Biogas Upgradation in Second Generation Anaerobic Digestion: External Hydrogen-Mediated Biomethanation in Microbial Electrolysis System Coupled with Methanogenic Reactor.

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A thesis submitted to the Department of Microbiology, Quaid-I-Azam University, Islamabad in the partial fulfillment of the requirements for the degree of

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In the name of Allah, the Most Merciful, the Most Kind

DECLARATION

The information and content contained in this thesis is my original work. I have not recently introduced any piece of this work somewhere else for any degree. All facts and content of this thesis are unique to me.

Arooj ul Mishqat

DEDICATION

I want to dedicate my work to my parents who have always pushed me to work harder in my field of interest and give it my all.

Arooj ul Mishqat

CERTIFICATE

It is certified that the research work presented in this thesis was completed by **Arooj Ul Mishqat** under the supervision of **Dr. Malik Badshah**. This report is submitted to the Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad; in the partial fulfillment of the requirements for the degree of Master of Philosophy (M.Phil) in Microbiology.

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List of Abbreviations

GHG	Greenhouse gas
WTE	Waste-to-energy
VFAs	Volatile fatty acids
HRT	Hydraulic retention time
CN	Carbon, nitrogen
CH_4	Methane
CO_2	Carbon dioxide
AD	Anaerobic digestion
BES	Bio electrochemical system
MES	Microbial electrolysis system
MEC	Microbial electrolysis cell
MEC-WAS	MEC- Wastewater activated sludge.
MEC-AD	Microbial electrolysis cell coupled with anaerobic digestion.
RETs	Renewable energy technologies
CHP	Combined heat and power plants
FA	Fre Ammonia
H_2	Hydrogen
O_2	Oxygen
H_2O	Water
H_2S	Hydrogen sulfide
N_2	Nitrogen
NH_3	Ammonia
CHP	Combined heat and power
LCFAs	Long chain fatty acids
OLR	Organic loading rate
COD	Chemical oxygen demand
PSA	Pressure swing adsorption
FA	Free ammonia

GC	Gas chromatography
SOWs	Solid organic wastes
LFD	Liquid fraction of the digestate
HPLC	High-performance liquid chromatography
SSE	Single set electrodes
DSE	Double set electrodes
BOD	Biological oxygen demand
S/I	Substrate to inoculum
C/N	Carbon to Nitrogen
C/N/P/S	Carbon/Nitrogen/Sulfur/Phosphorus
VS	Volatile solid
TS	Total solid
FVW	Fruit and vegetable waste
WWTP	Wastewater treatment process
LR	Liquid recirculation
BEAMR	Bio electrochemically aided microbial reactor
DIET	Direct interspecies electron transfer
WAS	Waste-activated sludge
UASB	Up-flow anaerobic sludge blanket
SBBL	Sustainable bioenergy and bio refinery lab
P2G	Power-to-gas
PEM	Proton exchange membrane
AEM	Anion exchange membrane
DET	Direct electron transfer
DIET	Direct interspecies electron transfer
EMR	Electromethanogenic reactor
ml, mm	Milliliter, millimeter
mg/L	Milligram per liter
ml/min	Milliliter per minute
V, mg	Volts, milligram
g VS/L	Gram volatile solids per liter

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	is reactor setup with a single set of electrodes applied with voltage, and R6 is reactor setup with a double set of electrodes applied with voltage in their electromethanogenic reactors.	

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ABSTRACT

Biogas is a cutting-edge renewable energy source with significant market potential due to the widespread availability of organic biomass and is capable of assisting countries to achieve their sustainable development goals by facilitating the formation and availability of renewable energy sources. It has the potential to be utilized in order to generate electricity that can be delivered to electrical grids, or it can be used as a fuel for automobiles. Its low methane content is one of its limitations, despite its potential. So, to improve its efficiency, in-situ biogas upgradation can be done by the application of a next generation anaerobic digestion coupled microbial electrolysis system. MEC partially sustains the energy demand by electroactive oxidation of organic matter via a bioanode and facilitates CO_2 reduction into CH_4 by employing a biocathode as an electron donor. This study evaluates the impact of a microbial electrolysis system in an electromethanogenic reactor coupled with in situ biogas upgradation during two stage anaerobic digestion. The current study was performed in the reactor setups R3 having a single set of electrodes (SSE), R4 having a double set of electrodes (DSE), R5 having a single set of electrodes applied with voltage (MEC), and R6 having a double set of electrodes applied with voltage (MEC), in their methanogenic reactors, in which the gases were recirculated for the purpose of upgrading biogas, including hydrogen supplied from an external source and results were compared with control, R1 with no electrodes and no recirculation of gases (biogas and hydrogen), and R2 with recirculation of gases (biogas and hydrogen) with no electrodes. Improving the performance of the in-situ upgradation process involved optimization of the flow rate, recirculation time, and recirculation time with applied voltage. Methane content through recirculation recorded in R1, R2, R3, and R4 was 65, 87, 90, and 92% during interval-based time optimization. Maximum amount of methane content recoded was 95 and 99% in R5 and R6 with an applied voltage of 0.7V during interval-based time optimization. The conclusion of the study is that the addition of MEC with an enhanced surface area of electrodes can significantly enrich the methane content during upgradation in two stage anaerobic digestion.

Chapter No. 1

Introduction

Access to sufficient resources, primarily utilities related to energy, to meet the basic requirements of individuals and the needs of society to advance social welfare and the economy is a vital prerequisite for sustainable development. Economic advancement depends on energy as a raw material for products and services. Energy can be produced from both renewable and non-renewable sources. The most important non-renewable source of energy in the biosphere, fossil fuels (coal, gas, and oil) account for 81.2% of total energy use (Soltani et al., 2013). The majority of the fossil fuels are used to generate electricity, which is needed to maintain the commercial energy supply. The load balance of the energy network is heavily dependent on fossil fuel power facilities for stability. Fossil fuels produce 70% of the electricity generated globally (Hasanuzzaman et al., 2017).

There is a greater demand for energy as a result of population growth, economic expansion, and industrialization. As of right now, fossil fuels are mostly used to meet this requirement. Despite being essential to economic growth, the natural ecology suffers when these resources are continuously used to generate electricity. There are two major effects that are recognized: greenhouse gas emissions and global warming. By 2040, global net electricity generation is expected to have increased by 93%, according to the text (Newell et al., 2019). It cautions against relying solely on fossil fuels, as this will make environmental issues worse.

There are environmental concerns, particularly in emerging nations, as a result of the fast-rising amount of waste from diverse sources, such as domestic sources, industries, and agriculture (Rasheed et al., 2021). It's getting harder to control this much waste. Transforming waste into energy, especially using processes involving anaerobic digestion (AD), is believed to be a practical and sustainable way to address environmental issues and lessen dependency on fossil fuels (Ali et al., 2020).

According to the Conference (2019), Pakistan is currently the sixth most populated nation in the world (Biresselioglu et al., 2019). Its population will exceed 333.1 million by 2050, growing at a rate of around 2.4% per year. It is also anticipated that by 2025, the rate of

industrialization will have increased to 52%. The average annual growth rate of energy consumption in Pakistan has been 8%. Power outages accounted for almost 30% of Pakistan's total power generation in 2018, which was 120,785 GWh (NTDC, 2013). To achieve the demand and supply difference for that particular year, 51,765 GWh of energy has to be produced (Yaqoob et al., 2021).

Pakistan's residential, agricultural, commercial, and industrial sectors suffer greatly from daily electricity outages, which can last up to 12 hours during the summer and even longer in rural areas (Rauf et al., 2015). Economic growth is hampered by these energy constraints. In order to tackle this issue, Pakistan, which possesses substantial biomass resources, particularly in its rural regions, can employ waste-to-energy (WTE) programs to convert organic waste, including manure and other wastes from animals, into biogas (Korai et al., 2016). This method tackles the environmental challenges related to garbage disposal in addition to helping to resolve power outages (Ram et al., 2021).

The global focus on the development of less polluted, alternative energy sources has increased due to the negative effects that utilizing conventional biomass and fossil fuels has on the environment, society, and human health (Jaiswal et al., 2022). The transition to more efficient biomass-derived fuels, such as biogas, has demonstrated some success in reducing the catastrophic ecological effects and the associated health and socioeconomic repercussions for humans; however, much more work is required. Sustainable living and climate goals may benefit from biogas production (Obaideen et al., 2022).

After 2050, 50% of the energy in the biosphere is predicted to originate from renewable energy sources (Gielen et al., 2019). Fossil fuels are putting strain on the planet, as fossil fuels contribute to greenhouse gas emissions, global warming, and air pollution. The hunt for alternate energy sources is critically important for the future. Bioenergy (biomass), nuclear energy, and solar energy are the most advanced emerging alternative energy sources. The most promising, always available, and economical of these is bioenergy (Tareen et al., 2018).

Biogas is produced by the biological conversion of waste or organic materials via anaerobic digestion, employing a method widely used for the treatment of organic waste. It is the energy of the future; it is regenerative and sustainable. Apart from being an important source of energy, especially for rural communities, it also helps mitigate the effects of climate change by reducing greenhouse gas emissions caused by the decomposition of organic matter and supports the maintenance of old, widely used ecosystem services (Dhanya et al., 2020).

A particular type of biofuel, referred to as biogas, is derived from biogenic material. It is generally described as a gas produced by bacteria fermenting organic matter in an anaerobic environment (one in which oxygen is absent). It may be composed of a wide variety of readily available wastes and organic materials, such as sewage sludge, animal dung, and municipal organic waste. Materials such as straw, sugarcane, manure, energy crops that are developed specifically for energy generation, biodegradable waste products, and leftovers from agricultural and industrial activities could also be used to produce energy (Bharathiraja et al., 2018).

As a reliable and sustainable alternative to fossil fuels, the environmental benefits of biogas technology are widely highlighted. Biogas has the potential to enhance energy security when it is accompanied by a reduction in greenhouse gas emissions. In comparison to combustion-based methods for such biomasses, it has a negligible effect on air quality and allows for the use of municipal, zoo technical, and agricultural wastes as a sustainable energy source. In short, biogas can be upgraded to "bio-methane," which can then be properly used as vehicle fuel or injected into natural gas grids (Sawyerr et al., 2019).

For processing biodegradable organic waste in order to generate biogas, anaerobic digestion is a more straightforward method that is widely employed. "Hydrolysis, acidogenesis, acetogenesis, and methanogenesis" are the four chemical and biological steps that comprise the anaerobic digestion process. Many microbial communities carry out the various stages of degradation. These bacteria operate in partially syntrophic relationships and impose different

requirements on their surroundings. Anaerobic fermentation has four stages that are discussed below in order to achieve the production of biogas (Schnürer, 2016).

The anaerobic digestion process and the production of biogas are influenced by a number of parameters, including the type of feedstock, temperature, the total amount of solids present, the ratio of carbon to nitrogen, the pH, alkalinity, uniform feeding, volatile fatty acids, hydraulic retention time, organic loading rate, and the concentration of macro- and micronutrients. By modifying metabolic pathways, microbial community activity composition, process diversity, thermal stability, and temperature, influence methane generation and process stability. Thermophilic anaerobic digestion may function at temperatures between 55 and 60 °C and has a number of benefits, including a high rate of degradation and a significant decrease in waste and methane production. However, their disadvantages include greater energy requirements and instability when compared to mesophilic processes. The preference for mesophilic anaerobic digestion stems from its rapid rate of degradation in high-temperature situations, which may lead to the production of a significant amount of VFAs that reduce pH and may hinder the process (Meegoda et al., 2018).

For anaerobic digestion, a carbon-to-nitrogen ratio of 25 to 30 is ideal. The carbon-to-nitrogen ratio reveals a substrate's nutritional content for anaerobic digestion. Anaerobic digestion fails as a result of process instability brought on by a high C:N ratio, which produces a high concentration of ammonia that is poisonous to microbial cells. While mesophilic microbes require an HRT ranging from 10 to 40 days, aerobic digestion requires an average HRT of over 14 days. Feedstock composition and OLR have an impact on HRT. High VFAs deposition is the result of low retention time (Van et al., 2020).

Cattle manure's high "methanogen" level and well-exhibited buffering ability make it crucial for the start of anaerobic digestion. When cattle dung is utilized for mono-digestion, anaerobic digestion performs inadequately and is less stable. Because of its improved C:N ratio, co-digestion of green organic waste with cattle manure may improve the viability of anaerobic

digesters. Reduced sulfide and elevated ammonia concentrations may have an inhibiting effect when co-digestion occurs (Rocha-Meneses et al., 2022).

Due to their high "volatile solids" content and low total solids, the hydrolysis of vegetable and fruit wastes occurs quickly, potentially increasing biogas output. Since volatile fatty acids can operate as a buffer solution to reduce the effects of ammonia and increase the production of biogas, having an optimal carbon content can help avoid ammonia. This has been demonstrated in recent studies. modifying the C/N proportions required for an anaerobic digestion process that is stable and long-lasting. Therefore, increasing emphasis is being paid to the anaerobic cattle manure for co-digestion process with green wastes in order to reduce waste and produce biogas (González et al., 2022).

Acidogenesis, hydrolysis, methanogenesis, and acetogenesis are all complete biological events that typically occur in the same reactor during anaerobic digestion. Less labor is needed, the design is simpler, and the cost is reduced. If the acid phase grows quicker than the slow-growing methanogens, difficulties could arise within a single stage. Methanogenesis and acidogenesis coexist in a single vessel in a "one-stage system." The acidogenic action, which mostly consists of CO_2, acetate, and hydrogen generation, increases with an increase in substrate feeding rate. However, the population of methanogens is unable to increase this action to the same extent. High organic loading rates have the benefit of allowing for the treatment of more waste in the same amount of area, which could reduce the reactor's overall cost. In anaerobic digestion, VFAs are eventually converted to CO_2 and CH_4 by species of methanogenic bacteria. However, a greater rate of organic loading may cause VFAs accumulation. Because of this, pH decreases, which could potentially cause the AD to fail (Ometto et al., 2019).

Acidogenesis and methanogenesis require their own reactors to avoid the issues of pH and growth requirements, which are the solutions to the concerns discussed previously. For rapid digestion at a high rate of organic loading and a stable process, a two-stage anaerobic digestion technique is therefore required. By regulating acidogenesis and shielding the methanogens

from overabundance and low pH shock, the two-stage mechanism reduces the likelihood of hazardous material accumulation. This explains why two-stage procedures can yield higher biogas production. Nevertheless, due to the accumulation of more "volatile fatty acids," methanogen efficiency decreases and is hindered at increasing rates of organic loading. Methanogen activity is inhibited when pH falls outside of the range (Ometto et al., 2019).

Anaerobic digestion is a process that is widely used to treat waste and produce biofuel, or biogas. Many strategies have been used to improve AD because of the frequent occurrence of instability and lower levels of production. To enhance and upgrade biomass, various strategies have been employed. Biogas has a lower coefficient of heating than natural gas because it contains more pollutants, such as methane (CH_4), in addition to 25–55% carbon dioxide (Bonse & Beyene, 2021). In light of the fact that biogas represents a significant advancement over fossil fuels in terms of application facilitation, purification, or upgrading of the gas, it is therefore required to ease its transportation and storage, attain a certain standard for direct injection into the natural gas grid, and increase its electricity conversion efficiency. Furthermore, specifically in comparison to fossil fuels, in AD systems, biogas is more environmentally beneficial because it removes or sequesters CO_2. A few energy-intensive commercially used methods for biogas upgrading include membrane separation, water-amine scrubbing, cryogenic separation, pressure swing adsorption, and cryogenic separation. A relatively new method of biogas valorization is being upgraded based on biotechnology. Biogas upgradation is accomplished by using microorganisms that convert the CO_2 content of the biogas into either methane (chemoautotrophic) or algal biomass (photoautotrophic), as opposed to the physio-chemical technique, which focuses primarily on the removal of carbon dioxide from the gas. The biological approach is thought to be more advanced than the conventional ones because the overall energy content of the gas that is fed is smaller than the cumulative energy content of the products. That being said, the biotechnological approach offers lower energy consumption and operating costs in comparison to physico-chemical upgrading approaches (Paolini et al., 2018).

Methane is produced when hydrogen is fed into an anaerobic digester during the in-situ biological upgrading process. This reaction occurs through the metabolism of hydrogenotrophic methanogens, which use available CO_2. Alternatively, during ex-situ biological upgradation, the CO_2 part of the biogas immediately undergoes conversion to CH_4 by alternatively enriched or pure hydrogenotrophic archaea when the biogas from the exogenous hydrogen and AD is introduced into the bioreactor separately (Voelklein et al., 2019).

Microbial electrolysis cells, or bio-electrochemical systems, have drawn a lot of attention in recent times. According to its definition, a bio-electrode (bio-cathode and bio-anode) is the electrode at which a redox reaction takes place in an electrochemical system in which at least one of the reactions is aided by microbes. MEC catalyzes substrate into byproducts by using an external power source. Protons, electrons, and carbon dioxide are produced when "electrochemically active" bacterial species oxidize organic materials within MEC. Protons are released into the solution when bacteria transfer electrons to the anode. The solution contains a variety of microbes that use H_2 and CO_2 in a variety of ways to create CH_4 (Hua et al., 2019).

Numerous studies have demonstrated how using "MEC to AD (MEC-AD)" can increase the pace of substrate breakdown (including that of resistant chemicals) and alter the AD microbial population by enriching methanogens and exoelectrogens, which increases the amount of biogas produced. Once stable microbial communities were established, the methane generation of "MEC-AD" was improved. Using electric signal detection that corresponded linearly with substrate concentration, real-time "MEC-AD" monitoring was possible. MEC has the benefit of enhancing the hydrogenotrophic methanogens on the cathode and breaking down more substrate. By lessening the accumulation of shorter-chain VFAs and altering the inhibitory effects of various harmful and refractory substances, "MEC-AD" coupled systems have also shown enhanced process stability as compared to antiquated anaerobic digesters. In order to better optimize the circumstances for each of the aforementioned microbial populations, the current study will be conducted in two-stage reactors, i.e., isolating the acidogenic reactor separately from the methanogenic reactor. The methanogenic reactor will have a MEC

installed, which will accelerate the breakdown of VFAs at the anode and enhance the growth of hydrogenotrophic methanogens at the cathode, improving process stability and efficiency (Yu et al., 2018).

In this study, graphite electrodes were inserted in methanogenic reactors with a single set of electrodes (SSE) in reactor setup R3, a double set of electrodes (DSE) in reactor setup R4, a single set of electrodes (SSE) with applied voltage in reactor setup R5, and a double set of electrodes (DSE) with applied voltage in reactor setup R6. The methane content was enhanced by the recirculation of biogas along with external hydrogen in methanogenic reactor for various time durations, and the effect of external applied voltage in methanogenic reactor during two-stage anaerobic digestion coupled with MEC was also studied during biogas upgradation.

Aim and Objectives

Aim:

The aim of this study is “Biogas Upgradation in Second Generation Anaerobic Digestion: External Hydrogen-Mediated Biomethanation in Microbial Electrolysis System Coupled with Methanogenic Reactor.”

Objectives:

This research was conducted to study the following objectives:

- Effect of flow rate, and duration of recirculation on biomethane content during suspended and attached growth methanogenic reactors.
- Effect of electrodes’ surface area on biogas upgradation.
- Effect of different intervals of gas feeding on biogas upgradation at optimized conditions.
- Effect of applied voltage during biogas upgradation employing a microbial electrolysis system coupled with two stage anaerobic digestion.

Chapter No. 2

Literature Review

The ability to produce energy is a prerequisite for the growth and development of every community or nation. The cost of energy has an impact on prices of goods and services. Consequently, there is a need to provide energy that is dependable, affordable, and appropriate while having the fewest negative environmental effects. Statistics indicate a gradual increase in energy needs between "2010 and 2040." With "non-renewable energy" making up the largest portion of the global energy supply, ecological problems are caused by increased greenhouse gas emissions. A literature review demonstrated that, between "1987 to 2012," fossil fuels accounted for the highest amount of energy, rising by over 84%, while renewable energy constituted the minimum (Sovacool et al., 2013). Aremu and Agarry said that transportation, distribution, and energy generation ("80–90%") come from fossil fuels. Energy security and reliance on non-renewable energy sources could be resolved with the help of renewable energy sources, which include geothermal power, hydropower, solar energy, and wind energy. With fossil fuels serving as the main energy source, the demand for energy has increased exponentially as a result of the expansion of the world's population (Agarry, 2017).

On the other hand, these fossil fuels contribute significantly to climate change, health problems, sea level rise, and ecological changes through the release of greenhouse gases (GHGs) like carbon dioxide and methane (Gahlawat & Lakra, 2020). In an effort to lessen these effects, nations have started using tactics like increasing the efficiency of technology, creating cutting-edge, eco-friendly devices, and switching to renewable energy sources. In order to completely replace fuels generated from fossil fuels, alternative energy sources must be created on a global scale. In response to the drawbacks of energy generation based on fossil fuels, renewable energy technologies (RETs) have become extensively embraced (Darmani et al., 2014). Global bioenergy generation potential is demonstrated by the extensive usage of anaerobic digestion (AD) techniques for cooking and power production. Biogas is an organic energy source that is renewable and can be upgraded to fulfill certain standards for direct injection into the natural gas grid, reduce the amount of natural gas needed for conversion, and facilitate storage and transportation. Biogas has a lower heating value than natural gas. Pakistan has the ability to produce biogas through anaerobic digestion of animal feces. Research and

development on biogas, which not only generates new employment prospects but also has a good effect on the ecosystem, is carried out in Pakistan by the Pakistan Council of Renewable Energy Technologies, Pakistan Council for Appropriate Technologies, and Pakistan Renewable Energy Society. Produced from organic waste, biogas is produced following the breakdown in the presence of anaerobic bacteria and is utilized in a number of sectors, such as thermal energy, and electricity (Kamran, 2018).

