$, NaOH and combine ($BiFeO_3$ + NaOH) pretreatment on cumulative methane yield for 75 days is shown in figure 4.10. The cumulative methane yield of untreated rice straw was observed to be 247.2 Nml/g VS. In case of rice straw pretreated with $BiFeO_3$ nanoparticles, highest methane yield of 345.8 Nml/g VS was observed at 0.25 g/L which is 39.9% more than the control group. This significant enhancement of methane yield was due to more availability of cellulose for microbial digestion after pretreatment. The dose 0.125, 0.5 and 1 g/L produced cumulative methane yield of 286.5, 293 and 279.8 Nml/g VS respectively. The higher doses produced lesser cumulative methane yield because of less removal of lignin and hemicellulose as high amount of nanoparticles blocked the light to pass through the solution (Mahdavi et al., 2022; Chang et al., 2018).

For alkaline (NaOH) pretreatment, all the doses have shown positive results in the enhancement of methane yield. The maximum methane yield enhancement was observed to be 40.3% at concentration of 0.6% which produced 346.7 Nml/g VS cumulative methane yield. The concentrations of 0.3, 0.9 and 1.2% produced 272.5, 322.8 and 274.7 Nml/g VS cumulative methane yield respectively. The increasing concentrations of NaOH (0.9% and 1.2%) reduced the methane yield this could be due to excessive swelling of microfibers at high concentrations of NaOH that might prevent separation of hemicellulose from fibrous structure of the cell (Rambabu et al., 2016).

In case of combined ($BiFeO_3$ + NaOH) pretreatment, the highest methane yield of 381.1 Nml/g VS was observed at the combination of 0.125 g/L $BiFeO_3$ and 0.6% NaOH. This optimum dose of combined pretreatment showed methane yield enhancement of 54.3% which is 10.3% and 10% more than the optimum doses of $BiFeO_3$ and NaOH pretreatment alone. Increased dose of $BiFeO_3$ nanoparticles in combination with 0.6% NaOH showed negative results in the methane yield production. The dose of 0.25, 0.5 and 1 g/L produced methane yield of 163.1, 184.2 and 146.7 Nml/g VS which is 34, 25.3 and 40.7% less than the control group respectively. This could be possible because of the toxicity caused by bismuth during anaerobic digestion after releasing metal ion (Bi^+) due to dissolution of nanoparticle (Gonzalez-Estrella et al., 2013).

