# Effect of varying organic loading on the production of biogas from pretreated wheat straw in batch reactor

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

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# Dedication

*This research is dedicated to my loving, caring, and industrious 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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Praised be Allah! [27:59] and: If you would count the blessings of Allah, you would not be able to reckon them, [14:34] and: Of the blessings of your Lord, speak out, [93:11] and: Remember Me, and I will remember you, give thanks to Me, [2:152]

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# LIST OF ABBREVIATION OR KEYWORDS

| | |
|---|---|
| AD | Anaerobic Digestion |
| CD | Cow Dung |
| MC | Moisture Content |
| NaOH | Sodium Hydroxide |
| OL | Organic Loading |
| TA | Total Alkalinity |
| $TiO_2$ | Titanium Dioxide |
| TS | Total Solids |
| VFA | Volatile Fatty Acids |
| VS | Volatile Solids |
| WS | Wheat Straw |

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# Abstract

Pakistan is a developing country and is experiencing a shortage of energy due to its rapidly growing population. Therefore, there is a need to explore the untapped energy potential of lignocellulosic biomass. But its complex structure is the main hurdle to biodegradation. Pretreatment helps in breaking this recalcitrant structure which improves degradability. Digestion parameters are important factors for smooth anerobic digestion. Optimum organic loading is vital to reduce or avoid acid accumulation, improve reactor stability, and increase biogas yield. This study, therefore, carried out to assess the impact of varying organic loading (OL) on production of biogas from pretreated wheat straw in batch reactor. Wheat straw (WS) was used as substrate and subjected to sodium hydroxide (NaOH), titanium dioxide ($TiO_{2)}$ and combined pretreatment. Pretreated WS was fed to reactors based on volatile solids at four different loadings of 10, 20 30 and 40 gVS/L. The results revealed that 10 gVS/L has the maximum biogas production in all the three pretreatments. The NaOH pretreated wheat straw at 10 gVS/L has produced 56%, 146% and 172% more biogas compared to OL 20, 30 and 40 gVS/L respectively. Similarly, $TiO_2$ pretreated wheat straw had high biogas yield at organic loading of 10 gVS/L, which was 74%, 214% and 292% more than the higher organic loadings respectively. Combined pretreatment had maximum biogas yield than NaOH and $TiO_2$ individually. Maximum total solids (TS) and volatile solids (VS) removal was also observed in case of combined pretreatment of WS at OL of 10 gVS/L. Biogas production, VS removal and stability of the reactor declined with an increase in OL from 10 gVS/L to 40 gVS/L, which can be attributed to increase in VFAs production in the higher organic loading.

## *Chapter 1*

# Introduction

## 1.1 Background

The escalating global population, rapid industrialization, and excessive reliance on fossil fuels have resulted in significant amount of greenhouse gas emissions. This alarming situation has led to widespread concerns worldwide. To combat these challenges and address the issue of fluctuating energy prices, there has been a growing adoption of clean and sustainable energy alternatives (Nosratpour et al., 2018).

Pakistan is currently facing a huge energy deficit and energy crises, which has prompted the country to actively explore various renewable energy solutions. Being an agrarian nation, Pakistan benefits from its substantial agricultural sector, which generates significant amounts of biomass. The agricultural crop residues produced in the country reach a staggering annual volume of approximately sixty-nine thousand metric tons, offering a vast resource to produce bioenergy. Among these residues, wheat straw (WS) stands out as a prominent example, accounting for an impressive quantity of around twenty-five thousand metric tons each year. Unfortunately, a considerable portion of this valuable agricultural by-product is presently utilized as feed for livestock, while the remaining WS is often burned in the open, resulting in severe environmental pollution (Rajput & Sheikh, 2019).

Pakistan possesses vast untapped potential for harnessing renewable energy from crop residues, particularly wheat straw. Wheat is one of the major crops cultivated in the country, and the straw leftover after harvest holds significant value as a renewable energy resource. With a considerable agricultural sector and substantial wheat production, Pakistan stands to benefit from utilizing this abundant biomass for energy generation. Wheat straw, which is often considered an agricultural waste, can be converted into bioenergy through various technologies such as anaerobic digestion, thermal conversion, or biochemical processes (Kashif et al., 2020). Anaerobic digestion (AD) has emerged as a widely endorsed and auspicious approach for the treatment of organic waste. The utilization of this technology presents a multitude of advantages, including the production of biogas that is abundant in methane, the efficient recycling of essential nutrients, the mitigation of greenhouse gas emissions, and the effective management of odorous compounds. Anaerobic digestion

(AD) is a multifaceted phenomenon that transpires within an environment characterized by a deficiency of oxygen. In this context, microorganisms undertake the decomposition of biodegradable substances, resulting in the production of carbon dioxide ($CO_2$) and methane ($CH_4$). The utilization of methane gas, which is generated via the process of anaerobic digestion (AD), presents significant potential due to its substantial energy content. This versatile resource can be effectively employed in two primary manners. The substance in question possesses the capacity to undergo combustion, thereby liberating thermal energy, or alternatively, it can be harnessed to generate electrical power through the utilization of internal combustion engines. The AD process encompasses a series of four distinct steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During the hydrolysis process, bacteria effectively degrade complex organic compounds into smaller, more soluble constituents such as glucose, fatty acids, and amino acids. The subsequent stage is acidogenesis, during which the generation of volatile fatty acids (VFAs) occurs, accompanied by the formation of by-products such as $NH_3$, $CO_2$, and $H_2S$. Subsequent to their production, the volatile fatty acids (VFAs) undergo a process known as acetogenesis, wherein they are transformed into acetate, carbon dioxide ($CO_2$), hydrogen ($H_2$), and various other compounds. In the conclusive phase, methanogens engage in the conversion of acetate, thereby facilitating the generation of biomethane through the process of methanogenesis. Within the array of sequential stages, it is noteworthy that hydrolysis, being the most protracted, exerts a pivotal influence on the overall tempo of the process. The anaerobic digestion (AD) process plays a pivotal role in the efficient management of waste, thereby making a significant contribution towards environmental cleanliness. This process involves the extraction of energy from organic waste while simultaneously reducing the release of detrimental emissions. The production of biogas presents a viable option for harnessing renewable energy, as highlighted by Zhang et al. (2019).

The renewable energy potential of wheat straw in Pakistan is promising on multiple fronts. Its utilization can help address the growing energy demand in the country, reduce dependence on fossil fuels, and mitigate greenhouse gas emissions. The conversion of wheat straw into bioenergy presents economic opportunities, as it can provide a source of income for farmers and create job opportunities along the value chain. The utilization of wheat straw for energy purposes can also contribute to waste management by reducing agricultural residues and minimizing open burning, which is a common practice in many rural areas (Kashif et al., 2020).

Wheat straw is an abundant and cost-effective resource that can be used sustainably for biofuel production. However, like other similar plant materials, the conversion of wheat straw into biogas faces challenges due to its complex structure. Factors such as the surface area available for reactions, the crystalline nature of cellulose, and the presence of lignin restrict the breakdown of the lignocellulosic material. To address this, a pretreatment step is necessary before anaerobic digestion (AD) to enhance the hydrolysis process and overcome these limitations (Mancini et al., 2018). Numerous approaches have been explored to treat plant-based materials before biogas production in order to improve the process. These methods can be classified as physical, chemical, or biological. Chemical treatments have garnered considerable attention due to their cost-effectiveness, faster reaction rates, and superior efficiency in breaking down the complex organic substances. Recently, advanced oxidation processes (AOPs) have emerged as promising techniques for pretreatment, showing success in augmenting biogas yields from plant-based materials. Furthermore, alkaline pretreatments have been extensively studied for their capacity to enhance the accessibility of carbohydrates to microorganisms, thereby leading to increased methane production (Mancini et al., 2018).

Lignocellulosic biomass comprises of three primary components: cellulose, hemicelluloses, and lignin. Cellulose is made up of chains of D-glucose units and has both organized crystalline regions and disordered amorphous regions. These cellulose strands form cellulose fibrils or bundles. Hemicelluloses, on the other hand, are complex mixtures of xylose, mannose, galactose, rhamnose, arabinose, glucose, and uranic acids. They lack a defined structure and are more easily broken down compared to cellulose. Lignin is composed of hydrophobic heteropolymers derived from three phenylpropane alcohols: p-coumaryl (H), coniferyl (G), and sinapyl (S). It has an amorphous structure and provides plants with resistance against microbial attacks. Lignin molecules are chemically bonded to the polysaccharides in the cell walls through lignin-carbohydrate complexes, which can impede the biodegradation of cellulose and hemicellulose (Carrere et al., 2016). The application of alkaline pretreatment, specifically using sodium hydroxide (NaOH), has been utilized to treat various lignocellulosic materials. This pretreatment method effectively enhances the biodegradability of the raw material by removing lignin and increasing porosity. These changes contribute to improved hydrolysis, ultimately resulting in higher yields of biogas production (Mancini et al., 2018).

In recent times, researchers have shown significant interest in the utilization of advanced oxidation processes (AOPs) to enhance the productivity of biofuels derived from organic substrates. AOPs involve the application of strong radicals generated from chemical reactions to oxidize inert or stubborn compounds present in the substrate. One common feature among all AOPs is the production of hydroxyl radicals, which play a crucial role in the oxidation process. The pre-treatment of substrates using AOPs has immense potential in breaking down the complex structure of lignocellulosic biomass, thereby increasing its hydrolyzability and solubility, leading to improved biodegradability. This, in turn, results in higher productivity of biofuels. AOPs offer advantages such as faster reaction rates compared to physical, chemical, and biological methods, thereby yielding higher conversion efficiencies. Some commonly employed AOPs with promising applications in bioenergy production include the Fenton process, ozonation, photocatalysis, ultraviolet radiation, ultrasound, electrochemical oxidation, hydrogen peroxide oxidation, wet air oxidation, and microwave enhanced AOP (M'Arimi et al., 2020).

Photocatalytic oxidation, a potential alternative for mild depolymerization of lignin, utilizes titanium dioxide ($TiO_2$) as the preferred catalyst due to its remarkable efficiency, stability, wide availability, and affordability. Although other semiconductor materials like $ZnO_2$ and CdS have been explored, $TiO_2$ remains the most commonly used. In this process, $TiO_2$ absorbs ultraviolet (UV) light to initiate the photooxidative degradation of lignin. The high energy and short wavelength of UV light facilitate two distinct pathways: electron-hole reactions and OH radical oxidation, leading to the complete breakdown of lignin through photolysis. As a result, aromatic aldehydes and carboxylic acids are the primary products generated from oxidative degradation. Notably, the valuable compound vanillin is a significant product obtained during the oxidative deconstruction of lignin, with yields ranging from 5% to 15% by weight in relation to the original lignin source (Li et al., 2016).

Titanium dioxide ($TiO_2$) has been extensively studied for its ability to degrade complex organic compounds in wastewater treatment. By employing $TiO_2$ as a pretreatment method under mild operational conditions, the formation of inhibitory substances can be minimized, and energy consumption can be reduced. Additionally, exploring the integration of photocatalytic processes with conventional methods such as physical-chemical or biological processes can lead to cost reductions in different studies and can be explored further (Alvarado-Morales et al., 2017). Anaerobic digestion of wheat straw offers a sustainable and efficient approach for converting this agricultural residue into valuable biogas. The process involves the breakdown of organic matter in the absence of oxygen by a diverse community of anaerobic microorganisms. The organic loading rate, which represents the quantity of substrate introduced into the anaerobic digester per unit time, is a critical parameter in the anaerobic digestion process (Kothari et al., 2014). Organic loading rates play a crucial role in the performance and stability of anaerobic digestion systems. When considering wheat straw as a substrate, finding the optimal organic loading rate is essential to achieve high biogas production rates and maintain process efficiency. The loading rate should provide sufficient organic matter to support microbial growth and biogas generation while avoiding overloading the system, which can lead to process instability, acidification, and reduced biogas yields (Leung & Wang, 2016). The selection of organic loading rates depends on several factors, including the characteristics of the wheat straw, the design and capacity of the anaerobic digester, and the desired biogas production. Low organic loading rates may be suitable for systems with limited capacity or when the digestibility of wheat straw is low. On the other hand, higher organic loading rates can be employed in systems designed to handle greater substrate inputs and can lead to increased biogas production. However, it is crucial to monitor and control the process closely when operating at higher loading rates to prevent potential issues such as process imbalance or accumulation of inhibitory compounds (Zealand et al., 2017).

## 1.1 Objectives of the study

1. Effect of different organic loading on the production of biogas from pre-treated wheat straw.
2. Effect of organic loading on digestion parameters of reactor during anaerobic digestion of pre-treated wheat straw.

# *Chapter 2*

# Literature Review

The following chapter gives a brief discussion about the available literature regarding anaerobic digestion of lignocellulosic biomass, its pretreatment methods and impact on biogas production under different conditions.

## 2.1 Lignocellulosic Biomass

Lignocellulosic biomass, including materials like corn straw, corn stover, sugar cane bagasse, cotton stalks, rice straw, wheat straw, and rice husks, is a plentiful organic resource that is readily available at a relatively low cost. It holds great potential for sustainable production of bioenergy and biofuels, such as biogas. Typically, lignocellulosic biomass consists of lignin (10–25%), cellulose (35–50%), hemicelluloses (20–35%), and small amounts of extractives (Ghaemi et al., 2019). After undergoing enzymatic hydrolysis, the hemicellulose and cellulose components can be fermented, making lignocellulosic biomass an ideal substrate for biogas production. It is important to note that the proportions of these constituents vary based on maturation, growth conditions, and the specific species involved.

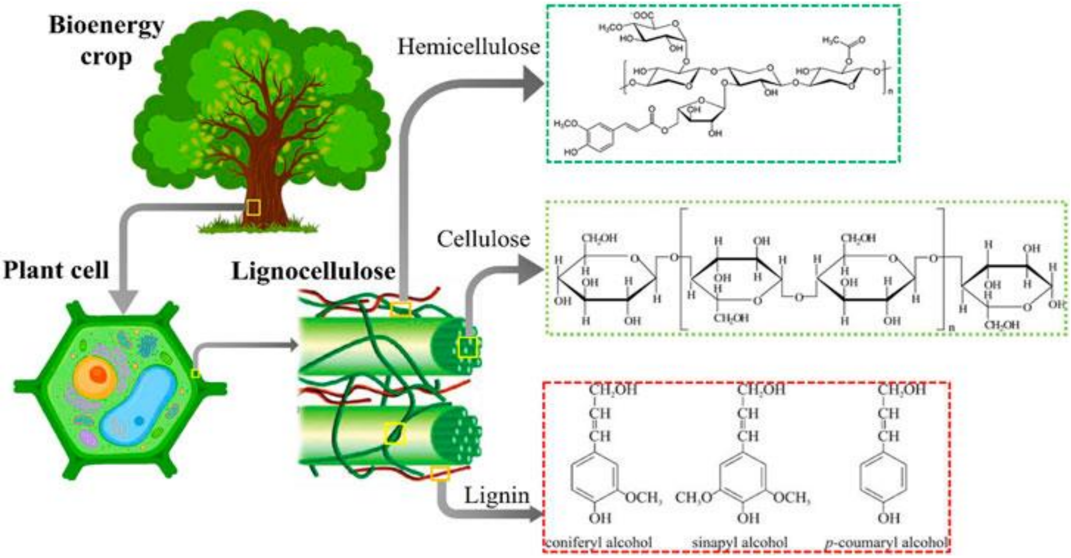


**Figure 2.1 Lignocellulosic biomass structure of plant cell wall**

## 2.2 Pretreatment Methods

The inherent resistance of lignocellulosic biomass hinders its biodegradation, resulting in a decrease in biogas production. Enhancing the breakdown of these resistant structures would improve the biodegradability of lignocellulosic biomass. Numerous factors, including accessible surface area, cellulose crystallinity and polymerization, degree of hemicellulose acetylation, and the presence of hemicellulose and lignin, have been found to influence the biodegradability of lignocellulosic feedstock. Therefore, the primary objective of pretreatment is to modify these properties in order to increase biodegradation. Through pretreatment, components such as hemicellulose and cellulose in lignocellulosic biomass are converted into simpler organic substances that can be readily biodegraded by microorganisms during the anaerobic digestion (AD) process (Yang et al., 2015).