2.1 Biogas and its Composition:

One could manufacture biogas from a variety of wastes and organic sources. Because it produces valuable fuel gas and disposes of the majority of the wastes afterward, anaerobic digestion is a guaranteed alternative for treating biodegradable wastes. The generation of biogas is a significant component in waste management. It helps to fight against “Global warming”. Methane combustion is substantially cleaner than coal burning, and it can provide the required energy with lower environmental carbon dioxide emissions (Bharathiraja et al., 2018). When fossil fuels are burned, a minute concentration of carbon is emitted to the atmosphere, but photosynthetic plants absorb the released carbon from biogas. The use of bio-methane reduces pollution of the air, land, and water. With reduced concentrations of harmful greenhouse gases, it serves as an alternative "source" of heat and energy and contributes to the preservation of forests and biodiversity. Additionally, as CO_2 and other gases that contribute to greenhouse effects are released into the environment during the decomposition of organic matter, its use does not increase the amount of greenhouse gases in the atmosphere. The increased need for energy can only be addressed by biomethane, which is simply an excellent method of doing so. Table 2.1 shows main constituents of biogas (Herout et al., 2012).

Table 2.1: The Composition of Biogas

S/No	Constituents	Concentration (v/v)
1	CO_2	15-60 %
3	N_2	0-5 %
2	CH_4	40-75 %
5	H_2S	0-5000 ppm
4	H_2O	1-5 %
7	Trace gases	<2 %
6	H_2	Traces
8	O_2	<2 %

2.2 Anaerobic Digestion and its Stages:

Anaerobic digestion has been extensively studied by several researchers in a variety of ways. In 2010, Arsova defined it as a naturally occurring process in which bacterial species break down raw materials to produce end products primarily digestate and biogas while oxygen is absent (Arsova, 2010). Furthermore, it is a method that relies on microorganisms to decompose materials without the assistance of light or oxygen to produce methane gas suitable for energy conversion. The process known as "anaerobic digestion," according to (Parawira et al., 2007), entails a variety of biological processes in which the byproducts of one stage of microbial activity serve as the starting point for a different substrate in a subsequent step. This results in changes to organic matter that combine carbon dioxide and methane. Due to the removal of human and animal waste for biogas applications and the production of digestate, which contains nitrogen and is used as fertilizer on farms, this process—anaerobic digestion—is very stimulating. Anaerobic digestion produces fewer greenhouse gases when compared to other waste disposal methods like landfilling and manure. This method provides digestate and biogas, making it appropriate for energy recovery. Furthermore, energy production could also be achieved in "combined heat and power (CHP)" plants. In addition, biogas might be used to supplement the use of coal in the grid's energy production (Tang et al., 2023).

"Hydrolysis, acidogenesis, acetogenesis, and methanogenesis" are the four steps that make up the anaerobic digestion process. The four phases listed above are carried out via relationships between different microorganisms, which constitute the basis of the AD mechanism.

2.2.1 Hydrolysis:

The hydrolytic bacterial species involved in this process have the ability to secrete extracellular enzymes that can convert proteins, lipids, and carbohydrates into amino acids, sugars, and long-chain fatty acids, respectively (Menzel et al., 2020). The hydrolysis products can diffuse across the cellular membranes of acidogenic microorganisms after being broken down by enzymes. While there is no evidence of improved hydrolytic activity below a pH range of 7, the ideal temperature and pH range for this process are between "30–50 ºC" and 5-7 (Batstone et al., 2009). However, it's important to remember that a number of substrata, including hemicellulose, cellulose, and lignin, may be difficult to break down and may be inaccessible to microbes due to complex assemblies; adding enzymes is a common practice to facilitate the hydrolysis of these compounds. The hydrolysis reaction is illustrated in Equation 1. The transformation of organic materials into glucose is demonstrated. The adsorption, enzyme synthesis, biomass accumulation, surface area, and material form and size are the components that are depended upon in this process.

$$C_6H_{10}O_4 + 2H_2O \text{ --------- } C_6H_{12}O_6 + 2H_2 \quad [1]$$

2.2.2 Acidogenesis:

Similarly, the fermentation process is the name given to this second stage of AD. Acidogenic microorganisms are able to produce transitory VFAs, or volatile fatty acids, as well as other compounds by absorbing hydrolysis products through their cell membranes. Acidogenesis is typically assumed to proceed at a quicker rate than subsequent AD stages, in contrast to subsequent processes. Acetic acid is assumed to be the primary organic acid in this reaction. The formation of VFAs increases with pH greater than 5, but the creation of a large amount of ethanol occurs at pH lower than 5. This behavior is consistent with the acidogenesis process. When the pH is less than 4, the processes may stop (Kim et al., 2003). Examples of reactions leading to acidogenesis are shown as

[$C_6H_{12}O_6$ ---------- $2CH_3CH_2OH + 2CO_2$ [2]]

[$C_6H_{12}O_6 + 2H_2$ --------- $2CH_3CH_2COOH + 2H_2O$ [3]]

[$C_6H_{12}O_6$ ---------$3CH_3COOH$ [4]]

Equation (2) shows how glucose and ethanol react with one another. The conversion of glucose from the aforementioned reactions results in propionate in equation (3) and acetic acids in equation (4). Glucose is currently recognized as the primary product. The acidogenesis process's principal products are butyric acid (CH_3CH_2COOH), formic acid (HCOOH), ethanol (C_2H_5OH), methanol (CH_3OH), propionic acid (CH_3CH_2COOH), acetic acid (CH_3COOH), and lactic acid ($C_2H_6O_3$) (Cord-Ruwisch & others, 2019).

2.2.3 Acetogenesis:

This is phase three of anaerobic digestion, and hydrogen plays a big role in this process. The "methanogenesis" stage of anaerobic digestion that comes after the acetogenesis process when it is thought to be appropriately connected. In order to produce CO_2, H_2, and acetate before the final conversion into CH_4, it caused the loss of electrons, which included VFAs and alcohol. The hydrogen that creates acetogenic bacteria also produces carbon dioxide, hydrogen, and acetate through the interaction of alcohol and VFAs (Sterling Jr et al., 2001). Homoacetogenic bacterial species produce acetate from hydrogen and carbon dioxide (Liebetrau et al., 2019).

Moreover, acetate produces hydrogen, which is used by acetogenic bacterial species. The number of final products from the acidification step procedure is determined by the substrate starts. The final products include propionic acids, butyric acids, chain fatty acids, and polymer substrates (Montag & Schink, 2016). Acetogenic microorganisms turn substrates into acetic acid, H_2, and CO_2 by use of a mixture of glycerol, alcohols, and lactic acids. Equations [5] to [7] show several examples of acetogenesis reactions. Equations [6] and [7] carry out the conversion of ethanol into acetic acid, while equation [5] converts propionate into glucose and acetic acid.

[$CH_3CH_2COO + 3H_2O$ -------- $CH_3COO + H + HCO_3 + 3H_2$ [5]]

[$C_6H_{12}O_6 + 2H_2O$ ---------- $2CH_3COOH + 2CO_2 + 4H_2$ [6]]

[$CH_3CH_2OH + 2H_2O$ ---------- $CH_3COO + 2H_2 + H$ [7]]

2.2.4 Methanogenesis:

Methanogenesis is the final stage. Methane is produced during this phase as a result of the methanogenic bacteria using the available intermediates. There are several factors that impede this slower phase, including temperature, pH, organic loading rate, and substrate. "*Methanococcus vannielli*" and "*Methanococcus voltae*" were found to exhibit 99% cell death within 10 hours of exposure to oxygen, indicating their sensitivity to the gas (PASSARIS et al., 2018). With "hydrogenotrophic methanogenesis" accounting for the remaining "1/3," methane synthesis is primarily attributed to "acetoclastic methanogenesis from acetate," accounting for nearly "2/3" (Zhang et al., 2023).

Methanogens appear to regenerate in anaerobic digestion at a remarkably slower rate than other bacteria, potentially taking anywhere from five to sixteen days. However, a number of hydrogenotrophic species have been reported to have a two-hour doubling time, such as *Methanococcus maripaludis*. The process of mechanization is what produces methane. The mechanization process is characterized by the responses described in equations [8] through [10].

[$CO_2 + 4H_2$ --------- $CH_4 + 2H_2O$ [8]]

[$2C_2H_5OH + CO_2$ --------- $CH_4 + 2CH_3COOH$ [9]]

[CH_3COOH ---------- $CH_4 + CO_2$ [10]]

Methane and carbon dioxide are produced as byproducts of the reactions shown in equations [8] through [10].

2.3 Anaerobic Reactors and its Types:

Anaerobic reactors were studied for biogas recovery through waste breakdown, categorized into two groups based on reactor number and phases involved.

2.3.1 Single Stage Anaerobic Reactors:

Methanogenesis, acetogenesis, and hydrolysis are only a few examples of the complete biological reactions that occur in a single stage within another reactor. The advantages are lower costs, simpler designs, and less work. Problems arise when the rate of acidification exceeds the rate at which methanogens rise more slowly. If methanogens and acidogens are combined in a single vessel in a one-stage system, hydrogen produced by acidogenic metabolism is produced, while a higher hydrogen partial pressure inhibits acetogens (Azbar & Speece, 2001). While the population of methanogens could not enhance its activity to the same degree, the acidogenic action, which mostly consists of hydrogen, carbon dioxide, and acetate formation, is increased when the substrate feeding rate is increased. Higher organic loading rates cause hydrogen-consuming processes to become saturated. This accumulation of hydrogen partially prevents hydrogen from being generated further, leading to the creation of a large organic electron sink, which explains methane generation imbalances and termination. However, a high organic loading rate has the benefit of allowing for the treatment of a large volume of waste in a space of comparable size, which could reduce the cost of the reactor as a whole (Shamurad et al., 2020).

Methanogenic and acetogenic bacterial species are often responsible for converting the volatile fatty acids produced during anaerobic digestion into carbon dioxide and H_4. Nevertheless, an increased organic loading rate helps to explain the buildup of VFAs. The pH falls as a result, and it may even cause failure of anaerobic digestion process. It is necessary to use these volatile fatty acids because they are eventually converting into methane (Yin et al., 2019).

2.3.2 Two Stage Anaerobic Reactors:

This procedure controls acidogenesis to protect the methanogens from lower pH shock and overload while achieving methanogenesis and acidogenesis in separate reactors. As a result, the possibility of a fatal material accumulation is reduced. Hence, a two-stage process produces more biogas for this reason. However, as OLR increases, methanogen efficacy decreases and subsequently, elevated volatile fatty acids accumulation leads to their suppression. As a result

of pH falling outside of the accepted range, methanogen activity is inhibited (Azbar & Speece, 2001).

2.4 Factors Affecting Anaerobic Digestion:

It is important to consider the most beneficial conditions when designing an anaerobic digestion system. Here, we'll talk about the effects of feed stock, pH, temperature, carbon/nitrogen ratio, HRT, and OLR and vice versa (Lohani & Havukainen, 2018).

2.4.1 Moisture Content:

Anaerobic digestion processes are influenced by moisture, which is a critical component of microbial metabolism. In the case of solid compositions less than 15%, wet anaerobic digestion takes place, but dry anaerobic digestion takes place when solid compositions exceed 15% (Colazo et al., 2015). Less retention time, more reduction in volatile solids (VS), lower inoculum volume, and increased methane output are some benefits of wet digestion. Conversely, fermenter capacity is smaller, digestate handling is simpler, and dry anaerobic digestion uses less energy (Zhang & Banks, 2013).

2.4.2 Feed Stock:

The treatment of animal compost and sewage sludge was previously accomplished using anaerobic digestion. Nevertheless, new methods of waste management were needed due to the emergence of environmental issues. Therefore, the field of anaerobic digestion is expanding rapidly in the present day to handle a variety of industrial, agricultural, and municipal wastes. The amount of lignin in feedstock causes degradation to become more difficult.

Feedstock composition changes suggestively for anaerobic digestion. In the form of a slurry with a total solids content of 3-12%, the waste of animals is collected. In contrast, the total solid content of chicken waste is 30% (Prado et al., 2022). Dry matter waste from agriculture changes greatly. The waste produced by the agricultural sector makes less than 1% of all solids. The anaerobic digestion process is impacted by some materials, including straw, wood, and inorganic debris like plastic, glass, and so on. The AD process often fails due to such undesired

components. The main ways that the VFAs vary are in the way that these wastes are handled, stored, and handled as a slurry. The concentration of VFAs in cattle manure is lower than in pig dung. The animal slurry VFAs contents generally do not exhibit any inhibitory effects (Dennehy et al., 2018).

Food waste often contains higher concentrations of soluble organic matter, which is easily broken down. This can result in higher concentrations of volatile fatty acids (VFAs) during the early stages of digestion, which could cause a rapid drop in pH and inhibit or reduce the process of methanogenesis. By co-digesting feedstock with a higher buffering capacity, this inhibition is lessened by increasing the formation of VFAs during the early stages of the digestive process. The anaerobic digestion process may benefit from increased stability as a result (Prado et al., 2022).

2.4.3 Particle Size:

Particle size affects how substrates are broken down because more free surface area allows hydrolases to do their biodegradation. The breakdown process is aided and biomethane synthesis is enhanced by the large surface area that small particles provide for the first adsorption of exoenzymes. Anaerobic digestion is improved by reducing substrate size since it shortens the digestion process and produces more biogas. The production of biomethane and biodegradability are improved when particle sizes are reduced from 100 to 3 mm. Nevertheless, incredibly small particle sizes could cause foam to form, which could cause the operation to fail (Rawoof et al., 2021).

2.4.4 Total Solids (TS) Content:

For dry materials, a similar-sized optimized anaerobic digestion reactor might handle a greater number of wastes than a liquid AD reactor (Li et al., 2020). When municipal solid wastes are in "mesophilic" batch conditions, the concentration of substrate primarily affects the AD. When total solids concentration increased from 20–30%, COD elimination decreased from 80.69–69.05% over that period (Zabed et al., 2019).

2.4.6 Feeding:

Uniform feeding is necessary to keep the microorganism in relatively constant contact with waste, which will improve digestion. Thus, feeding needs to be consistent in terms of both quantity and quality, and it needs to be done at the same interval of time (Appels et al., 2008).

2.4.7 Temperature:

The two distinct temperature ranges for anaerobic digestion separation are "thermophilic AD around (55 to 70°C) " and "mesophilic AD around 25–40°C," as was previously discussed. A wide variety of temperatures is beneficial to "thermophilic AD," and it often results in faster reaction rates. In thermophilic anaerobic digestion, however, acidification may occur, which would hinder this process. Additionally, the mechanism is less stable overall and more susceptible to ecological variations when the temperature range is larger. There are several intrinsic reasons why thermophilic anaerobic digestion is less remarkable, including increased energy input, increased investments, increased toxicity, and susceptibility to environmental conditions. Mesophilic systems exhibit a great deal of stability, but they also often produce less methane, have limited biodegradability, and have challenges related to nutritional imbalance. Thus, ideal conditions for anaerobic digestion might include hydrolysis and acidogenesis that are thermophilic, followed by methanogenesis that is mesophilic. This could be planned as a two-step AD process (Nie et al., 2021).

2.4.8 pH:

The pH of the AD reactor has an impact on the digesting process, products, and bacterial colonies. Ideally, the pH range for AD is 6.8–7.4. Since free ammonia (FA) concentrations are higher when pH is not controlled, it is also important to reduce ammonia toxicity. Growing microorganisms will help break down a few FA and get rid of the inhibition they cause when pH is maintained within an appropriate range. Acidogenic and methanogenic bacteria have different ideal pH levels, which must be considered. Methanogenesis exhibits its optimum

effectiveness in pH ranges of 6.5-8.2, with 7.0 being the ideal range. For acidogenesis, a pH of 5.5–6.5 is ideal (Lisowyj & Wright, 2020).

2.4.9 Organic Loading Rate (OLR):

Within a continuous feeding system, it describes whole volatile solids that are fed into a digester daily. The researchers have found that, but only to a limited extent, biogas generation increases in tandem with an increase in organic loading rate. The AD reactor's conditions can change with the significant amount of fresh materials added every day, which ultimately causes the bacteria's activity to be inhibited. Bacterial acidogenesis, hydrolysis, and methanogenesis activities are all increased when compared to a significantly higher OLR. Increased production of volatile fatty acids (VFAs) is the result of this inhibition, and this increase ultimately explains the acidification process. For the purpose of promoting a beneficial AD system, OLR calculation should be done carefully (Jiang et al., 2013).

2.4.10 C/N (Carbon/Nitrogen) Ratio:

The C/N ratio is crucial for biomethane generation as it provides essential nutrients for microbial growth. A low C/N ratio inhibits bacterial growth, while a higher C/N ratio indicates soluble acid intermediate production. Maintaining the C/N ratio below inhibitory threshold values can reduce total ammonia nitrogen levels. Improper C/N ratios can result in byproducts, increased total ammonia nitrogen liberation, and VFA accumulation. Excessive C/N ratios can lead to nitrogen depletion and reduced biogas production. High C/N ratios can also cause increased pH levels, decreased microbial activity, and increased carbon concentration. To achieve the ideal C/N ratio, stable substrate compositions should be mixed with various substrates. C/N ratio recommended range is 16 to 27, while C/N/P/S ratio recommended range is 500 to 1000:15 to 20:5 to 3 (Mao et al., 2015).

2.4.11 Retention Time:

Calculating the hydraulic retention time (HRT) involves dividing the capacity of the biological reactor by the influent flow rate. The solid retention time represents the mean duration that the bacteria and solids spend inside the digester. The duration of solid retention under mesophilic

conditions ranges from 15 to 30 days. The OLR and substrate composition are necessary to calculate an operational HRT. Even though a longer than ideal HRT is not an efficient use of digester resources, it does result in the creation of VFAs when taken down. Therefore, in order to achieve maximum and continuous methane production, a longer HRT and a lower OLR are the best strategies (Mart\'\in-Pascual et al., 2014).

2.4.12 Volatile Fatty Acids:

Acetic, butyric, propionic, and valeric acids are the primary components of VFAs. The main organic waste intermediate products during AD are these. Generally, acetogenic and methanogenic bacteria are responsible for converting the VFAs produced during AD into CH4 and "carbon dioxide." Yet, with a higher rate of organic loading, VFA buildup may happen. This causes a drop in the pH range and could even cause the AD to fail. Particularly important in the production of biogas are the acids propionic and acetic among those listed above. An acetic acid concentration more than 0.8 g/L has been shown to cause AD failure, according to earlier study. A prior study indicated that if the "propionic to acetic acid" ratio is more than 1.4, AD will not function. This means that the "propionic to acetic acid" ratio may be used as a sign of an AD disturbance. A variety of traditional techniques, including gas chromatography with relatively simple pretreatment, ion exchange, and HPLC, are used to determine VFAs. VFAs are a crucial factor that also affects AD and determines pH. While methanogens require a pH range of 6.6-7.6, acidogenesis requires a pH value of 5.5-6.5. Propionic and acetic acids, respectively, are present at higher pH values or close to neutrality, whereas butyric and acetic acids are the primary VFAs at low pH levels. pH regulation allows for the maintenance of both the type and quantity of bacteria that produce acid (Mkhize et al., 2014).

2.4.13 Ratio: Substrate to Inoculum:

The substrate to inoculum ratio (S/I), which plays a critical role in anaerobic digestion, is dependent upon the properties of the feedstock. When food waste is digested, soluble acid intermediates accumulate because food waste has a high biodegradability. By contrast, because of their buffering properties, other substrates can lessen the build-up of volatile acids. Using municipal waste or the inoculum already present in a setup are two ways to handle this, both

of that can shorten the plant's lag phase. A greater S/I ratio is necessary for thermophilic conditions required for anaerobic digestion, but a desired S/I ratio around 2 and 3 for VS is often advised for mesophilic bacteria (Khadka et al., 2022).

2.4.14 Stirring:

Considering that, the bacteria in the reactor have limited access to food sources. For them to acquire their food supply, the digestate in the reactor needs to be combined properly. The digestion process has been found to be improved with some mixing. But research has shown that forceful shaking may actually slow down the AD (Lindmark et al., 2014).

2.4.15 Ammonia:

The formation of ammonia occurs during the breakdown of proteins and additional organic substrates that are rich in nitrogen. The two primary forms are free ammonia and ammonium ions. Although it is useful as a nutrient that microorganisms need to develop, large amounts of it may be fatal. In the stabilizing ratio of C/N, ammonia plays a crucial role that may have an impact on AD performance. Decrease in methane yield and the efficacy of AD are the results of decreased ammonia levels when the C/N ratio is greater than thirty. Ammonia is essential for increasing the buffering capacity of AD because it neutralizes the effect of VFAs produced during AD, according to earlier study (Karthikeyan et al., 2012).

In contrast to greater concentrations, which impede bacterial development, lower ammonia levels are necessary for bacterial growth. The methane output decreases by 50% when the ammonia level exceeds 1.7–1.4g/L. The presence of free ammonia (NH_3) is accurately established to be the source of the inhibition of digestion, which occurs when the concentration of NH_3 is between "1.7-1.8 g/L". Variations in feedstock composition, environmental conditions, inoculums, and periods of acclimation all influence the wide range of ammonia concentrations that can lead to failure. Ammonia emissions from wastewater are a consequence of both the suppression of AD and the greater ammonia content (Mlinar et al., 2022).

