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Review on the Pre-treatment Advancements of Biogas Production Barriers

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Abstract

Biogas production is a promising renewable energy source that can reduce greenhouse gas emissions and improve environmental health. Substrate pre-treatment methods, including physical, chemical, and biological methods can increase biogas yields and reduce operational costs. This review assessed the advancements in substrate pre-treatment methods for biogas production, while exploring potential benefits and drawbacks of various techniques. Physical pre-treatment methods, such as chopping, grinding, steam explosion, and high-pressure homogenization, have been found to increase biogas yield despite requiring high energy consumption and expensive equipment. Chemical pre-treatment methods involving acid and alkaline hydrolysis have been effective, but can be costly and generate hazardous wastes. The biological pre-treatment methods utilized microorganisms or enzymes, have advantages of higher biogas yields, shorter process time, and eco-friendliness. Future research can focus on developing more efficient and targeted pre-treatment methods using nanotechnology and genetic engineering, optimizing existing methods, and combining multiple pre-treatment methods to enhance efficiency. Improving pre-treatment methods can lead to benefits such as increased biogas production, reduced costs, and improved waste management practices.

Keywords: Biogas production, Waste management, Pre-treatment advancements, Hazardous wastes, Future prospects

INTRODUCTION

Biogas production is a promising renewable energy source that has gained increasing attention worldwide. It is produced through the anaerobic digestion of organic materials, including animal waste, food scraps and agricultural residue, which are broken down by bacteria in the absence of oxygen (Atelge *et al.,* 2020). This process produces a mixture of methane, carbon dioxide and other trace gases, which can be used as a fuel for heat and electricity generation or as a transportation fuel (Thiruselvi *et al.,* 2021; Amoo *et al.,* 2023a; Paranjpe *et al.,* 2023). The type of substrate used can have a significant impact on biogas production, as some substrates are more easily degradable than others (Nwokolo *et al.,* 2020). Biogas production plays a crucial role in the transition to a sustainable energy future, as it provides a reliable and renewable energy source that is both environmentally friendly and economically feasible (Suman, 2021; Amoo *et al.,* 2023b). Recent studies have shown that biogas production has the potential to significantly reduce greenhouse gas emissions compared to fossil fuels (Siddiki *et al.,* 2021;Burg *et al.,* 2018). Biogas production has been shown to improve soil health through the production of nutrient-rich fertilizer known as digestate (Lee *et al.,* 2021). Biogas production has been reported to provide significant economic benefits by reducing household energy costs and the costs of manure management as well as generating income from the sale of excess biogas and digestate (Lu & Gao, 2021).

In addition, some studies have shown that biogas production can create jobs in the agricultural and energy sectors, including installation, maintenance and operation of biogas systems, as well as the production and marketing of digestate (Petravić-Tominac *et al.,* 2020).

Substrates with high carbohydrate and protein content typically produce more biogas than those with high lignin content (Garciaet *al.,* 2019). This is because, substrates with higher lignin content are more difficult to digest by biogas-producing microorganisms (Tsavkelova *et al.,* 2018). Substrate pre-treatment is therefore necessary to improve the efficiency and effectiveness of biogas production from such substrates (Gunes *et al.,* 2019). Pre-treatment methods can help to increase the surface area and accessibility of the organic material, as well as break down complex molecules into simpler forms that are more easily digestible by microorganisms (Ab Rasid *et al.,* 2021). This can lead to higher biogas yields and shorter retention times, which can help to reduce operational costs and increase overall productivity (Zhang *et al.,* 2019). Substrate pre-treatment methods can be classified into three main categories: physical, chemical, and biological (Stanley *et al.,* 2022; Wagle *et al.,* 2022). Physical methods involve the use of mechanical, thermal, or other physical processes to alter the physical or chemical properties of the substrate (Atelge *et al.,* 2020). Chemical methods involve the use of acids, alkalis, or other chemicals to break down or modify the substrate (Nguyen et al., 2021). Biological methods involve the use of enzymes or microorganisms to break down or modify the substrate (Ferdeș *et al.,* 2020). This review aims to evaluate advancements in substrate pre-treatment methods for biogas production, identifying and addressing any challenges and exploring the potential benefits and drawbacks of various techniques. It also assesses the effectiveness of these methods in increasing biogas production and highlights areas where further research is needed to improve efficiency and feasibility.

PHYSICAL PRETREATMENT METHODS

Physical pre-treatment methods are an important step in biogas production as they help to increase the efficiency of the process and reduce the time and cost required for the production (Atelge *et al.,* 2020). The advancements in physical pre-treatment methods have been aimed at increasing the efficiency and effectiveness of the methods and reducing the environmental impact of the process (Stanley *et al.,* 2022).

Mechanical pre-treatment

Mechanical pre-treatment involves the physical disruption and size reduction of substrates used in biogas production to increase their surface area and facilitate microbial degradation (Atelge *et al.,* 2020). This technique encompasses various equipment and processes to achieve efficient substrate preparation, leading to improved biogas yields and process stability. For example, one study found that grinding and crushing of sludge increased biogas yield by 25% compared to untreated sludge (Gu *et al.,* 2021). Another study found that chopping and grinding of straw resulted in a 33.6% increase in biogas yield compared to untreated straw (Wu *et al.,* 2022). These improvements were attributed to the increased surface area and improved substrate solubilisation, allowing for better accessibility by microorganisms and increased release of soluble organic compounds (Stanley *et al.,* 2022). The equipment used in mechanical pre-treatment varies depending on the specific substrate characteristics and desired treatment intensity. Common equipment includes shredders, crushers, grinders, chippers, and mixers (Raseetha *et al.,* 2022). Shredders and crushers are typically used for coarse particle size reduction, while grinders and chippers are employed for finer grinding or chipping (Bergström & Di Fulvio, 2019). Mixers ensure uniform substrate blending and homogeneity (Singh *et al.,* 2020). Mechanical pre-treatment methods have several limitations that need to be considered. These include high cost of the equipment used in reducing the particle size of the substrates, noise pollution produced by the equipment used, high energy consumption of the equipment, and low effectiveness in treating substrates with high lignocellulosic content (Onumaegbu *et al.,* 2018). The noise produced by the machines used in mechanical pre-treatment can have a negative environmental impact, particularly in areas with high population density or sensitive ecosystems (Zhang & Poon, 2018)

The cost of mechanical pre-treatment is influenced by factors such as the scale of operation, substrate characteristics, required equipment, and maintenance expenses (Kumar *et al.,* 2020). Capital costs involve the initial investment in equipment, while operational costs include maintenance, electricity, and labour (Kiptoo *et al.,* 2020). The cost range varies widely, from few to hundreds of thousands of dollars, depending on the scale and complexity of the system (Yang et al., 2018). The time required for mechanical pre-treatment depends on the substrate type, initial particle size, desired particle size distribution, and the specific equipment employed (Garuti *et al.,* 2022). It can range from a few minutes to several hours (Al Afif & Pfeifer, 2021). Additional time may be needed for system setup, substrate loading/unloading, and equipment cleaning. Mechanical pre-treatment consumes energy mainly through the operation of the equipment. The energy consumption depends on factors such as equipment specifications, substrate characteristics, and the particle size reduction intensity (Singh *et al.,* 2020). Electric motors powering the equipment are the primary energy consumers (Trianni *et al.,* 2019). The specific energy consumption can vary significantly depending on the equipment efficiency, substrate properties, and operational parameters (Panigrahi & Dubey, 2019). Furthermore, the high energy consumption can result in a significant carbon footprint (Sharif *et al.,* 2019). To ensure that mechanical pre-treatment methods are environmentally optimized, it is important to minimize both noise pollution and energy consumption during the process (Filipe *et al.,* 2019). Achieving this can be done by selecting suitable equipment and optimizing processing parameters, such as reducing the speed of equipment, or using equipment that produces less noise (Dey & Yodo, 2019). It is also crucial to evaluate the carbon release resulting from the technique to have a comprehensive assessment of its environmental impact (Sharif *et al.,* 2019).