Pretreatment techniques can be categorized into three main groups: physical methods including liquid hot water, pyrolysis, microwave, ultrasound, irradiation, extrusion, and comminution, chemical approaches such as acid, alkaline, ionic liquids, ozonolysis, wet oxidation, and catalyzed steam-explosion, and biological methods involving enzymes, bacterial consortium, and fungi.

## 2.3 Anaerobic Digestion Process

AD is a versatile technology that transforms organic wastes into valuable forms of energy using a diverse range of microorganisms in an oxygen-deficient environment. The resulting end products consist of organic residue, biogas (comprising 60-70% $CH_4$), $CO_2$ (30-40%), and small quantities of other gases like nitrogen ($N_2$), ammonia ($NH_3$), hydrogen sulfide ($H_2S$), hydrogen ($H_2$), and water vapor ($H_2O$). The specific composition of the produced biogas depends on the conditions of digestion and the type of substrate employed. The generation of biogas takes place through a collaborative process involving a consortium of microbes, progressing through four distinct phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These phases are given below in Fig 2.2 (Metcalf & Eddy, 2003).

### 2.3.1 Hydrolysis

Firstly, the substrate undergoes a hydrolysis reaction, where intricate insoluble organic components including proteins, carbohydrates, lipids, and nucleic acids are broken down by extracellular enzymes. This degradation process transforms them into simpler forms such as amino acids, soluble sugars, fatty acids, and pyrimidines and purines, respectively.

### 2.3.2 Acidogenesis

During this phase, fermentative bacteria facilitate the conversion of the reduced compounds into various products, including hydrogen, carbon dioxide, acetate, propionate, butyrate, formate, methanol, and methylamines, among others.

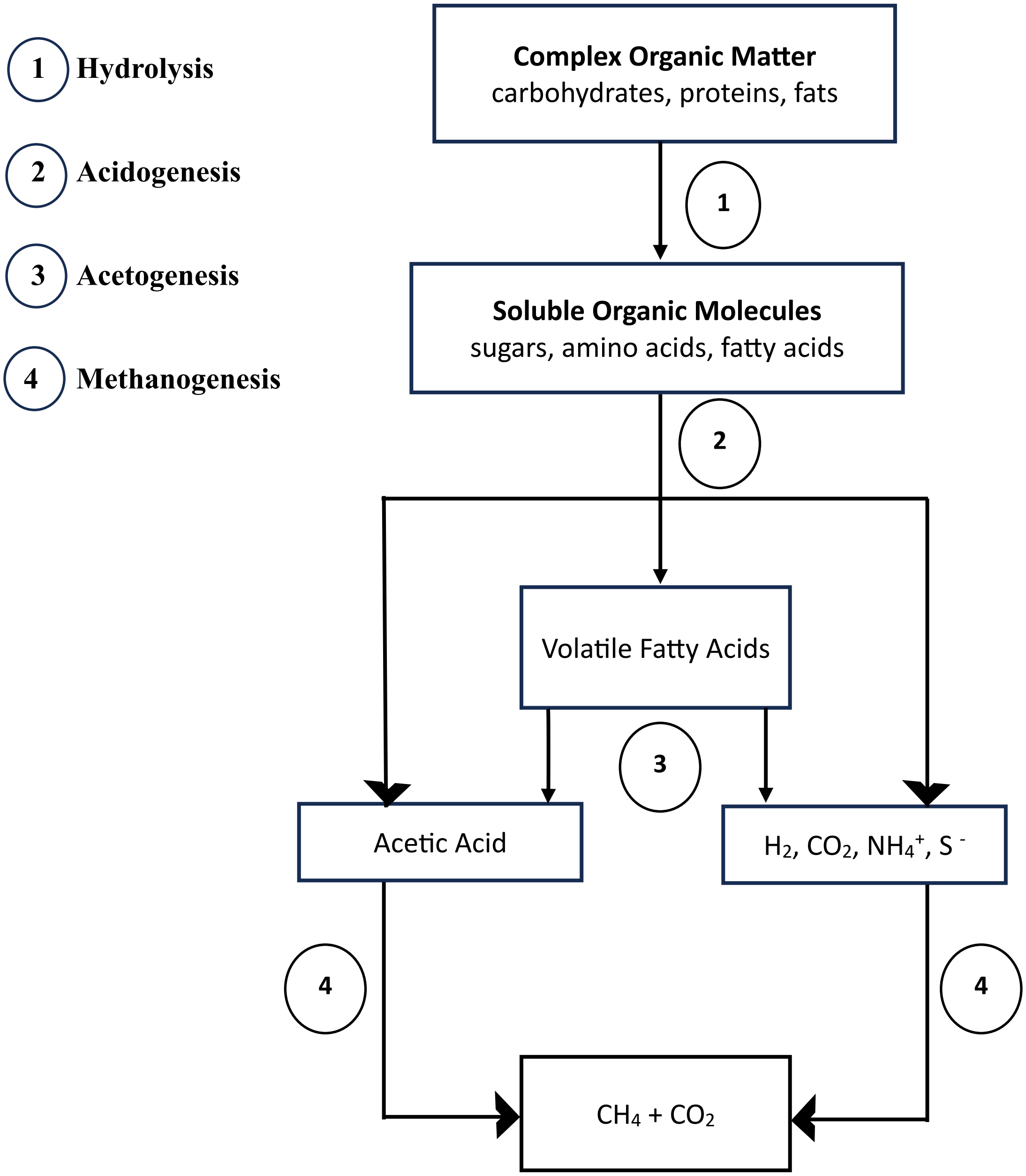


**Fig. 2.2 Mechanism of anaerobic digestion process**

### 2.3.3 Acetogenesis

During this phase, acetogens play a crucial role in further breaking down the organic acids into hydrogen, carbon dioxide, and acetate. These compounds are then utilized directly for the generation of methane.

### 2.3.4 Methanogenesis

In the last phase, the production of methane is facilitated by two distinct microbial consortia: Acetoclastic methanogens, which consume acetate, and Hydrogenotrophic methanogens, which utilize $H_2/CO_2$ as their energy source. Acetotrophic methanogens convert acetate into carbon dioxide and methane, while Hydrogenotrophic methanogens convert $CO_2$ into methane by employing hydrogen as the electron donor.

## 2.4 Factors affecting Anaerobic Digestion

### 2.4.1 pH

The pH is a critical parameter that directly influences the performance and stability of anaerobic digestion (AD). Generally, AD systems exhibit optimal functioning within a pH range of 6.8-7.4. Variations in pH can have a significant impact on the growth rate of methanogenic microbes, leading to instability in AD performance. The ideal pH for methanogenic bacteria lies within the range of 7-7.5, ensuring their optimal activity. If the pH falls below 6.8, the activity of methanogenic bacteria diminishes, while on contrary high alkalinity levels result in reduced methane production (Hagos et al., 2017).

### 2.4.2 Temperature

The temperature of anaerobic digesters is a crucial factor in the process of anaerobic digestion. Typically, anaerobic digesters operate within the mesophilic temperature range, which is around 30 to 40°C. Alternatively, they may function in the medium thermophilic range. While the best results in anaerobic digestion are obtained at higher thermophilic temperatures, it is important to consider the economic aspect as achieving and maintaining the thermophilic range requires additional energy. Operating at a temperature range of 50-60°C offers a more balanced performance and improved efficiency compared to the mesophilic range. Although mesophilic setups demonstrate sufficient process stability and bacterial growth when compared to thermophilic systems, they yield lower methane production and exhibit limitations in biodegradability, as well as nutrient imbalance-related challenges (Kothari et al., 2014).

### 2.4.3 VFAs

VFAs serve as intermediate byproducts in the production of methane. These acids can be utilized as indicators to assess the performance and stability of AD systems. If VFAs accumulate, they can lower the pH below 6, resulting in acidification within the AD reactor. This acidification hampers the activity of methanogenic bacteria, leading to the generation of toxic compounds and ultimately causing AD failure. To prevent disruption of the AD process, it is crucial to maintain VFAs within a range of approximately 1500-2000 mg/L (Bah et al., 2014).

### 2.4.4 Alkalinity

Alkalinity serves as a crucial parameter for evaluating the stability and performance of an anaerobic digestion (AD) system. In an ideal AD process, methane production occurs at a pH of 7. However, maintaining this pH value against the production of $CO_2$ and VFAs during the process requires feedstock with higher alkalinity levels. Failure to properly maintain the pH of the system can result in the accumulation of VFAs, which may halt the digestion process (Neshat et al., 2017).

### 2.4.5 Ammonia

Nutrients play a crucial role in preserving the nutritional composition of anaerobic microorganisms. During the anaerobic digestion (AD) process, organic nitrogen is transformed into ammonia nitrogen ($NH_3$ and $NH_4$). This conversion provides alkalinity to the AD system, ensuring the efficient operation of the digester. However, it is important to note that an excessive concentration of ammonia nitrogen can have detrimental effects on anaerobic microbes, leading to instability in the anaerobic digester. This is particularly observed in thermophilic anaerobic digestion. An optimal concentration of ammonia nitrogen is essential for maintaining sufficient buffering capacity of methanogenic bacteria in anaerobic digestion (AD). This, in turn, enhances the stability of the AD process and reduces inhibition. Ammonia plays a significant role in the overall anaerobic digestion process. However, it is important to note that ammonia can also be a major factor contributing to instability and decreased biogas production in the AD process (Shi et al., 2020).

### 2.4.6 Sulfides

Sulfur functions as an essential element for the growth of methanogenic bacteria, particularly prominent in methanogen cells that exhibit higher sulfur content compared to other anaerobic organisms. Challenges arise in anaerobic digesters when an excess of sulfur is present, leading to sulfide-related inhibition. This predicament commonly arises when dealing with substrates abundant in sulfides, notably in industrial wastewaters like those from rubber latex and slaughterhouse facilities. Within anaerobic environments, sulfate-reducing bacteria (SRB) play a pivotal role in converting sulfate into sulfides, a process crucial for substrate digestion.

A significant concern emerges when sulfate is introduced into an anaerobic digester. The activity of SRBs in converting sulfate to sulfide contributes to the inhibition of methane-forming bacteria. This inhibition manifests as a reduction in both methane ($CH_4$) production and substrate breakdown. The toxicity resulting from sulfide is intrinsically linked to the pH level. Empirical observations indicate that sulfide toxicity becomes prominent within the pH range of 6.4 to 7.2. The threshold for severe inhibition in anaerobic digesters is documented to be approximately 100 to 800 mg/L of dissolved sulfide, or around 50 to 400 mg/L of dissociated $H_2S$ (Maillacheruvu et al., 2015).

A study by Chen et al. (2008) provides insights into the mechanism of sulfide inhibition. It identifies hydrogen sulfide ($H_2S$) as the primary culprit behind this inhibition, as it can breach microbial cells and disrupt polypeptide chains, leading to the degradation of proteins within these anaerobic microbes. This process ultimately diminishes cell metabolism. Sulfate reduction, a fundamental step in anaerobic digestion, is facilitated by two main groups of sulfate-reducing bacteria: incomplete and complete oxidizer bacteria.

In a pioneering investigation, Yuan et al. (2020) explored a novel strategy to mitigate sulfide-related inhibition. They accomplished this by coupling microbial electrolysis with anaerobic digestion, resulting in augmented biogas production even in environments rich in sulfides.

### 2.4.7 Carbon to Nitrogen (C/N) Ratio

The C/N ratio plays a critical role in the anaerobic digestion (AD) technique as it is essential for microbial growth. It has been reported that a C/N value within the range of 20-30 is optimal for the AD process. Low C/N ratios can result in a carbon shortage, increasing the risk of volatile fatty acid (VFA) and ammonia ($NH_3$) accumulation in the digester, thereby inhibiting microbial growth (Li et al., 2011). Conversely, a high C/N ratio provides insufficient nitrogen ($N_2$) for microorganism growth, leading to reduced methane production and AD process failure. Lignocellulosic substrate has a very high C/N ratio, making it unsuitable as a sole feedstock for the AD process. On the other hand, animal dung exhibits a low C/N ratio, which poses a challenge to an efficient AD process. To address this issue, the co-digestion of lignocellulosic biomass and animal dung can be a viable solution to enhance biogas production (Risberg et al., 2013).

### 2.4.8 Mixing

The anaerobic digestion process is notably influenced by the aspect of mixing. Researchers have conducted numerous investigations to explore the impact of mixing in both laboratory-scale and pilot-scale systems. Effective mixing is crucial within an anaerobic digester to ensure the optimal interaction between microbes and the substrate medium. The necessity of mixing has garnered support from several scholarly inquiries (Bridgeman, 2012; Gerardi, 2003; Conklin et al., 2008; Halalsheh et al., 2011), yet it has also faced challenges from opposing viewpoints (Cuetos et al., 2017; Kim et al., 2002; Ward et al., 2008).

Karim et al. (2005) delved into the impact of mixing on reactor performance. Their study concluded that inadequate mixing led to the formation of hydraulic dead zones, resulting in reduced hydraulic retention time (HRT) and subpar digester performance. Conversely, Kim et al. (2002) observed enhanced performance in an anaerobic digester that lacked stirring, opting for an unstirred, continuously fed lab-scale biogas reactor. This unstirred configuration facilitated a quicker startup phase, albeit with comparatively lower biogas production in the long run when compared to mixed digesters (Karim et al., 2005).

Mixing intensity, too, has been scrutinized for its impact on biogas production. Karapaju and Rintala (2008) noted that excessive mixing resulted in minimal biogas output, while a gentler mixing approach led to enhanced biogas production. A novel approach introduced by Yang & Deng (2020) demonstrated an innovative perspective. They reported an increase in methane production of 6.4%, 11.9%, and 19.6% through the utilization of air as a mixing source, contrasting with traditional methods such as biogas-based mixing and mechanical mixers, or even the absence of mixing altogether.

### 2.4.9 Retention Time

The duration of time that microbes and substrate spend in the digester is crucial for ensuring the complete anaerobic degradation of the added substrate. This duration is often referred to as the total time or average time substrate spends in the reactor. In the anaerobic digestion process, retention time is categorized into two groups: solid retention time (SRT) and hydraulic retention time (HRT). SRT represents the total period during which the solid biomass remains inside the digestion system. On the other hand, HRT refers to the time the semi-solid or liquid portion of the substrate or sludge spends in the reactor (Metcalf & Eddy, 2003).

The SRT is designed to maintain a bacterial population within the digester, ensuring effective waste degradation and stabilization. According to Mao et al. (2015), the development, growth, and retention of microbes are influenced by the anaerobic digestion temperature, substrate composition, and organic matter loading rate. In general, HRT corresponds to a higher loading rate in the digester. A study suggests that a retention period of 10-40 days is necessary for the digestion of organic waste in a mesophilic temperature-operated digester.