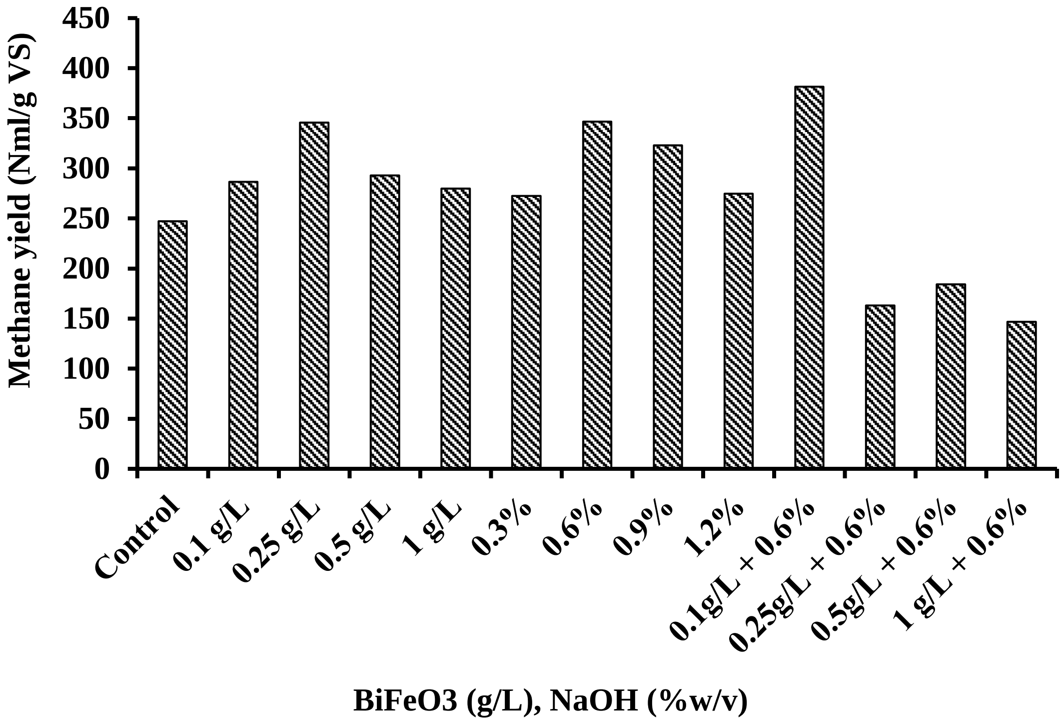

Figure 4:11 Effect of pretreatment on cumulative methane yield

4.6 Effect of pretreatment on solid (TS and VS) removal

The effectiveness of anaerobic digestion is determined through volatile solid (VS) removal. The effect of $BiFeO_3$, NaOH and combine ($BiFeO_3$ + NaOH) pretreatment on VS removal is shown in figure 4.11. The VS removal of control group after 75 days of biogas production was observed to be 24.28%. The maximum VS removal of all pretreatments was observed from combined pretreatment of 0.125 g/L $BiFeO_3$ with 0.6% NaOH showing 57.56% VS removal. This removal of VS was observed to be 136.6% more than the control group. For photo-catalytic ($BiFeO_3$) pretreatment, maximum VS removal of 41% was shown by 0.25 g/L. While, for alkaline (NaOH) pretreatment, 0.6% showed the highest VS removal of 50.11%. The increase in solid removal after pretreatment was reported in previous studies (Maryam et al., 2021; Sabeeh et al., 2020; Khalid et al., 2019). Maryam et al. (2021) reported the increase of VS removal upto 7.9% after pretreatment of dewatered waste activated sludge (DWAS) with 0.6 g/L TiO_2 at 37°C for 3 h under UV light. Khalid et al. (2019) reported 21% more VS removal from the rice straw pretreated with 2% NaOH at 37°C for 5 d.

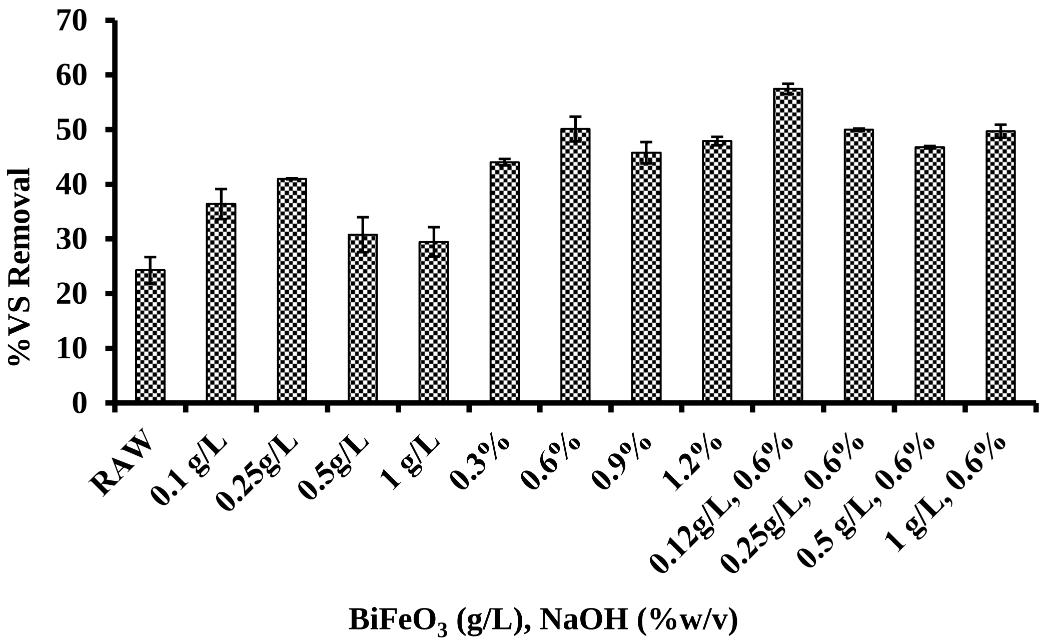

Figure 4:12 Effect of pretreatment on solid removal

Overall, the VS removal were in accordance to the biogas and methane production. The high VS removal at 0.25 g/L, 0.6% and 0.125 g/L + 0.6% concentrations showed that more solids were utilized in the biogas and methane production due to more availability of cellulose.

4.7 Effect of pretreatments on reactor stability

The reactor stability parameters including pH, alkalinity, volatile fatty acids (VFA) and VFA/alkalinity is shown in table 4.2. pH is one of the most important parameter in reactor stability. It was observed that all the reactors after anaerobic digestion was in neutral range showing the proper working of methanogens. The optimum pH for the working of methanogens is reported to be 6.8-7.2 (Ye et al., 2013) VFA/TA ratio is another important factor to determine the reactor stability. VFA/TA ratio of all the reactors were ranging from 0.13-0.69. For reactor stability the optimum VFA/alkalinity is recommended to be < 0.4 (Wang et al., 2016).