2.4.16 Micro- and Macronutrients:

Numerous macro- and micronutrients are required for the survival and effective growth of microorganisms. Nitrogen, sulfur, carbon, and phosphorus are macronutrients. A ratio of "C:N:P:S" or "600:15:5:1" indicates sufficient nutrients (Mao et al., 2015). Important micronutrients that should be supplied to increase the pace at which microorganisms proliferate include molybdenum, iron, cobalt, tungsten, nickel, and selenium. The micronutrients are required for biogas production when energy crops are utilized exclusively. As co-factor F430 synthesis, which is involved in the creation of methane, depends on nickel, all methanogens are dependent on it. A cobalt-containing corrinide factor III was synthesized by the cell. Trace elements are necessary for only a small number of methanogenic bacterial growths. For these "micronutrients," the concentration range is smaller and falls between 0.05 and 0.06 mg/L. Of the micronutrients needed at high concentrations (between 1 and 10 mg/L), only iron is needed. Higher loadings and a steady process for ammonia fermentation require the inclusion of micronutrients (Menon et al., 2017).

2.5 Biogas Up-Gradation:

After being processed to remove carbon dioxide and certain other gases, biogas—which is frequently used for cooking, heating, and power generation—can be used as an automobile fuel. noted that CO_2 is removed from biogas on a wide scale by chemical or physical processes such as membrane filtering, pressure swinging, water cleansers, chemical scrubbers, and adsorption. The use of pricey chemicals and filters, the requirement for a significant amount of water during scrubbing, and the roughly 1-8% methane loss during the process are the drawbacks of these methods. These limitations have been addressed by ex-situ (in an externally linked reactor) or in-situ (within a methanogenic digester) biological upgrading. Utilizing CO_2 as CH_4 instead of eliminating it, reducing atmospheric CO_2, and boosting the synthesis of CH_4 during anaerobic digestion are the two most evident advantages of bio-upgradation. Ex-situ biogas up-grading involves releasing H_2 into the environment and transferring biogas from an anaerobic digester that contains hydrogenotrophic microorganisms to another anaerobic digester where it is converted to CH_4 (Thiruselvi et al., 2021). One drawback of the ex-situ upgrading approach is that it necessitates the use of an additional reactor for biogas upgrading,

increasing the process's running costs. In-situ biogas upgradation entails feeding additional H_2 and organic substrate to the same digester that generates biogas, where hydrogenotrophic methanogens convert CO_2 and H_2 to CH_4. In contrast, part of the CO_2 is converted to CH_4 simultaneously in the exact same reactor by H_2 supply before being gradually upgraded ex-situ in the hybrid biogas upgradation process, which combines in-situ and ex-situ techniques. In-situ biogas upgrading is probably the most efficient of all of these methods since it permits the utilization of hydrogenotrophic methanogens and reduces the requirement for infrastructure modifications for post-gas treatment. Furthermore, this gas-to-power technology is reasonably priced (Mishra et al., 2021).

2.6 Technologies for Upgrading Biogas:

2.6.1 Technologies Involving Chemical and Physical Methods:

Biogas upgradation converts CO_2 into methane using physical and chemical processes. Five commercial technologies, including advanced hydrogenation and cryogenic procedures, can achieve methane recovery rates above 96%, requiring higher temperatures and pressures (Muñoz et al., 2015).

2.6.1.1 Water Scrubbing Technology, A Physical Absorption Technique:

The physical absorption approach is a popular method for upgrading and cleaning biogas by separating hydrogen sulfide and carbon dioxide from the biogas. This process involves compressing and pumping the biogas into a column, improving mass transfer. A flush column circulates the water phase, and dissolved CH_4 is recovered. Regenerative absorption and single pass scrubbing are commercial methods for reusing water, with regeneration involving air stripping and inert gas or steam for high H_2S concentrations (Cozma et al., 2015).

2.6.1.2 Use of Organic Solvents in the Physical Absorption Method:

Hydrogen sulfide (H_2S) and carbon dioxide (CO_2) are absorbed from biogas using organic solvents like methanol and polyethylene glycol dimethyl ether. Genosorb® and Selexol® are popular products for this process. Organic solvents like Selexol® have higher solubility for

CO_2, but regeneration is challenging due to CO_2's high solubility (Kapoor et al., 2019). To reduce energy usage, H2S is removed before exposure. Raw biogas is compressed, cooled, and heated to 80°C for regeneration. The process can reach up to 98% methane content (Al Mamun & Torii, 2015).

2.6.1.3 Amine Solution-Based Chemical Absorption Technique:

Chemical scrubbers extract CO_2 molecules from biogas using aqueous amine solutions like mono-, di-, or tri-ethanolamine. They also absorb H_2S. The process involves an exothermic process, resulting in a high CO_2 and H_2S amine solution. The stripping column uses heat to dissolve bonds, and trapped CO_2 is released. Alkaline salts like calcium, sodium, and potassium sodium hydroxides react with CO_2, but aqueous alkaline salts are preferred due to their affordability (KHEMKA, 2023).

2.6.1.4 PSA: Pressure Swing Adsorption:

PSA is a technology that splits gases in biogas using high surface area materials, activated carbon, carbon molecular sieves, and zeolites. The process involves pressurization, adsorption, blow-down, and purging. Methane passes through an adsorption tank, selectively holding N_2, CO_2, H_2O, O_2, and H_2S. The process requires several adsorption columns and H_2S extraction. The technology is small, low energy, and safe, with a maximum methane loss of 4% (Augelletti et al., 2017).

2.6.1.5 Membrane Separation:

Membrane technology is a method for upgrading biogas by separating components based on their penetration rates. This technique uses polymeric materials like cellulose acetate and polyimide in wet and dry separation processes. Permeation rates affect mobility-selectivity and are influenced by gas sorption coefficients and membrane materials. The type and material of the membrane affect CO_2 separation effectiveness. Gas/gas membrane cascades come in single stage, two stage, and three stage configurations. Improved biogas typically has a CH_4 content of 95% or higher. Micro-porous membranes distinguish dry and wet processes, combining membrane technology and absorption methods. Wet membrane technology allows gas

molecules to permeate and consume liquid media, producing pure CO_2 for industrial applications. However, it has disadvantages like expensive membranes and brittleness.

2.6.1.6 Process of Cryogenic Separation:

Liquefied methane (CH_4) is separated from biogas using this process that reduces temperature to around 110°C. This process recovers nearly 97% of biomethane and eliminates contaminants like water, siloxanes, H_2S, carbon dioxides, and halogens. Despite its promising results, the cryogenic separation technique faces challenges like methane losses, high operating costs, and operational issues (Baena-Moreno et al., 2019).

2.6.1.7 Process of Chemical Hydrogenation:

The Sabatier reaction and chemical hydrogenation processes, involving ruthenium and nickel catalysts, can reduce CO_2 using H_2. However, these methods have limitations, including the need for new catalysts, the need for pure gases, and high energy costs (Adnan et al., 2019).

2.6.2 Biological Technologies:

2.6.2.1 Techniques to Biologically Upgrade Biogas:

Chemically autotrophic and photosynthetic biological biogas upgrading processes are being validated for full-scale implementation, transforming CO_2 into energy-containing commodities under benign conditions, significantly contributing to a sustainable, bio-based circular economy (Angelidaki et al., 2018).

2.6.2.2 Chemoautotrophic Methods:

Hydrogenotrophic methanogens use H_2 to convert CO_2 to CH_4 as part of the chemoautotrophic biogas upgrading processes. The focus here is on using renewable electricity to electrolyze water in order to produce hydrogen gas (H_2), so making this process renewable. The power-to-gas (P2G) method enables the storage of excess energy from solar panels or wind turbines as H_2. Although water electrolysis offers a greener alternative for H_2 generation, batteries have constraints pertaining to capacity and materials. Given that P2G technology integrates renewable energy with a biogas technology, it is appealing to convert H_2 to CH_4, in spite of

H_2's low volumetric energy density. The electricity can be transformed through this process into a higher energy chemical energy carrier (CH_4) that can be stored in the natural gas infrastructure that is already in place. Lowering the original investment costs, the integration also takes place inside the biogas plant facilities. The conversion of CO_2 to CH_4 during chemoautotrophic processes is significant because it raises the overall energy value rather than separating it. In line with strategies to separate the production of biogas from the availability of biomass, this technology promotes the sustainability of the biogas output. Hybrid designs are still being developed, but in-situ and ex-situ procedures have been shown to work in experiments. In-situ configurations are available for hydrogen-assisted biogas upgrading (Lóránt & Tardy, 2022).

2.6.2.3 In situ Biological Biogas Upgradation:

the in-situ biogas upgrading process, in which methanogenic archaea in a biogas reactor utilizes hydrogen (H_2) to combine with endogenous carbon dioxide (CO_2) and produce methane (CH_4). If operating parameters are closely watched, the procedure can recover methane up to 99% of the time. The elimination of bicarbonate causes the pH to rise over 8.5, which hinders methanogenesis. This presents a problem. There are several solutions to this problem, including co-digestion involving acidic waste or pH control techniques. The oxidation of alcohols and VFAs is another problem (Sarker et al., 2018). This can only occur at low H_2 concentrations; at higher H_2 levels (> 10 Pa), anaerobic digestion is inhibited, which results in the accumulation of electron sinks. Unexpectedly high H_2 concentrations could affect the balance of the system by causing problems with VFAs breakdown. For efficient in-situ biogas upgradation, it is essential to solubilize H_2 in the liquid phase. Other critical factors include reactor design, gas recirculation, and injection module selection. Several studies have looked into various techniques to get the resulting biogas output to have a methane content between 89% and 96%. These techniques include the use of porous devices including ceramic sponges and hollow fiber membranes (Wahid et al., 2019).

2.6.2.4 Ex situ Biological Biogas Upgradation:

According to the ex-situ biogas upgrade idea, H_2 and CO_2 are supplied from outside sources and converted to CH_4 in an anaerobic reactor containing a hydrogenotrophic culture. The simplicity, stability, independence from biomass, compatibility with external CO_2 sources, and capacity to provide electricity to remote places are benefits of this approach over the in-situ process (Kougias et al., 2017). Methane percentage can range from 79% to 98%, and the process is capable of handling large gas volumes with effective upgrading. The low mass transfer rate of gas to liquid, particularly for H_2, presents a technical problem. Diffusion devices, stirring intensity, reactor layout, gas recirculation flow, and other factors all affect this rate. Temperature is a key factor in the novel concepts being investigated to improve the efficiency of biomethanation. Mesophilic cultures exhibit a bioconversion of around 60% lower than enriched thermophilic colonies (Angelidaki et al., 2018). Microorganisms require an adaptation period before working at higher temperatures, particularly 65 °C, which improves efficiency. Biogas upgrading is influenced by different types of reactors, gas recirculation, and blending techniques. High methane concentrations at the output gas are demonstrated by bubble column reactors or up flow series reactors, employing trickling bed reactor systems, which can achieve 98–99% efficiency. Ideal kinetics and gas composition are influenced by vigorous stirring rates and diffusion devices that have particular pore diameters. In summary, the results emphasize the possibilities and difficulties of upgrading ex-situ biogas for the purpose of producing methane sustainably (Thapa et al., 2023).

2.6.2.5 Biological Biogas Upgradation Systems Employing Microbial Communities:

Two processes in particular are the focus of biological upgrading of biogas in anaerobic digestion. Hydrogenotrophic methanogenic archaea use external H_2 as an electron source to convert CO_2 to CH_4 in the first phase. At pH 7, this mechanism, called hydrogenotrophic methanogenesis, is competitively advantageous. In the second phase, acetoclastic methanogenic archaea metabolizes acetate into CH_4 after homoacetogenic bacteria use the Wood-Ljungdahl pathway to convert CO_2 to acetate. Low H_2 partial pressure is essential

for both energy conservation and proton reduction under typical methanogenic conditions, as H_2 is produced by the oxidation of acetate. Syntrophic acetogens and syntrophic acetate oxidizers are inhibited by external H_2 addition, but homoacetogenic species and hydrogenotrophic methanogens are supported in microbial communities (Omar et al., 2019). Methanogens that are hydrogenotrophic are essential for the effective upgrading of biogas. Techniques like endogenous enrichment and bioaugmentation can be used to boost their abundance. Because mixed adapted cultures are more resilient and economical than pure cultures, using them is beneficial. *Methanomicrobium, Methanobacterium, Methanothermobacter* and *Methanoculleus*,are common hydrogenotrophic methanogenic species that are revealed by microbial investigation during biogas upgrading (Corbellini et al., 2021).

2.6.2.6 Photoautotrophic Methods:

Photosynthetic biogas upgrading is a method for storing CO_2 to produce methane-rich gas using phototrophic organisms like algae in photo bioreactors. Open systems are more resource-efficient but have higher costs and energy demands. Microalgae absorb CO_2 efficiently, generating biomass and increasing CH_4 content. This process can recover up to 97% of methane, potentially benefiting a circular economy. High photosynthetic efficiency can be achieved using cyanobacteria and microalgae. Efficiency depends on operating parameters like dissolved oxygen concentrations, temperature, wavelength, light intensity, and time for gas retention (Bose et al., 2019).

2.6.2.7 Upgradation of Biogas with Additional Fermentation Methods:

The studies explore the potential of biogas upgrading by producing liquid products like medium chain fatty acids, ethanol, butyrate, and acetate from carbon dioxide (CO_2) and hydrogen (H_2). Mixed culture fermentation is suggested for its nutrient-rich benefits and lack of sanitation. Medium chain fatty acid production, particularly caproate and caprylate, is suggested. However, challenges include limited specificity and energy intensive separation. The study also explores the viability of bio succinic acid synthesis, despite the need for inexpensive H_2 sources and contaminants (Omar et al., 2019).

2.6.3 Employing Microbial Electrochemical Techniques to Upgrade Biogas:

Microbial electrochemical systems (MECs) are used to remove carbon dioxide (CO_2) from biogas and create methane (CH_4). Bacteria release electrons into the atmosphere, which react with protons in the cathode electrode to produce hydrogen. This hydrogen can be used for biogas upgrading, with up to 80% of the process's total energy efficiency documented. MECs can produce methane by decreasing CO_2 in the cathode, influenced by the cathode's set voltage. In-situ biogas upgrading, using MEC in methanogenic reactors, is more effective than ex-situ upgraded techniques (Aryal et al., 2022). The ion exchange membrane (AEM) and proton exchange membrane (PEM) equipped MECs for CO_2 removal have been compared, with PEM-MEC having a higher rate of methane synthesis and better COD removal efficacy but also consuming more energy per unit of CO_2 removed. MECs can produce acetate and formic acid, two valuable liquid compounds from CO_2. The microbial electrochemical method is an environmentally sustainable way to combine multiple benefits, including CO_2 consumption, COD removal in the anode, and the generation of high-value gas and liquid products (Tartakovsky et al., 2021).

Using an external electric current, MEC-AD is a novel approach that primarily uses microbe-electrode interactions to transform organic molecules into hydrogen or methane. Potentially used for in-situ biogas upgrading, this method is especially intriguing. An external power source, preferably from limited or restricted renewable energy, powers two electrodes that are directly installed into the AD reactor in a hybrid MEC-AD system. Both DET and DIET methods can be used to transmit the free electrons from the breakdown of organic materials to the cathode for the reduction of CO_2 (X.-Z. Fu et al., 2020).

Essential elements of the MEC upgradation process are columbic efficiency, current density, and cathode material. By improving digestion capacity, increasing VFAs production, and encouraging further VFAs conversion to methane, the integrated MEC-AD system may enhance bio electrochemical performance. Due to decreased external voltage and production of oxygen, internal resistance and power consumption may be reduced, making it potentially more efficient. Appropriate electrode design and strong MEC-AD system construction can be

aided by an understanding of the fundamentals of high electrical potential (Zakaria et al., 2020).

2.7 Microbial Electrolysis Cells (MECs):

The first proposal for the idea of a MEC (microbial electrolysis cell) was made in 2005. Two different research groups that operated roughly simultaneously on separate projects claim that hydrogen gas is produced as the final product in an electrolysis-type methodology. "Bio electrochemically aided microbial reactor (BEAMR)" was the initial name of this method. Later, it was referred to as "bio-catalyzed electrolysis" (Rousseau et al., 2020). It was subsequently categorized as "microbial electrolysis" or "electrohydrogenesis," nevertheless. The process known as "electro-methanogenesis" was eventually coined to describe the capacity of an electrolysis cell to change CO_2 into CH_4. Concerns like the depletion of fossil fuels and environmental hazards seem to have an answer in the MEC. It is capable of reducing CO2 levels, cleaning up waste and contaminants, and producing clean, sustainable electro-fuels through bio electrochemical synthesis (Gautam et al., 2023). With additional research, MEC has moved from a concept to a technology, albeit its actual application is still being studied. The utility of MEC is limited by the numerous unsolved technological issues.

The application of various "microbial electrochemical technologies" has been the subject of an increasing number of studies during the past few decades. Specifically, research is being done to improve the recovery of bio-methane from wastes with exceptional strength using microbial electrolysis cells (MECs) and AD, also referred to as the MEC-AD system. These studies demonstrate that MEC-AD systems have the potential to overcome the encounters with conservative digesters outlined above. But anaerobic technologies are mostly recommended for feedstocks with higher strengths. Exoelectrogens and methanogens are enhanced by MEC-AD, which can accelerate substrate breakdown and alter the AD microbial community to produce more biogas. Stable microbial communities were generated, which improved MEC-AD for methane generation. Real-time monitoring of the MEC-AD process is possible by the detection of electric signals that exhibit a linear correlation with substrate concentrations (Ghangrekar et al., 2023).

Several specific electroactive bacterial species oxidize the simpler organic acid(s) produced by the hydrolysis of composite biopolymers and fermentation in the MEC-AD system. Methane is produced in four ways. Several electrotrophic methanogens directly use CO_2, protons, and electrons to create CH_4, a process known as electromethanogenesis; protons (H+) are reduced into H_2 gas through a cathodic electrochemical reaction; formerly, hydrogenotrophic methanogens utilized the H_2; Conventional acetoclastic methanogenesis and "syntrophic fermentative" bacterial species produce H_2 gas that was previously utilized by (hydrogenotrophic) methanogens. An established cathode voltage is necessary for the contribution of these two cathode-related activities. For instance, "methanogenesis from electrochemically generated hydrogen could be aided by the electro-methanogenesis via direct electron transport". Anodic electroactive bacterial species can outcompete acetoclastic methanogens due to their slower growth kinetics, which may aid in methanogenesis. In particular, increased H_2 generation may benefit hydrogenotrophic methanogens, which are less susceptible to decreased temperatures and ammonia inhibition. Consequently, MEC-AD systems may present favorable conditions for increased methanogen activity from a thermodynamic and kinetic perspective (W. Wang et al., 2022).

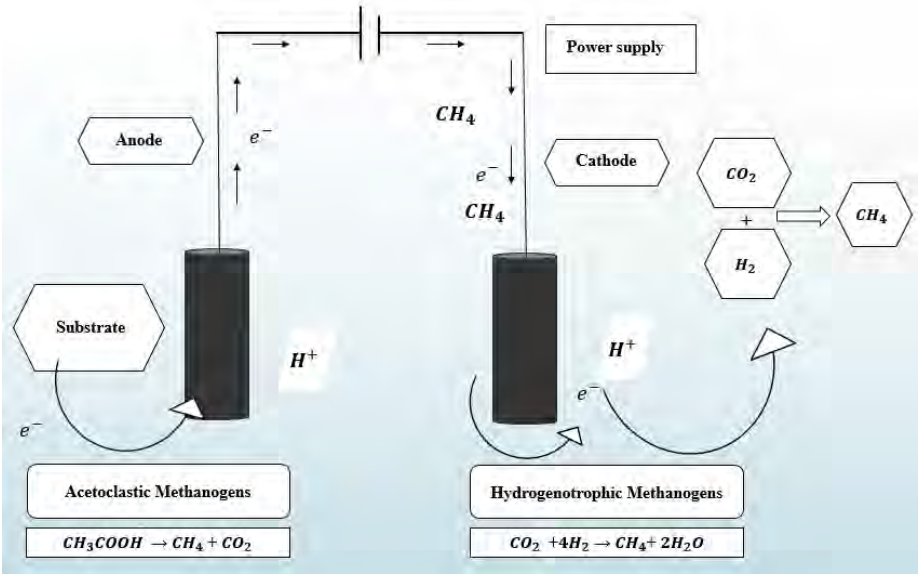

Figure 2.1: The process of anaerobic digestion at anode and cathode of MEC installed within a reactor.

2.7.1 Microbial Communities:

Electrogenic bacterial species populations and methanogens are influenced by a MEC. For substrate degradation and biogas generation in an AD system, the diversity of microbes is essential. In contrast to anaerobic digestion, the microbial community was altered by the applied voltage, and the bacterial populations within "MEC-AD" increased. Biogas generation was raised to varied degrees during the MEC-AD process, and the microbial population changed. The substrate's degradation is accelerated and enhanced by electrogenic bacteria. Faster substrate breakdown is the outcome of "MEC-AD" due to the enhanced proliferation of exoelectrogenic bacterial species, especially Geobacter species. A wide range of organic compounds, particularly aromatic hydrocarbons and VFAs, can be used as anaerobic substrates by Geobacter species, which are the most common exoelectrogens in MEC-AD (H. Wang et al., 2021). It is possible to transfer the electrons produced during the oxidation of organic substances to another species or an electrode. Similarly, MEC-AD enriches other populations associated with substrate use. The percentage of methane formed increases when conductive components are used because they hasten the substrate's breakdown. It is possible to use conductive components that enhance direct DIET within MEC-AD to hasten substrate breakdown and increase methane generation, as evidenced by the finding of DIET (direct interspecies electron transfer) in Geobacter and other species. Biogas generation is increased, and substrate breakdown is accelerated by the microbial populations that are altered by microbial electrolysis cells. During MEC-AD, the population of microbes experiences variations, and the nature of organization and composition of the microbial community within MECs fluctuate depending on the substrate (Zhu et al., 2024).