### 2.4.10 Organic Loading Rate (OLR)

The production of $CH_4$ is significantly influenced by one crucial operational parameter known as the Organic Loading Rate (OLR). The OLR represents the total amount of dry solids loaded into the digester per unit of volume, and it determines the volume of feedstock required for the anaerobic digestion process. Overfeeding the digester can have adverse effects, such as severe acidification and inhibition of anaerobic digestion due to the unsuitable acidic environment that hinders microbial survival. Accumulation of Volatile Fatty Acids (VFAs) also contributes to the inhibition of anaerobic digestion. Hence, it is crucial to maintain an appropriate organic loading rate to prevent anaerobic inhibition (Kothari et al., 2014).

Moreover, increasing the OLR while decreasing the HRT can result in a significant reduction in net methane yield. Leung and Wang (2016) conducted a study that concluded that lowering the OLR below the optimum level, along with a high HRT, can also lead to a decrease in methane production. This reduction is attributed to the insufficient buffering capacity within the digester. While maintaining a higher OLR leads to shorter HRTs, it can result in the washout of microbes, ultimately leading to decreased biogas production.

## 2.5 Studies on impact of OLR on biogas production

Organic loading rates play a crucial role in the efficient production of biogas. The organic loading rate refers to the amount of substrate that is introduced into the anaerobic digestion process per unit volume or time. Several studies have investigated the impact of different organic loading rates on biogas production and have demonstrated the significance of optimizing this parameter. Higher organic loading rates can enhance biogas production by increasing the availability of fermentable organic matter, thereby promoting the activity of methanogenic microorganisms. However, it is essential to strike a balance, as excessively high loading rates can lead to process instability and reduced biogas yields due to incomplete degradation or accumulation of inhibitory compounds. Thus, careful consideration of organic loading rates is necessary to maximize biogas production while ensuring process stability and efficiency. Extensive research has been conducted to examine the effects of varying organic loading rates (OLRs) on the generation of biogas. Elevated organic loading rates (OLRs) have been observed to positively impact the rate of biogas generation. However, it is important to note that this intensified biogas production may be accompanied by the potential for process inhibition arising from the accumulation of specific compounds. A comprehensive research inquiry was conducted to examine the synergistic effects of co-digesting goose manure and wheat straw under anaerobic conditions. The findings of the investigation demonstrated that the augmentation of the Organic Loading Rate (OLR) from 1.5 grams of Volatile Solids (VS) per liter per day to 4.5 grams of VS per liter per day resulted in a significant increase in methane production during the initial phase of the experimental period. However, the increase in methane production became insignificant as the organic loading rate (OLR) was further increased. The study also observed a comparatively lower methane production rate in Continuous Stirred Tank Reactors (CSTRs) as compared to batch experiments. The study conducted by

Hassan et al. (2017) observed that the maximum methane production occurred when the Organic Loading Rate (OLR) reached 4.5 grams of Volatile Solids (VS) per liter per day. This optimal condition led to a methane yield of 254.65 milliliters per gram of Volatile Solids.

A subsequent investigation was undertaken to examine the co-digestion of canola straw and buffalo dung, with a particular focus on the impact of different organic loading rates (OLRs) on methane generation. The primary objective was to determine the optimal OLR that would yield the highest methane production. According to the available report, it has been observed that the efficacy of the anaerobic digestion process experiences a decline when the Organic Loading Rate (OLR) exceeds optimal levels or falls below them. The investigation determined that the most favorable organic loading rate (OLR) for achieving the highest methane production through the co-digestion of canola straw and buffalo dung was identified as 2.66 gVS L-1 day-1. The highest level of methane production was attained during this operational load range (OLR). The findings of this study indicate that elevated organic loading rates (OLRs) may result in a reduction in methane production potential, potentially attributable to an insufficient quantity of inoculum. Furthermore, the significance of optimizing organic loading rates (OLRs) in order to enhance the efficiency of biogas production is underscored by Sahito et al. (2016). The presence of low organic loads within a reactor system can give rise to diminished metabolic activity and an inadequate production of gas. Conversely, the existence of high organic loads can induce the buildup of volatile fatty acids, thereby engendering a toxic environment within the reactor. The present study sought to examine the impact of volatile solids (VS) organic loads on methane production, specifically in the context of co-digestion involving primary sludge and wheat straw. The results of the study indicate that the purified biogas exhibited the highest methane content when subjected to organic loads of 6.0 and 7.50 gVS/L, whereas the lowest methane content was observed at an organic load of 3.0 gVS/L. The investigation additionally exhibited that the maximum cumulative methane yield was achieved when the organic load reached 7.50 gVS/L, thereby signifying the optimal exploitation of the feedstock (Elsayed et al., 2016).

In a study conducted by Jabeen et al. (2015), an investigation was carried out on the high-solids anaerobic co-digestion of food waste and rice husk. The researchers examined this process under various organic loading rates (OLRs) of 5, 6, and 9 kg VS/m3/d. The present study aimed to investigate the influence of various organic loading rates (OLRs) on the production of biogas. The findings of this investigation revealed that the highest daily biogas yield, amounting to 196 L/d, was achieved when the OLR reached 6 kg VS/m3/d. Nevertheless, when operating at an Organic Loading Rate (OLR) of 9 kilograms of Volatile Solids per cubic meter per day (kg VS/m3/d), the daily biogas production experienced a significant reduction, amounting to 136 liters per day (L/d). In addition to the measurements, an assessment of the specific biogas yield (SBY) was conducted. The investigation revealed that the SBY attained its maximum value when subjected to an organic loading rate (OLR) of 5 kg volatile solids (VS) per cubic meter per day. Remarkably, the average SBY recorded at this OLR was determined to be 446 liters per kilogram of volatile solids (L/kg VS). The specific biogas yield (SBY) exhibited a negative correlation with the organic loading rate (OLR) at levels of 6 and 9 kg volatile solids (VS) per cubic meter per day (kg VS/m3/d), resulting in average yields of 399 L/kg VS and 215 L/kg VS, respectively.

A study was carried out to assess the influence of varying organic loading rates (OLRs) on the generation of biogas while performing anaerobic digestion on rice straw. The OLRs that underwent testing were 1.22, 1.46, 1.70, and 2.00 kilograms of Volatile Solids (VS) substrate per cubic meter per day. The investigation disclosed that as the OLR was elevated to 2.00 kilograms of VS substrate per cubic meter per day, the production of biogas displayed a corresponding increase. The rates of biogas production were recorded as 332.8, 327.6, 324.6, and 319.3 cubic meters per metric ton of dry rice straw for OLRs of 1.22, 1.46, 1.70, and 2.00 kilograms of VS substrate per cubic meter per day, sequentially. The cumulative rate of biogas production throughout the entire process reached 323 cubic meters per metric ton of dry rice straw. Furthermore, the study noted a divergence in the microbial communities found in association with rice straw and slurry. Microorganisms specialized in breaking down rice straw were predominantly situated in the straw itself, while the slurry exhibited an abundance of methanogenic microbes. Within the rice straw anaerobic digestion system, the hydrogenotrophic pathway was identified as the primary biochemical route of methanogenesis (Zhou et al., 2017).

In their latest research, Ünyay et al. (2022) conducted an investigation to assess the influence of diverse organic loading rates (OLRs) on the generation of biogas from switchgrass through the process of anaerobic digestion. The researchers further explored the effects of varying substrate to inoculum ratios on this biogas production. The operational loading rates (OLRs) that were examined in this study encompassed values of 0.75, 1.0, and 1.5 grams of volatile solids per liter per day (gVS/L.d). The findings of the study revealed a positive correlation between biogas production and organic loading rate (OLR), indicating that as the OLR increased, so did the biogas production. However, it is important to note that this relationship exhibited a threshold beyond which further increases in OLR did not result in a proportional increase in biogas production. The batch system, when operated at an organic loading rate (OLR) of 1.1, exhibited the most significant methane yield of 204 mLCH4/gVS. In the semi-continuous configuration, it was observed that methane yields exhibited values of approximately 148, 157, and 60 mLCH4/gVS at organic loading rates (OLRs) of 0.75, 1.0, and 1.5 gVS/L.d, respectively. At the maximum organic loading rate (OLR) of 1.5 gVS/L.d, a significant reduction in methane production was observed. In the semi-continuous operational mode, an optimal organic loading rate (OLR) of 1.0 grams of volatile solids per liter per day (gVS/L.d) was determined, resulting in a methane yield of 35%.

## 2.6 Studies on types of biomass for biogas production

Biomass, a renewable energy resource derived from organic matter, plays a crucial role in the global energy landscape. It encompasses a wide range of materials, such as agricultural residues, forestry by-products, organic wastes, and dedicated energy crops. One of the most promising applications of biomass is in biogas production, where organic matter is converted into biogas through anaerobic digestion. Biogas, primarily composed of methane and carbon dioxide, is a versatile and clean energy source that can be used for electricity generation, heating, and cooking.

According to the International Energy Agency (IEA) biomass accounts for a significant portion of global renewable energy production. In 2021, biomass-based energy sources contributed approximately 48% of the total renewable energy supply, making it a leading contender in the renewable energy sector (IEA, 2019).

The types of biomass suitable for biogas production are diverse and regionally dependent. Common feedstocks include animal manure, food waste, energy crops, and agricultural residues. Among these, straw from crops like wheat, rice, and other grains holds immense potential. Pakistan, being an agricultural country, generates a substantial amount of straw biomass from the cultivation of crops like wheat, rice, and barley. Wheat and rice are the staple crops, contributing significantly to the agricultural sector. The straw residues left behind after harvesting these crops represent a vast and readily available source of biomass for biogas production. Wheat is one of the major cereal crops in Pakistan, and its straw is abundantly available after the harvest season. Wheat straw is composed of lignocellulosic material, making it suitable for anaerobic digestion in biogas plants. The estimated annual production of wheat straw in Pakistan is around 35-40 million tons, presenting a substantial opportunity for biogas generation. Rice is another crucial staple crop in Pakistan, and the country ranks among the top producers globally. Rice straw, left behind after rice harvesting, is another potential feedstock for biogas production. It contains a higher silica content, making it more challenging to digest compared to wheat straw. However, with the right biogas technology and process optimization, rice straw can contribute significantly to the biogas potential in Pakistan. The annual production of rice straw in Pakistan is approximately 25-30 million tons (Yaqoob et al., 2021).

Wheat straw stands out as an ideal biomass feedstock for biogas production due to its unique composition and availability. Unlike many other biomass types, wheat straw is abundantly available in large quantities after the wheat harvest season, making it a readily accessible and reliable resource for biogas plants. This easy availability helps ensure a consistent supply of feedstock, enhancing the stability and efficiency of biogas production processes.

Furthermore, wheat straw's lignocellulosic nature makes it a valuable candidate for biogas production. Lignocellulosic materials consist of complex organic compounds, including cellulose, hemicellulose, and lignin. While lignin poses challenges in some biogas production processes, wheat straw's relatively lower lignin content compared to other biomass types makes it more amenable to anaerobic digestion. The cellulose and hemicellulose components of wheat straw can be readily broken down by microorganisms during the biogas fermentation process, leading to the production of biogas rich in methane.

Its use as biomass for biogas production brings significant environmental benefits. By diverting wheat straw from open field burning, a common practice in some regions, biogas plants can help mitigate air pollution and greenhouse gas emissions. Burning straw releases harmful particulate matter and contributes to climate change. By converting wheat straw into biogas through anaerobic digestion, methane emissions are captured and utilized as an energy source, effectively reducing the carbon footprint associated with straw disposal (Rahmani et al., 2022).

## 2.7 Studies on pretreatment of lignocellulosic biomass for biogas production

The presence of hemicellulose and lignin in WS acts as barriers, limiting the bioconversion of cellulose to fermentable sugars to only 20%, especially when the cellulose is not fragmented by any pretreatment technology. Consequently, it is essential to break down the recalcitrant structure before initiating any bioconversion process. The primary goal of pretreatment is to alter the compact structure and improve the digestibility of sugars in lignocellulosic biomass.

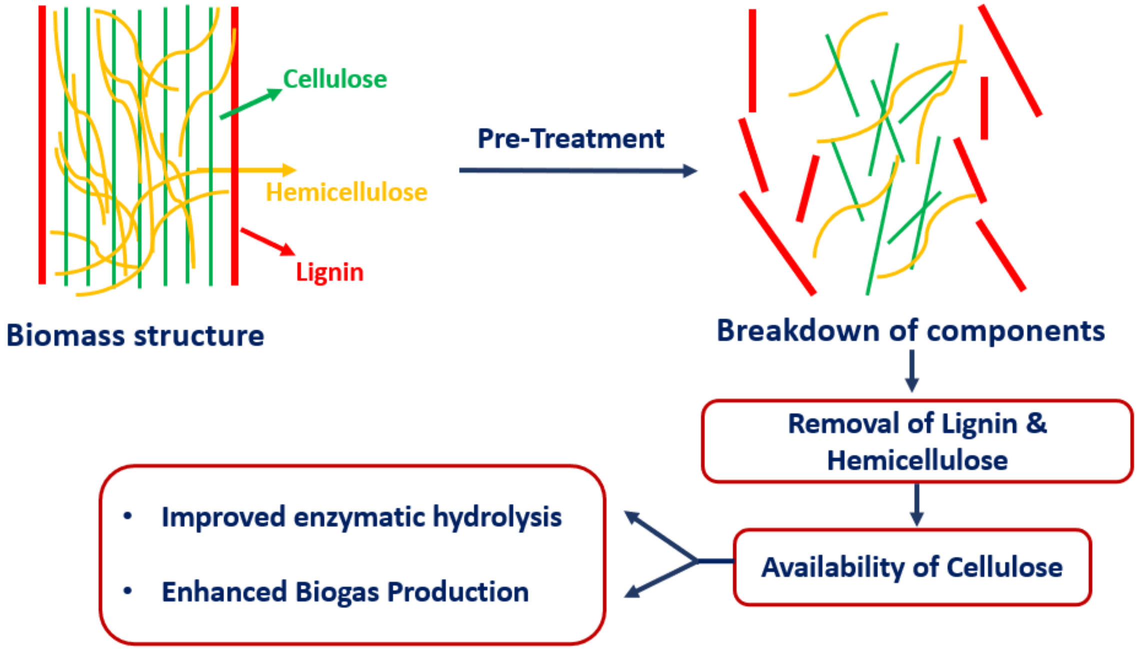


Fig 2.3 Pretreatment: Mode of action

Numerous pretreatment approaches, ranging from physical and chemical to physicochemical and biological methods, have been explored in previous research to improve the accessibility of cellulose for microbial or enzymatic degradation. Physical pretreatment methods involve techniques like chipping, extrusion, shredding, grinding, milling, and irradiation. Chemical pretreatment encompasses the use of alkalis, acids, ionic liquids, organic solvents, and more. Physicochemical pretreatment methods include hot water treatment, ammonia fiber explosion (AFEX), steam explosion, and $CO_2$ explosion. On the other hand, the environmentally friendly biological approach employs fungi (such as soft rot, brown and white fungi), microbial consortia, and enzymes to effectively break down the recalcitrant components in lignocellulosic feedstock (Bharathiraja et al., 2017).