Table 4.2 Effect of pretreatments in reactor stability

Pretreatment doses		pH	TA (mg/L)	VFA (mg/L)	VFA/TA
$BiFeO_3$ (g/L)	**NaOH %w/v**				
Raw RS		7.14	800	175	0.22
0.125	-	7.20	775	225	0.29
0.25	-	7.25	1125	200	0.18
0.5	-	7.12	975	225	0.23
1	-	7.13	1300	825	0.63
-	0.3	7.28	875	125	0.14
-	0.6	7.45	1125	175	0.16
-	0.9	7.39	950	150	0.16
-	1.2	7.51	1200	150	0.13
0.125	0.6	7.54	1250	425	0.34
0.25	0.6	7.30	3175	1975	0.62
0.5	0.6	7.32	2450	1700	0.69
1	0.6	7.17	1650	875	0.53

In the study, most of the reactors, with high biogas production, were observed to be < 0.4, showing the stability of the reactors while, the ones with high amount of $BiFeO_3$ nanoparticles (1 g/L, 0.25 g/L + 0.6%, 0.5 g/L + 0.6% and 1 g/L + 0.6%) showed VFA/TA ratio of 0.63, 0.62, 0.69 and 0.53 representing the unstable behavior of the reactors. This could be due to the toxicity caused by Bi^+ metal ions released after dissolution of nanoparticles (Gonzalez-Estrella et al., 2013).

4.8 Biogas production data validation for raw and pretreated rice straw

Modified Gompertz Kinetic model was run on the measured biogas data (Hm) to check the data validation. The results of kinetic model for all the pretreatments is shown in table 4.3.

Table 4.3 Kinetic parameters of Modified Gompertz Model for pretreated rice straw

Pretreatment Doses		Hm (Nml/g VS)	Hp (NmLgVS)	λ (days)	Rm (Nml/g VS/d)	R^2
$BiFeO_3$ (g/L)	NaOH %w/v					
Raw RS		409.9	400.68	0.5	15	0.995
0.125	-	457.5	437.7	0.9	19.9	0.992
0.25	-	499.0	499.02	2.8	15.2	0.999
0.5	-	428.2	420.56	4.1	16.6	0.992
1	-	430.4	394.65	7.3	21.6	0.988
-	0.3	380.8	363.12	4	23.39	0.992
-	0.6	490.5	498.42	0.4	18.5	0.998
-	0.9	487.2	484.05	0.15	21.6	0.997
-	1.2	431.2	444	0.18	12.3	0.994
0.125	0.6	511.9	512.9	0.12	18.2	0.997
0.25	0.6	377.5	380.9	0.14	18.1	0.974
0.5	0.6	333.7	309.6	0.17	29.1	0.954
1	0.6	272.1	267.49	11.4	15.1	0.989

It can be seen from the data that the predicted values (Hp) match well with the measured values showing coefficient correlation between 0.954-0.999. The maximum lag phase (λ) of 7.3 and 11.4 days was observed for the reactors having maximum amount of $BiFeO_3$ nanoparticles i.e. 1 g/L and 1 g/L,0.6% respectively. This was because of the inhibition caused by bismuth in nanoparticles. It was observed that the lag phase (λ) of untreated rice straw was shorter as compared to many pretreated ones due to lack of inhibitors (Liu et al., 2015). Rajput et al. (2018) compared different kinetic models to check the best fit to the produced results. It was reported that, modified Gomperz model showed better fitting to the produced biogas data rather than logistic functions and transference models.

CHAPTER 5

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

- Dose of 0.25 g/L bismuth ferrite was observed to be optimum with 39.9% methane yield enhancement. This enhancement is due to 68.32% cellulose increase after the pretreatment.
- The concentration of 0.6% NaOH was observed to be optimum with 135.23% cellulose increase and enhancement of methane yield upto 40.3%
- The combine dose of 0.125 g/L bismuth ferrite with 0.6% NaOH showed maximum cellulose increase of 144.4% among all the pretreatments. Because of this, methane yield enhancement of 54.3% was observed to be maximum among all other pretreatments.
- Data fitting of Modified Gompertz Kinetic Model showed that the predicted values (Hp) match well with the measured values showing the validation of the biogas data.

5.2 Recommendations

Based on the study, following recommendations are made.

- In combine pretreatment, effect of lower doses of bismuth ferrite nanoparticles should be studied.
- Effect of bismuth ferrite nanoparticles on other lignocellulosic material should be studied.
- Further research should use stronger neodymium magnet to separate bismuth ferrite magnetic nanoparticles.
- After combine pretreatment, first wash the rice straw properly then recover $BiFeO_3$ with magnet to maximize recovery of nanoparticles.

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