Steam explosion pre-treatment

Steam explosion pre-treatment involves the application of high-pressure steam followed by a rapid depressurization process to break down lignocellulosic biomass into its constituent components (Yu *et al.,* 2022). This technique enhances enzymatic digestibility, increases sugar yields, and improves overall efficiency in subsequent bioconversion processes (Mihiretu *et al.,* 2019). For example, a study found that steam explosion significantly increased biogas production by improving the accessibility of enzymes to the substrate. However, they also noted that the process required high energy consumption and expensive equipment (Kaldis *et al.,* 2022). In another study, steam explosion significantly improved biogas production by increasing the solubilisation of organic matter in pig manure. However, they also highlighted the need for further research to optimize the process and reduce the associated costs (Mulat *et al.,* 2018). The primary equipment required for steam explosion pre-treatment includes a steam generator, a pressure vessel (digester), a steam delivery system, a rapid depressurization mechanism, and a collection system for the treated biomass (Weber *et al.,*2019). The authors stated further that, steam generator produces high-pressure steam, which is then transported to the digester using a network of pipes. The digester serves as the main vessel for steam explosion, where biomass is subjected to controlled steam explosion conditions (Aghbashlo *et al.,* 2019).

The cost of steam explosion pre-treatment depends on several factors, including the scale of operation, biomass feedstock, and equipment specifications (Bhatia *et al.,* 2020). Capital costs involve the initial investment in equipment, while operational costs include steam generation, maintenance, and labor (Tobin *et al.,* 2020). Generally, steam explosion pre-treatment equipment costs can range from tens of thousands to several million dollars, depending on the size and complexity of the system (Ahmed *et al.,* 2021). The time required for steam explosion pre-treatment depends on various factors, such as the type of biomass, operating conditions, and desired degree of biomass disruption (Stanley *et al.,* 2022). Typically, the process duration ranges from a few seconds to a few minutes (Onumaegbu *et al.,* 2018). However, additional time may be required for system setup, loading/unloading biomass, and equipment cleaning. Steam explosion pre-treatment is an energy-intensive process due to the requirement for steam generation and maintaining high-pressure conditions (Walker *et al.,* 2018). The energy consumption depends on the size of the system, operating pressure, and steam quality (Yu *et al.,* 2022). The steam generation process is the most energy-consuming aspect, and the energy source (e.g., fossil fuels, electricity, biomass) used for steam generation greatly influences the overall energy consumption of the process (Stanley *et al.,* 2022).

High-pressure homogenization pre-treatment

High-pressure homogenization is a mechanical pre-treatment technique that subjects substrates to high pressure and shear forces, breaking down their structure and improving microbial access to organic matter (Panigrahi & Dubey, 2019). In this technique, organic wastes, agricultural residues, or energy crops are collected and chopped or ground into smaller pieces. The prepared substrate is loaded into the feed system for continuous supply during the homogenization process. A high-pressure pump generates the required pressure within the system by forcing the substrate through it. The substrate passes through narrow gaps or nozzles in the homogenizing valve, creating intense shear forces and turbulence. The substrate experiences mechanical stress, breaking down its structure, increasing surface area, and improving microbial activity.

The pre-treated substrate exits the homogenizing valve and is collected separately and transferred to an anaerobic digester or further processed for biogas production (Kamperidou & Terzopoulou, 2021). The advantages of high-pressure homogenization include enhanced biodegradability, improved digestion kinetics, increased biogas yields, and enhanced process stability (Wang *et al.,* 2023). In a study, high-pressure homogenization significantly improved biogas production by increasing the solubilisation of lignocellulosic compounds in corn straw. However, the study also found that high-pressure homogenization caused a significant increase in the temperature of the substrate, which negatively affected the microbial community (Olatunji *et al.,* 2019). Another study showed that high-pressure homogenization significantly increased biogas production by improving the accessibility of enzymes to the substrate. The researchers also highlighted the energy-intensive nature of the process as a potential challenge (Poddar *et al.,* 2022).

The cost of high-pressure homogenization pre-treatment depends on several factors, including the scale of operation, substrate characteristics, required equipment, and maintenance expenses (Sidana & Yadav, 2022). Capital costs involve the initial investment in the high-pressure homogenizer and associated equipment, while operational costs include maintenance, electricity, and labour (Strobel *et al.,* 2020). The cost range can vary significantly, from hundreds to thousands of dollars, depending on the scale and complexity of the system (Strobel *et al.,* 2020). The time required for high-pressure homogenization pre-treatment depends on various factors, including the substrate type, desired treatment intensity, and equipment specifications (Barhoum *et al.,* 2020). The process duration typically ranges from a few seconds to a few minutes (Barhoum *et al.,* 2020). However, additional time may be needed for system setup, substrate loading/unloading, and equipment cleaning. High-pressure homogenization pre-treatment consumes energy primarily through the operation of the high-pressure pump (Nabi *et al.,* 2020). The energy consumption depends on factors such as equipment specifications, treatment intensity, and substrate properties (Drévillon *et al.,* 2018). The specific energy consumption can vary significantly depending on the equipment efficiency, operating pressure, and the characteristics of the substrate (Onumaegbu *et al.,* 2018).

Microwave pre-treatment

Microwave pre-treatment involves the application of microwave energy to substrates before anaerobic digestion, promoting the breakdown of complex organic compounds and facilitating microbial activity (Ambrose *et al.,* 2020). The equipment required for microwave pre-treatment includes a microwave generator, a suitable vessel or reactor, and a mixing or stirring mechanism (Yue *et al.,* 2021). Microwave generators produce and deliver microwave energy to the substrate, while the vessel provides containment and ensures safety during the process (Yue *et al.,* 2021). The mixing or stirring mechanism promotes uniform heating and treatment (Li *et al.,* 2019). The substrate, which can include organic wastes, energy crops, or agricultural residues, is collected and prepared for pre-treatment. It is typically chopped or ground into smaller pieces to ensure uniformity and enhance microwave penetration (Atelge *et al.,* 2020). The prepared substrate is loaded into a suitable vessel or reactor that is microwave-safe and allows for efficient energy transfer (Yue *et al.,* 2021). The vessel containing the substrate is exposed to microwave irradiation. Microwaves generate heat by exciting water molecules present in the substrate, leading to thermal and non-thermal effects (Ambrose *et al.,* 2020). The authors reiterated further that, these effects contribute to the breakdown of complex organic compounds, lignocellulosic structures, and microbial cell walls. During microwave irradiation, the substrate is often mixed or stirred to ensure uniform heating and treatment throughout. This helps in maximizing the exposure of the substrate to the microwave energy and improving treatment effectiveness (Li *et al.,* 2019). After the pre-determined treatment duration, the vessel is cooled down, and the pre-treated substrate is discharged for further processing, typically into an anaerobic digester (Li *et al.,* 2019).

This technique offers potential for improving the efficiency and effectiveness of biogas production processes (Yue *et al.,* 2021). For instance, a study showed that microwave pre-treatment significantly increased biogas production by solubilizing the lignocellulosic components of food waste. The study also found that microwave pre-treatment required less time compared to other pre-treatment methods, making it suitable for industrial applications (Begum *et al.,* 2021). Similarly, another study showed that microwave pre-treatment significantly improved biogas production by breaking down the complex organic matter in the dairy manure. However, the study also highlighted the challenge of uneven distribution of microwave radiation within the substrate, which can result in incomplete pre-treatment and decreased efficiency (Bundhoo, 2018).