2.7.2 Improved Stability Via MECs:

Substrate hydrolysis, acidogenesis, acetogenesis, and methanogenesis, are the four phases of the polyphase mechanism known as anaerobic digestion. Therefore, cooperation within a complex microbial population is essential to the efficiency and stability of anaerobic digestion. Nonetheless, AD frequently experiences instability, especially when handling excessively concentrated organic substrates. A suboptimal C/N ratio, OLR, buffer system,

temperature, volatile fatty acid concentration, reactor type, ammonium ion occurrence, toxicity, and/or other factors can all cause unsteadiness. As a result, the AD process results in substrate degradation and inconsistent biogas synthesis. FVW in particular, are substrates with higher levels of moisture and organic content that can form VFAs quickly. As a result, the pH is lowered, disrupting fermentation, and preventing the biogas production. By integrating a "MEC with AD" and using highly concentrated food wastes as substrate, one team of researchers successfully shortened the time it took for methane production to stabilize. Furthermore, because of the rapid transfer of VFA and elimination of COD, the overall rate of methane output of the "MEC-AD" reactor was approximately 1.7 times higher than that of the AD reactor (Yu et al., 2018).

Methanosarcina thermophile and *Methanobacterium formicicum* were identified to be the crucial microbes within "MEC-AD" after a thorough analysis of the microbe community. These two species are capable of converting a variety of substrates, including carbon dioxide, methanol, formate, hydrogen, and acetate, into methane (Cai et al., 2019).

2.7.3 Optimum Voltage Accelerates Substrate Degradation and Methane Production:

A research team used waste-activated sludge as a substrate in an AD reactor, with varying suspended solids and pH. They monitored COD elimination and methane generation rate, achieving a removal efficacy of 56.5% and increasing methane generation to 147.1 ± 29.2 mL within 72 hours (Park et al., 2020).

Studies show that MEC-AD systems have greater rates of substrate breakdown compared to AD systems. When used to manage waste-activated sludge (MEC-WAS), the elimination efficacy of total COD increases from 10.7% to 31.5%. Protein removal rates also increase, and the rate of COD elimination is 100% within 72 hours of applying acetate to produce biogas. The lowest percentage of carbon dioxide in total biogas is obtained in a MEC-AD reactor with 1V, compared to 43.2% ± 2.3% in AD reactors. Applied voltage affects the fermentation process of MEC-AD for CH4 production, with an optimal external voltage of 0.8 V responsible for energy recovery from waste-activated sludge (Yu et al., 2018).

2.7.4 MECs are Used to Transform Communities of Electrogenic Bacteria and Methanogens:

The production of biogas and the breakdown of substrates in an AD system are greatly aided by the community of bacteria present (Jadhav et al., 2019). Recent studies using metagenomics have looked at secondary reactor samples that were either in mesophilic (35 ± 1 °C) or thermophilic (55 ± 1 °C) conditions. In terms of the total number of cells, or sequence reads, "*Firmicutes*" accounted for 60% of the community and was the top-ranking phylum among the 236 genome bins. Within the two types of bacteria listed below, there are two different methanogens for the synthesis of methane throughout AD: (I) "Acetoclastic methanogens," which convert acetate to carbon dioxide and methane, and (II) "Hydrogenotrophic methanogens," which produce methane by using hydrogen and carbon dioxide. *Methanobacteria* and *Methanomicrobia* dominated the archaeal community and were responsible for the generation of methane. *Geobacter* populations are the most prevalent microorganisms in MECs. Many researchers found that, at various applied voltages, *Geobacter* species are the most prevalent bacterial species in MECs. "*Geobacter sulfurreducens*" was found to be abundant within MEC (72%) and capable of produce hydrogen in an alternate experiment. By varying the voltage, the microbial community was altered, and in comparison, to anaerobic digestion, the bacterial population increased significantly in MEC-AD. Biogas yield was supported to several limitations, and changes in the microbial community occurred during the MEC-AD process. Accelerated substrate breakdown was observed along with the enrichment of "electrogenic" bacterial species. Accelerated substrate decomposition is the outcome of enhanced exoelectrogenic bacterial growth, especially in *Geobacter* species, within MEC-AD. *Geobacter* species are the most prevalent exoelectrogens in MEC-AD, and they may anaerobically use a wide range of organic compounds as substrates, including aromatic hydrocarbons and VFAs. Electrons produced during the oxidation of organic materials may be transported to an electrode or another species. Also concentrated within MEC-AD are additional populations linked to the use of particular substrates. These populations, which work with *Geobacter sulfurreducens* to degrade propionate, are responsible for the metabolism of complex organic waste types into acetate and H_2 in a MEC-AD reactor. The abundance of

these populations is significantly higher than that of the control system. Clostridium may help reduce Fe (III) by transferring electrons from organic metabolism to solid Fe (III); this method demonstrates Clostridium's capacity to act as a "metabolizing substrate as an electron donor for an anode" and, as a result, improve COD removal. The microbial community is now changed to electro-active groups by applied voltage during the "MEC-AD" process, which also enriches the bacteria involved in substrate degradation. *Methanobrevibacter, Methanosaeta,* and *Methanosarcina* are the distinctive members of the acetoclastic methanogen group, while *Methanospirillum Methanobacterium* is the characteristic group of hydrogenotrophic methanogens (Yu et al., 2018).

2.7.4 Increased Substrate Decomposition and Methane Production with the Addition of Conductive Materials:

Co-cultures of *Geobacter* species with *Methanosarcina* or *Methanosaeta* indicated "direct electron connection" of *Geobacter* with additional species, also known as "DIET (i.e. direct interspecies electron transfer)." Using magnetite, granular activated carbon, carbon felt, or other conductive materials, several researchers have attempted to promote "DIET" among fermentative methanogens and bacteria in order to increase methane generation. The rate of substrate breakdown increases when 5 or 10 mM magnetite is added. *Methanosaeta* and *Geobacter* species were found to be more abundant on the surface of biochar when the communities of bacteria were studied (Sun et al., 2023). This change was responsible for the increase in methane generation. Anaerobic digestion also uses other carbon-based conductive materials, such as carbon cloth and carbon felt, whose effects on substrate breakdown and methane output have been seen on a variety of substrates. The data demonstrate that carbon-based conductive materials can accelerate substrate breakdown and increase methane output. Such conductive components, especially carbon-based ones, are also used in MEC; examples of these include carbon cloth, graphite felt, and granular activated carbon, all of which have been shown to increase methane manufacturing (Martins et al., 2018). The findings indicate that the components that promote DIET may be employed in "MEC-AD" to speed up substrate breakdown and improve methane production. Through the alteration of microbial populations,

MEC accelerates substrate breakdown and boosts biogas output. In contrast, different substrates cause the structure and makeup of the microbe population within MECs to change, and the changes that occur within the microbe community throughout MEC-AD are subtle. Furthermore, the process of enriching these bacteria using a MEC takes a considerable amount of time, but it is faster than conservative AD (Yang et al., 2024).

2.7.5 MECs Facilitates the Breakdown of Complex Substrates and Recalcitrant Compounds:

Lignocellulosic substances like hemicellulose, lignin, and cellulose are resistant to disintegration, making them unsuitable for energy recovery. Hydrogen, with a higher energy content than gasoline, is an effective and greener energy resource (Purahong et al., 2016). Various methods for producing hydrogen include thermal cracking, chemical catalysis, electrolysis, and biological techniques. However, energy recovery effectiveness remains underwhelming. An electrohydrogenesis mechanism within a MEC-AD has been proposed to convert resistant materials into hydrogen gas at higher rates and yields. A two-step technique called "MEC-AD" has been proposed for lignocellulose breakdown, producing four times more hydrogen than traditional methods (Ndayisenga et al., 2021).

Chapter No 3

Materials and Methods

The Sustainable Bioenergy and Biorefinery Lab (Annexi) (SBBL), Department of Microbiology, Quaid-i-Azam University, Islamabad, was the site of this current research project. Standard microbiological techniques were followed throughout the entire research project.

3.1 Feedstock for Anaerobic Co-digestion:

Co-digestion of fruit and vegetable waste with cattle manure waste was the focus of the current study project. Past research conducted in our lab indicates that a statistically significant ratio of FVW, which is fruit and vegetable waste with manure from cattle, was 1:1. About thirty kilograms of a mixture of cow and buffalo dung were gathered from a dairy farm. Following that, the debris was separated from manure and stored at 4ºC until needed. 56.5 kg of FVW was brought from the Bara Kahu Murree Road market in Islamabad to the microbiological research facility. The fruit and vegetable waste (FVW) were then grinded using a grinder into fine particles. Following grinding, the uniform blend was preserved at -4ºC in anticipation of additional examination.

Cattle manure and FVW were co-digested as substrates in two stage anaerobic digesters connected to MEC. To make a homogenous combination, FVW and manure were thoroughly combined. Subsequently, the two substrates' total solids and volatile solids were calculated.

3.1.1 Volatile Solids (VS) and Total Solids (TS) Determination:

The amount of total solids and volatile solids was determined after the grinded fruit and vegetables were combined evenly. Similar to what the (Mahmoodi et al., 2018) study suggests, the TS and VS of the manure were also calculated.

In order to determine the substrate's total solids content:

- To make sure that all organic material attached to the crucibles evaporates and that they are prepared for the experiment, we first heat them to 550°C in a muffle furnace.

- Following a period of cooling to room temperature, the crucibles are weighed on a precise weighing balance, and the results are noted.
- Then, in order to record the results, we will weigh the crucibles precisely after adding our substrate to them.
- One can calculate the weight of the sample by deducting the weight of the empty crucible from the weight of the crucible containing the sample.
- To remove all of the moisture from the oven, we will then bake the crucibles for a full day (24 hours) at 105°C.
- One day after we removed them from the oven, we let them come down to room temperature.
- After that, weigh them precisely on a balance and record the results.
- The weight of the crucible containing the dry sample can be subtracted from the total weight of the empty crucible to find the dry weight.
- Every sample was taken in triplicate.
- We may calculate TS by using the following formula:

$$\text{TS (\%) of the sample} = \frac{\text{Weight of dried sample}}{\text{Weight of initial sample}} \times 100$$

The mean value of the sample's TS was utilized after it was calculated in triplicate.

How much volatile solid is in the sample?

- A muffle furnace was used to measure the volatile solid content of the dried sample by heating it to 550°C for two hours. That means that only inorganic particles remain after the sample's organic material evaporates.
- Following their removal from the muffle furnace, the crucibles cool to room temperature. The weight of them was then calculated using precision balancing.
- We calculated the weight of ash by deducting the overall weight of an empty crucible from the weight of a crucible that contained ash.
- To calculate VS, utilize the following formula:

$$\text{VS (\%) of TS} = \frac{\text{weight of dried sample} - \text{weight of ash}}{\text{Weight of dried sample}} \times 100$$

3.1.2 Development of Inoculum:

An anaerobic digester cannot be launched until the inoculum is generated. The inoculum can be obtained from a cattle manure slurry high in anaerobic microorganisms or from an anaerobic digester that is currently in operation. In the subsequent study, we generated our own inoculum based on the provided technique.

- Three to seven parts water and manure were mixed together. Until it was entirely homogeneous, the slurry was thoroughly combined.
- Afterwards, the mixture was placed inside a digester and corked shut.
- A gas collection hole, a feeding hole, and a digestate removal hole allowed pipes to pass through the cork and into the reactor.
- A gas line was connected to the gas bag for biogas collection.
- We then placed the complete system in an incubator at 37°C.
- Three days later, gas production from the inoculum starts.
- Using the previously described method, the TS and VS of the inoculum were then determined.

3.2 Configuration of the Reactors:

A microbial electrolysis cell, or electrolysis assembly, was part of the reactor, a simple anaerobic digester. A pair of 0.5-volt AA cell batteries served as the power source. There were five different types of connections: feeding, digestate removal, gas outflow (where a bag is attached to collect biogas), anode and cathode connections, and a hydrogen recirculation connection. The first setup was supplied with 3 g VS/L of organic load and acted as the control mechanism for the entire experimental setup. Experimental setup 2 was installed with MEC with two electrodes (one anode and one cathode) within the reactor. Furthermore, MEC with

four electrodes—two anodes and two cathodes—was installed within the reactor of experimental setup 3.

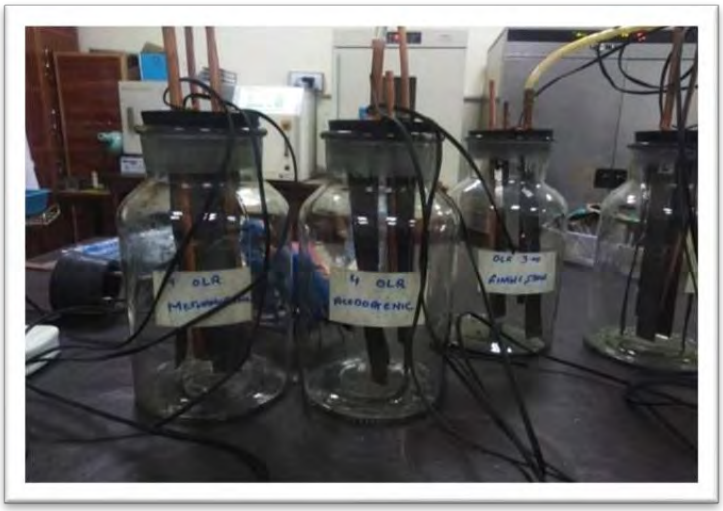

Figure 3.1: Reactor setup

3.2.1 Assembly and Construction of Microbial Electrolysis Cell:

Through an external battery source, an electrolysis assembly was linked. Attached to this component is the single step of anaerobic digestion (methanogenesis). Purifying biogas to produce only methane is MEC's objective. Graphite sheets were utilized as an anode and cathode. Two identical-sized sheets were made from them. The breadth of each electrode measured 2.54 cm, and its length was 10.16 cm, resulting in a total area of 10.16 cm × 2.54 cm for length and width. There is a 3.18-cm gap between the anode and cathode. Similar to the reactor arrangement with four electrodes, each electrode in the four-electrode configuration is 3.18 cm from the electrode in the region in front of it. This is the case with the reactor setup, coupled with two electrodes. The two electrodes that are in opposition to one another in a reactor with four electrodes are in parallel to one another, as are the other two electrodes. Graphite sheets were fastened to the exterior power source using steel clamps along with bolts that passed through cork. One end of the plug was attached to the electrolysis assembly of

plates two and four, whereas the other end was attached to the negative and positive terminals of the battery outside the digester.

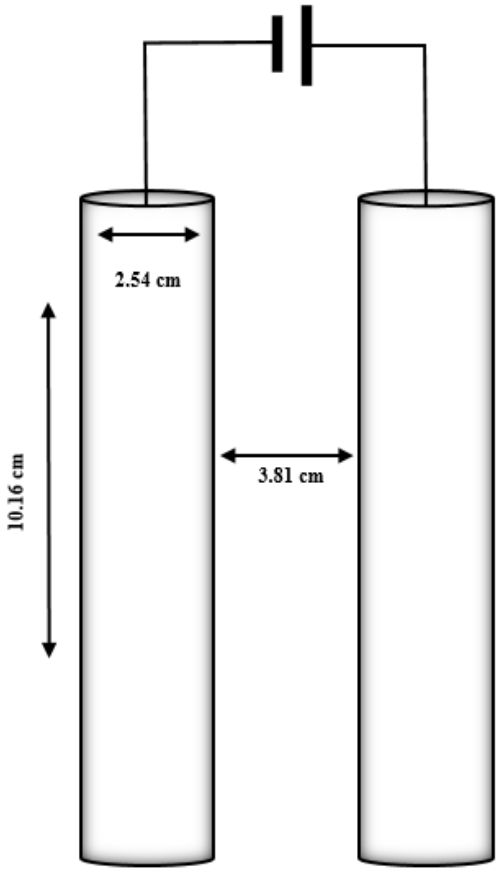

Figure 3.2: MEC assembly comprising a single set of Electrodes.

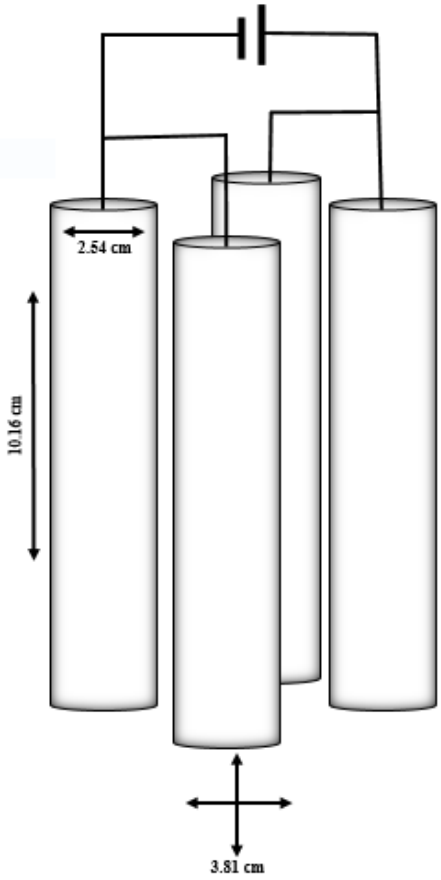

Figure 3.3: MEC assembly comprising a double set of Electrode.

3.3 Anaerobic Digestion Process:

The study examined the process of two-stage anaerobic digestion at a 1:1 ratio in the context of VS for the digestion of fruit and vegetable waste and animal manure. 37 °C was the consistent temperature that was maintained for each experimental set. With a working volume of 1500 ml, the reactors' total capacity was 2500 ml for each reactor.

3.3.2 Parameters of Operation:

3.3.2.1 Hydraulic Retention Time:

The hydraulic retention period for the reactors was 10 days. This duration surpasses the doubling time of methanogens, and it is sufficient to decompose the majority of organic compounds.

3.3.2.2 Organic Loading Rate:

In the reactors, 3 g of Vs/L of feedstock was fed daily at a flow rate of 150 ml.

3.3.2.3 Flow Rate:

A 150 milliliter per day flow rate was determined. Utilizing the provided method and a 1500 mL working volume, it was calculated for a retention time of 10 days.

Flow rate = working volume of reactor
/HRT
Working volume of reactor = 1500 ml
HRT = 10 days
So,

Flow rate = 1500 ml/10 days

Flow rate = 150 ml/day

3.3.2.4 Concentrations:

OLR = Concentration × (Flow rate / Volume of reactor)
Therefore, Conc. = OLR × (Volume of reactor / Flow rate).

Concentration at OLR 3gVS/L:

OLR = 3 g VS/L

Flow rate = 150 ml

Reactor Volume = 1500 ml

Therefore,

Conc. =3× (1500/150) ml

Conc. = 3 × 10 Concentration

= 30g VS/L

3.3.2.5 Temperature:

The incubator was maintained at 37°C in this work to maintain a steady temperature. I was aware of the severe temperature sensitivity of methanogens and other anaerobic microbes in general, which can result in large variations in findings even with a one-degree temperature difference.

3.3.2.6 pH:

As the experiment went on, the pH changed over time based on the rate of loading and operational conditions, but it was initially maintained at 7.3. Daily measurements and records were made of the pH values in the methanogenic and hydrolytic reactors.

3.3.2.7 Two Stage Anaerobic Digestion:

Both reactors have been filled with a 1.5 liter inoculum to start the anaerobic co-digestion experiment. The dissolved oxygen was then removed with nitrogen gas to create an anaerobic environment. Following that, feeding was continued for 96 hours without removing the digestate up to a 1.5-liter volume within the acidification reactor. 150 mL of the sample was removed from the same reactor after 96 hours and moved to a methanogen reactor before being moved to an acidification reactor. Without removing any digestate from the methanogenic reactor until the operating volume reached 1.5 liters, the same process was repeated for up to 8 days. The methane-generating reactor's digested 150 ml was withdrawn every day. Next, the recovered acidogenic reactor effluent was transferred to the methanogenic reactor, where it was subsequently fed. During the course of the more than twenty days that the experiment was conducted, each stage of the two-stage anaerobic co-digestion process had a hydraulic retention

period of ten days. CH_4 concentration is determined for biogas after reaching a steady state. Alkalinity, volatile solids reduction, and VFAs were examined after three days, and pH was measured daily for both reactors. At a concentration of 3 g VS L-1 day-1, organic loading was done.

3.4 Up-gradation of Biogas:

The present investigation is an objective of an ongoing project whereby past investigations' conditions were optimized as mentioned above. This study aims to optimize the conditions for biogas upgradation by employing MEC (Microbial Electrolysis Cell) coupled with two stage anaerobic digestion along with the recirculation of supplied hydrogen and biogas in order to increase the methane content.

Two distinct types of reactor setups have been developed specifically for the project. These two types of experimental setups were: R3 with a single set of electrodes (SSE) and R4 with a double set of electrodes (DSE) in methanogenic reactors of both reactor setups. A comparison analysis was also conducted using two control configurations. Control (R1) was operated under normal conditions of biogas production without recirculation of gases (biogas and hydrogen) and electrodes, while both biogas and external hydrogen recirculation were incorporated in Control (R2) with no graphite electrodes in its methanogenic reactor. Due to this, the impact of the voltage supply to electrodes (MEC) could be assessed. When voltage was supplied, the experimental configurations were designated as R5 and R6, where R5 had a single set of electrodes with voltage and R6 had a double set of electrodes with voltage. Initially, biogas was required to be brought to a steady state. Subsequently, before starting the upgrading process, the methane content of the gas was determined by passing it through a scrubbing solution (5M NaOH). The values for hydrogen that was supplied externally were then calculated for a 1200 ml biogas recirculation volume.

After achieving a steady state and after evaluating biogas production and its methane content at each phase of the biogas upgradation process, every external condition that was provided was thoroughly evaluated for more than 15 days. The biogas production was assessed for

methane content before the upgrading process began, both with and without the applied voltage, to assess how MEC affects the methane content of biogas. The experimental work was carried out in four distinct phases, which are explained below.