### 2.7.1 Impact of Alkaline Pretreatment on lignocellulosic biomass and biogas production

Alkaline pretreatment involves the use of bases such as NaOH, KOH, $Ca(OH)_2$, and $NH_4OH$ to dissolve mainly lignin, partially hemicellulose, and to some extent cellulose, making the biomass more susceptible to microbial degradation. Historically, alkaline pretreatment has been predominantly applied in the paper and pulp industry. The underlying principle of alkaline pretreatment is the breaking of linkages between carbohydrates and lignin. This removal of cross-links increases the substance's porosity and surface area, leading to the swelling of the crystalline structure and a reduction in crystallinity and degree of polymerization. NaOH is the most commonly used alkali and has been extensively studied to enhance biogas production from various lignocellulosic biomass sources, including corn stover, wheat straw, sugarcane bagasse, rice straw, woody materials, sunflower stalks, sludge, oil palm empty fruit bunches, and fruit branches (Sun et al., 2016). Numerous research studies have demonstrated the effectiveness of alkaline pretreatment in breaking down the lignocellulose complex, resulting in an increase in biogas production and methane yield.

According to a study that aimed to investigate the effect of alkaline pretreatment on the production of biogas or methane from Pennisetum hybrid, a specific type of grass. The researchers performed the alkaline pretreatment using varying concentrations of NaOH solution (ranging from 2% to 8% w/w) at three distinct temperatures (35, 55, and 121 °C) and durations (24, 24, and 1 hour). The samples that had undergone pretreatment as well as those that had not undergone any form of treatment were then subjected to anaerobic digestion, a procedure conducted under mesophilic conditions at 37 °C in order to produce biogas. In sealed beakers, samples were submerged in a solution of sodium hydroxide (NaOH) as part of the alkaline pretreatment procedure. Pretreatments were conducted on the samples at temperatures of 35 and 55 °C, respectively. The samples were then incubated for 24 hours in a thermostatic water bath without agitation. The samples were subjected to pretreatments at a temperature of 121 °C, during which they were positioned precisely within an autoclave for one hour. In order to facilitate the methanogenesis process, the pH of the samples was adjusted to 7.0 via the addition of hydrochloric acid (HCl) following the pretreatment procedure. The results of the study indicate that the administration of alkaline pretreatment had a significant effect on methane production. It was determined that the modified Gompertz equation, which was used to analyze the data, possessed a high degree of reliability. The observed trend in methane yield was positively correlated with increases in both NaOH concentration and temperature. Using a NaOH concentration of 8% and maintaining a temperature of 121 °C, the optimal conditions for attaining the highest methane yield were observed, according to the results of the experiment. According to Kang et al. (2018), the use of pretreatment techniques increased the conversion efficiency and kinetic properties of Pennisetum hybrid, thereby facilitating an increase in methane production.

In a separate study conducted by Shetty et al. (2017), the primary objective was to improve the rice straw bio methanation process by employing an alkali pretreatment technique. The ultimate aim of this study was to reduce the alkali demand and eliminate the need for heating during the pretreatment process in order to improve the hydrolysis and bio methanation of rice straw. The ability of alkali pretreatment to disrupt the ester bonds that exist between lignin, hemicellulose, and cellulose within biomass has been extensively acknowledged. Ultimately, this perturbation improves the biomass's enzymatic accessibility during the anaerobic digestion process. Nevertheless, the majority of alkali pretreatment procedures involve the application of heat and the use of substantial quantities of chemicals, incurring significant costs and having a significant impact on the environment. In the present study, researchers utilized a 1% sodium hydroxide (NaOH) pretreatment at room temperature to enhance the rice fiber biomethanation process. The implementation of this pretreatment procedure resulted in a methane yield that was significantly greater than that of untreated rice straw, specifically by more than 34%. Under optimized conditions, the maximal biogas production was measured at 514 liters per kilogram of volatile solids per day, with a methane concentration of 59%. Rice straw's hydrolysis and biomethanation processes were significantly enhanced by the administration of alkali pretreatment at room temperature for three hours. This treatment resulted in an increase in methane production. (Shetty et al., 2017) discovered that the alkali pretreatment procedure employed in this study was more cost-effective and time-efficient than previous protocols that required heating and longer treatment durations.

### 2.7.2 Effect of photocatalytic pretreatment on lignocellulosic biomass and biogas production

All pretreatment methods utilized to break down the compact structure of lignocellulosic biomass impose harsh operational conditions, such as high pressure and temperature requirements, and the use of high concentrations of alkalis, acids, and ionic liquids, which may introduce toxicity into the anaerobic digester and hinder biogas production. However, a promising approach to disintegrate and solubilize lignin under milder conditions involves the use of an Advanced Oxidation Process (AOP) that combines the photocatalyst $TiO_2$ and UV light. The objective of the Photocatalytic Oxidation process is to enhance the biodegradability of the biomass and boost biogas production in anaerobic digestion. The photocatalytic disintegration of lignin occurs when the TiO2 surface is exposed to high-energy and short-wavelength ultraviolet (UV) light. This UV light generates OH radicals and electron-hole reactions, which complete the photolysis process. The OH radicals play a crucial role in oxidatively breaking down carbohydrates and recalcitrant lignin by creating reactive oxygen species. The major products of this oxidative disintegration of lignin are carboxylic acids and aromatic aldehydes. Through the oxidative degradation of lignin, vanillin is produced in the range of 5-15 wt % relative to the lignin source. Previous research has investigated the degradation of lignin in materials such as wood flour, wheat straw, sugarcane bagasse, rice straw, and rice husk, resulting in valuable products like aldehydes, vanillin, acetic acid, succinic acid, and malonic acid, among others (Li et al., 2015). Various studies have employed $TiO_2$/UV as a pretreatment technology for degrading organic contaminants in water and air. The $TiO_2$/UV process has demonstrated successful applications in pretreating paper mill effluent, black liquor, and olive mill waste, as well as in air purification units. Additionally, the field of nanoscience has significantly expanded in science and technology, offering numerous advantages. In photocatalysis, researchers are extensively exploring nano-sized catalysts due to their added benefits, which include a decrease in the recombination of electron-hole pairs. The quick diffusion of charge carriers to the surface of the particles to be degraded and the increased surface area enhances the interaction of the target particles with the catalyst (Vorontsov & Tsybulya, 2018). According to Alvarado-Morales et al. (2016), their study revealed that $TiO_2$ photocatalytic pretreatment of wheat straw (WS) resulted in increased biodegradability and enhanced biogas production. In particular, wheat straw pretreated with 1.5% $TiO_2$ for 3 hours of UV irradiation showed a remarkable 37% increase in methane yield compared to the untreated control. The study concluded that the Advanced Oxidation Process (AOP) in the presence of $TiO_2$ and UV light holds promise as a pretreatment method for effectively disrupting lignin-rich substrates under mild conditions. In the study conducted by (Jafari & Zilouei, 2016), explored the pretreatment of bagasse using $TiO_2$ in conjunction with UV light, followed by dilute sulfuric acid hydrolysis. This pretreatment approach demonstrated significant improvements in biomethane and biohydrogen production in AD and consecutive dark fermentation process. Notably, the highest hydrogen yield of 101.5 mL/g (VS) was achieved when using 1 g of nano $TiO_2$ per liter with 120 minutes of UV light exposure, followed by 30 minutes of sulfuric acid hydrolysis. The researchers concluded that the nano-$TiO_2$ pretreatment effectively disrupted the surface morphology and reduced the degree of crystallinity, contributing to the observed enhancements in gas production.

### 2.7.3 Influence of combined pretreatment on lignocellulosic biomass and biogas production

In their pioneering work, Kobayakawa et al. (1989) were the first to explore the oxidative degradation of lignin and discovered that the complete breakdown of lignin could be achieved by reacting hydrogen peroxide with either UV light or ferric ions. Similarly, Ohnishi et al. (1989) conducted a comparative study on various photocatalysts for lignin degradation. They found that the photocatalytic process using TiO2 could be enhanced by incorporating metals such as Pt, Au, and Ag. Studies have shown that utilizing a 10% Cu/$TiO_2$ photocatalyst, along with solar radiation as the light source and air as the oxidizing agent, resulted in improved biodegradability and solubility of coffee pulp. This enhanced biodegradability, in turn, promotes increased biogas production during anaerobic digestion (Corro et al., 2014).

Chang et al. (2018) investigated the impact of a combined pretreatment method on rice straw and biogas production. The researchers used a $TiO_2$/UV/$H_2O_2$ pretreatment to improve the enzymatic hydrolysis of rice straw and enhance biogas production. They examined the optimal conditions for the pretreatment and analyzed the composition and characteristics of the untreated and pretreated rice straw. The results showed that the $TiO_2$/UV/$H_2O_2$ pretreatment effectively removed lignin and hemicellulose from the rice straw, leading to increased enzymatic hydrolysis and the release of reducing sugars. The pretreatment also resulted in changes in the morphology of the rice straw. The researchers found that adding 13 mM $H_2O_2$ at pH 4, with an irradiation time of 3 hours and a concentration of 0.50% $TiO_2$, was the optimal condition for pretreatment. According to Anjum et al. (2018), the utilization of carbon nitride/Titania nanotubes ($C_3N_4$/$TiO_2$ NTs) as a pretreatment for sludge, in the presence of visible light, led to a significant enhancement in methane potential, reaching up to 723.4 ml $kg^{-}1$ VS. This value was 1.37 times higher than the photolytic sludge and 1.6 times higher than the raw sludge, indicating the considerable improvement achieved through this novel approach.

## *Chapter 3*

# Materials and Methods

This chapter provides a detailed explanation on the materials used, the experimental setup, as well as the methodological approach that was used in this investigation. The primary objective of this study is to evaluate the effect of loading rates on the production of biogas from wheat straw (WS) that has been subjected to one or more of the following: treatment with NaOH; treatment with $TiO_2$; and treatment that combines treatment with both NaOH and $TiO_2$. Methodological approach is illustrated in Figure 3.1 below.

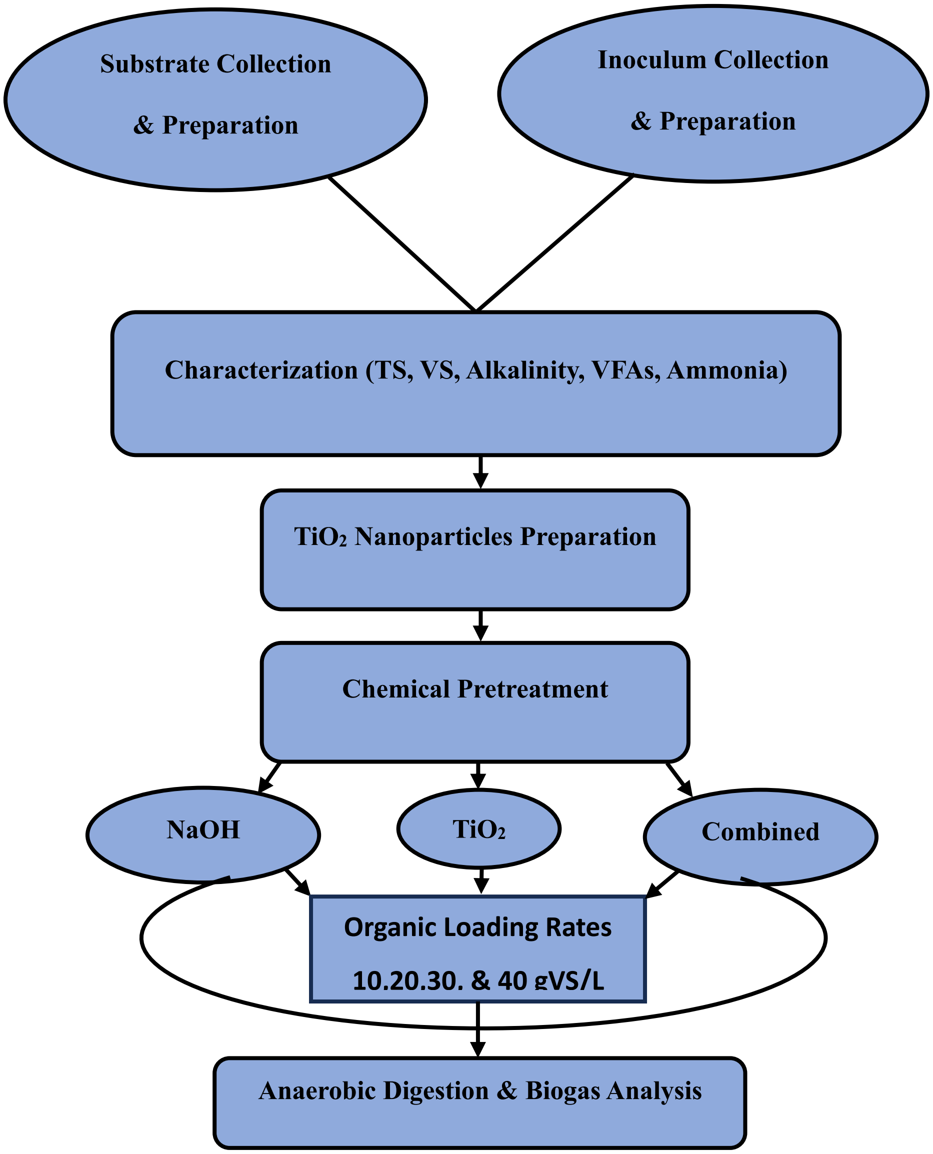


**Fig. 3.1 Methodological Approach**

## 3.1 Substrate and Inoculum Preparation

In this study, wheat straw was used as a substrate. The WS was obtained from a field in Village Chamkani, near Peshawar, and was carefully transported to the laboratory for initial analysis. To achieve the desired biomass size, the straw was shredded using a small-scale shredder and then passed through an 18-mesh sieve, resulting in biomass particles ranging from 3 mm to 5 mm. The sieved wheat straw was stored and later utilized for subsequent analysis.

**Fig. 3.2 Crushed Wheat straw**

Cow dung (CD) was used as inoculum in the anaerobic process. CD was collected from a nearby farm located in Sector H-13 Islamabad. Before it was being used, CD was kept for degassing at 37 °C in an anerobic condition for 20 days and gas was collected twice a day.

**Fig. 3.3 Cow dung as an inoculum**

## 3.2 Primary Characterization and Fiber Analysis

During the process of treating wheat straw and cow dung, a number of straightforward characteristics, such as total solids (TS), volatile solids (VS), alkalinity, and volatile fatty acids (VFAs), were measured. All the tests were carried out in accordance with (APHA, 2017) procedures. In order to get a comprehensive understanding of the chemical make-up of WS, the extractives and structural components of the substance, such as lignin, hemicellulose, and cellulose, were subjected to the processes developed by Li et al. (2004).

## 3.3 Nanoparticles Preparation

To produce nanoparticles, Titanium (IV) Oxide Anatase (CAS NO. 13463-67-7) obtained from DAEJUNG CHEMICAL & METALS CO., LTD. Korea was utilized as the source of Titania. The liquid impregnation method, as described by (Husnain et al., 2016), was employed to achieve a nano-sized crystal structure. For the preparation of the photocatalyst, 100 grams of Titanium (IV) Oxide was dissolved in 600 mL of distilled water and stirred for 24 hours. The resulting $TiO_2$ solution was allowed to settle for 24 hours and then dried in an oven at 105 °C for 24 hours. The dried $TiO_2$ slurry was crushed into powder form and subjected to calcination in a muffle furnace (JSR, JSMF-270H, Korea) at 550 °C for approximately 6 hours. The calcined $TiO_2$ was then slowly cooled down to obtain a nano-sized crystal structure.