The initial investment cost for microwave pre-treatment equipment can be relatively high, especially for large-scale applications (Ramos *et al.,* 2022). The cost of microwave pre-treatment depends on various factors, including the scale of operation, equipment specifications, and energy consumption (Halder *et al.,* 2019). Initial investment costs include the microwave generator, vessel/reactor, and mixing mechanism, while operational costs involve energy consumption, maintenance, and labour expenses (Hassan *et al.,*2018). Microwave pre-treatment reduces the retention time required in the anaerobic digester, allowing for higher throughput and increased process efficiency (Yue *et al.,* 2021). However, the duration of microwave pre-treatment varies depending on several factors, including the substrate characteristics, desired treatment intensity, microwave power, and equipment specifications (Yue *et al.,* 2021). Shorter treatment durations are typically preferred to minimize energy consumption and maximize process efficiency (Yue *et al.,* 2021). The treatment time can range from a few seconds to several minutes, but it is crucial to optimize the duration to achieve the desired level of substrate breakdown without excessive energy usage or substrate overheating (Begum *et al.,* 2021). Microwave pre-treatment requires a significant amount of energy to generate microwaves and heat the substrate (Pilli *et al.,* 2020). The energy consumption is influenced by factors such as the power rating of the microwave generator, treatment duration, and substrate properties (Bundhoo, 2018). The specific energy consumption can vary widely, depending on the scale of operation and the efficiency of the microwave equipment (Ramos *et al.,* 2022). Optimization strategies, such as adjusting microwave power and treatment time, can help minimize energy consumption while maintaining effective pre-treatment (Munoz-Almagro *et al.,* 2021).

Ultra sonication pre-treatment

Ultrasound pre-treatment involves the application of high-frequency sound waves to substrates before anaerobic digestion, promoting the disruption of complex organic compounds and facilitating microbial activity (Arman *et al.,* 2023). The equipment required for ultrasound pre-treatment includes an ultrasound generator, transducers or sonotrodes, a vessel or reactor, and mixing or stirring mechanisms (Askarniya *et al.,* 2023). The ultrasound generator produces the high-frequency sound waves, which are transmitted to the substrate through transducers or sonotrodes (Arman *et al.,* 2023). The vessel or reactor provides containment and efficient energy transfer, while mixing or stirring mechanisms ensure uniform treatment (Askarniya *et al.,* 2023). In this technique, the substrate, which can include organic wastes, agricultural residues, or energy crops, is collected and prepared for pre-treatment. It may undergo size reduction or chopping to ensure uniformity and facilitate efficient ultrasound penetration (Atelge *et al.,* 2020). The prepared substrate is loaded into a suitable vessel or reactor capable of withstanding ultrasound exposure. The vessel is designed to efficiently transmit ultrasound energy to the substrate (Kazimierowicz *et al.,* 2023). Ultrasound energy is applied to the substrate through the use of transducers or sonotrodes (Xu *et al.,* 2021). These devices emit high-frequency sound waves into the substrate, causing cavitation, microstreaming, and acoustic pressure effects (Kazimierowicz *et al.,* 2023). These effects disrupt the substrate structure and enhance its biodegradability (Xu *et al.,* 2021). During ultrasound pre-treatment, it is common to employ mixing or stirring mechanisms to ensure uniform exposure of the substrate to ultrasound energy. This helps to maximize the treatment effectiveness and enhance substrate breakdown (Strieder *et al.,* 2021). After the pre-determined treatment duration, the ultrasound energy is ceased, and the substrate is cooled down. The pre-treated substrate is then discharged for further processing, typically into an anaerobic digester (Askarniya *et al.,* 2023).

This technique holds promise for improving the efficiency and effectiveness of biogas production processes. Ultrasound pre-treatment can decrease the retention time required in the anaerobic digester, increasing process throughput and efficiency (Pramanik *et al.,* 2019). A study showed that ultra-sonication pre-treatment significantly improved biogas production by breaking down the complex organic matter in food waste (Zia *et al.,* 2022). In addition, the study also highlighted that ultra-sonication pre-treatment has a low environmental impact and is suitable for small-scale applications. In another study, ultra-sonication pre-treatment significantly improved biogas production by increasing the solubilisation of organic matter in manure. However, the study also found that the limited effectiveness of ultra-sonication in treating substrates with high lignocellulosic content can be a challenge for this method (Lee *et al.,* 2019). The initial capital investment for The cost of ultrasound pre-treatment comprises both capital and operational expenses (Dalton *et al.,* 2022). Capital costs include the purchase and installation of ultrasound equipment, including the generator, transducers or sonotrodes, and the vessel or reactor (Dauknys *et al.,* 2020). The duration of ultrasound pre-treatment varies depending on the substrate characteristics, desired treatment intensity, and equipment specifications (Askarniya *et al.,* 2023). Shorter treatment durations are typically favoured to minimize energy consumption and optimize process efficiency (Kazimierowicz *et al.,* 2023). The treatment time can range from a few minutes to several hours, but it is crucial to find the right balance between treatment duration and substrate breakdown to avoid excessive energy usage or substrate degradation (Xu *et al.,* 2021). The energy consumption is influenced by factors such as treatment duration, power rating of the equipment, and system efficiency (Arman *et al.,* 2023). Ultrasound pre-treatment requires electricity to power the ultrasound generator and transducers/sonotrodes (Strieder *et al.,*2021). The authors stated further that, it is important to optimize the process parameters to minimize energy consumption while maintaining effective pre-treatment.

Plasma pre-treatment

Plasma pre-treatment involves the application of plasma discharge to substrates before anaerobic digestion, promoting the breakdown of complex organic compounds and enhancing microbial activity (Stanley *et al.,* 2022). In addition, the plasma discharge interacts with the substrate, leading to the dissociation of complex organic compounds, the formation of reactive species, and the enhancement of substrate biodegradability. The equipment required for plasma pre-treatment includes a plasma generator, a reactor or chamber, gas supply systems, and mixing or stirring mechanisms (Asghari *et al.,* 2022). The plasma generator provides the necessary electrical discharge or microwave energy to generate plasma (Bashir *et al.,* 2022). The reactor or chamber allows for efficient plasma treatment and containment of the substrate (Asghari *et al.,* 2022). Gas supply systems supply the appropriate gas medium for plasma formation, and mixing or stirring mechanisms ensure uniform treatment (Bashir *et al.,* 2022). In this technique, the substrate, which can include organic wastes, agricultural residues, or energy

ultrasound equipment can be relatively high, especially for larger-scale applications (Dauknys *et al.,* 2020).

crops, is collected and prepared for pre-treatment. It may undergo size reduction or chopping to ensure uniformity and facilitate efficient plasma treatment (Atelge *et al.,* 2020). The prepared substrate is loaded into a suitable plasma reactor or chamber, designed to facilitate efficient plasma discharge and ensure safety during the process (Wright *et al.,* 2020). Plasma is generated by applying an electrical discharge or microwave energy to a gas medium, creating a high-energy plasma field (Arelli *et al.,* 2018). During plasma pre-treatment, it is common to employ mixing or stirring mechanisms to ensure uniform exposure of the substrate to plasma discharge. This helps to maximize the treatment effectiveness and enhance substrate breakdown (Wright *et al.,* 2020). After the pre-determined treatment duration, the plasma discharge is ceased, and the substrate is cooled down. The pre-treated substrate is then discharged for further processing, typically into an anaerobic digester (Arelli *et al.,* 2018).