- During phase 1, hydrogen was introduced to the gas collecting system and recirculated at various flow rates of 32, 64, 96, and 128 ml/min for four hours. Each flow rate was carried for more than 15 days, which maximized the flow rate for gas recirculation.
- During phase 2, the gases were recirculated daily for durations of 4, 6, and 8 hours, and each duration was carried for more than 15 days at an optimal flow rate of 32 ml/min in two-stage anaerobic digestion.
- During phase 3, the optimization of interval-based time during the upgradation of biogas was carried out through biogas recirculation with external hydrogen supply during two-stage anaerobic digestion in interval-based time durations of 4, 6, and 8 hours at a flow rate of 32 ml/min. Short intervals have been employed to operate the peristaltic pump in a cyclic manner. It had an alternative on-off cycle, going through two hours of activity followed by an hour of inactivity. The peristaltic pump was cycled for the durations of 4, 6, and 8 hours in interval-based recirculation for more than 15 days for each interval-based duration to maximize biogas upgradation.
- The two experimental setups (R3 and R4) were connected to the battery source (referred to as R5 and R6) during phase 4. The methanogenic reactor electrodes in the interval-based recirculation system were subjected to a voltage of 0.7 V throughout an alternate on-off cycle lasting 4, 6, and 8 hours, fed at a flow rate of 32 ml/min. By using interval-based time optimization and applying voltage, it was completed. During each interval-based duration, it was extensively examined for over 15 days.

Below are the schematic designs (Fig. 3.6, 3.7, and 3.8) and the entire experimental setup for the biogas upgrade (Fig. 3.4, and 3.5).

Figure 3.4: Experimental setup for the biogas upgradation without MECs

Figure 3.5: Experimental setup for the biogas upgradation with MECs

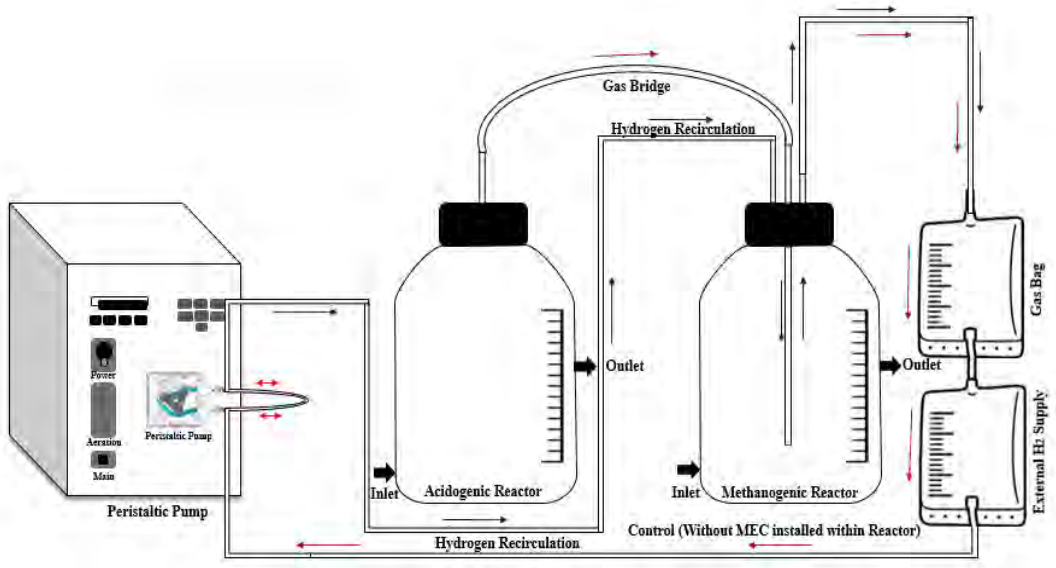

Figure 3.6: Schematic diagram for upgradation of biogas in control

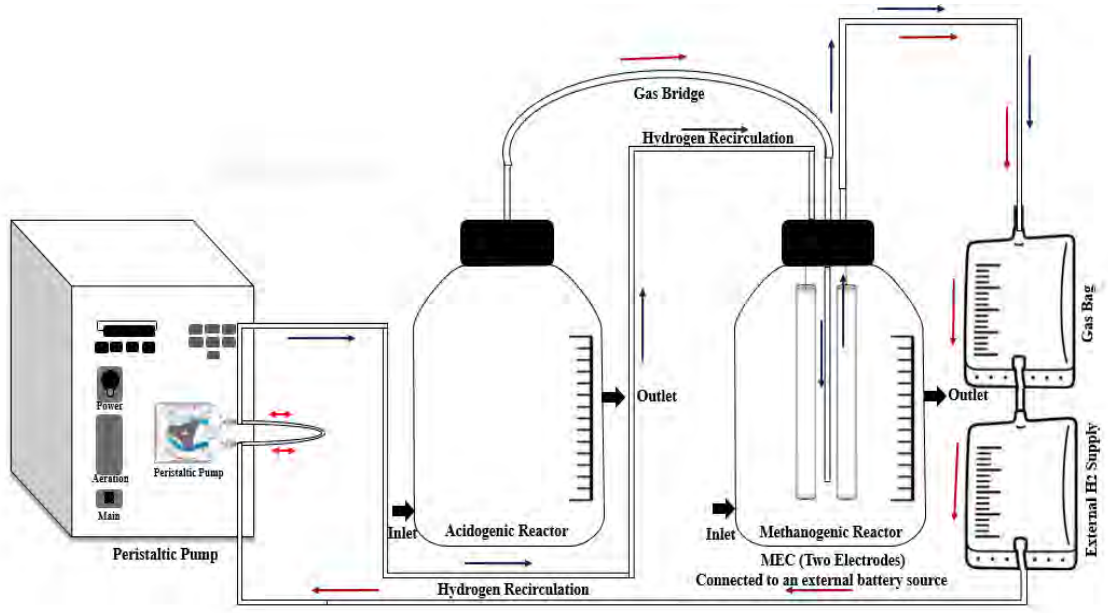

Figure 3.7: Schematic diagram for upgradation of biogas in experimental setup having single set of electrodes.

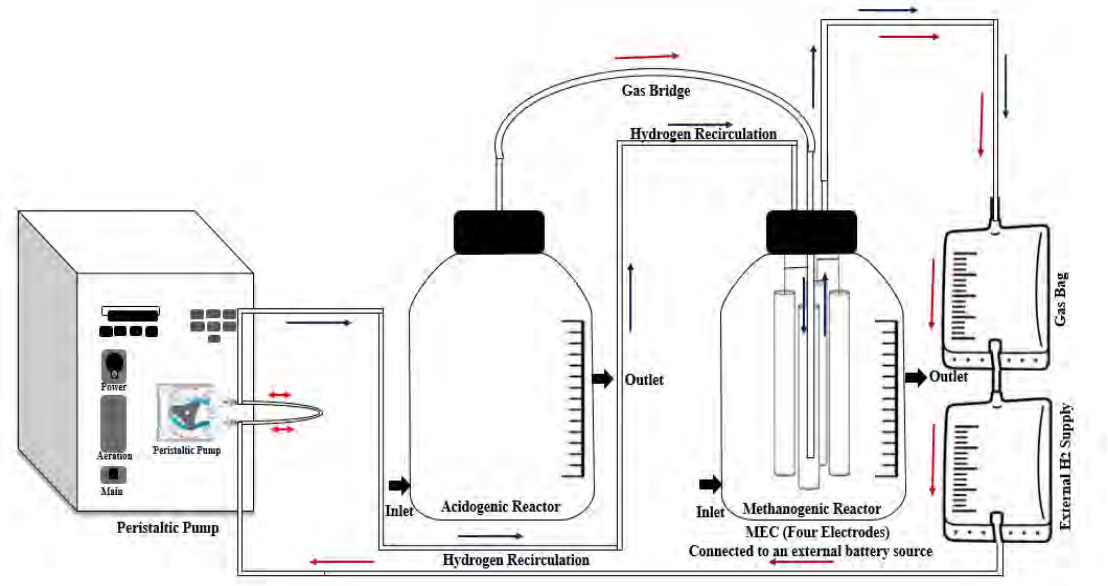

Figure 3.8: Schematic diagram for upgradation of biogas in experimental setup having double set of electrodes.

3.5 Measurement of Biogas:

After being collected in biogas bags that are fastened to the reactors, the biogas from two-staged reactors is measured with syringes, and the results are recorded. Given its portability and ability to be placed in incubators alongside reactors to maintain reactor temperature, gas bags are being used inside the incubator.

3.6 Measurement of Methane Content:

The biogas is scrubbed through a 5M NaOH solution to perform scrubbing in order to measure the amount of methane present. The NaOH solution will absorb CO_2, leaving only CH_4 in the syringes. Consequently, we can use that information to calculate the overall amount of methane the reactor has created.

3.7 Determining Alkalinity and VFAs:

Using APHA Standard Methods, 20th ed., p. 2-27, method 2320B (1998), the effluent's VFAs and alkalinity were ascertained.

Here are the steps that will occur subsequent:

- A 250ml beaker was filled with 10 milliliters of sample.
- To find the sample's initial pH, a pH meter was utilized.
- Once the pH was brought down to 4.3 by adding 0.1 normalized H_2SO_4, the amount of acid used was noted for the sake of alkalinity calculations.
- After that, more acid was added to the sample until its pH reached 3.5.
- After that, the sample spent three minutes boiling on a hot plate.
- Subsequently, the sample was permitted to reach room temperature.
- Next, 0.1 normalized NaOH was added to the sample to get its pH up to 7.
- The VFAs of the system is ascertained by the amount of NaOH.

Alkalinity and VFAs calculations by using formula:

$$\textbf{Alkalinity (mg/L)} = \frac{\text{V ml of acid consumed X Normality of the acid used X 50000}}{\text{V } ml\ of\ sample}$$

$$\textbf{VFA (mg/L)} = \frac{\text{V ml of alkali consumed X Normality of the alkali used X 50000}}{\text{V } ml\ of\ sample}$$

Chapter No.4

Results

The goal of the current study was to improve the amount of methane in the biogas by converting carbon dioxide into methane in a two-stage anaerobic digestion process coupled with MEC in interval-based time during in-situ biogas upgradation. For this purpose, the hydrogen was provided from an external source, and the gases were recirculated to increase the contact time between gases and methanogens and increase the conversion rate. The gases were recirculated at varying flow rates and times during optimization in order to achieve a high methane content. Working with R1, R2, R3, R4, R5, and R6, the goal was to ascertain how MEC affected the amount of methane during in-situ biogas upgradation. To maximize the flow rate for recirculation, the gases were circulated at 32, 64, 96, and 128 ml/min throughout the study's first phase. In order to optimize the duration for recirculation, the gases (biogas + supplied hydrogen) were continuously recirculated for 4, 6, and 8 hours at an optimized flow rate of 32 ml/min in the second phase, and during the third phase, the interval-based duration for recirculation was optimized at a flow rate of 32 ml/min for 4, 6, and 8 hours. In the study's fourth phase, biogas upgradation was performed under optimized conditions with an interval-based duration for recirculation of 4, 6, and 8 hours and an applied voltage (MEC) of 0.7V at a flow rate of 32 ml/min.

Two distinct experimental settings, which are described below, were used for the investigation.

- In experimental setup 1 (R3), a single set of graphite electrodes was mounted in the methanogenic reactor. When voltage was applied, the setup was denoted by R5.
- In Experimental Setup 2 (R4), a double set of graphite electrodes was mounted in the methanogenic reactor. When voltage was applied, the setup was denoted by R6.
- For the experiment, two controls were used: R1 without graphite electrodes with no biogas and H_2 recirculation, which was kept under normal conditions of biogas production throughout the experiment. And R2 control, in which the methanogenic reactor was not mounted with graphite electrodes, but gases (biogas and hydrogen) were recirculated.

4.1 Characteristics of Feedstock:

In the current investigation, the biogas was produced by the co-digestion of fruit and vegetable waste in a 1:1 ratio with cattle manure. Table 4.1 displays the substrate's total solids and volatile solids, which were calculated. 17.55% of the total solids in the cattle manure have a moisture content of 82.45%, and 13.13% volatile solids. There were 4.92% total solids, 4.51% volatile solids, and 95.08% moisture content in the fruit and vegetable waste.

Table 4.1: Characteristics of Feedstock

Biomass	TS (%)	VS of TS (%)	VS of Sample (%)	Moisture (%)
Fruit and vegetable Waste	4.92%	91.80%	4.51%	95.08%
Cattle manure	17.55%	74.79%	13.13%	82.45%

4.2 Biogas and its Composition:

The biogas production achieved a steady state at the start of the experiment, and the total amount of biogas as well as the carbon dioxide and methane contents were measured. The total amount of biogas obtained after achieving steady state in R1, R3, R4, R5 and R6 was approximately 1500, 1570, 1630, 1740, and 1790 ml; the total amount of methane present in the total biogas produced in R1, R3, R4, R5, and R6 was recorded to be 960, 1041, 1108, 1235, and 1288 ml, respectively. Whereas, the concentration of carbon dioxide recorded in R1, R3, R6, R5, and R6 was 540, 529, 522, 505, and 502 ml, respectively.

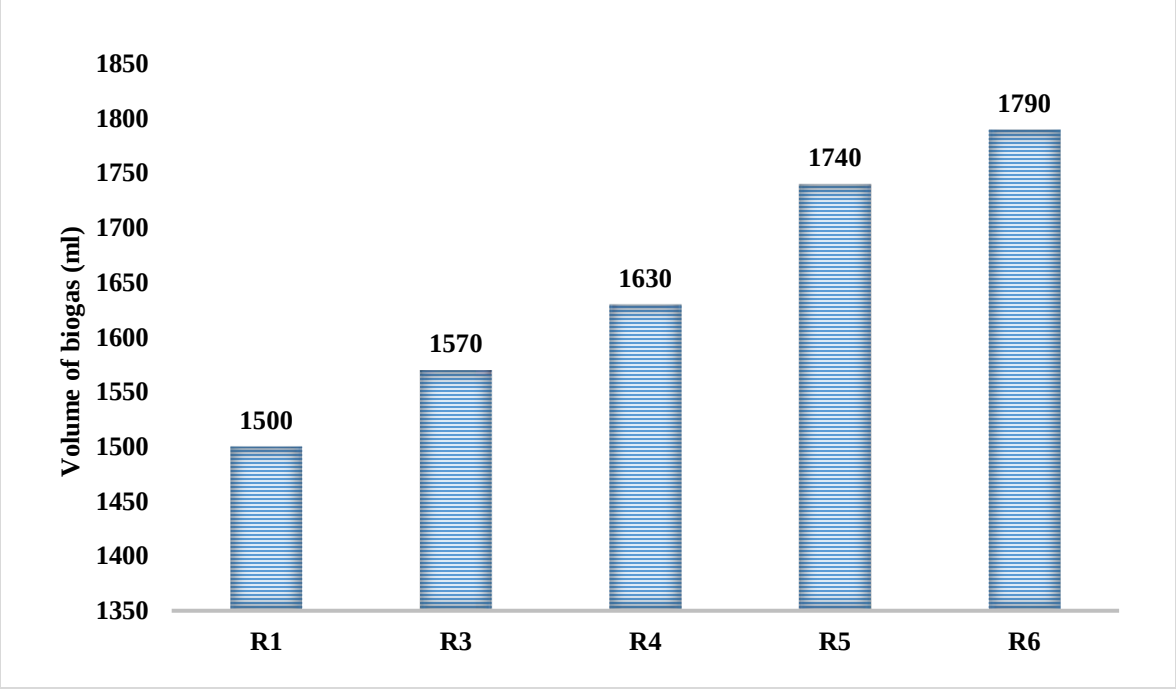

Figure 4.1: Biogas and its composition in (ml), where R1 is control with no recirculation of gases (biogas and hydrogen) and electrodes, Whereas R3 is reactor setup having a single set of electrodes, and R4 is reactor setup with a double set of electrodes in their methanogenic reactors, and R5 is reactor setup with a single set of electrodes applied with voltage, and R6 is reactor setup with a double set of electrodes applied with voltage in their electromethanogenic reactors.

4.3 Effect of MEC on Methane Content:

Prior to the biogas upgradation through external hydrogen supply and recirculation of gases, both SSE and DSE were operated first under normal conditions without recirculation and without applying voltage to electrodes, and then voltage was applied (MEC). The methane content with no upgradation and without applied voltage was recorded to be 64% in R1, 66% in R3, and 68% in R4, compared to the methane content obtained by means of 0.7V of applied voltage, which was 71% in R5 and 72% in R6, respectively.

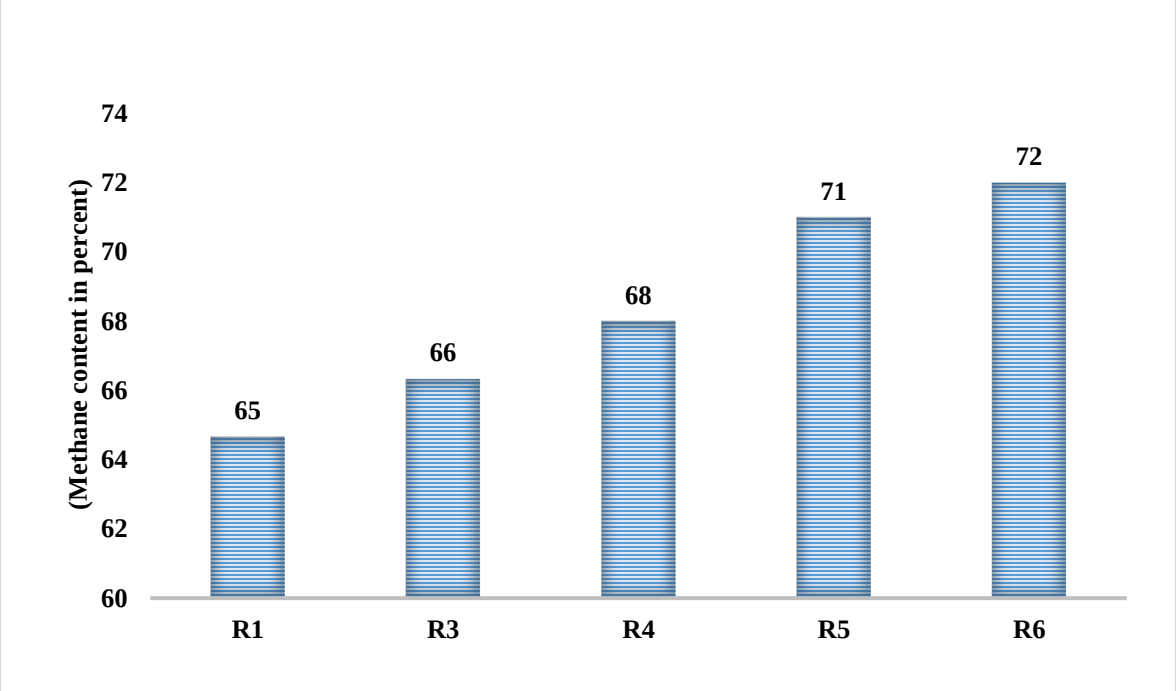

Figure 4.2: Methane Content in (%) without recirculation of gases (biogas and external hydrogen), where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, Whereas, R3 is reactor setup having a single set of electrodes, and R4 is reactor setup having a double set of electrodes in their methanogenic reactors, and R5 is reactor setup with a single set of electrodes applied with voltage, and R6 is reactor setup with a double set of electrodes applied with voltage in their electromethanogenic reactors.

4.4 Optimization of Flow Rate:

For maximizing the methane content of biogas recirculated, biogas and supplied hydrogen were recirculated in R2, R3, and R4 and the biogas upgrade was compared with R1. The maximum methane content of biogas was recorded as 81 and 84% in R3 and R4 at 32 ml/min during optimization of flow rate through four hours of recirculation. R1 had only 65% methane content throughout the experiment. The methane content of biogas recorded was 68, 70, 73, and 76% in R2; 70, 73, 77, and 81% in R3, and 73, 76, 80, and 84 in R4, at flow rates of 128, 96, 64, and 32 ml/min, respectively.

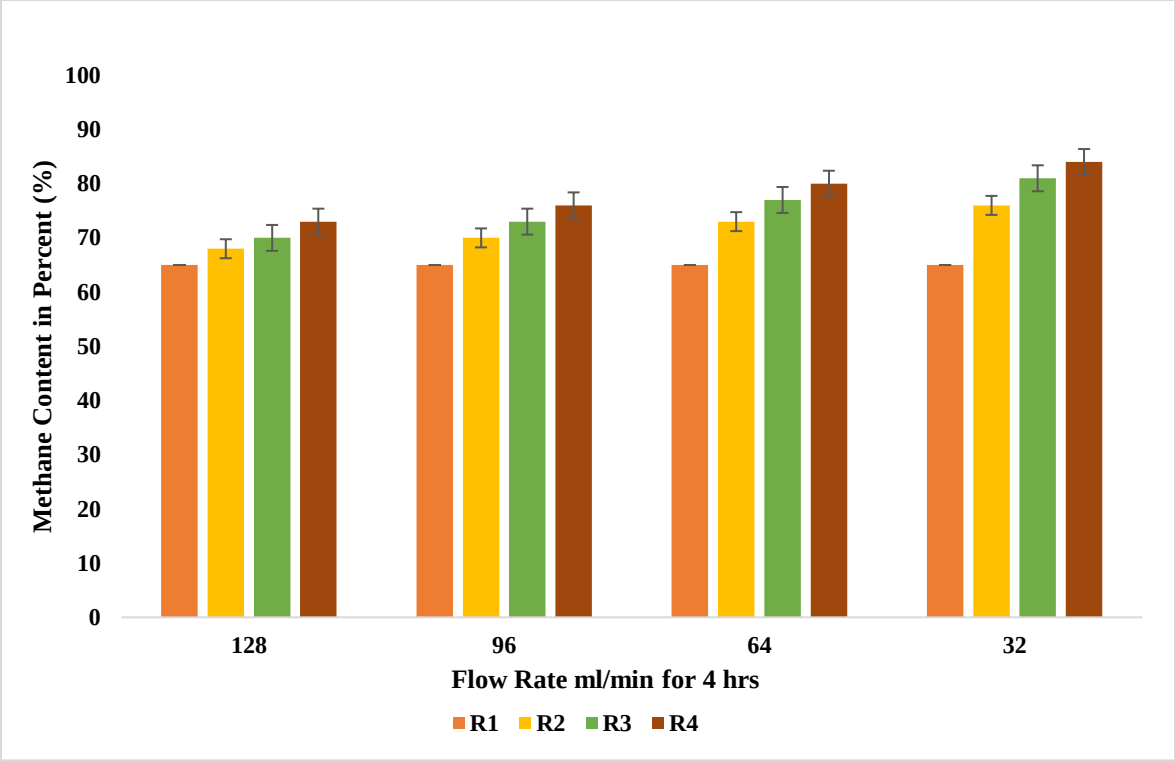

Figure 4.3: Effect of flow rates (128, 96, 64, and 32 ml/min) on Methane Content in (%) during recirculation for 4 hours daily, where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R3 is reactor setup with a single set of electrodes, and R4 is reactor setup having a double set of electrodes in their methanogenic reactors.