(a) Crushed $TiO_2$

(b) Calcined $TiO_2$

**Fig. 3.4 Preparation of nanoparticles (a) Crushed $TiO_2$ (b) Calcined $TiO_2$**

## 3.4 Chemical Pretreatment of Substrate

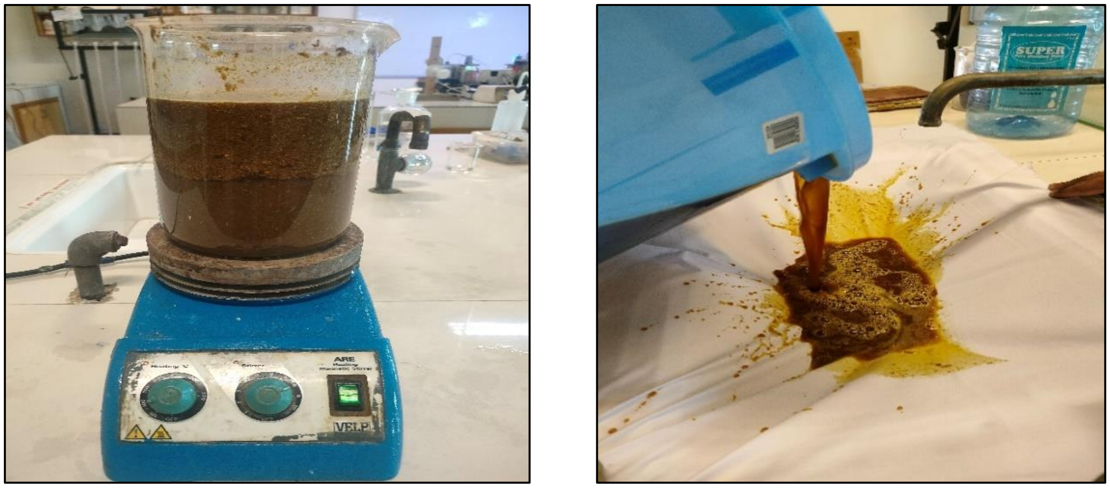
Wheat straw was chemically pretreated by the following methods before it was used for the anaerobic digestion process.

i. Alkaline Pretreatment
ii. Photocatalytic Pretreatment
iii. Combined Pretreatment

### 3.4.1 Alkaline Pretreatment

Alkaline pretreatment of WS was done through NaOH. The treatment was carried out in 2-liter beakers at 40 °C for 3 hours. NaOH concentration of 1% (w/v) was taken and WS and NaOH solution was mixed in a ratio of 1:20. Each gram of WS was mixed with 20 mL of 1% NaOH solution. During the treatment process, the mixture was continuously stirred with the help of magnetic stirrer at 200 rpm. After completion, the pretreated WS was cooled, filtered, and washed with distilled water until neutral pH was obtained. At the end, was dried in oven at 105 °C for 24 hours. The dried WS was then used for further analysis.

**(a) NaOH pellets** **(b) WS and NaOH solution**



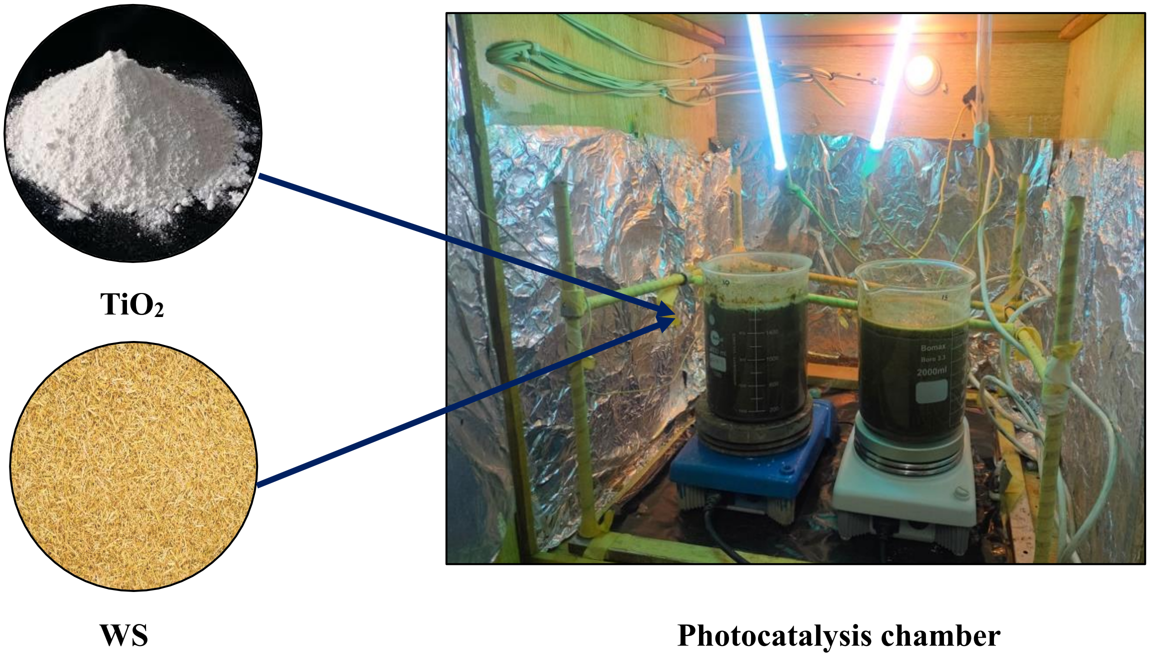
**(c ) Heating and stirring** **(d) Filtering and washing**

**(e) NaOH pretreated WS**

**Fig. 3.5 (a-e) Alkaline pretreatment of WS with NaOH**

### 3.4.2 Photocatalytic Pretreatment

Photocatalytic pretreatment of WS was carried out in photocatalysis chamber using $TiO_2$. The photocatalysis chamber used in the pretreatment process consisted of four 100 watts UV-A Lamps having a wavelength of interest ranging from 300-320 nm. $TiO_2$ concentration of 1.5 g/L was taken and WS to $TiO_2$ solution ratio was set as 1:20. The lamps were fixed at the top of beakers containing WS and $TiO_2$ solution and were exposed to UV irradiation at 40 °C for 3 hours. During the treatment process, the WS sample was continuously stirred at 200 rpm with the help of magnetic stirrer. After completion, the pretreated WS was cooled, filtered, and washed with distilled water to obtain neutral pH. At the end, the pretreated WS was dried at 105 °C for 24 hours in oven and used for subsequent analysis.



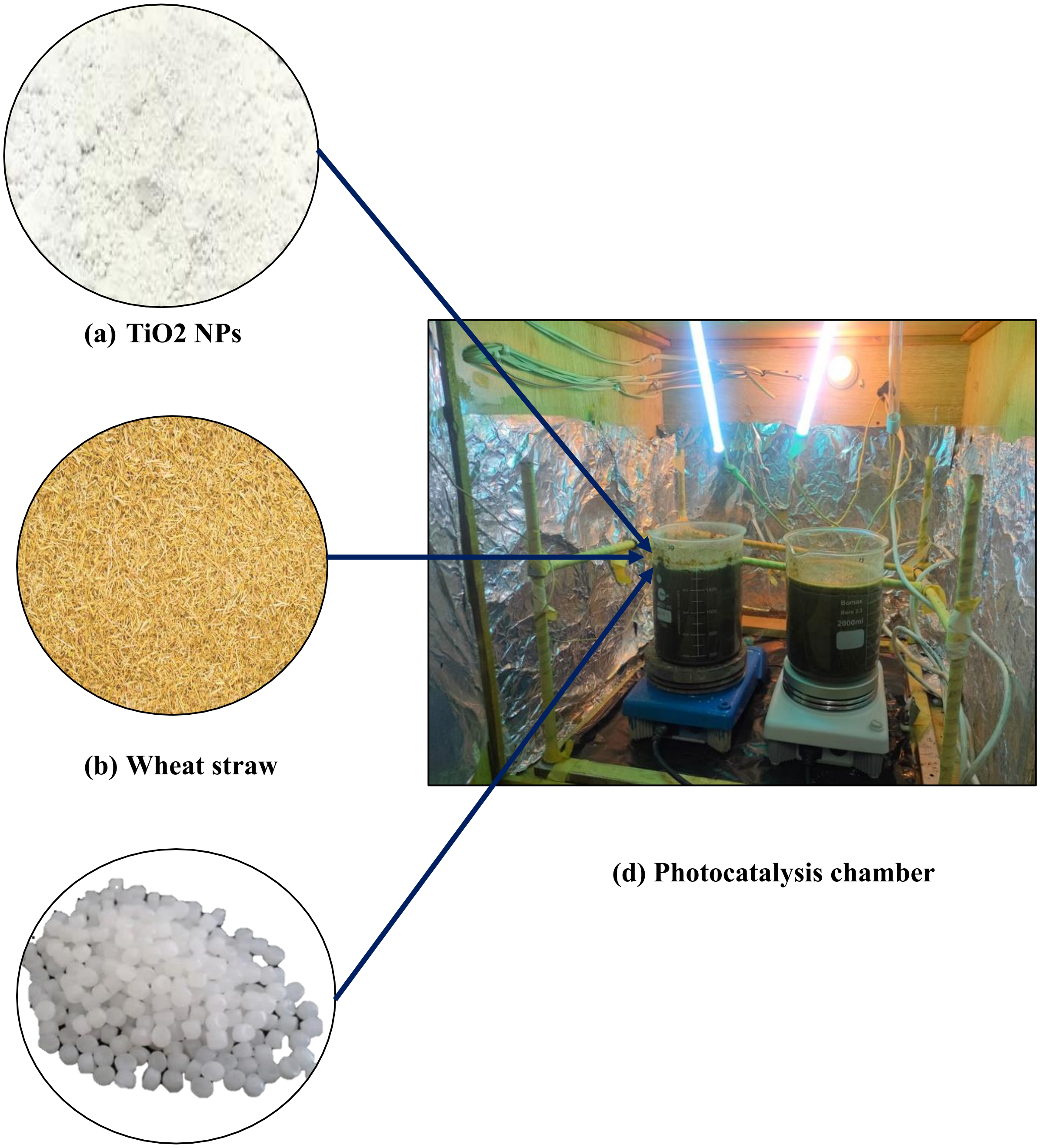
**(a) Pretreatment of WS in Photocatalysis chamber**

**(b) $TiO_2$ Pretreated WS after washing**

**Fig. 3.6 (a-b) Photocatalytic pretreatment of WS using $TiO_2$**

### 3.4.3 Combined Pretreatment

Combined pretreatment of WS was carried out in photocatalysis chamber using NaOH combined $TiO_2$. The photocatalysis chamber used in the pretreatment process consisted of four 100 watts UV-A Lamps having a wavelength of interest ranging from 300-320 nm. NaOH Concentration of 1% (w/v) along with $TiO_2$ concentration of 1.5 g/L was taken. The ratio of WS to solution was kept as 1:20. The lamps were fixed at the top of beakers containing WS and $TiO_2$ solution and were exposed to UV irradiation at 40 °C for 3 hours. During the treatment process, the WS sample was continuously stirred at 200 rpm with the help of magnetic stirrer. After completion, the pretreated WS was cooled, filtered, and washed with distilled water to obtain neutral pH. At the end, the pretreated WS was dried at 105 °C for 24 hours in oven and used for subsequent analysis.



**(a) TiO2 NPs**

**(b) Wheat straw**

**(c) NaOH pellets**

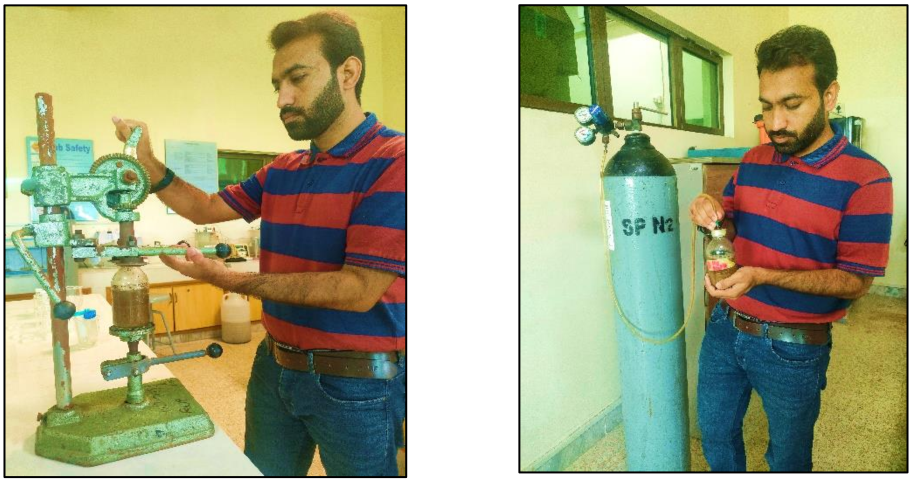
**(d) Photocatalysis chamber**

(e) Combined pretreated WS after washing

Fig. 3.7 (a-e) Combined pretreatment of WS using NaOH and $TiO_2$

## 3.5 Anerobic Digestion Setup

A fixed amount of wheat straw, either pretreated or untreated, was introduced into glass reactors functioning as anaerobic digesters. These reactors operated in batch mode and had a capacity of 300 ml. The inoculum and substrate were mixed in a 1:1 ratio based on their volatile solids (VS) content to achieve a final working volume of 225 ml. The substrate loading rates varied at 10, 20, 30, and 40 gVS/L. Wheat straw was pretreated with three types of pre-treatments including NaOH (1%), $TiO_2$ (1.5 g/L) and Combined (1% NaOH + 1.5 g/L $TiO_2$). To reach the working volume of 225 ml, distilled water was added. The pH was maintained between 7.2 and 7.3 by using a sodium bicarbonate buffer. All the anaerobic digesters were sealed tightly with rubber septa and screw caps, and the headspaces were purged with pure $N_2$ gas for approximately 2 minutes to establish anaerobic conditions. The reactors were placed in a mesophilic incubator at a temperature of 37±1 ⁰C for a duration of 30 days. Twice daily, the AD reactors were manually shaken for 1 minute to ensure mixing. Each digestion material consisted of a pretreated wheat straw sample, with an untreated wheat straw sample serving as the control. Additionally, a blank AD reactor containing cow dung without any wheat straw sample was included to measure methane yield from the cow dung. All AD reactors were operated in triplicate. A detailed experimental design showing organic loading rates, pretreatment types and the number of AD reactors in triplicates is given in Fig. 3.9.