This technique holds promise for improving the efficiency and effectiveness of biogas production processes. Plasma pre-treatment can decrease the retention time required in the anaerobic digester, increasing process throughput and efficiency (Wright *et al.,* 2020). One study found that plasma pre-treatment significantly improved the biogas yield and reduced digestion time, demonstrating the potential of this method for enhancing biogas production from lignocellulosic substrates (Maneein *et al.,* 2018). Another study investigated the use of a hybrid plasma-catalytic system for the pre-treatment of food waste and found that the hybrid system significantly improved the solubilisation and methane production of the food waste (Arelli *et al.,* 2018). The cost of plasma pre-treatment involves both capital and operational expenses. Capital costs include the purchase and installation of plasma equipment, reactor/chamber, gas supply systems, and mixing mechanisms. Operational costs include energy consumption, maintenance, and labour (Koniuszewska *et al.,* 2020). The scale and complexity of the system will influence the overall capital investment required for plasma pre-treatment (Arelli *et al.,* 2018). The initial capital investment for plasma equipment can be relatively high, especially for larger-scale applications (Wright *et al.,* 2020). The duration of plasma pre-treatment can vary depending on factors such as the substrate characteristics, desired treatment intensity, and equipment specifications (Stanley *et al.,* 2022). Treatment times typically range from a few minutes to several hours (Bashir et al., 2022). It is important to optimize the treatment duration to achieve the desired level of substrate breakdown without excessive energy consumption or substrate degradation (Gunes *et al.,* 2021).

Plasma pre-treatment requires electricity to power the plasma generator and maintain the plasma discharge (Ramamoorthy *et al.,* 2020). The energy consumption is influenced by factors such as treatment duration, power rating of the equipment, and system efficiency (Back et al., 2018). It is important to optimize the process parameters to minimize energy consumption while maintaining effective substrate breakdown (Gunes *et al.,* 2021).

Pulse electromagnetic field (PEMF) pre-treatment

PEMF pre-treatment involves the application of pulsed electromagnetic waves to substrates before anaerobic digestion, promoting the breakdown of complex organic compounds and enhancing microbial activity (Szwarc & Głowacka, 2021). The equipment required for PEMF pre-treatment includes an electromagnetic field generator, a treatment vessel or reactor, and mixing or stirring mechanisms (Safavi & Unnthorsson, 2018). The electromagnetic field generator generates pulsed electromagnetic waves of specific frequencies and intensities (Szwarc & Szwarc, 2020). The treatment vessel or reactor facilitates efficient exposure of the substrate to the electromagnetic field, and the mixing mechanisms ensure uniform treatment (Szwarc *et al.,* 2022). In this technique, the substrate, such as organic wastes or agricultural residues, is collected and prepared for pre-treatment. This may involve size reduction or chopping to ensure uniformity and facilitate efficient PEMF treatment (Atelge *et al.,* 2020). The prepared substrate is loaded into the treatment vessel or reactor that allows for efficient exposure to the pulsed electromagnetic field (Zia *et al.,* 2022). Pulsed electromagnetic waves are applied to the substrate using specialized electromagnetic field generators (Kovačić *et al.,* 2021). The electromagnetic waves induce electrical currents and vibrations within the substrate, leading to the breakdown of complex organic compounds and enhancing substrate biodegradability (Capodaglio, 2021). During PEMF pre-treatment, it is common to employ mixing or stirring mechanisms to ensure uniform exposure of the substrate to the electromagnetic field. This helps maximize treatment effectiveness and enhance substrate breakdown (Begum *et al.,* 2021). After the pre-determined treatment duration, the PEMF treatment is ceased, and the pre-treated substrate is discharged for further processing, typically into an anaerobic digester (Capodaglio, 2021).

This technique has gained attention as a potential means to improve the efficiency and effectiveness of biogas production processes because it can decrease the retention time required in the anaerobic digester (Kovačić *et al.,* 2021). It promotes the breakdown of complex organic compounds, enhancing substrate biodegradability and microbial accessibility, which leads to increased biogas production and enhanced methane yields (Szwarc *et al.,* 2022). A study found that pulse electromagnetic field pre-treatment significantly improved the solubilisation and methane production from food waste, indicating its potential as an effective method for enhancing biogas production (Szwarc & Głowacka, 2021). The authors further noted that the method had low energy consumption and a low environmental impact, making it a promising technology for small-scale applications. Another study also found that pulse electromagnetic field pre-treatment significantly improved biogas yield and reduced digestion time, indicating its potential as an effective method for biogas production from lignocellulosic substrates. The study also noted that the method had a low environmental impact, making it suitable for small-scale applications (Safavi & Unnthorsson, 2018).

The cost of PEMF pre-treatment involves both capital and operational expenses. Capital costs include the purchase and installation of the electromagnetic field generator, treatment vessel or reactor, and mixing mechanisms (Safavi & Unnthorsson, 2018). Operational costs include energy consumption, maintenance, and labour (Szwarc *et al.,* 2022). The scale and complexity of the system will influence the overall capital investment required for PEMF pre-treatment. The initial capital investment for PEMF equipment can be relatively high, especially for larger-scale applications (Capodaglio, 2021). The duration of PEMF pre-treatment can vary depending on factors such as the substrate characteristics, desired treatment intensity, and equipment specifications (Kovačić *et al.,* 2021). Treatment times typically range from minutes to hours (Szwarc & Szwarc, 2020). It is important to optimize the treatment duration to achieve the desired level of substrate breakdown without excessive energy consumption or substrate degradation (Gunes *et al.,* 2021). PEMF pre-treatment requires electricity to power the electromagnetic field generator (Szwarc *et al.,* 2022). The energy consumption is influenced by factors such as treatment duration, power rating of the equipment, and system efficiency (Zia *et al.,* 2022).

CHEMICAL PRETREATMENT METHODS

Chemical pre-treatment methods involve the use of chemicals to break down or modify the structure of lignocellulosic biomass, making it easier to extract the desired products (Kumar *et al.,* 2020).

This step is crucial in improving the efficiency of the anaerobic digestion process and reducing the overall cost and time required for biogas production (Wagle *et al.,* 2022). In this section, we will discuss the key chemical pre-treatment methods used in the enhancement of biogas production and their effectiveness in improving the efficiency and sustainability of the process.

Acid hydrolysis pre-treatment

Acid hydrolysis pre-treatment involves the use of acid solutions to break down complex organic compounds into simpler and more biodegradable forms (Zafar *et al.,* 2022). In this technique, the substrate, such as lignocellulosic biomass or organic waste, is collected and prepared for pre-treatment. This may involve size reduction or chopping to increase surface area and facilitate acid penetration (Atelge *et al.,* 2020). A suitable acid, such as sulphuric acid or hydrochloric acid, is added to the substrate in a predetermined concentration. The acid concentration depends on the substrate composition and desired pre-treatment intensity (Nava-Valente *et al.,* 2023). The acid and substrate are thoroughly mixed to ensure uniform contact and the mixture is then allowed to react under controlled conditions, including temperature, pressure, and reaction time (Gomes *et al.,* 2022). The reaction time can vary depending on the substrate characteristics and desired degree of hydrolysis (Nava-Valente *et al.,*2023). After the desired reaction time, the acid is neutralized using a base, such as sodium hydroxide or calcium hydroxide, to bring the pH back to neutral or near-neutral levels (Marks *et al.,* 2020). Neutralization is crucial to ensure the subsequent anaerobic digestion process is not adversely affected (Gomes et al., 2022). The pre-treated slurry is separated into a liquid fraction and a solid residue (Sun *et al.,* 2021). The authors stated further that various separation techniques, such as centrifugation, filtration, or sedimentation, can be employed to achieve efficient solid-liquid separation. The pre-treated solid fraction is directed to the anaerobic digester for biogas production, while the liquid fraction may undergo further treatment or be used for other applications (Mulat *et al.,* 2018). In addition, this process enhances the accessibility of microorganisms to the substrate, leading to improved biogas yields and process efficiency. In a study, it was found that acid hydrolysis pre-treatment of food waste increased the production of biogas by up to 27.8% compared to untreated waste (Gunes *et al.,* 2019). Another study found that acid hydrolysis pre-treatment significantly improved the yield of biogas, with an increase of up to 61.5% compared to untreated rice straw (Liu *et al.,* 2021).