4.3.1 pH of Methanogenic Reactor:

One of the most important factors in determining the stability of the process is the reactor's pH change. The pH of the methanogenic reactor was assessed every three days while the flow rate in the in-situ biogas upgrading process was optimized. The table below shows the range of pH values for R3 and R4 at flow rates of 128, 96, 64, and 32 ml/min in comparison to control R1 and R2 (Table 4.2).

Table 4.2: pH during optimization of flow rate.

Flow Rate	R1 (No Recirculation, No electrodes)	R2 (Recirculation, No electrodes)	R3 (Single set of electrodes)	R4 (Double set of electrodes)
128 ml/min	6.91	7.26	7.25	7.24
96 ml/min	6.91	7.24	7.24	7.23
64 ml/min	6.91	7.24	7.22	7.21
32 ml/min	6.91	7.21	7.20	7.19

4.3.2 Alkalinity and VFAs Accumulation:

The internal parameter to be considered to assess the stability of the methanogenesis process is the alkalinity and accumulation of VFAs. As the methanogenic reactor was operating for optimization of flow rate during upgradation, the alkalinity recorded in R1, R2, R3, and R4 was 2250, 2320, 2350, and 2380 mg/L at a flow rate of 128 ml/min, 2250, 2420, and 2390 mg/L at a flow rate of 96 ml/min, 2250, 2480, 2450, and 2420 mg/L at a flow rate of 64 ml/min, and 2250, 2520, 2490, and 2460 mg/L at a flow rate of 32 ml/min. Accumulated VFAs recorded in R1, R2, R3, and R4 were 1480, 1450, 1432, and 1421 mg/L at a flow rate of 128 ml/min; 1480, 1430, 1420, and 1399 mg/L at a flow rate of 96 ml/min; 1480, 1409, 1406; and 1380

mg/L at a flow rate of 64 ml/min; and 1480, 1399, 1375, and 1365 mg/ml at a flow rate of 32 ml/min.

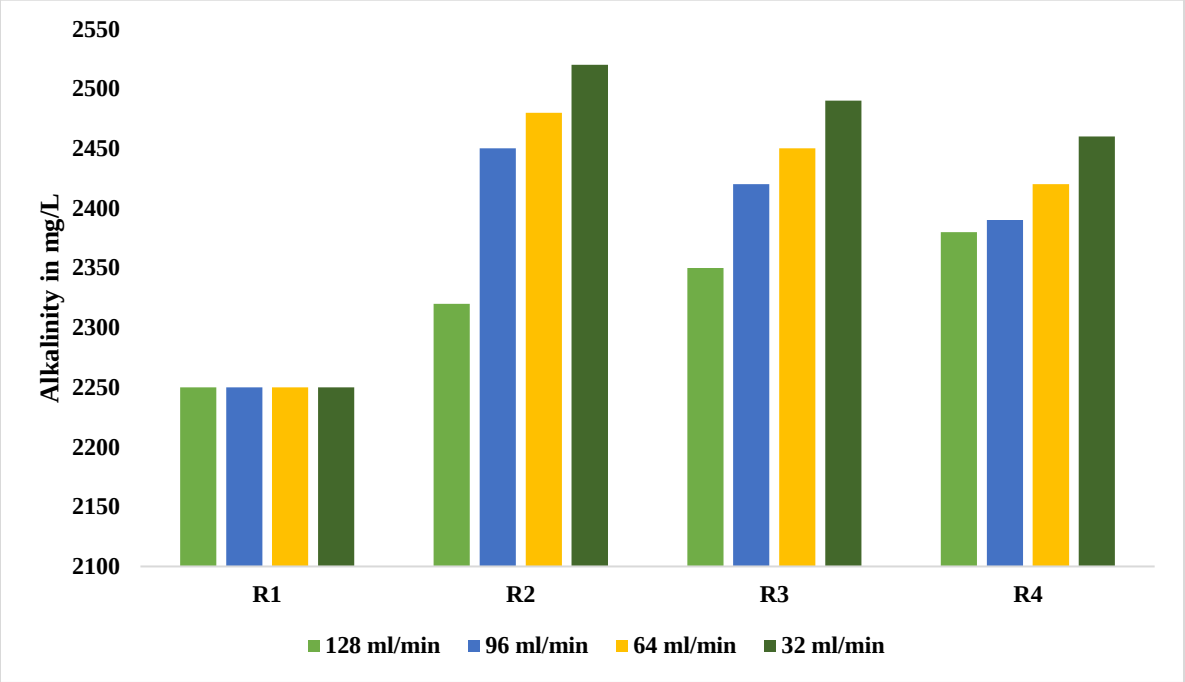

Figure 4.4: Alkalinity in methanogenic reactors of R2, R3, and R4 at flow rates of 128, 96, 64, and 32 ml/min during optimization of flow rate. Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes Whereas R3 is reactor setup with a single set of electrodes, and R4 is reactor setup with a double set of electrodes in their methanogenic reactors.

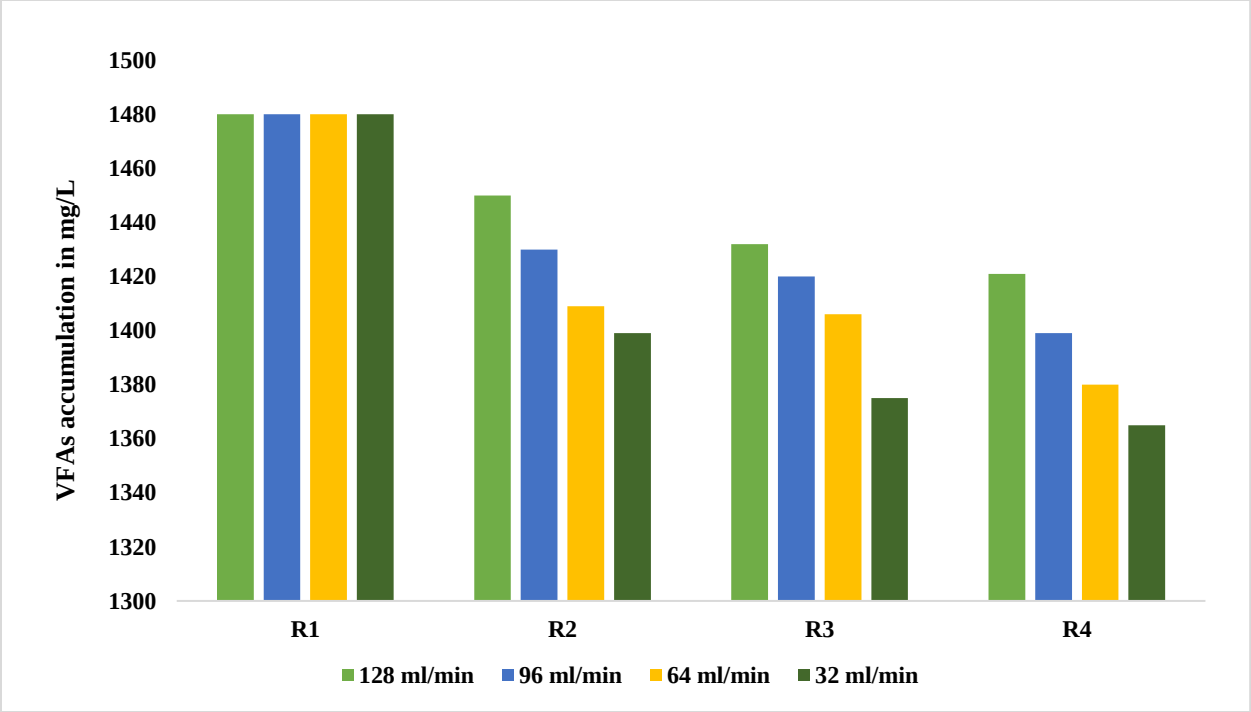

Figure 4.5: VFAs accumulation in methanogenic reactors of R2, R3, and R4 at flow rates of 128, 96, 64, and 32 ml/min during optimization of flow rate. Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R3 is reactor setup with a single set of electrodes, and R4 is reactor setup with a double set of electrodes in their methanogenic reactors.

4.3.3 VFAs to Alkalinity Ratio:

As with the previous three internal essential parameters to assess process stability, the VFAs-to-alkalinity ratio is also considered significant. The methanogenic reactor's VFAs to alkalinity ratio was measured during recirculation in R1, R2, R3, and R4 during the flow rate optimization process for in-situ biogas upgradation.

Table 4.3: VFAs to Alkalinity ratio during optimization of flow rate.

Flow Rate	R1 (No Recirculation, No electrodes)	R2 (Recirculation, No electrodes)	R3 (Single set of electrodes)	R4 (Double set of electrodes)
128 ml/min	0.6	0.6	0.6	0.5
96 ml/min	0.6	0.5	0.5	0.5
64 ml/min	0.6	0.5	0.5	0.5
32 ml/min	0.6	0.5	0.5	0.5

4.4 Time Optimization:

For optimizing the recirculation time for biogas upgradation, the recirculation was conducted for three different durations (i.e., 4, 6, and 8 hours), and the results were compared with R1 and R2, where the methane content obtained was 65% throughout the experiment in R1. During optimization of recirculation time for biogas upgradation, in R2, the methane content was 76, 80, and 84; 81, 86, and 89% in R3; and 84, 88, and 92% in R4 for 4, 6, and 8 hours of continuous recirculation at a flow rate of 32 ml/min.

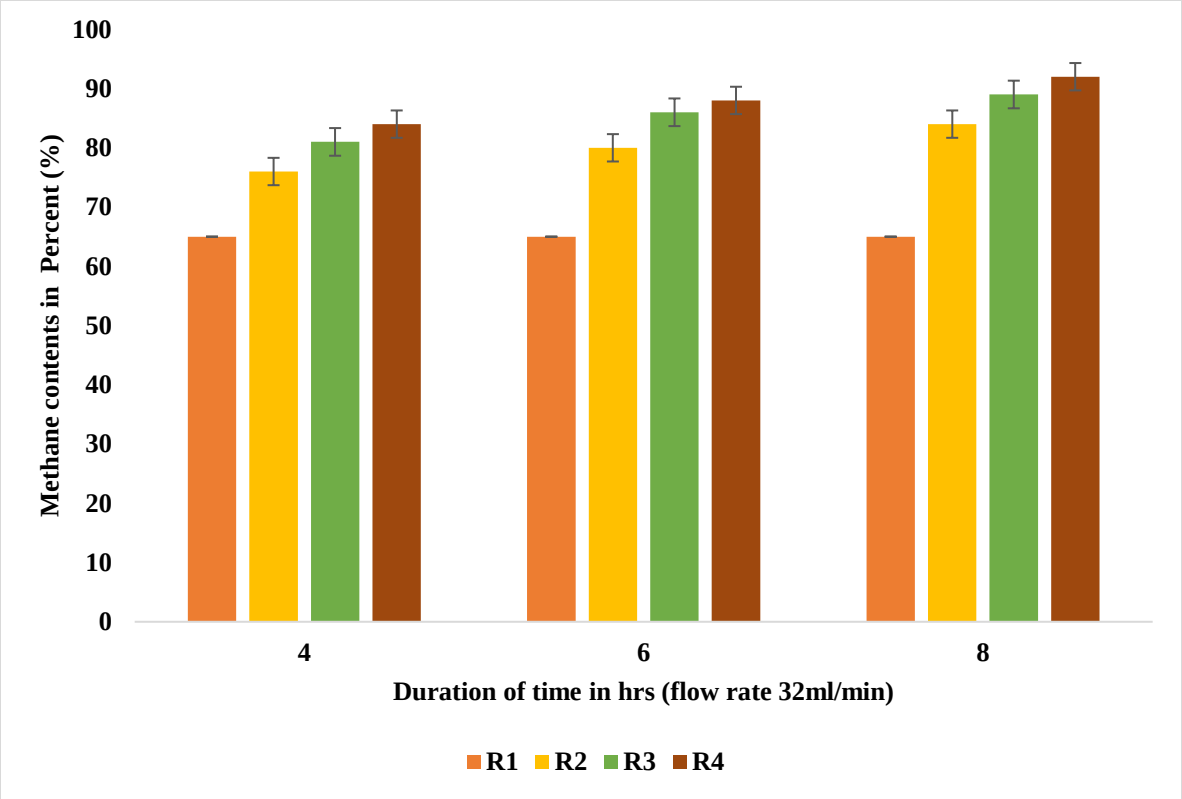

Figure 4.6: Effect of continuous durations of recirculation (4, 6, and 8 hours) on methane content (%) during time optimization. Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R3 is reactor setup with a single set of electrodes), and R4 is reactor setup with a double set of electrodes in their methanogenic reactors.

4.4.1 pH of Methanogenic Reactor:

One important parameter for assessing the stability of the process is the reactor's pH change. The pH of the methanogenic reactor was monitored during optimization of time for in-situ biogas upgradation. Following 4, 6, and 8 hours of continuous recirculation, the pH ranges of R3 and R4 relative to R2 and R1 are shown in Table 4.4.

Table 4.4: pH during time optimization

Duration of recirculation (continuous)	R1 (No Recirculation, No electrodes)	R2 (Recirculation , No electrodes)	R3 (Single set of electrodes)	R4 (Double set of electrodes)
4 h	6.91	7.29	7.25	7.24
6 h	6.91	7.30	7.26	7.25
8 h	6.91	7.31	7.27	7.26

4.4.2 Alkalinity and VFAs Accumulation:

Other internal parameters to be considered to assess the stability of the methanogenesis process are the alkalinity and accumulation of VFAs. As the methanogenic reactor was operating for optimizing time during upgradation, the alkalinity recorded in R1, R2, R3, and R4 was 2250, 2390, 2360, and 2330 mg/L at 4 hours of continuous recirculation, 2250, 2450, and 2420, and 2390 mg/L at 6 hours of continuous recirculation, and 2250, 2490, 2460, and 2430 mg/L at 8 hours of continuous recirculation. The accumulated VFAs recorded in R1, R2, R3, and R4 was 1480, 1450, 1438, and 1426 mg/L at 4 hours of continuous recirculation; 1480, 1440, 1404, and 1395 mg/L at 6 hours of continuous recirculation; and 1480, 1424, 1377, and 1374 mg/L at 8 hours of continuous recirculation, respectively.

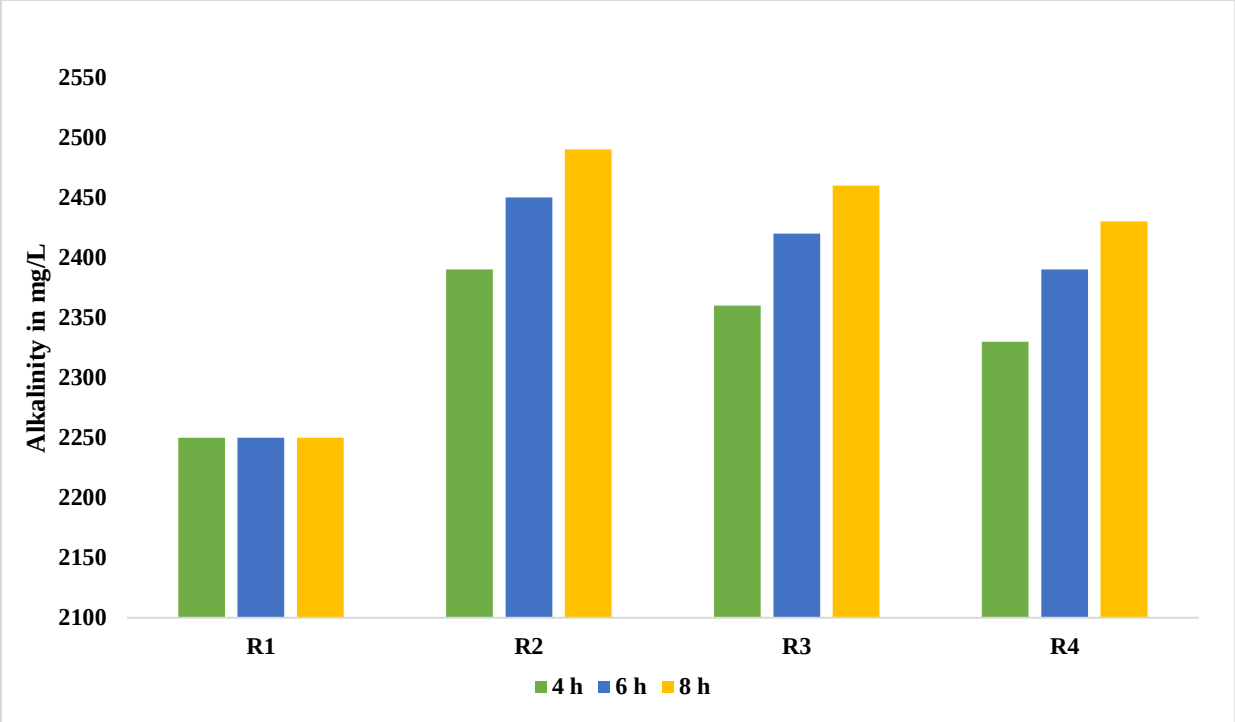

Figure 4.7: Alkalinity in methanogenic reactors of R2, R3, and R4 due to continuous recirculation for 4, 6, and 8 hours during time optimization Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R3 is reactor setup with a single set of electrodes, and R4 is reactor setup with a double set of electrodes in their methanogenic reactors.

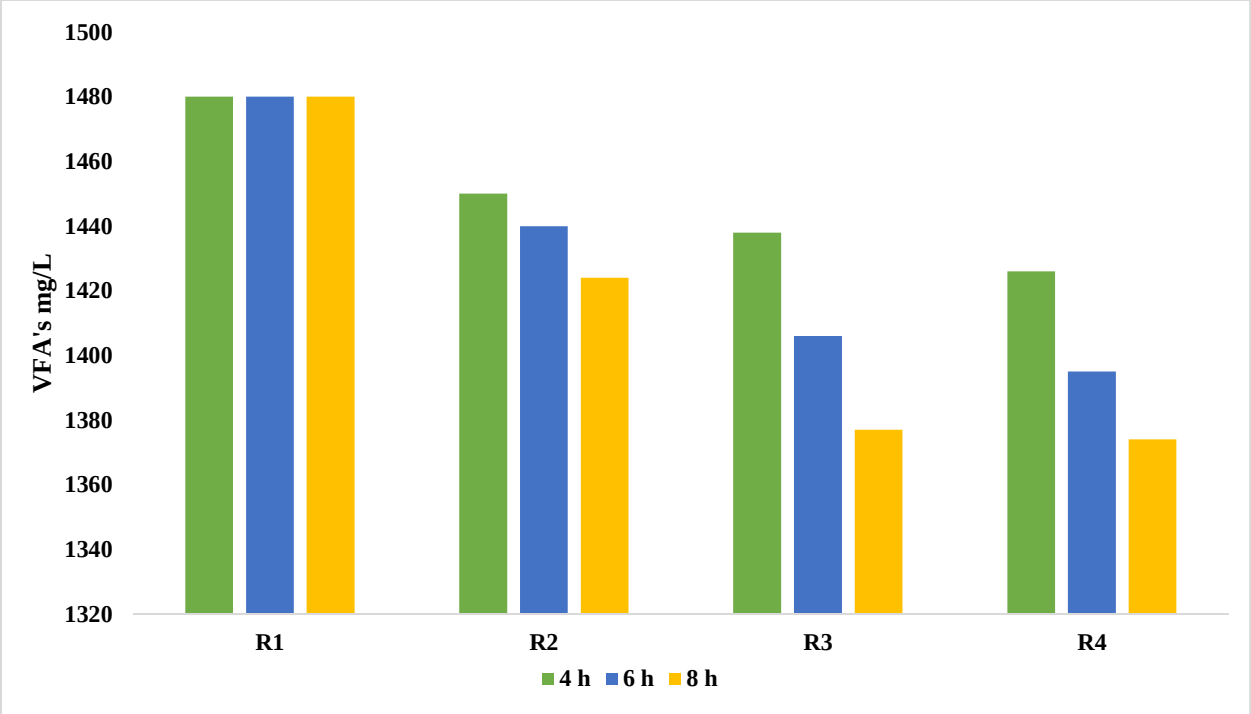

Figure 4.8: VFAs accumulation in methanogenic reactors of R1, R2, R3, and R4 due to continuous recirculation for 4, 6, and 8 hours during time optimization Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R3 is reactor setup with a single set of electrodes, and R4 is reactor setup with a double set of electrodes in their methanogenic reactors.