**(a) Aluminum Caps** **(b) Nitrogen Purging**

**(c) Incubation**

**Figure 3.8 Preparation of Digesters and Nitrogen Purging**

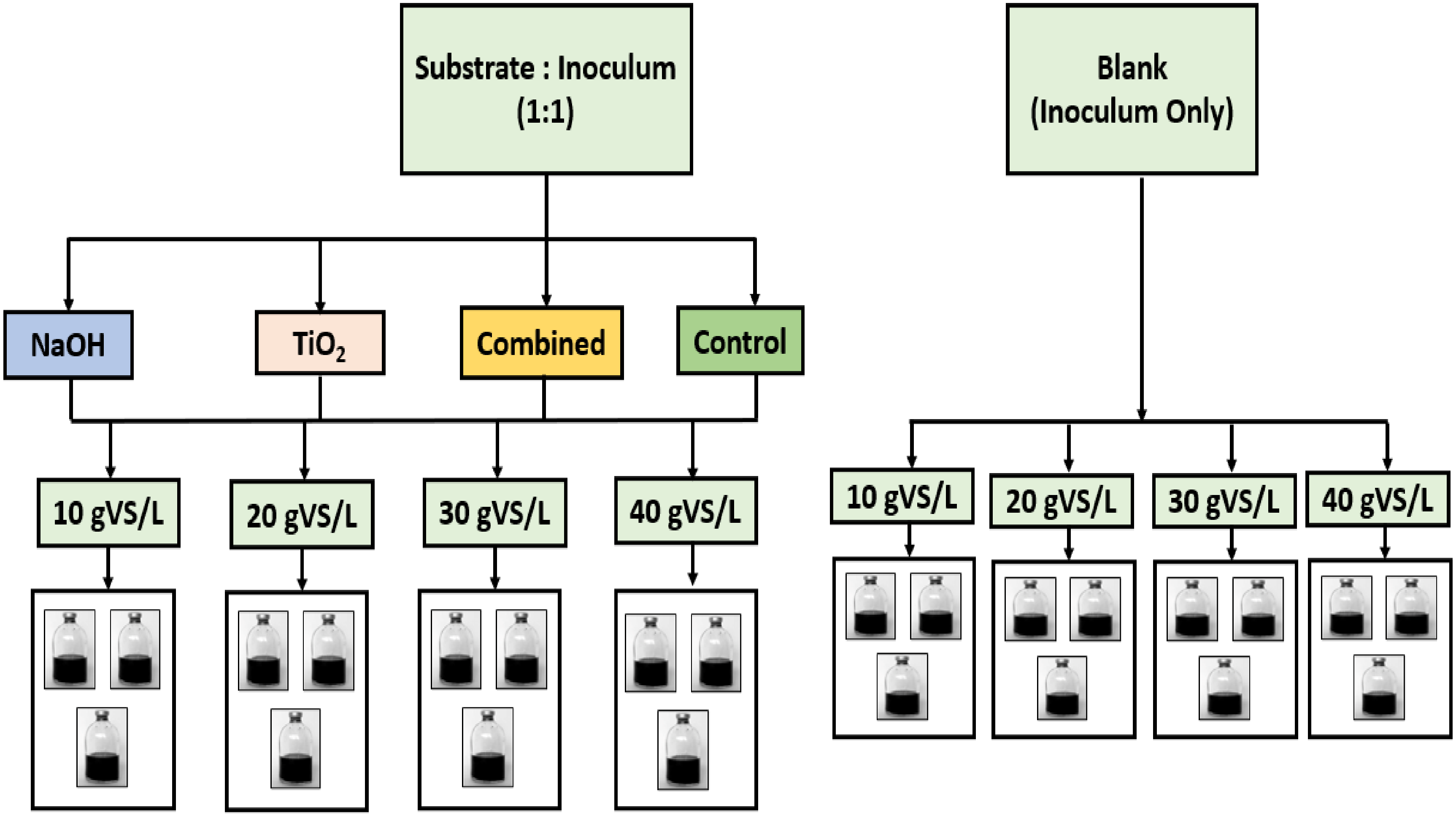


Fig. 3.9 Experimental Design

## 3.6 Substrate Characterization

Primary analysis of raw and pretreated WS was done by analyzing different parameters such as TS, VS and MC following the guidelines provide by (APHA, 2017). Fiber analysis including extractives, lignin, hemicellulose, and cellulose of untreated and treated WS was carried out following the prescribed method by (Li et al., 2004). The experiments were performed in triplicates to get satisfactory results.

### 3.6.1 Total Solids

For the determination of total solids in WS samples, the china dish was washed with distilled water and dried at 105 °C for an hour in oven. The oven fried china dish was then cooled in the desiccator and 50 g of WS was transferred to the dish. The china dish was placed in the oven at 105 °C for 24 hours for TS content. After completion, the dish was taken out from the oven. Cooled in the desiccator and weighed using analytical balance. The quantity of wheat straw remaining in the dish after drying was TS content and was calculated by the following equation 3.1.

$$TS(\%) = \frac{(W1-W2)}{(W3-W2)} X100 \qquad (3.1)$$

Where, $w_1$ = weight of china dish after evaporation + weight of dried WS residue

$w_2$ = weight of china dish

$w_3$ = weight of wet WS sample + weight of china dish

### 3.6.2 Moisture Content

The quantity loss while drying the sample at 105 °C is the moisture content and can be calculated by the following equation 3.2.

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

### 3.6.3 Volatile Solids

Determination of volatile solids involves ignition of the already dried WS sample for TS in a muffle furnace at 550 °C for 30 minutes. After completion, the WS sample was cooled in a desiccator and weighed to get the value of volatile solids by the following equation 3.3.

$$VS(\%) = \frac{(W1-W4)}{(W1-W2)} X100 \qquad (3.3)$$

Where, $w_4$ = weight of WS sample + china dish after ignition

### 3.6.4 Extractives

For the determination of extractives amount in the WS sample, solvent extraction method using 60 mL of acetone for each gram of wheat straw was used. The sample was heated at 90 °C for 2 hours. After completion, the WS sample was oven dried at 105 °C and the amount of extractives were calculated as the difference in weight before and after the extraction process by the following equation 3.4.

$$Extractives\ (\%) = \frac{W0-W1}{(W0)}\ X100 \quad (3.4)$$

Where, $w_0$ = weight of dried WS sample before extraction

$w_1$ = weight of WS sample after extraction

### 3.6.5 Hemicellulose

Determination of hemicellulose was carried out by adding 150 mL of 0.5 mol/L NaOH solution to each gram of extractives-free WS sample and heated at 80 °C for 3.5 hours. After completion, the sample was cooled down and washed with distilled water until neutral pH value is obtained. After that, the sample was dried to a constant weight and the amount of hemicellulose was calculated by the difference in weight before and after this process by the following equation 3.5.

$$Hemicellulose\ (\%) = \frac{W1-W2}{(W1)}\ X100 \quad (3.5)$$

Where, $w_1$ = weight of extractives-free WS sample

$w_2$ = weight of WS sample after heating

### 3.6.6 Lignin

Lignin content present in WS sample was determined by adding 30 mL of 98% sulfuric acid for each gram of extractives-free WS sample and kept for 24 hours in ambient temperature. After this, the sample was boiled at 100 °C for 1 hour. The boiled sample was then cooled, filtered, and washed with distilled water to remove remaining sulfate ions. For the detection of sulfate ions, the WS sample was titrated with 10% barium chloride. It was then dried to a constant weight and residue left was calculated as lignin content by the following equation 3.6.

$$Lignin\ (\%) = \frac{W4\ [1-\left(\frac{Extractives}{100}\right)]}{(W3)}\ X100 \qquad (3.6)$$

Where,

$w_3$ = weight of extractives-free WS sample after boiling

$w_4$ = weight of dried WS sample after titration with barium chloride

### 3.6.7 Cellulose

The cellulose content can be determined by the difference assuming that extractives, hemicellulose, and lignin are the only components of entire biomass and can be calculated by the following equation 3.7.

$$Cellulose\ (\%) = 100 - (Extrictives + Hemicellulose + Lignin) \qquad (3.7)$$

## 3.7 Analytical approach to Anaerobic Digestion

Biogas volume was assessed daily with the help of water displacement technique. The daily measured biogas volume was converted to normal milliliter (NmL) (dry gas, P = 100 kPa = 760 mm Hg, T = 0 ºC) according to equation 3.8.

$$V_{NmL} = (V \times 273 \times (760 - P_w\times))/(273 + T) \times 760) \qquad (3.8)$$

Where, $V_{NmL}$ = dry biogas volume 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)

Throughout the initial week of the digestive process, biogas samples were systematically gathered twice per week, with the intention of conducting a comprehensive analysis of their composition. Subsequently, the frequency of sampling was diminished to a weekly basis. The quantification of methane content in the biogas was accomplished through the utilization of a gas chromatograph (GC-2010 PLUS SHIMADZU) that was equipped with a Molecular sieve 5A PLOT (Porous layer open tubular) column and a thermal conductivity detector (TCD). The carrier gases employed in this study were helium and nitrogen. The pH, total alkalinity (TA), volatile fatty acids (VFAs), total solids (TS), and volatile solids (VS) were assessed at the commencement and culmination of the digestion phase. A supplementary group of anaerobic digestion (AD) reactors was prepared using the identical methodology employed for the experimental reactors. These additional reactors were subsequently utilized for analysis prior to the commencement of the digestion process. The pH measurements of all the reactors were directly assessed on day 1. In order to conduct alkalinity and volatile fatty acid (VFA) analysis, a 20 g portion of the wet anaerobic digestion (AD) sample was obtained and subsequently subjected to centrifugation at a speed of 6000 revolutions per minute (rpm) for a duration of 5 minutes. Later, the supernatant was used for alkalinity and VFA analysis while the pellet was transferred back to the AD reactor with an addition of required amount of water to maintain a previous solid to liquid ratio. After that, TS analysis was performed by initially weighing the reactors and putting them in oven at 105 °C for 24 hours. After drying, the dried material from the reactors were shifted to china dishes and kept in muffle furnace for VS analysis at 550 °C for 30 minutes.

## *Chapter 4*

# Results and Discussion

This chapter gives a detailed discussion of the results acquired during the study. The results include characteristics of substrate and inoculum, pretreatment of wheat straw, the effect of different organic loading on biogas yield and reactor's stability.

## 4.1 Characteristics of wheat straw and cow dung

Table 1 shows characteristics of wheat straw and cow dung. WS had comparatively higher TS and VS content than cow dung due to less moisture content present in the dried biomass. Cow dung has more moisture content and hence lower TS and VS content.

**Table 1 Characteristics of substrate and inoculum**

| **Parameters** | **Wheat Straw** | **Inoculum** |
|---|---|---|
| Moisture Content (%) | 8 | 90.41 |
| Total Solids (%) | 92 | 9.59 |
| Volatile Solids (% of TS) | 80 | 73.48 |
| Volatile Fatty Acids (mg/L) | --- | 1150 |
| Alkalinity (mg/L) | --- | 5200 |
| VFA/Alkalinity | --- | 0.22 |

## 4.2 Effect of OL on biogas production from untreated WS

The daily biogas production from untreated wheat straw under different organic loading is depicted in Fig 4.1. The organic loading of 40 gVS/L has maximum daily biogas production of 2002 mL followed by 30, 20 and 10 gVS/L with a daily biogas production of 1948, 1934, and 1765 mL respectively. However, in case of cumulative biogas yield, 10 gVS/L organic loading has the maximum biogas yield of 784 mL/gVS. Organic loading 20, 30 and 40 gVS/L has a cumulative biogas yield of 429, 288, and 222 mL/gVS respectively. The biogas yield declines with an increase in organic loading. The cumulative biogas yield from untreated WS under different organic loading is given in Fig. 4.2.

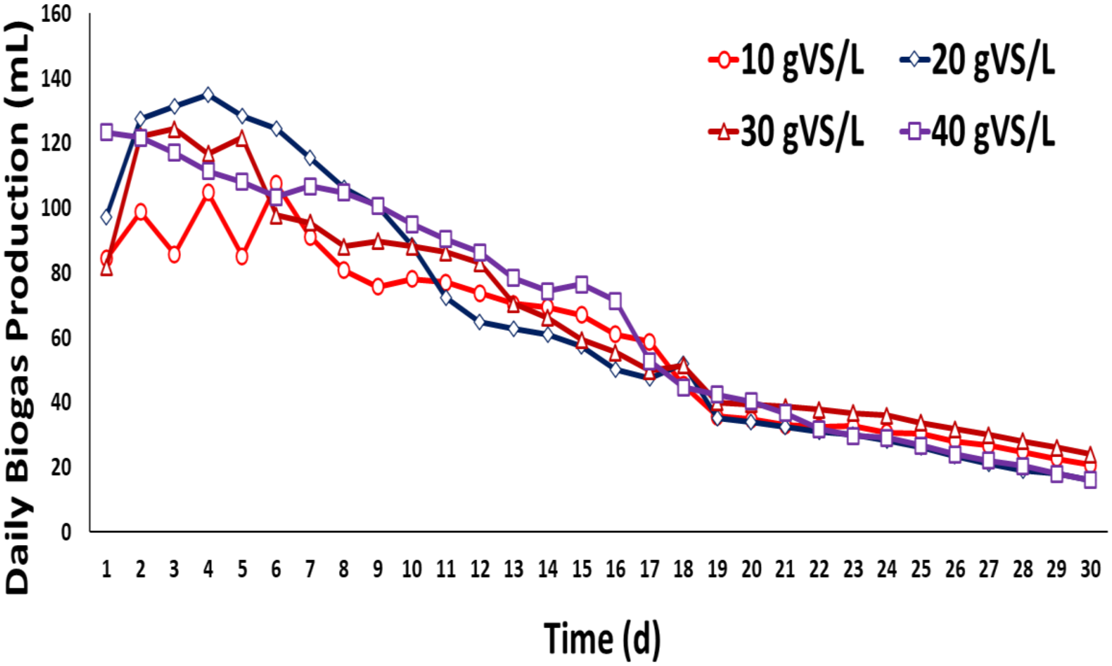


**Fig. 4.1 Effect of organic loading on daily biogas production from untreated WS**

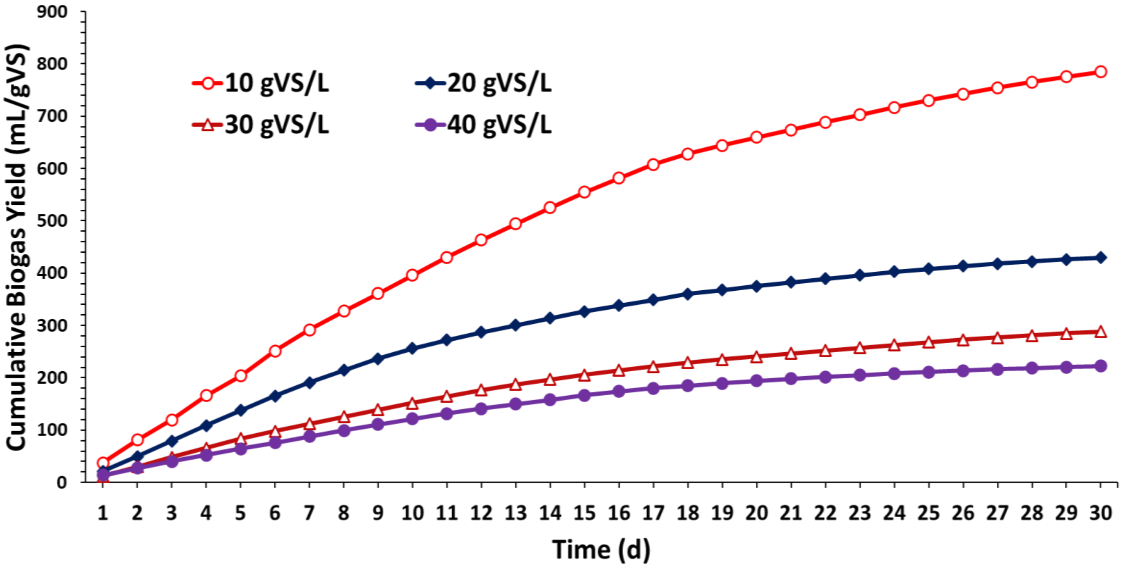


**Fig. 4.2 Effect of organic loading on cumulative biogas yield from untreated WS**

## 4.3 Effect of OL on biogas production from NaOH pretreated WS

In the case of NaOH pretreatment, the organic loading of 40 gVS/L has produced the highest daily biogas of 3195 mL, followed by 30, 20 and 10 gVS/L with a daily biogas production of 2781, 2653 and 2180 mL, respectively. The daily biogas production from pretreated WS is given in Fig. 4.3.