However, acid hydrolysis can be costly due to capital and operational expenses (Rosales-Calderon *et al.,* 2021). The costs may vary depending on the scale and complexity of the system. Capital costs involve the purchase and installation of equipment, including reactors, acid storage tanks, dosing systems, neutralization units, and solid-liquid separation units (Cheng *et al.,* 2019). Operational costs include acid and base consumption, energy requirements for mixing and heating, maintenance, and labour (Vasconcelos *et al.,* 2020). The cost of acids and bases depends on their availability and market prices. Furthermore, the duration of acid hydrolysis pre-treatment depends on several factors, including the substrate characteristics, acid concentration, temperature, and desired degree of hydrolysis (Mirmohamadsadeghi *et al.,* 2021). Treatment times typically range from a few hours to several days (Zafar *et al.,* 2022). It is important to optimize the treatment times to achieve the desired level of substrate hydrolysis without excessive energy consumption or substrate degradation (Gomes *et al.,* 2022). In addition, energy consumption in acid hydrolysis pre-treatment is influenced by several factors, including mixing requirements, heating of the reaction vessel, and solid-liquid separation (Nava-Valente *et al.,* 2023). The energy needed for mixing is dependent on the intensity and duration of mixing (Marks *et al.,* 2020). Heating is required to maintain the desired reaction temperature, which can vary depending on the substrate and acid used (Rosales-Calderon *et al.,,* 2021). Energy requirements for solid-liquid separation depend on the chosen separation technique (Cheng *et al.,* 2019). Optimizing process parameters, such as mixing intensity and temperature control, can help minimize energy consumption and improve overall efficiency (Olatunji *et al.,* 2021). Implementing energysaving measures, such as heat recovery systems, can also contribute to reducing energy consumption during acid hydrolysis pre-treatment (Maktabifard *et al.*, 2018).

Alkaline hydrolysis pre-treatment

Alkaline hydrolysis pre-treatment involves the use of alkaline solutions to break down complex organic compounds such as lignocellulose into simpler forms such as cellulose and sugars, thus enhancing the accessibility of microorganisms to the substrate (Ahmed *et al.*, 2022). In this technique, the substrate, such as lignocellulosic biomass or organic waste, is collected and prepared for pre-treatment. This may include size reduction or chopping to increase the surface area and facilitate alkaline penetration (Atelge *et al.*, 2020).

An appropriate alkaline solution, such as sodium hydroxide (NaOH) or potassium hydroxide (KOH), is added to the substrate. The concentration of alkaline solution depends on the substrate composition and desired pre-treatment intensity (Arias *et al.*, 2018). The substrate and alkaline solution are thoroughly mixed to ensure uniform contact and then allowed to react under controlled conditions, including temperature, pressure, and reaction time (Abudi *et al.*, 2020). The reaction time can vary depending on the substrate characteristics and desired degree of hydrolysis (Donkor *et al.*, 2022). After the desired reaction time, the alkaline solution is neutralized using an acid, such as sulphuric acid or citric acid, to bring the pH back to neutral or near-neutral levels (Marks *et al.*, 2020). Neutralization is crucial to ensure the subsequent anaerobic digestion process is not adversely affected (Jankovičová *et al.*, 2022). The pre-treated slurry is separated into a liquid fraction and a solid residue, and various separation techniques, such as centrifugation, filtration, or sedimentation, can be employed to achieve efficient solid-liquid separation (Arias *et al.*, 2018). The pre-treated solid fraction is directed to the anaerobic digester for biogas production, while the liquid fraction may undergo further treatment or be used for other applications (Abudi *et al.*, 2020). This process promotes improved biogas yields and process efficiency. For example, a study found that alkaline pre-treatment resulted in a significant increase in biogas production compared to untreated manure, with an increase of up to 80% (Zahan & Othman, 2019). Another study found that alkaline pre-treatment significantly improved the yield of biogas, with an increase of up to 33.8% compared to untreated corn stover (Wahid *et al.*, 2020).

Alkaline hydrolysis pre-treatment can be costly due to capital and operational expenses, and the cost may vary depending on the scale and complexity of the system (Usmani *et al.*, 2021). Capital costs include the purchase and installation of equipment, such as reactors, alkaline solution storage tanks, dosing systems, neutralization units, and solid-liquid separation units (Arias *et al.*, 2018). Operational costs include alkaline solution and acid consumption, energy requirements for mixing and heating, maintenance, and labour (Abudi *et al.*, 2020). The cost of alkaline solutions depends on their availability and market prices. Furthermore, the duration of alkaline hydrolysis pre-treatment can vary depending on substrate characteristics, alkaline concentration, temperature, and desired degree of hydrolysis (Jankovičová *et al.*,2022). Generally, the treatment time ranges from a few hours to several days (Wahid *et al.*, 2020; Zahan & Othman, 2019). It is important to optimize the reaction time to achieve the desired level of substrate hydrolysis without excessive energy consumption or substrate degradation (Olatunji *et al.*, 2021). Finally, energy consumption in alkaline hydrolysis pre-treatment is influenced by several factors, including mixing requirements, heating of the reaction vessel, and solid-liquid separation (Halder*et al.*,2019). The energy needed for mixing depends on the intensity and duration of mixing (Singh *et al.*, 2020). Heating is required to maintain the desired reaction temperature, which can vary depending on the substrate and alkaline solution used (Makamure *et al.*,2021). Energy requirements for solid-liquid separation depend on the chosen separation technique (Singh & Patidar, 2018). Efficient mixing systems, such as agitators or recirculation pumps, can help reduce energy consumption during alkaline hydrolysis pre-treatment (Maktabifard *et al.*, 2018). Additionally, incorporating heat recovery systems and optimizing temperature control can contribute to minimizing energy requirements (Zamri *et al.*, 2021).

Ozonation pre-treatment

Ozonation pre-treatment involves the use of ozone gas (O_3) to break down complex organic compounds such as lignocellulosic biomass to extract the cellulose and hemicellulose fractions, and facilitate the biodegradation of substrates in anaerobic digestion (Rahmani *et al.*, 2022). In this technique, the substrate, such as organic waste or lignocellulosic biomass, is collected and prepared for pre-treatment. This may involve size reduction or chopping to increase the surface area and facilitate ozone penetration (Atelge *et al.*, 2020). Ozone gas is introduced into the pre-treatment vessel or reactor containing the substrate. The ozone gas is distributed evenly throughout the substrate by efficient mixing or sparging methods (M'Arimi*et al.*, 2020). The ozone and substrate mixture undergoes a reaction for a specific residence time. The residence time can vary depending on the substrate characteristics, desired degree of pre-treatment, and ozone concentration (Den *et al.*, 2018). After the desired reaction time, the ozone gas is decomposed or removed from the system. This can be achieved through various methods, such as ozone destructors or activated carbon filters, to prevent ozone release into the environment (Karuppiah & Azariah, 2019). The pre-treated substrate, enriched with readily biodegradable compounds, is directed to the anaerobic digester for biogas production (Mozhiarasi, 2022). This process enhances the biogas yield and improves the overall efficiency of the biogas production process. digestion of corn stover (Ab Rasid *et al.*, 2021). In another study, this pre-treatment method was used to improve the biodegradability and biogas production of microalgae biomass (Vats *et al.*, 2020). Furthermore, ozonation pre-treatment was found to improve the biogas yield from anaerobic digestion of rice straw (Patil *et al.*,2021).