4.4.3 VFAs to Alkalinity Ratio:

The VFAs-to-alkalinity ratio is regarded as an additional internal important indicator to evaluate the process stability of the methanogenesis process. In the methanogenic reactors of R2, R3, and R4 gases were continuously recirculated for 4, 6, and 8 hours, respectively, in order to quantify the VFAs to alkalinity ratio and determine the optimal time period for in-situ biogas upgradation (Table 4.5).

Table 4.5: VFAs to Alkalinity Ratio during time optimization

Duration for recirculation (continuous)	R1 (No Recirculation, No electrodes)	R2 (Recirculation, No electrodes)	R3 (Single set of electrodes)	R4 (Double set of electrodes)
4 h	0.6	0.6	0.6	0.6
6 h	0.6	0.5	0.5	0.5
8 h	0.6	0.5	0.5	0.5

4.5 Interval-Based Time Optimization:

After optimizing the time required for recirculation during in-situ biogas upgradation in order to maximize the methane content of biogas, Further experimentation was conducted to maximize methane production in interval-based time optimization for biogas recirculation at three different durations: 4, 6, and 8 hours of total hydrogen feeding in an alternative on-off cycle of the peristaltic pump with 2 hours of feeding in between one hour of not feeding. The maximum methane content obtained was 90 and 95% in R3 and R4 for 8 hours of interval-based recirculation at a flow rate of 32 ml/min. During interval-based optimization of time for biogas upgradation, the methane content recorded in R2 was 78, 83, and 87%; 81, 87, and 90% in R3; and 86, 92, and 95% in R4 at interval-based recirculation times of 4, 6, and 8 hours, respectively, at a flow rate of 32 ml/min. Compared to R1 where the methane content obtained was 65%.

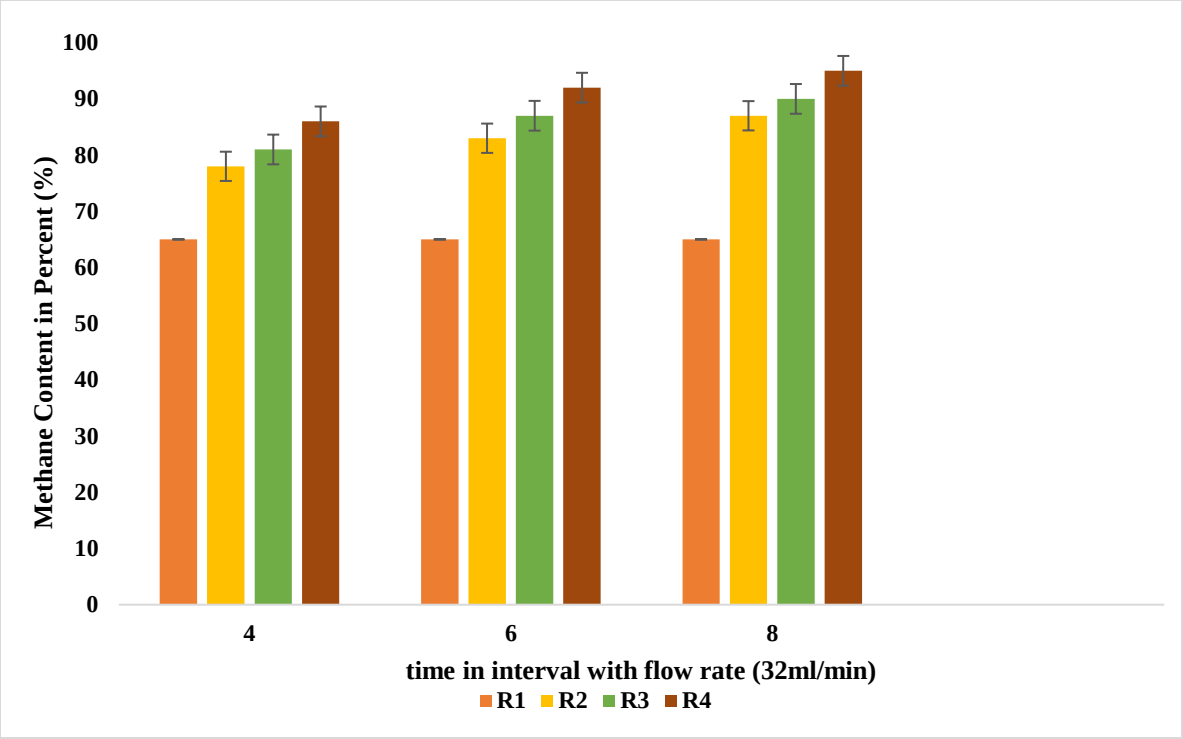

Figure 4.9: Effect of interval-based time (alternative On-Off cycle with 1 hour of interval) for total durations (4, 6, and 8 hours) due to recirculation on methane content (%) in interval-based time optimization Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R3 is reactor setup with a single set of electrodes, and R4 is reactor setup with a double set of electrodes in their methanogenic reactors.

4.5.1 pH of Methanogenic Reactor:

The change in pH of the reactor is considered a key parameter to determine the process's stability. During the interval-based time optimization in in-situ biogas upgradation, the pH of the methanogenic reactor was measured every three days. The pH of R3 and R4 compared to R2 during 4, 6, and 8 hours of interval-based recirculation had a range given below in table 4.6.

Table 4.6: pH during interval-based time optimization.

Time Duration (Intervals)	R1 (No Recirculation, No electrodes)	R2 (Recirculation, No electrodes)	R3 (Single set of electrodes)	R4 (Double set of electrodes)
4 h	6.91	7.21	7.20	7.19
6 h	6.91	7.22	7.21	7.20
8 h	6.91	7.23	7.22	7.21

4.5.2 Alkalinity and VFAs Accumulation:

Internal parameters considered to assess the stability of the methanogenesis process include alkalinity and the accumulation of VFAs. As the methanogenic reactor was operating for optimizing interval-based time during upgradation of biogas, the alkalinity recorded in R1, R2, R3, and R4 was 2250, 2380, 2360, and 2330 mg/L at 4 hours of interval-based recirculation, 2250, 2410, 2380, and 2350 mg/L at 6 hours of interval-based recirculation, and 2250, 2450, 2410, and 2380 mg/L at 8 hours of interval-based recirculation. The accumulated VFAs recorded in R1, R2, R3, and R4 were 1480, 1420, 1398, and 1387 mg/L at the time of 4 hours of interval-based recirculation; 1480, 1390, 1381, and 1360 mg/L at the time of 6 hours of continuous recirculation; and 1480, 1374, 1359, and 1341 mg/L at the time of 8 hours of interval-based recirculation.

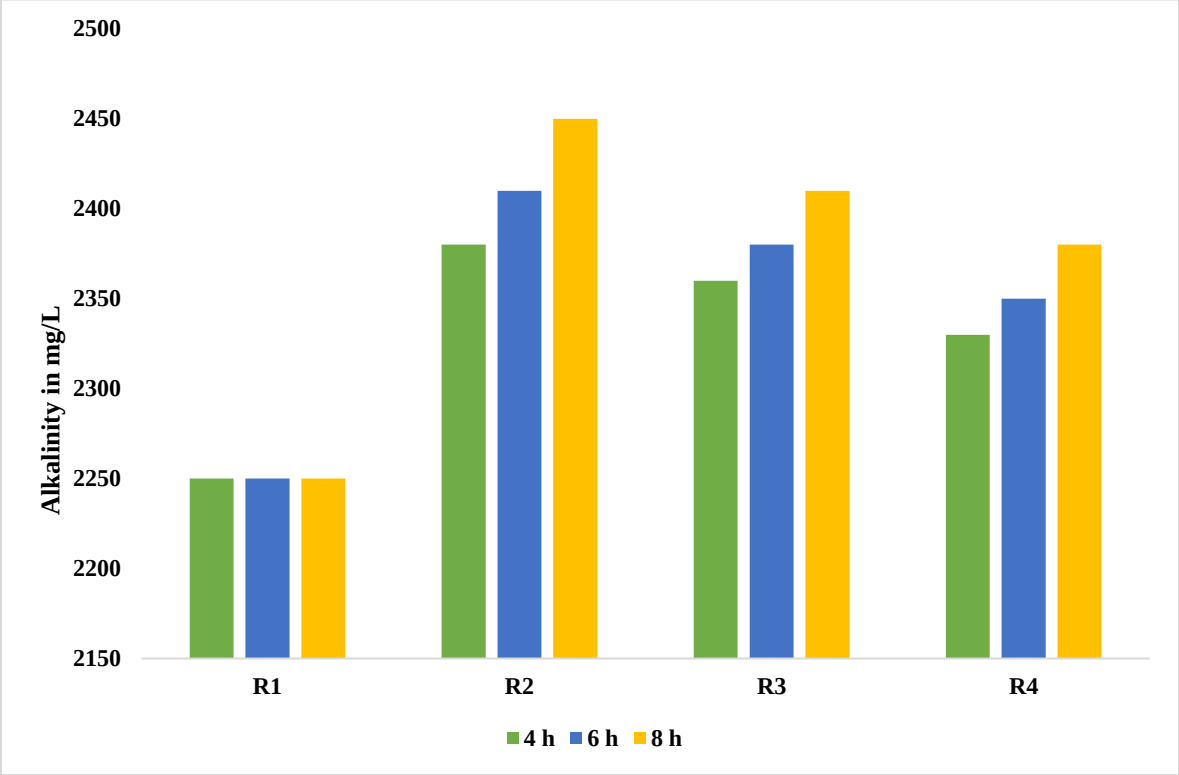

Figure 4.10: Alkalinity in methanogenic reactors of R2, R3, and R4 due to interval-based recirculation for 4, 6, and 8 hours during interval-based time optimization. Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R3 is reactor setup with a single set of electrodes, and R4 is reactor setup with a double set of electrodes in their methanogenic reactors.

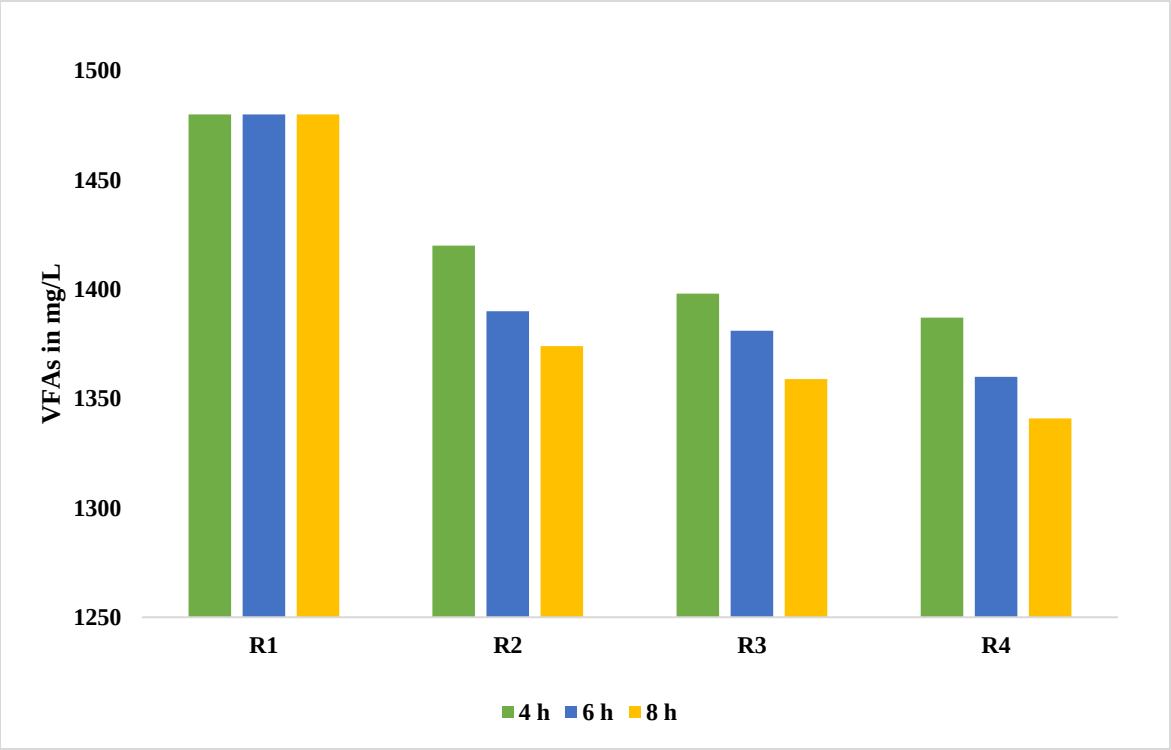

Figure 4.11: VFAs Accumulation in methanogenic reactors of R2, R3, and R4 due to interval-based recirculation for 4, 6, and 8 hours during interval-based time optimization. Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R3 is reactor setup with a single set of electrodes, and R4 is reactor setup with a double set of electrodes in their methanogenic reactors.

4.5.3 VFAs to Alkalinity Ratio:

The VFAs to alkalinity ratio in the methanogenic reactor was measured for 4, 6, and 8 hours of interval-based recirculation in R2, R3, and R4, respectively, in order to assess the process stability during the interval-based time optimization in in-situ biogas upgradation. Table 4.7 below provides the range.

Table 4.7: VFAs to Alkalinity ratio during interval-based time optimization.

Time Duration (Intervals)	R1 (No Recirculation, No electrodes)	R2 (Recirculation, No electrodes)	R3 (Single set of electrodes)	R4 (Double set of electrodes)
4 h	0.6	0.5	0.5	0.5
6 h	0.6	0.5	0.5	0.5
8 h	0.6	0.5	0.5	0.5

4.6 Interval-Based Time Optimization with Applied Voltage:

In phase four of upgrading biogas, the reactors were equipped with MECs in R3 and R4, and a voltage of 0.7V was applied (referred to as R5 and R6). Interval-based recirculation was performed for the duration of 4, 6, and 8 hours of feeding supplied hydrogen, and the results were compared with R1 and R2. The maximum methane content obtained was 95 and 99% in R5 and R6 for 8 hours of interval-based recirculation at a flow rate of 32 ml/min when voltage was applied. In R1 where the methane content obtained was 65% throughout the experiment. During interval-based optimization of time for biogas upgradation, voltage was applied. The methane content in R2 (without MEC) was 78, 83, and 87%, whereas methane content recorded was 83, 89, and 92% in R5 (i.e., R3 with voltage applied) compared to methane content obtained in R3, i.e., 81, 87, and 90% and 90, 96, and 99% of methane content in R6 (i.e., R4 with voltage applied) compared to R4, where methane content was 86, 92, and 95% during interval-based recirculation of gases for 4, 6, and 8 hours of duration at a flow rate of 32 ml/min.

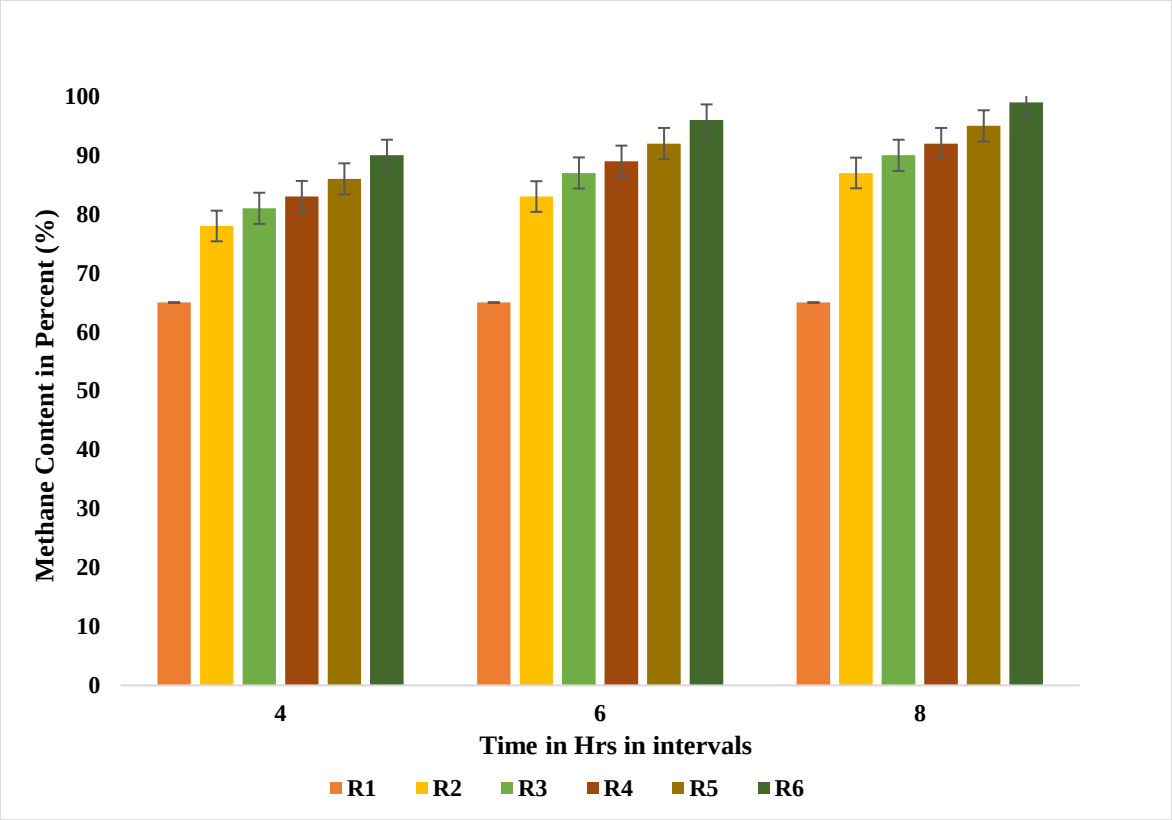

Figure 4.12: Effect of interval-Based recirculation (alternative On-Off cycle with 1 hour of interval) for total durations (4, 6, and 8 hours) with 0.7V of applied voltage on methane content (%) during interval-based time optimization with applied voltage (with MEC). Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R5 is reactor setup with a single set of electrodes applied with voltage, and R6 is reactor setup with a double set of electrodes applied with voltage in their electromethanogenic reactors.

4.6.1 pH of Methanogenic Reactor:

The pH of the methanogenic reactor was checked every three days to assess the stability of the process during the interval-based time optimization with applied voltage in in-situ biogas upgradation. Following 4, 6, and 8 hours of interval-based recirculation, the pH ranges of R5 and R6 relative to R2 are shown in table 4.8.

Table 4.8: pH during interval-based time optimization with applied voltage.

Time Duration (Intervals)	R1 (No Recirculation, No electrodes)	R2 (Recirculation, No electrodes)	R5 (Single set of electrodes with voltage)	R6 (Double set of electrodes with voltage)
4 h	6.91	7.20	7.18	7.16
6 h	6.91	7.21	7.19	7.17
8 h	6.91	7.22	7.20	7.18

4.6.2 Alkalinity and VFAs Accumulation:

Internal parameters to be considered to assess the stability of the methanogenesis process also include alkalinity and the accumulation of VFAs. As the methanogenic reactor was operating for optimizing interval-based time with applied voltage during upgradation of biogas, the alkalinity recorded in R1, R2, R5, and R6 was 2250, 2410, 2380, and 2350 mg/L at 4 hours of interval-based recirculation at 0.7V, 2250, 2430, 2400, and 2370 mg/L at 6 hours of interval-based recirculation at 0.7V, and 2250, 2490, 2460, and 2430 mg/L at 8 hours of interval-based recirculation at 0.7V. The accumulated VFAs recorded in R1, R2, R5, and R6 were 1480, 1454, 1415, and 1395 mg/L at 4 hours of interval-based recirculation, 1480, 1436, 1380, and 1350 mg/L at 6 hours of interval-based recirculation, and 1480, 1424, 1345, and 1321 mg/L at 8 hours of interval-based recirculation.

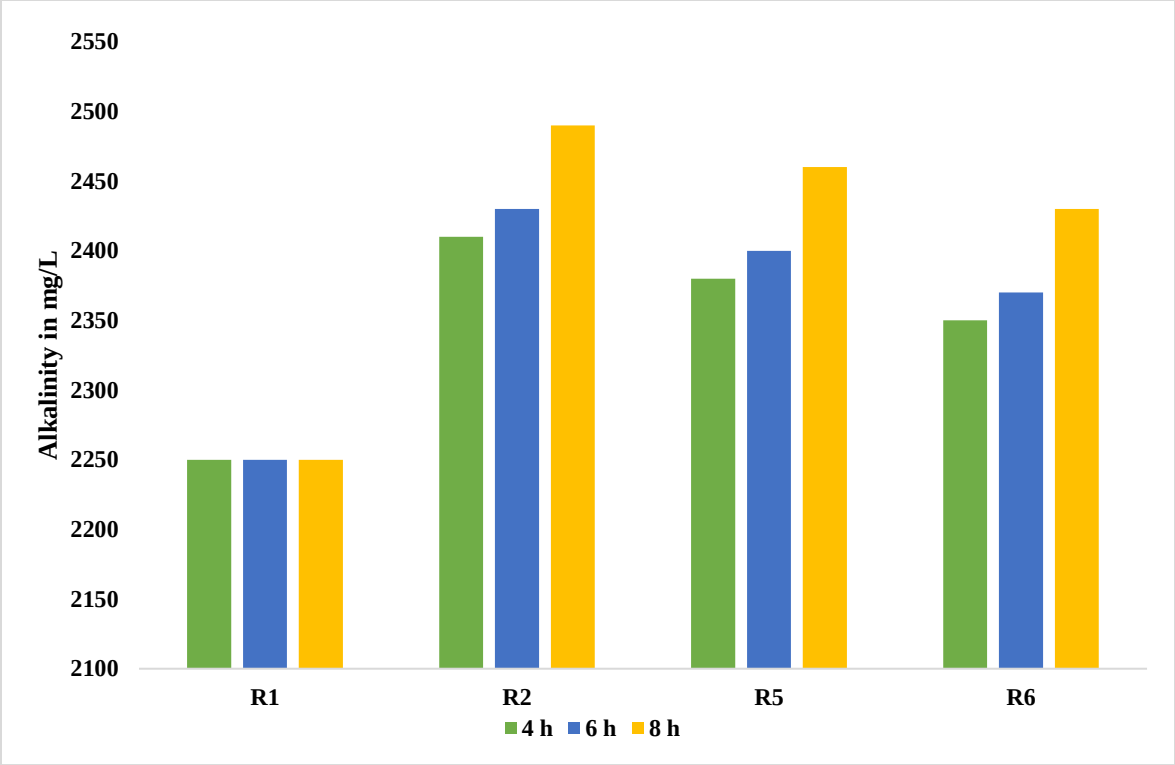

Figure 4.13: Alkalinity in methanogenic reactors of R2, R5, and R6 due to interval-based recirculation for 4, 6, and 8 hours during interval-based time optimization with applied voltage. Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R5 is reactor setup with a single set of electrodes applied with voltage, and R6 is reactor setup with a double set of electrodes applied with voltage in their electromethanogenic reactors.