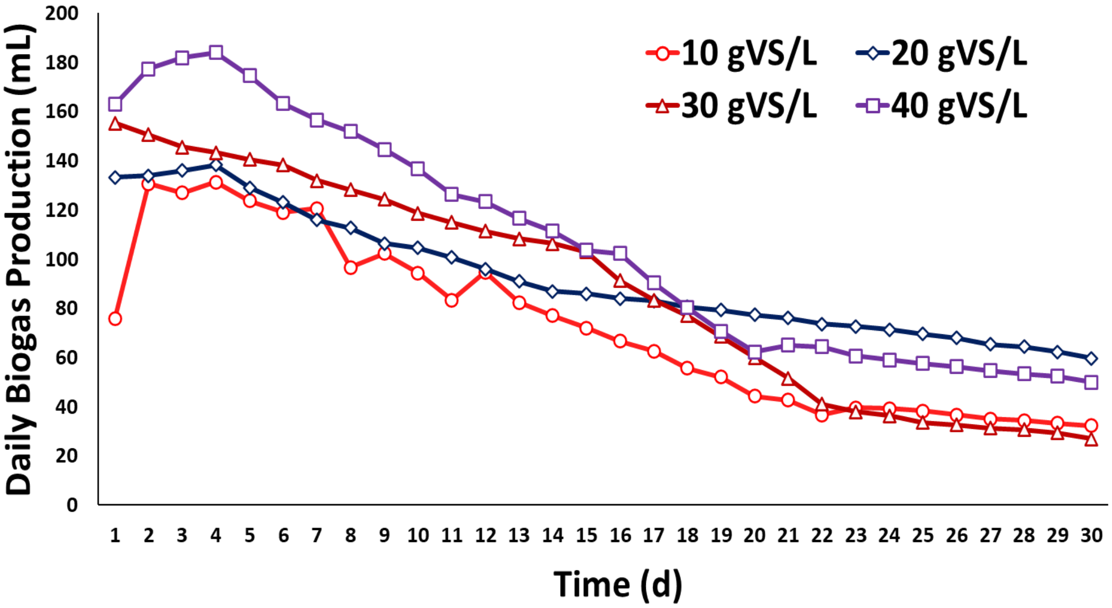


**Fig. 4.3 Effect of organic loading on daily biogas yield from NaOH pretreated WS**

The effect of organic loading on cumulative biogas yield from NaOH pretreated WS was also studied. The NaOH pretreated WS at 10 gVS/L gave 56%, 146% and 172% higher biogas yield than higher organic loadings. The biogas yield was high at low organic loadings, however, it declined with the increase in organic loading. These results align with the results of (Chandra et al., 2012), who reported that, NaOH pretreated WS produced 87% more biogas at OLRs 6-8 gVS/L. The cumulative biogas production from NaOH pretreated WS under different organic loading is depicted in Fig. 4.4.

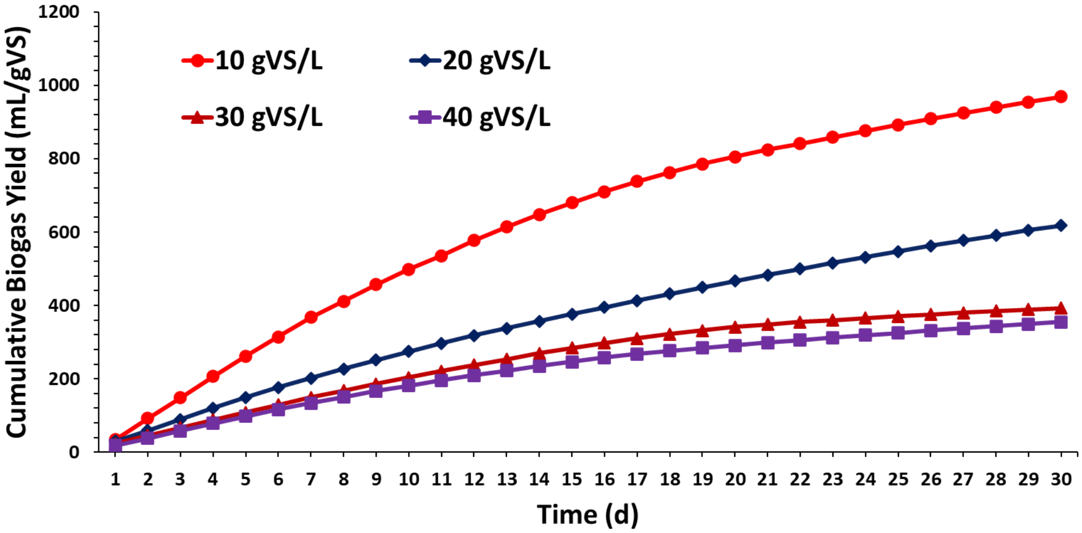


**Fig. 4.4 Effect of organic loading on cumulative biogas yield from NaOH pretreated WS**

## 4.4 Effect of OL on biogas production from $TiO_2$ pretreated WS

The effect of organic loading on daily biogas production from Tio2 pretreated wheat straw was observed as 20 gVS/L has produced more biogas than Organic loading 10, 30 and 40 gVS/L. At organic loading 20 gVS/L, the $TiO_2$ pretreated WS has produced 2659 mL biogas. However, organic loading 40, 10 and 30 gVS/L has produced 2363, 2314, and 2210 mL biogas, respectively. The daily biogas production from $TiO_2$ pretreated WS under different organic loading is given in Fig. 4.5 below.

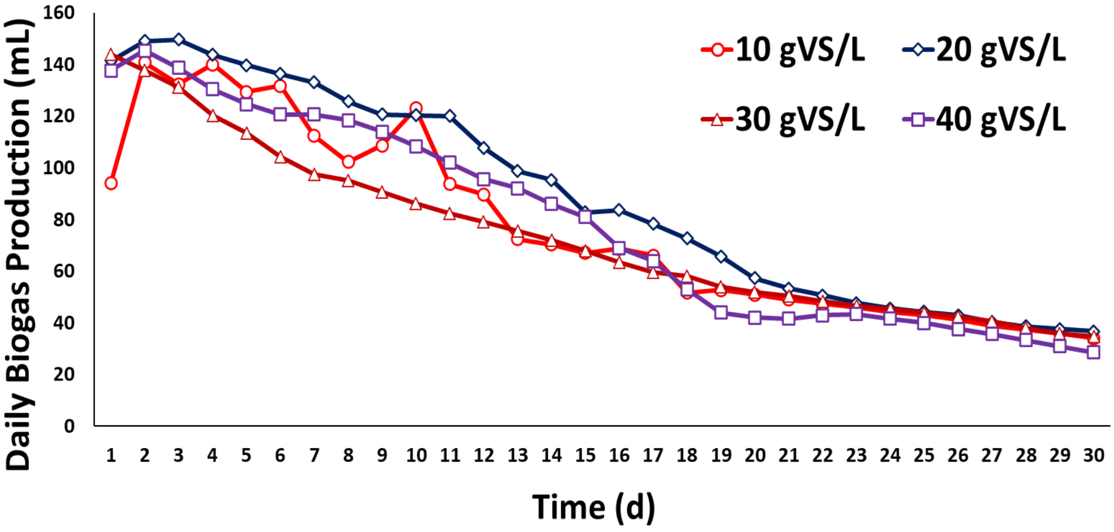


Fig. 4.5 Effect of organic loading on daily biogas yield from $TiO_2$ pretreated WS

The effect of organic loading on cumulative biogas yield from $TiO_2$ pretreated wheat straw revealed that 10gVS/L has produced 74%, 214% and 292% more biogas yield than 20, 30 and 40 organic loading. These results align with the results from (Alvarado-Morales et al., 2017). They have reported 37% improvement in biogas yield at 10gVS/L loading when pretreated with 1.5 g $TiO_2$/L. The biogas yield declined at higher organic loading. Fig. 4.6 depicts the impact of OL on cumulative biogas yield from $TiO_2$ pretreated WS.

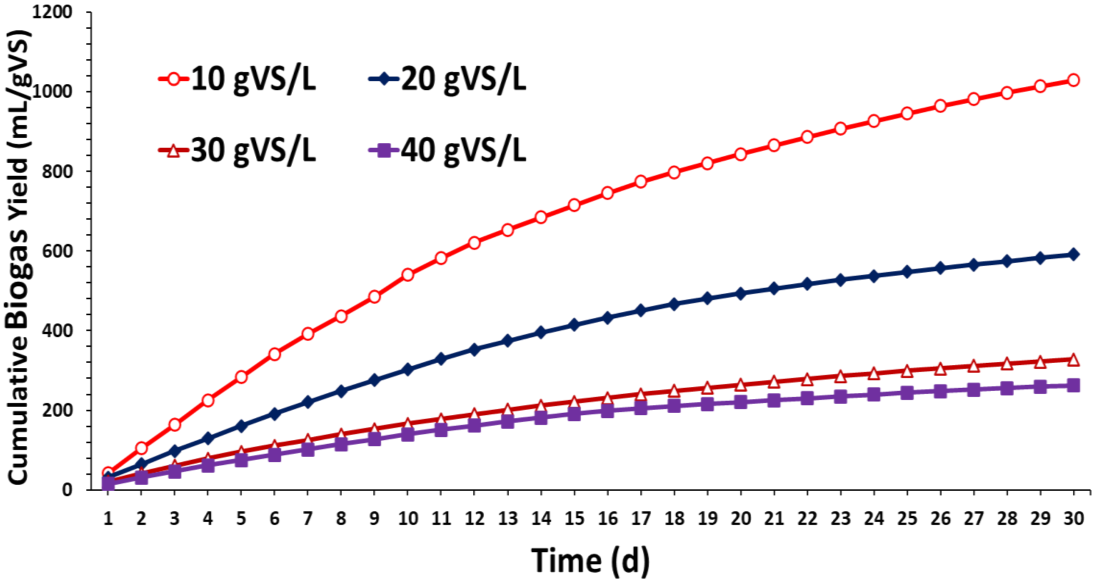


Fig. 4.6 Effect of organic loading on cumulative biogas yield from $TiO_2$ pretreated WS

## 4.5 Effect of OL on the production of biogas from Combined pretreated WS

In case of daily biogas production from combined pretreated wheat straw, the organic loading 40 gVS/L has produced high biogas of 3610 mL, followed by 30 20 and 10 gVS/L. Daily biogas production from combined pretreated WS under the effect of organic loading is given in Fig. 4.7.

The cumulative biogas yield obtained from combined pretreated wheat straw shows that 10gVS organic loading has increased the biogas yield 87%, 146% and 206% more than 20, 30 and 40 gVS/L organic loading. The combined pretreatment has maximum biogas yield than NaOH and $TiO_2$ individually.

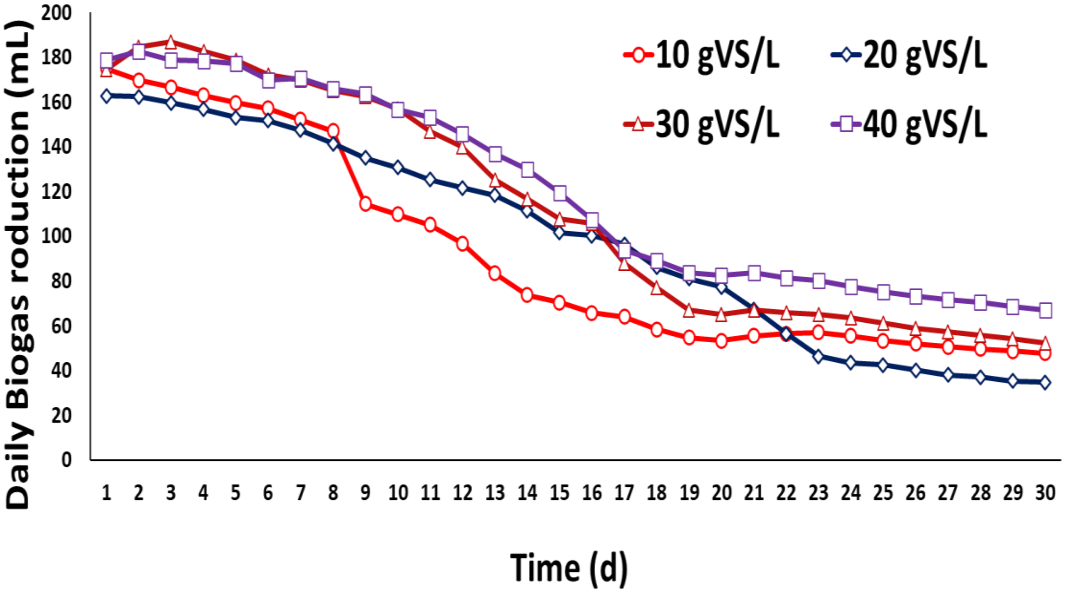


**Fig. 4.7 Effect of organic loading on daily biogas production from combined pretreated WS**

The biogas production was low at high organic loading while the low organic loading has produced the maximum biogas. The cumulative biogas production from combined pretreated WS under different organic loading is given in Fig. 4.8.

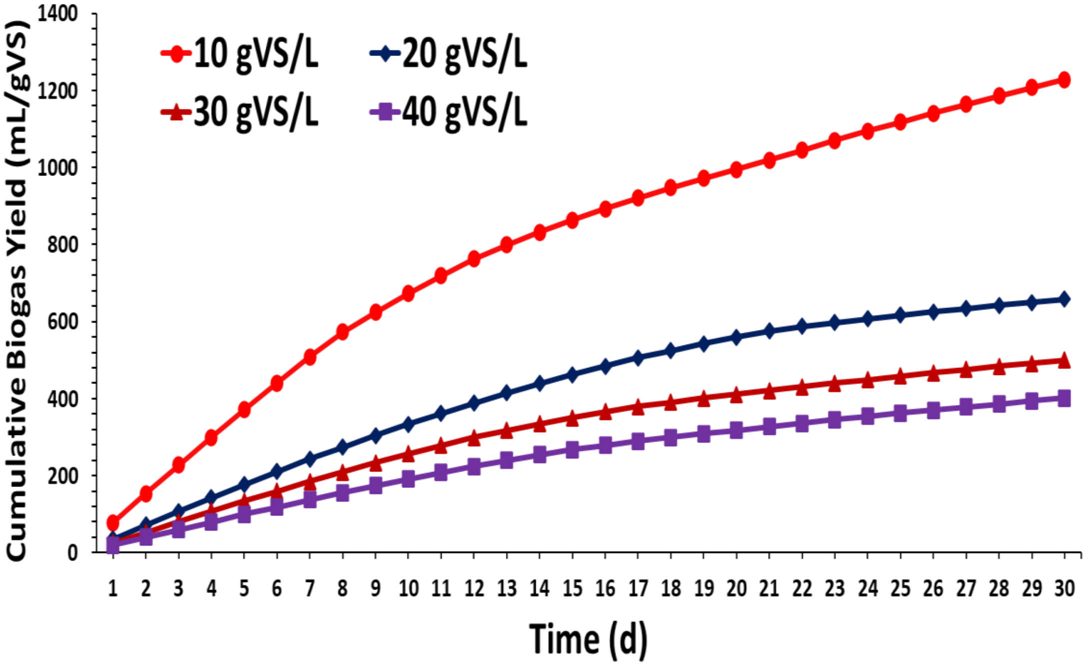


**Fig. 4.8 Effect of organic loading on cumulative biogas yield from combined pretreated WS**

## 4.6 Effect of organic loading on solids removal of WS

TS and VS removal are important factors in AD process. Among all the organic loading rates, the maximum TS and VS removal of 77 and 71% was observed in combined pretreated WS at 10 gVS/L. The impact of organic loading, in this case, was observed on solids removal. When the organic loading was low, the removal of solids was maximum as more biogas was produced and the conditions were stable. With the increase in organic loading, the solids removal decreased which can be attributed to the change observed in the reactor's stability. Acids formation took place which resulted in low biogas as well as low solids removal. These results are in agreement with study conducted by (Rajput & Sheikh, 2019), they have reported maximum VS removal of 78% at low OL during AD of Sunflower meal & digested manure. TS and VS removal at all organic loadings for pretreated WS are present in Fig. 4.9.