Ozonation pre-treatment can be costly due to capital and operational expenses, and the cost may vary depending on the scale and complexity of the system (Sudalyandi & Jeyakumar, 2022). Capital costs include the purchase and installation of equipment, such as ozonation vessels, ozone generators, mixing systems, ozone decomposition or removal units, and separation units (Almomani *et al.*, 2019). Operational costs include ozone generation, maintenance, energy consumption for mixing and ozonation, and labour (Pilli *et al.*, 2020). Regular maintenance and periodic replacement of ozone-generating components should be considered for optimal system performance (Pilli *et al.*, 2020). In addition, the duration of ozonation pre-treatment can vary depending on the substrate characteristics, ozone concentration, desired degree of pre-treatment, and residence time (Vats *et al.*, 2020). The treatment time typically ranges from minutes to a few hours (Ab Rasid *et al.*, 2021). It is important to optimize the reaction time to achieve efficient substrate degradation without excessive energy consumption (Patil *et al.*, 2021). Energy consumption in ozonation pre-treatment is influenced by several factors, including mixing requirements, ozone generation, and separation (Karuppiah & Azariah, 2019). The energy needed for mixing depends on the intensity and duration of mixing (Den et al., 2018). Energy requirements for separation of the treated substrate from the spent gas depend on the chosen separation technique (Singh & Patidar, 2018). Efficient mixing systems, optimized ozone generation, and the use of energy-efficient equipment can help reduce energy consumption during ozonation pre-treatment (Hafeez *et al.*, 2020).

BIOLOGICAL PRETREATMENT METHODS

Biological pre-treatment methods involve the use of microorganisms such as bacteria and fungi or enzymes to breakdown the complex organic compounds in feedstock materials, leading to improved biogas production (Ferdeș *et al.*, 2020). Some of the advancements in biological pre-treatment of organic substrates for enhanced biogas production are discuss below.

Bacterial pre-treatment

Biogas production is a complex process that involves the anaerobic degradation of organic matter by microbial consortia (Vyas *et al.*, 2022). However, the presence of complex substrates with recalcitrant components can hinder the biogas production process (Chukwuma *et al.*, 2020). Bacterial pre-treatment techniques have shown promise in improving the degradation efficiency and biogas yield by breaking down complex substrates into simpler compounds (Chukwuma *et al.*, 2021). In this technique, suitable substrates, such as agricultural waste, food waste, or sewage sludge, are collected and characterized to determine their composition and suitability for biogas production (Almomani et al., 2019). Specific bacterial strains or consortia with high hydrolytic and fermentative abilities are selected and inoculated into the substrate (Chukwuma *et al.*, 2021). These bacteria produce extracellular enzymes that degrade complex organic compounds such as lignocellulose into soluble substances such as cellulose, sugars and fatty acids (Menzel *et al.*, 2020). The inoculated substrate is incubated under controlled anaerobic conditions, typically in a digester or fermenter (Ahmed *et al.*, 2022). Continuous or batch mixing is employed to ensure uniform distribution of bacteria and substrate, facilitating microbial growth and activity (Srivastava *et al.*,2021). Process parameters, such as pH, temperature, and substrate concentration, are monitored and adjusted to maintain optimal conditions for bacterial growth and activity. This step ensures efficient substrate degradation and biogas production (Sepehri *et al.,* 2019). The bacterial pre-treatment process requires specific equipment to operate. A digester or fermenter, equipped with mixing systems and monitoring instruments, provides the controlled anaerobic conditions necessary for bacterial growth and substrate degradation (Srivastava et al., 2021). Mechanical or hydraulic mixing systems are employed to ensure uniform distribution of bacteria and substrate, preventing the formation of dead zones and promoting efficient degradation (Singh et al., 2019). pH and temperature sensors, gas flow meters, and analytical devices for substrate characterization are essential for monitoring the process parameters and assessing its performance (Nasiri & Khosravani, 2020).

Several factors influence the effectiveness of bacterial pre-treatment. Substrate characteristics such the composition, particle size, and lignocellulosic content of the substrate significantly affect the degradation efficiency (Lee *et al.,* 2020).

In one study, ozonation pre-treatment was shown to enhance biogas production during anaerobic

Highly lignocellulosic substrates may require additional pre-treatment methods to enhance bacterial activity (Chukwuma *et al.,* 2020). The selection of appropriate bacterial strains or consortia with high hydrolytic and fermentative capabilities is crucial for effective pre-treatment (Almomani *et al.,* 2019). Strain optimization and microbial community engineering can further improve the process (Eng & Borenstein, 2019). The optimum pH range for biogas production is typically between 6.5 and 8.0, while the temperature range depends on the type of digestion; mesophilic (35-40°C) or thermophilic (50-60°C) as reported by Nsair *et al.* (2020). The authors stated further that, the specific optimal pH and temperature may vary depending on the substrate and microbial consortium. Regular monitoring and adjustments are crucial to maintain ideal conditions for biogas production (Eng & Borenstein, 2019). Deviations from the optimal pH and temperature range can affect the process efficiency (Nsair *et al.,* 2020). The cost, time, and energy consumption of bacterial pre-treatment depend on various factors, including the substrate type, scale of operation, and process optimization (Chukwuma *et al.,* 2021). In addition, the capital investment for equipment and infrastructure can be significant, but operational costs are relatively lower. The time required for pre-treatment varies depending on the substrate and microbial activity, typically ranging from several days to weeks (Sepehri *et al.,* 2019). Energy consumption is mainly associated with maintaining the desired temperature and mixing requirements (Nsair *et al.,* 2020).

The advantages of bacterial pre-treatment include higher biogas yields, shorter process time, eco-friendliness, easily isolation from different sources and the ability to degrade a wide range of feedstock materials (Chukwuma *et al.,* 2021; Menzel *et al.,* 2020). In a study, pre-treating food waste with a consortium of Bacillus, Clostridium and Streptomyces, resulted in higher biogas yields, shorter process time, and lower levels of organic matter compared to untreated samples (Periyasamy *et al.,* 2023). In another study, the use of biochar as a carrier for bacterial pre-treatment of rice straw resulted in higher biogas yields and shorter process time compared to traditional pre-treatment methods (Masebinu *et al.,* 2019). Nevertheless, bacterial pre-treatment has some challenges and limitations, such as the requirement of strict control of process conditions to avoid the production of inhibitors that can hinder the anaerobic digestion process, a high degree of variability in the efficiency of different bacterial strains and low yields of the pre-treatment process (Gunes *et al.,* 2019).

Fungal pre-treatment

Fungal pre-treatment of substrates is an innovative approach that improves the efficiency and effectiveness of biogas production by breaking down complex organic compounds such as lignocellulose into simpler forms such as cellulose and sugars (Kamperidou & Terzopoulou, 2021). Fungi such as *Trichoderma sp*. and *Aspergillus sp.* are commonly used for fungal pre-treatment (Abduh *et al.,* 2022; Zulkifli *et al.,* 2018). In this pretreatment technique, appropriate substrates, such as agricultural residues, food waste, or lignocellulosic materials, are selected based on their composition and availability (Kamperidou & Terzopoulou, 2021). The substrates are then prepared by size reduction and particle size adjustment to optimize fungal access and activity (Atelge *et al.,* 2020). Specific fungal strains or consortia known for their lignocellulolytic activities are selected and inoculated into the substrate (Abduh *et al.,* 2022; Zulkifli *et al.,* 2018). These fungi produce a range of extracellular enzymes that degrade complex organic compounds into simpler forms (Abduh *et al.,* 2022; Zulkifli *et al.,* 2018). The inoculated substrate is incubated under controlled conditions, typically at mesophilic temperatures, to promote fungal growth and enzymatic activity (Nahak *et al.,* 2022). Moisture levels are carefully controlled to ensure optimal fungal performance (Jaronski, 2023). Mechanical mixing or aeration is employed to ensure uniform distribution of fungi and substrates, facilitate oxygen transfer, and prevent the formation of anaerobic zones (Saeedian *et al.,* 2022). This step enhances fungal colonization and degradation efficiency (Jaronski, 2023). Process parameters such as temperature, moisture content, pH, and substrate concentration are monitored and adjusted to maintain optimal conditions for fungal growth and activity (Benyahya *et al.,* 2021). Regular monitoring allows for process optimization and improved biogas production (Wu *et al.,* 2021). The fungal pre-treatment process requires specific equipment. A reactor system, such as a bioreactor or fermenter, provides the controlled conditions required for fungal growth and substrate degradation (Kamperidou & Terzopoulou, 2021). The system is equipped with mixing devices, aeration systems, and temperature and moisture control mechanisms (Saeedian *et al.,* 2022). In addition, mechanical or hydraulic mixing devices are utilized to ensure thorough mixing of fungi and substrates, promoting uniform colonization and enzymatic degradation. Temperature sensors, moisture probes, pH meters, and analytical devices for substrate characterization are necessary for process monitoring and control (Wu *et al.,* 2021). Several factors influence the effectiveness of fungal pre-treatment. The composition of the substrate, including lignin content, carbohydrate composition, and particle size, significantly affects fungal degradation efficiency (Kamperidou & Terzopoulou, 2021). Substrates with high lignin content may require additional pre-treatment methods to enhance fungal access and activity (Kainthola *et al.,* 2021). The selection of suitable fungal strains or consortia with high lignocellulolytic capabilities is crucial for effective pre-treatment (Abduh *et al.,* 2022; Zulkifli *et al.,* 2018). Fungal strain optimization and consortia engineering can further enhance the process (Abduh *et al.,* 2022; Zulkifli *et al.,* 2018). Optimal temperature, moisture content, pH, and aeration are essential for fungal growth and activity. Deviations from the optimal range can negatively impact the pre-treatment process (Nadir *et al.,* 2019). Most fungi exhibit optimal growth within a specific temperature range. Mesophilic fungi typically thrive at temperatures ranging from 20 to 40°C, while thermophilic fungi prefer higher temperatures, often between 45 and 60°C (de Oliveira *et al.,* 2019). The authors reiterated further that, it is essential to maintain the temperature within the optimal range for the specific fungal species to ensure their growth and activity. Moisture content plays a crucial role in fungal growth and activity. Fungi require a certain level of moisture to thrive, as it supports enzymatic reactions and nutrient uptake (Nadir *et al.,* 2019). Generally, a moisture content of 60 to 80% is considered optimal for fungal growth, although some species may have specific requirements within this range (Tai *et al.,* 2019). The pH level affects fungal enzymatic activity and nutrient availability, and different fungal species have varying pH preferences, but most fungi prefer a slightly acidic (5.0) to neutral pH (7.0) range (Tedersoo *et al.,* 2020). Fungi require oxygen for respiration and metabolism, therefore, adequate aeration is crucial for supplying oxygen to the fungal cultures and preventing anaerobic conditions (Garcia-Ochoa *et al.,* 2020). Mixing or aeration systems are employed to ensure proper gas exchange and prevent the formation of anaerobic zones (Dewi *et al.,* 2021). Agitation methods can vary based on the scale of operation and the specific fungal pre-treatment system (Garcia-Ochoa *et al.,* 2020).

The cost, time, and energy consumption of fungal pre-treatment depend on various factors, including substrate type, scale of operation, and process optimization (Kamperidou & Terzopoulou, 2021). Capital investment for equipment and infrastructure can be substantial, while operational costs are generally lower (Abduh *et al.,* 2022). The duration of pre-treatment can range from several days to weeks, depending on substrate characteristics and fungal activity (Nadir *et al.,* 2019). Energy consumption is primarily associated with maintaining optimal temperature and aeration requirements (de Oliveira *et al.,* 2019). The advantages of fungal pre-treatment include cost-effectiveness, eco-friendliness, higher biogas yields, easy isolation from different sources, shorter process time and the ability to degrade lignocellulosic materials (Kainthola *et al.,* 2021). In one study conducted to evaluate the impact of *Trichoderma reesei* pre-treatment on biogas production from corn stover, fungal pre-treatment increased biogas production by 27.3% compared to the control (Zulkifli *et al.,* 2018). In another study, which investigated the potential of *Aspergillus oryzae* pre-treatment on biogas production from rice straw, pre-treatment significantly increased the biogas production by 30.8% compared to the control (Abduh *et al.,* 2022). However, fungal pre-treatment has some limitations, such as the requirement of specific environmental conditions and the potential production of toxic metabolites by some fungi strains (Kusi *et al.,* 2018). Nevertheless, these limitations can be addressed by optimizing the process conditions and selecting appropriate fungal strains (Wagle *et al.,* 2022).

Enzymatic pre-treatment

Enzymatic pre-treatment is a method that involves the use of enzymes to break down complex organic compounds into simpler compounds that can be easily digested by microorganisms during anaerobic digestion (Li *et al.,* 2019). Enzymes such as cellulases, hemicellulases, and ligninases are used to break down cellulose, hemicellulose, and lignin, respectively (Liang *et al.,* 2020). In this pretreatment method, suitable substrates, such as agricultural residues, food waste, or lignocellulosic materials, are selected based on their composition and availability (Singh, 2021). The substrates are prepared by size reduction and pretreated to optimize enzymatic access and activity (Atelge *et al.,* 2020). Specific enzymes with desired hydrolytic activities are selected based on the substrate composition and added to the substrate to facilitate the breakdown of complex organic compounds into simpler forms ((Liang *et al.,* 2020).

The enzyme-substrate mixture is incubated under controlled conditions, typically at mesophilic or thermophilic temperatures, to promote enzymatic activity (Mohapatra *et al.,* 2020). pH levels are carefully adjusted and maintained within the optimal range for enzyme efficiency (Wu *et al.,* 2022). Mechanical mixing or agitation is employed to ensure uniform distribution of enzymes and substrates, facilitating enzymatic access to the substrate surface and improving the degradation efficiency (Cebreiros *et al.,* 2021). According to Kumar *et al.* (2021), process parameters such as temperature, pH, substrate concentration, and enzyme dosage are monitored and adjusted to maintain optimal conditions for enzymatic activity). Furthermore, continuous monitoring allows for process optimization and improved biogas production. In a study by Chen *et al.* (2018), the enzymatic pre-treatment process requires specific equipment. A reactor system, such as a bioreactor or fermenter, equipped with mixing devices, temperature and pH control mechanisms, and monitoring instruments, provides the controlled conditions necessary for enzymatic activity and substrate degradation. Mechanical or hydraulic mixing devices are employed to ensure thorough mixing of enzymes and substrates, promoting uniform enzymatic access and improving degradation efficiency (Cebreiros *et al.,* 2021). Temperature sensors, pH meters, enzyme activity assays, and analytical devices for substrate characterization are necessary for process monitoring and control (Mohapatra *et al.,* 2020).

The composition, lignin content, carbohydrate structure, and particle size of the substrate significantly impact enzymatic degradation efficiency (Xu *et al.,* 2019). Substrates with high lignin content or complex structures may require additional pre-treatment methods to enhance enzyme access and activity (Lan *et al.,* 2020). Choosing the appropriate enzymes and determining the optimal dosage is crucial for efficient pre-treatment (Lan *et al.,* 2020; Xu *et al.,* 2019). Enzyme compatibility with the substrate and synergy between different enzymes play a vital role in achieving higher degradation rates (Wang *et al.,* 2018). Optimal pH and temperature conditions must be maintained to ensure enzymatic activity and stability (Chen *et al.,* 2018). The optimal enzyme dosage, pH, and temperature conditions for enzymatic pre-treatment of biogas substrates vary depending on the specific enzymes, substrate composition, and desired degradation level (Bhushan *et al.,* 2021). Generally, the enzyme dosage can be optimized through experimental testing to balance effective degradation without excessive costs (Ferdeș *et al.,* 2020). According to Bhushan *et al.*(2021), enzymes typically exhibit optimal activity and stability at slightly acidic to neutral pH ranges from 5.0 to 7.0. In addition, temperature optimal range from mesophilic (35 - 50°C)to thermophilic (50 - 70°C) for most lignocellulolytic enzymes. Specific enzyme guidelines and experimentation should be followed to determine the optimal conditions for a given enzymatic pre-treatment process (Hashemi *et al.,* 2021).