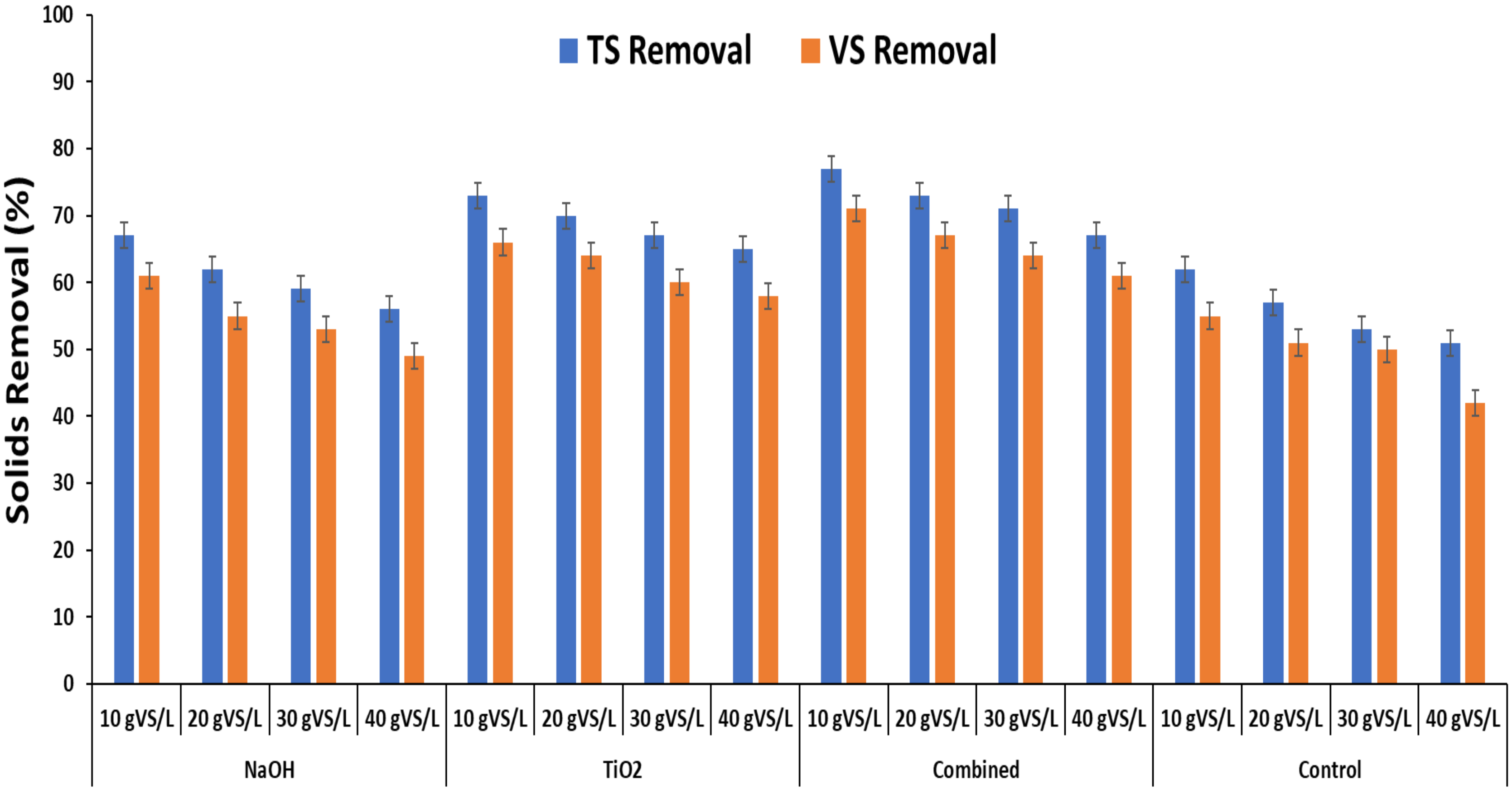


Fig. 4.9 Effect of organic loading on TS and VS removal of WS

## 4.7 Effect of organic loading on reactor stability

Reactor stability is of utmost importance in anerobic digestion process. To keep the digestion process going it is important to keep the stability parameters in optimum range.

### 4.7.1 pH

The sharp decline in pH resulting from the accumulation of volatile fatty acids (VFAs) has a direct impact on the activity of methanogenic bacteria and the overall stability of the anaerobic digester process, as highlighted by Neshat et al. (2017). Consequently, parameters such as pH, total alkalinity, volatile fatty acids concentration, and the ratio of VFAs to alkalinity serve as critical indicators of stress used for evaluating the performance of anaerobic digestion (AD). The pH level notably influences the growth rate of microorganisms, the progression of digestion, and the resultant products. The assessment of AD reactor efficiency involves the measurement of pH both before and after the digestion process. (Neshat et al., 2017) reported the pH of 6.7-7.3 as optimum for anerobic digestion. The pH of all the reactors ranged from 6.7-7.2. Fig. 4.10 shows the pH of all the reactors.

### 4.7.2 Effect of organic loading om VFA/TA ratio of WS

The VFA/TA ratio was increased in the final due to accumulation of acids at high organic loading which resulted in instability of the reactor and hence low biogas yield. The VFA/TA ratio for all the reactors ranged from 0.14-0.31. Although VFA/TA ratio did not exceed the limit which causes inhibition, but a significant change was observed in the ratio under high and low organic loading. At low organic loading, no instability was observed in the reactors and the biogas production was high. On the other hand, at high organic loading the ratio was increased due to acids formation which ultimately resulted in low biogas yield and stability was also compromised a bit. The results obtained are in agreement with the work from (Wang et al., 2016). They reported VFA/TA ratio of 0.4 as an optimum for anaerobic digestion. Fig 4.11 depicts the effect of organic loading on VFA/TA ration of WS.

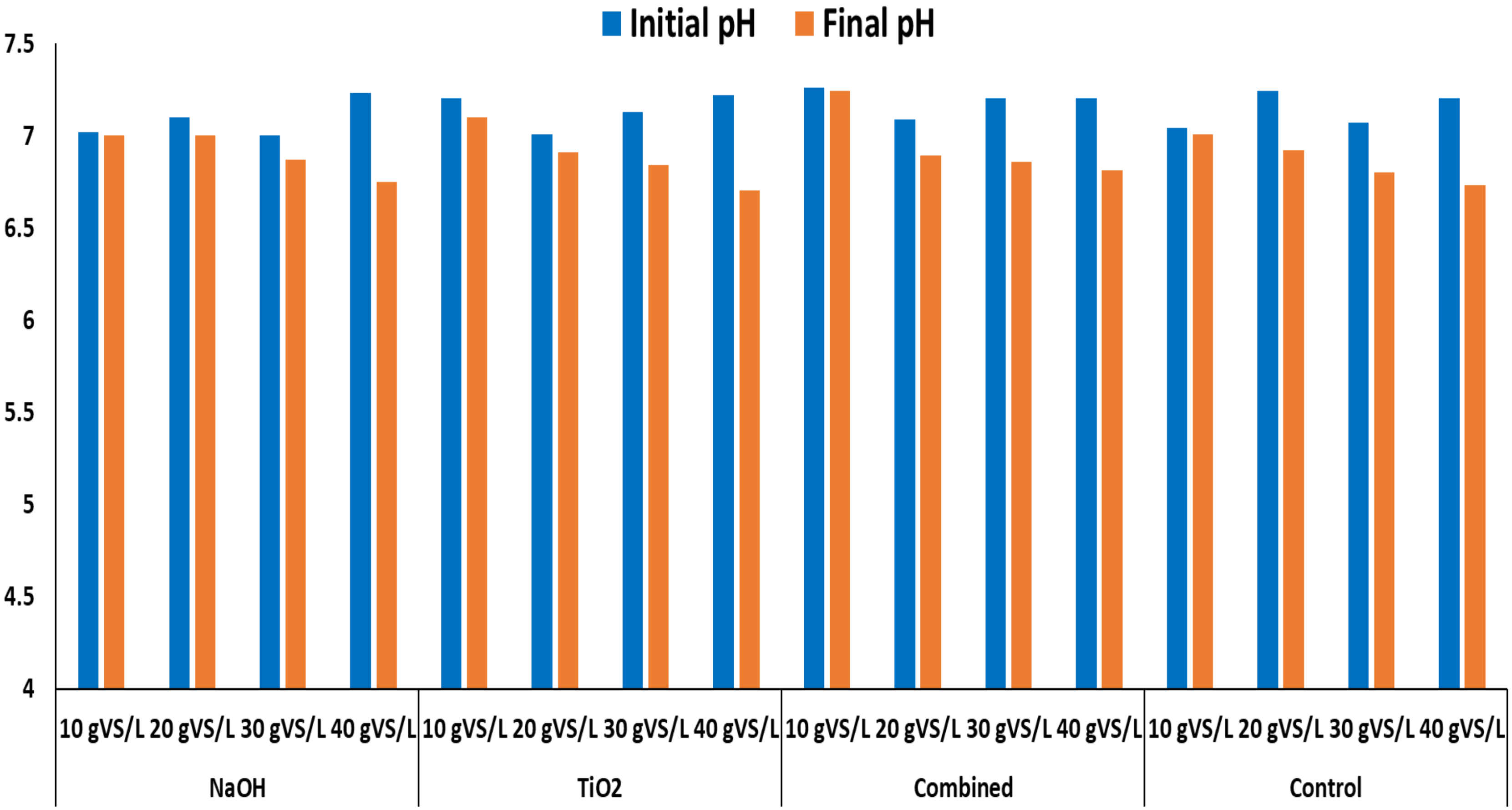


Fig. 4.10 Effect of organic loading on pH of WS

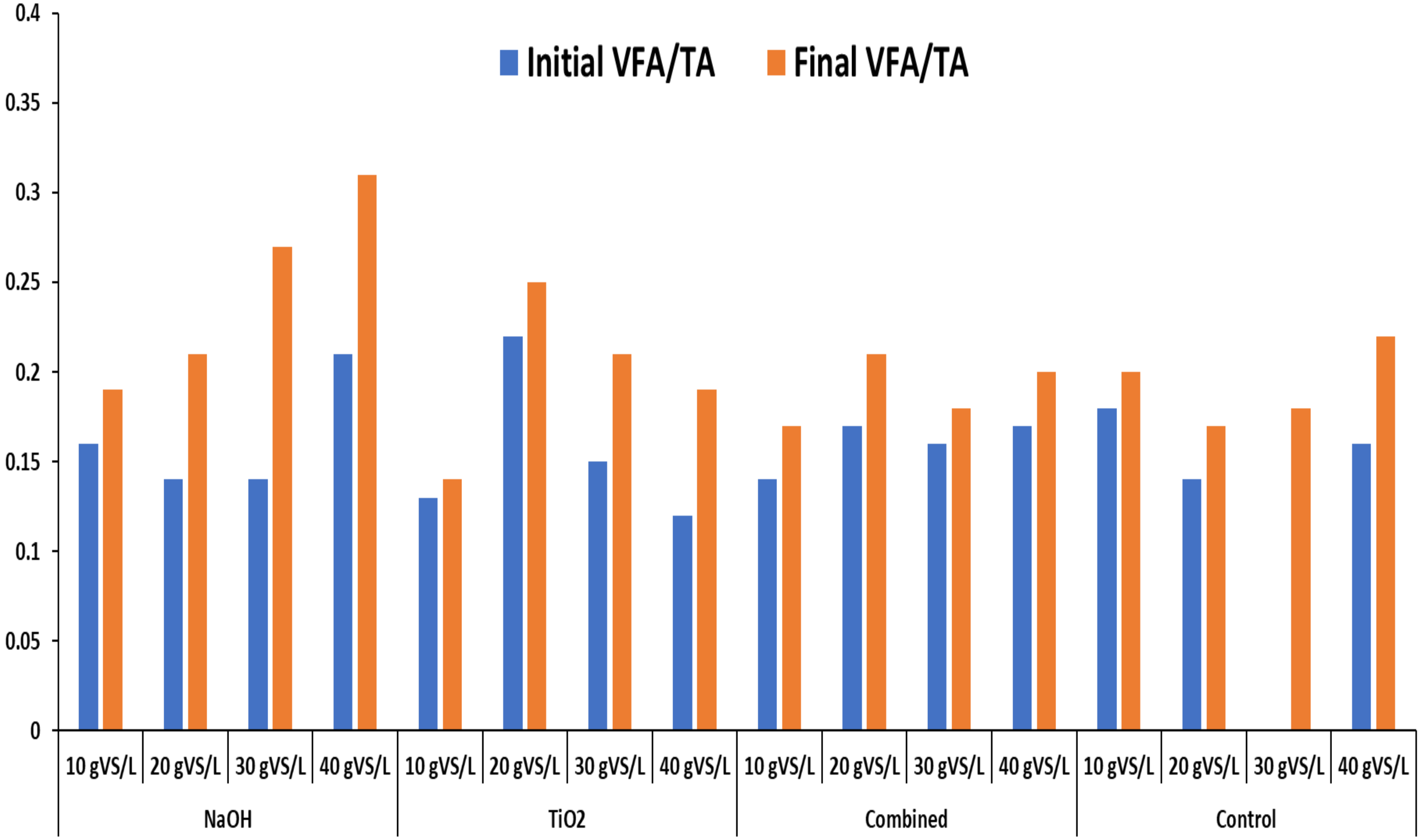


Fig. 4.11 Effect of organic loading on VFA/TA ratio of WS

## 4.8 Summary

### 4.8.1 NaOH

In the case of NaOH pretreatment, the organic loading 10 gVS/L has achieved maximum biogas production with an increase of 56%, 146% and 172%, compared to organic loading of 20, 30 and 40 gVS/L.

### 4.8.2 $TiO_2$

In case of $TiO_2$ pretreatment, the organic loading 10 gVS/L has achieved higher biogas production which was 74%, 214% and 292%, higher than organic loading of 20, 30 and 40 gVS/L.

### 4.8.3 Combined pretreatment

In the case of combined pretreatment, the organic loading 10 gVS/L has achieved maximum biogas production with an increase of 87%, 146% and 206%, compared to organic loading of 20, 30 and 40 gVS/L.

### 4.8.4 Reactor stability

The reactor showed stable performance at OLR 10 gVS/L, where VFA/TA ratio was ranged between 0.14-0.31 with corresponding pH range of 6.7-7.2.

# *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

- Biogas Yield was high in case of low organic loading whereas slight decline was observed at high organic loading.
- Maximum Biogas Yield was achieved at organic loading of 10 gVS/L under all three pretreatments.
- Maximum TS and VS removal was observed in case of combined pretreatment of WS at organic loading 10 gVS/L
- Biogas production, VS removal and stability of the reactor decline with an increase in Organic Loading from 10 gVS/L to 40 gVS/L, which can be attributed to excessive VFAs production.

## 5.2 Recommendations

- More studies should be conducted with different pretreatments to enhance biogas production.
- Co-digestion of wheat straw with other types of biomass should be studied under varying organic loading.
- Areas of green synthesis of nanoparticles should be explored to further minimize environmental impacts.

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