The cost, time, and energy consumption of enzymatic pre-treatment depend on various factors, including substrate type, enzyme selection, scale of operation, and process optimization (Ferreira *et al.,* 2021). The cost primarily comprises enzyme procurement and operational expenses (Liang *et al.,* 2020). The duration of pre-treatment varies based on substrate characteristics and enzymatic activity, ranging from several hours to days

(Kumar *et al.,* 2021). The advantages of enzymatic pre-treatment method include higher biogas yields, reduced process time, cost-effective, eco-friendly and increased efficiency of anaerobic digestion (Ferdeș *et al.,* 2020). In one study, cellulase and laccase pre-treatment of wheat straw significantly improved biogas production and methane yield following anaerobic digestion of the straw (Schimpf & Schulz, 2019). Similarly, another study showed that a cocktail of enzymes, including cellulase, xylanase and pectinase pre-treatment significantly increased biogas yield and reduced the process time, demonstrating the potential of the method for enhancing biogas production (Mlaik *et al.,* 2019). However, enzymatic pre-treatment has some limitations, such as the high cost of enzymes and low yields of the pre-treatment process (Onumaegbu *et al.,* 2018).

FUTURE PROSPECTS

Biogas production involves the conversion of organic materials into methane-rich gas through a series of microbial processes (Atelge *et al.,* 2020). However, the efficiency of biogas production is often limited by the accessibility of the organic materials to microbial degradation (Nwokolo *et al.,* 2020). Despite the various advancements in substrate pre-treatment techniques, there is still a need for further research in exploring better and new pre-treatment methods that can break down the complex organic molecules in feed stocks into simpler forms for enhanced biogas production (Stanley *et al.,* 2022; Wagle *et al.,* 2022). An area for further research can include a novel technology like nanotechnology (Nasrollahzadeh *et al.,* 2019).

Nanotechnology can be used to develop more efficient and targeted pre-treatment methods, such as the use of nanoparticles to increase the surface area of feedstocks, thereby enhancing their accessibility to microbial degradation (Govarthanan *et al.,* 2022; Sanusi *et al.,* 2021). One study investigated the use of metal-organic frameworks (MOFs) as nanocatalysts for the hydrolysis of lignocellulosic biomass and showed that MOFs significantly improved the hydrolysis rate and yield of biogas production compared to conventional methods (Liao *et al.,* 2018). Similarly, another study developed a nanocatalyst based on iron oxide nanoparticles for the pre-treatment of food waste, which resulted in improved biogas production efficiency (Wang & Astruc, 2019).

Further research can be conducted on optimizing the existing pre-treatment methods to increase their efficiency and decrease their environmental impact (Janke *et al.,* 2018). Optimizing the conditions of chemical and thermal pre-treatment methods can improve their effectiveness while reducing the energy and resource requirements. For example, a study investigated the effect of temperature and pH on the efficiency of acid pre-treatment of food waste for biogas production, and found that increasing the temperature and decreasing the pH of the acid pre-treatment, resulted in higher biogas production and improved process efficiency (Dasgupta & Chandel, 2020). Similarly, a study optimized the conditions of alkaline pre-treatment of corn stover using response surface methodology, resulting in increased biogas production and reduced chemical oxygen demand (COD) of the effluent (Wong *et al.,* 2018). Thermal pre-treatment methods can also be optimized for increased efficiency and reduced environmental impact. For example, a study investigated the effect of temperature and heating time on the efficiency of microwave-assisted thermal pre-treatment of cow manure. The study found that increasing the temperature and heating time resulted in improved biogas production and reduced solids content in the effluent (Shrestha *et al.,* 2020).

Combining multiple pre-treatment methods is a promising direction for enhancing the efficiency of biogas production (Hallaji *et al.,* 2019). Researchers are exploring the potential benefits of combining different pre-treatment techniques to develop more efficient and effective processes (Onumaegbu *et al.,* 2018). Mechanical, chemical, and biological pre-treatment methods are commonly used in biogas production, and their combination has been studied in recent years. For example, one study investigated the effect of a combination of thermal, mechanical, and chemical pre-treatment on the anaerobic digestion of microalgal biomass, and found that the combined pre-treatment resulted in higher biogas production and improved solubilisation of the biomass (Wagle *et al.,* 2022). Similarly, another study explored the effect of combining mechanical and biological pre-treatment on the anaerobic digestion of pig manure, and found that the combination of mechanical and biological pre-treatment resulted in higher biogas production and improved degradation of organic matter, compared to individual pre-treatment methods (Vats *et al.,* 2020). Another study, investigated the effect of combining chemical and biological pre-treatment on the anaerobic digestion of food waste, and found that the combined pre-treatment resulted in improved biogas production and reduced solid content, compared to individual pre-treatment methods (Zia *et al.,* 2022).

The implications of improving substrate pre-treatment methods for the biogas industry are significant. Improving substrate pre-treatment methods is crucial to enhance the efficiency of biogas production (Almomani *et al.,* 2019). The development of more sustainable and efficient pre-treatment methods can lead to various benefits, including increased biogas production, reduced costs, and improved waste management practices (Prajapati *et al.,* 2021). Pre-treatment can improve the accessibility of feedstocks to microbial degradation, thereby enhancing the efficiency of the anaerobic digestion process. One study showed that the use of ultrasound-assisted alkaline pre-treatment increased biogas production from food waste by 17.6% compared to untreated waste (Kumar *et al.,* 2020). Improvements in pre-treatment methods can also lead to reduced costs (Ab Rasid *et al.,* 2021). The use Overall, this passage presents a well-rounded perspective on the future prospects of substrate pre-treatment in biogas production. The suggestions for further research and development, such as nanotechnology, optimization of existing methods, and combination of techniques, provide valuable insights into potential avenues for improving biogas production efficiency and addressing the challenges identified by researchers.

CONCLUSION

Biogas production is a promising renewable energy source with significant potential to reduce greenhouse gas emissions (GHG). Pre-treatment methods have been shown to increase biogas yields and reduce operational costs, but choosing the appropriate method depends on the specific application and of efficient pre-treatment methods can reduce the amount of energy required for anaerobic digestion and decrease the use of expensive enzymes. For example, a study showed that the use of a combined mechanical and thermal pre-treatment method reduced the energy consumption of anaerobic digestion by 20.5% (Kannah *et al.,* 2021). In addition to economic benefits, improving pre-treatment methods can have environmental benefits. The use of efficient pre-treatment methods can reduce greenhouse gas emissions by decreasing the amount of waste that goes to landfills (Lindberg *et al.,* 2022). Pre-treatment can also improve the quality of digestate, which can be used as a fertilizer and reduce the use of synthetic fertilizers. A study showed that the use of a combination of microwave and alkaline pre-treatment reduced the total nitrogen content in the digestate by 60%, indicating the potential for improved fertilizer quality (Deng *et al.,* 2020).

feedstock material. While advancements in pre-treatment methods have brought us closer to realizing the full potential of biogas production, it is important to implement proper waste management practices to prevent environmental contamination. With continued research and innovation, we can further optimize and improve pre-treatment methods to make biogas production more efficient and feasible. The economic and environmental benefits of biogas production make it a crucial component in the transition towards a more sustainable future. As we continue to explore and enhance biogas production, it has the potential to become a widely adopted and accessible renewable energy source that can contribute significantly to meeting energy demands while mitigating climate change.

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