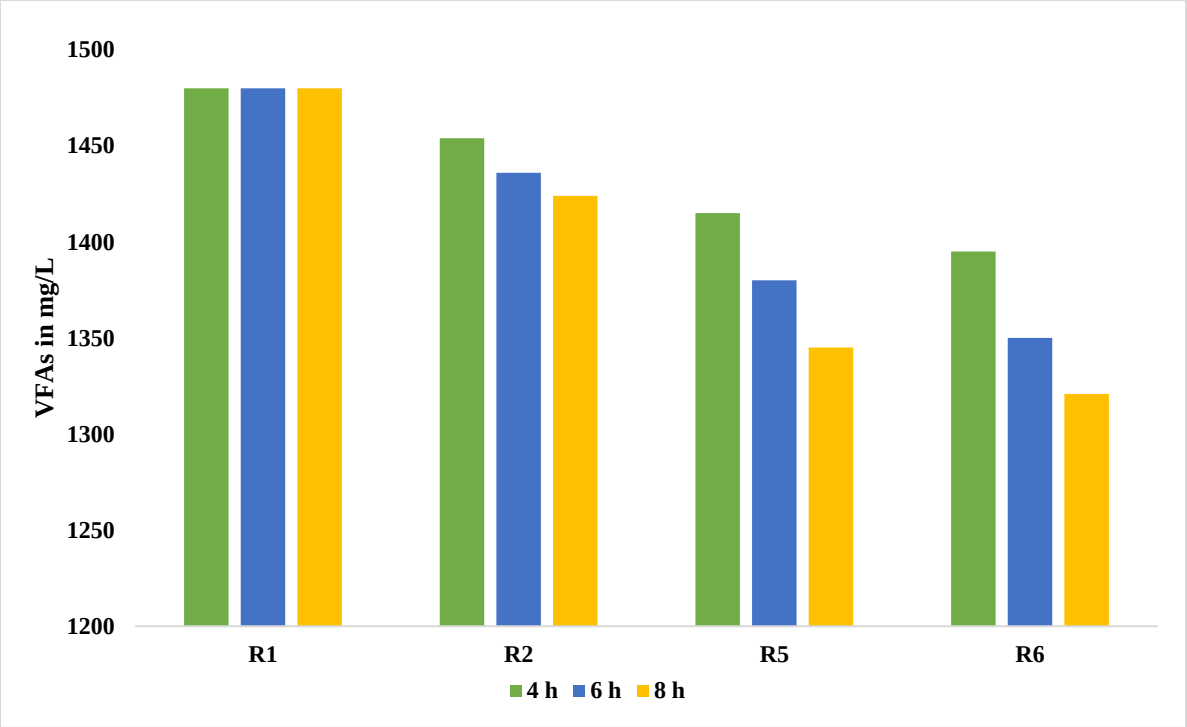

Figure 4.14: VFAs accumulation in methanogenic reactors of R2, R5, and R6 due to interval-based recirculation for 4, 6, and 8 hours during interval-based time optimization with applied voltage. Where R1 is control without recirculation of gases (biogas and hydrogen) and electrodes, and R2 is control with recirculation of gases and without electrodes. Whereas R5 is reactor setup with a single set of electrodes applied with voltage, and R6 is reactor setup with a double set of electrodes applied with voltage in their electromethanogenic reactors.

4.6.3 VFAs to Alkalinity Ratio:

Applying voltage (i.e., with MEC) in R5 and R6, the VFAs to alkalinity ratio in the methanogenic reactor was measured after 4, 6, and 8 hours of interval-based recirculation and compared with the R2 without MEC. The range is displayed in table 4.9 below.

Table 4.9: VFAs to Alkalinity ratio during interval-based time optimization with applied voltage.

Time Duration (Intervals)	R1 (No Recirculation, No electrodes)	R2 (Recirculation, No electrodes)	R5 (Single set of electrodes with voltage)	R6 (Double set of electrodes with voltage)
4 h	0.6	0.6	0.5	0.5
6 h	0.6	0.5	0.5	0.5
8 h	0.6	0.5	0.5	0.5

Chapter No. 5
Discussion

The study aimed to increase biogas methane content through a two-stage anaerobic digestion process during in-situ biogas upgradation, integrating MEC for studying its effects. The substrate used for biogas production was characterized by both TS and VS prior to biogas production. Cattle manure had 17.55% TS and 74.79% VS of TS, but green waste had 4.92% TS and 91.80% VS of TS. Reportedly, manure had 19.7 and 11.9% TS, and fruit and vegetable waste had 79% and 85% TS, respectively. However, the feedstock's composition and source determine its TS and VS. One of the key variables affecting the production of biogas is the C/N ratio. Based on VS, fruit and vegetable waste and cattle manure were co-digested in a 1:1 ratio to balance the C/N ratio within an ideal range. The C/N ratio in cattle manure is 15.5, which results in high biogas generation. During co-digestion, on the other hand, the C/N ratio is 30 (X. Wang et al., 2012). The composition of the manure is contingent upon its source and kind. Although the composition determines the C/N ratio for green grocery waste, which ranges from 20 to 60 (Fernández-Gómez et al., 2010).

During the initial phase of investigation, the biogas production achieved a steady state at the start of the experiment, and the total amount of biogas as well as the carbon dioxide and methane contents were measured. The total amount of biogas obtained after achieving steady state in R1, R3, R4, R5 and R6 was approximately 1500, 1570, 1630, 1740, and 1790 ml; the total amount of methane present in the total biogas produced in R1, R3, R4, R5, and R6 was recorded to be 960, 1041, 1108, 1235, and 1288 ml, respectively. Whereas, the concentration of carbon dioxide recorded in R1, R3, R6, R5, and R6 was 540, 529, 522, 505, and 502 ml, respectively.

Prior to upgrading the biogas, the biogas was monitored to evaluate the effect of MEC on methane content. The methane content obtained in R1, R3, and R4 was 64, 66, and 68%, compare to R5, R6 methane content was 71 and 72%, respectively. Upon the initiation of the study, MEC studies revealed a significant production of H_2, which subsequently decreased as the concentration of CH_4 increased. As long as they continue to use the generated H_2 with CO_2 to form methane, hydrogenotrophic methanogens have been found to be able to thrive in harsh

conditions. Furthermore, direct electron transfer, from MEC electrodes or H_2 by hydrogenotrophic methanogens has been demonstrated to boost CH_4 content. The acceptable methane percentage for automobiles is estimated to exceed 75%. The utilization of biogas is limited due to its high carbon dioxide levels (Subramanian et al., 2013). Biogas's potential as a natural gas replacement is increased when it is upgraded into biomethane. Biogas can be referred to as biomethane or bio-natural gas after its methane level is increased to at least 95% in order for it to satisfy natural gas criteria (Allegue et al., 2012). In this study, in-situ biogas upgradation was employed for biomethanation, which utilized acetoclastic methanogenesis via homoacetogenic acetate production, the direct electron transfer (DET) pathway, and the direct hydrogenotrophic methanogenesis pathway for in situ carbon dioxide-to-methane bioconversion.

During the first phase of this study, the methanogenic reactors of R2, R3, and R4 were supplied with external hydrogen and biogas were recirculated to optimize flow rate for high-rate biogas upgrading. The methane production and methane content of biogas were assessed. Throughout the process of optimizing flow rate, hydrogen was introduced to the biogas, and the mixture of gases was continuously circulated at rates of 128, 96, 64, and 32 ml/min for four hours per day. The study compared the methane content of R2, R3, and R4 with varying flow rates. Results indicated that recirculation at 128 ml/min recorded methane content obtained was 68, 70, and 73%, while recirculation at 96 ml/min recorded a methane content of 70, 73, and 76%. Recirculation at 64 ml/min methane content was73, 77, and 80%. At flow rate of 32 ml/min, the maximum methane content obtained was 74, 81, and 84%, respectively, in R2, R3, and R4 in comparison the R1 had methane content 65% during the entire operation. The residence time of the reactant gases passing within the methanogenic reactor would generally increase with a decrease in the flow rate of biogas. This leads to increased CH_4 production and a drop in H_2 and CO_2 fractions with flow rate reduction. In order to maximize biogas recovery as methane and ensure substrate to bacterium contact, mixing is essential. Moreover, mixing at 10 rpm increased methane output by 77%, supporting the idea that the mixing effect causes an increase in methane production. Mixing boosted substrate liquefaction, substrate mobility, and nutrient

transfer, which led to a rise in methane production (Nsair et al., 2019). On the other hand, methane output might have decreased because of the high-speed mixing at higher flow rates. Methane content increased as a result through an indirect pathway. Homoacetogenic bacteria first convert CO_2 into acetate, which is then broken down by acetoclastic methanogenic archaea to produce CH_4. Alternatively, homotrophic methanogenic archaea (hydrogenotrophic methanogens) drive the direct hydrogenotrophic methanogenesis pathway, which uses H_2 as an electron donor to reduce CO_2 directly into CH_4 (S. Fu et al., 2021). At a flow rate of 32 ml/min, the maximum methane content recorded increased by 70 to 81% in R3 and 73 to 84% in R4, respectively, compared to the highest applied flow rate of 128 ml/min. The partial pressure of H_2 rises with increased flow rate, which has an adverse effect on the microbial process and lowers CH_4 generation. Methane production is reduced, and VFAs accumulate as a result of acetoclastic methanogen inhibition brought on by the high partial pressure of hydrogen (Khan et al., 2022). Moreover, in methanogenic reactors, the solubilization of H_2 into the liquid phase is another important factor to be considered because the microbes need it to pass through the gas-liquid phase barrier to be accessible. Because of the high flow rate, gases have a low aqueous solubility, which restricts gas-liquid mass transfer and inhibits biomethanation (Jensen et al., 2021). Due to these reasons, biomethanation can be achieved at lower flow rates for recirculation.

The optimum daily duration necessary for attaining high methane content was optimized in the second phase by continuous recirculation of gases for three different durations of 4, 6, and 8 hours at an optimal flow rate of 32 ml/min. The methane content of biogas increased in both reactor configurations R3 and R4 compared to the R2. After recirculation in R2, R3, and R4 maximum methane content obtained was 76, 81, and 84% in 4 hours of continuous recirculation;80, 86, and 88% in 6 hours of continuous recirculation, and 84, 89, and 92% during 8 hours of continuous recirculation of biogas and supplied hydrogen relative to R1 with 65% of methane content throughout the experiment. The methane content in R3 and R4 increased by 89% and 92%, respectively, relative to the control during 8 hours of continuous recirculation. The study found that as recirculation duration increases, gas mixing increases,

leading to an elevation in the conversion process and a rise in methane content (Khan et al., 2022). The most effective continuous recirculation time for high-rate conversion was eight hours. However, the addition of hydrogen and its prolonged recirculation may inhibit the AD process. An extensive adaptation period is necessary for the reactor's microbial community to produce methane at its maximum capacity. Interval-based recirculation further reduced continuous recirculation for hours, indicating that the reactor's microbial community needs time to adapt to new substrate conditions.

In the third phase of in-situ biogas upgradation, the study involved interval-based recirculation of biogas and hydrogen supply for 4, 6, and 8 hours of interval-based hydrogen feeding in a cyclic manner of alternative on-off cycles at a flow rate of 32 ml/min. The methane content of biogas improved in both reactor configurations R3 and R4 compared to the R1, and R2. After recirculation in R2, R3 and R4, the maximum methane content obtained was 78, 81, and 86% in 4 hours of interval-based recirculation; 83, 87, and 92% in 6 hours of interval-based recirculation; and 87, 90, and 95% during 8 hours of interval-based recirculation of biogas and supplied hydrogen. The methane content in R3 and R4 increased by 90% and 95%, respectively, relative to the control during 8 hours of interval-based recirculation. Alternative on-off cycles resulted in more gas-to-gas contact with hydrogenotrophic methanogens, which increased the conversion process. Moreover, reactors' experience inhibited anaerobic digestion, leading to the accumulation of electron sinks like lactate, propionate, butyrate, and ethanol at high hydrogen partial pressure. This results in excessive acidity due to VFAs accumulation can lead to imbalance or deterioration, in continuous recirculation while interval-based recirculation can lower the reactor's hydrogen partial pressure. Also, low methane content and the accumulation of VFAs are caused by the inhibition of acetoclastic methanogens due to the high partial pressure of hydrogen caused by the continuous recirculation of supplied hydrogen (Angelidaki et al., 2018). Furthermore, the reactor's methane content was enhanced by feeding hydrogen at a flow rate of 32 ml/min, resulting in a high-rate conversion at 2 hours of recirculation and 1-hour interval for 8 hours' interval-based recirculation. Similarly, a point reaches at which methanogens may no longer effectively absorb further hydrogen when the

hydrogen cycle continues for a continued length of time. Interval-based time for recirculation provided an environment in which methanogens can progressively absorb hydrogen during the intervals, allowing them to adjust to conditions and avoiding surplus capacity. Similar to how enzymes' active sites become saturated during a chemical reaction.

The fourth phase of in-situ biogas upgradation was studied in order to optimize interval-based time for recirculation of gases and supplied hydrogen for 4, 6, and 8 hours with MEC (i.e., applied voltage) at a flow rate of 32 ml/min to study the effect of applied voltage on the upgradation of biogas during interval-based time optimization. R1 had 65% of methane content during the whole experiment. Whereas methane content in R2 was 78, 83, and 87% at 4, 6, and 8 hours of interval-based recirculation. The methane content of biogas increased in both reactor configurations R5 and R6 compared to R3 and R4. After recirculation of gases and supplied hydrogen in R5 and R4, the maximum methane content obtained was 83 and 90% in 4 hours of interval-based recirculation, 89 and 96% during 6 hours of interval-based recirculation, and 95 and 99% during 8 hours of interval-based recirculation of biogas and supplied hydrogen. The methane content in R5 and R6 increased by 95% and 99%, respectively, in 8 hours of interval-based recirculation with an applied voltage of 0.7V. However, R3 and R4 had methane contents of 90 and 92% during 8 hours of interval-based recirculation with no voltage applied.

For high-rate conversion, it has been concluded that recirculation should occur in interval-based time with MEC (applied voltage of 0.7V). Prior to applying voltage, the electrodes in methanogenic reactors of R5 and R6 formed a biofilm due to the hydrogenotrophic methanogens' adhesion to the surfaces of electrodes to form biofilm. In a methanogenic reactor, the biofilm keeps the population of methanogens high by preventing them from washing out with digestate. As the population of hydrogenotrophic methanogens increases, so does the concentration of methane in biogas. According to studies conducted, the methane content in SSE is lower than that in DSE due to the less surface area of a single set of electrodes compared to a double set of electrodes in DSE. Compared to R4 and R3, in R6 and R5 (electro-methanogenic reactors), there was a significant rise in methane content as a result of applied voltage in interval-based time during recirculation of gases due to enrichment of

exoelectrogens along with hydrogenotrophic methanogens. In earlier research for this project, it was shown that an applied voltage greater than 1.0 V would hinder biogas production. As there is a limit to how quickly substrate breakdown is accelerated by external voltage. The potential explanation is that the high voltage causes the microbes to be destroyed (Baek et al., 2021). In order to achieve high rates of pollutant removal, biogas production, and energy efficiency in a microbial electrolysis system, the ideal external voltage for certain substrates is therefore essential. The methane production improved when the external electric field was operated at 0.7V. A suitable voltage applied may prevent energy waste from excessive electron supply, raise the concentration of CH_4, and reduce CO_2 effectively.

Moreover, methanogenesis is inhibited when pH levels rise beyond 8.5, which is the primary technical obstacle to in-situ biogas upgradation. The loss of bicarbonate, a crucial buffer that regulates the anaerobic digestion process for biogas production, is the factor that is responsible for the pH increase (Angelidaki et al., 2018). Hydrogen (H^+) and bicarbonates are produced when CO_2 dissolves in the reactor's liquid phase. Due to the reduction in H^+ that takes place from the use of CO_2, the pH of the reactor rises as a result. A solution to this technological difficulty was removed by integrating the microbial electrolysis system in interval-based time for the recirculation of gases during upgrade. A novel approach to upgrading biogas involves employing a microbial electrolysis cell (MEC), wherein the bio-electro-methanogenesis process converts carbon dioxide to methane. Using a bio cathode to provide reducing power to a methanogenic biofilm developing on the electrode surface is the process by which the bio-electro-methanogenesis reaction takes place. Abiotic proton reduction produces hydrogen, which is subsequently consumed by the methanogenic microorganisms on the electrode surface. Alternatively, the hydrogen-mediated mechanism produces hydrogen through direct electron uptake from the electrode. These two limit mechanisms for the CO_2 reduction drive the bio-electro-methanogenesis reaction. Furthermore, considering the produced alkalinity, CO_2 sorption as HCO_3^- in the catholyte constitutes an additional mechanism for CO_2 removal in a MEC bio cathode. Other species than protons or hydroxyls migrate through the membrane to produce alkalinity. Ninety percent of the total CO_2 is removed by the primary mechanism

of CO_2 sorption. The CO_2 sorption in a bio cathode can be influenced by the surface area available to facilitate the mass transfer from the gas phase to the liquid phase, or it could be triggered by the chemical reaction that occurs between a hydroxyl ion and a CO_2 molecule (Zeppilli et al., 2020). This study, a fully biological MEC system was used in biogas upgradation. Numerous studies have clearly shown that bio-electrochemical reactions eliminate the majority of the inhibitory effects. We can assume that applied voltage speeds up the production of methane, maintains process stability, and removes organic waste. The average amount of methane produced was 99% of biogas upgraded with MEC. Furthermore, The MEC is designed to enhance the efficacy of removing refractory contaminants by combining electrochemical redox processes and microbial metabolism. On the other hand, it appears that the rate and amount of pollutant breakdown for the large-scale application of MEC for the elimination of pollutants. The construction and use of electrodes with high electrical conductivity and biocompatibility, the enrichment of hydrogenotrophic methanogens in biofilm via gas recirculation with external hydrogen during biogas upgradation, and the selection of microorganisms with excellent electron transport ability for electro-active biofilm formation are common strategies approached to improve electron transfer in MEC.

Changes in pH, alkalinity, VFAs accumulation, and the ratio of VFAs to alkalinity were found to indicate process stability. pH and other possible inhibitors weren't controlled in this study, but they were observed (Zhao et al., 2020). The majority of the previous investigations emphasized how pH variations affected the generation of biogas; they did not consider the impact of hydrogen addition or VFAs. Even if they are all related to one another, these criteria can still have an impact on one another. For example, increased production of VFAs results in a reduction in pH when the H_2 partial pressure is high. Acidogenesis and methanogenesis, two subsequent digesting processes, might be impacted by such pH variations in addition to biomass hydrolysis. The pH of the methanogenic reactor was within the range of 6.9 –7.2 for R2, R3, R4, R5, and R6 compared to R1, which had a pH of 6.91 throughout the process. During flow rate optimization, pH decreased with the decrease in flow rate; during time optimization in continuous recirculation, interval-based recirculation, and with applied voltage

R5 and R6, the pH increased as the time increased, but the pH obtained was in the optimal range. A pH of 7.1–7.2 is a potential threshold for methane production in our study. Ideally, the operational pH for effective methanogenesis ranges between 6.5–7.5, which falls between the slightly acidic towards neutral range with a moderate basic range (Ruggeri et al., 2015).

During the course of the investigation, R1's alkalinity was 2250 mg/L. Whereas, when hydrogen was supplied and gases were recirculated while optimizing the flow rate, the alkalinity of the methanogenic reactor R2, with alkalinity increasing from 2320 mg/L, 2450 mg/L, and 2480 mg/L to 2520 mg/L; in R3 increased from 2350 mg/L, 2420 mg/L, and 2450 mg/L to 2490 mg/L. And in R4, alkalinity increased to 2380 mg/L, 2390 mg/L, 2420 mg/L, and 2460 mg/L, respectively, at flow rates of 128, 96, 64, and 32 ml/min, respectively. Whereas, during optimization of time duration for biogas upgradation, the alkalinity of the methanogenic reactor in R2 increased by 2390 mg/L, 2450 mg/L, and 2490 mg/L; in R3 increased from 2390 mg/L to 2450 mg/L to 2490 mg/L. And in R4, alkalinity increased by 2360 mg/L, 2420 mg/L, and 2460 mg/L for 4, 6, and 8 hours of continuous recirculation. While optimizing the interval-based time for recirculation of gases, the alkalinity of the methanogenic reactor in R2, with alkalinity increased from 2380 mg/L, 2410 mg/L, and 2450 mg/L; in R3 increased from 2360 mg/L to 2380 mg/L to 2410 mg/L. And in R4, alkalinity increased from 2330 mg/L, 2350 mg/L, and 2380 mg/L, respectively, during 4, 6, and 8 hours of interval-based recirculation. During optimization the interval-based time for the recirculation of gases with the applied voltage, R2, had alkalinity increase to 2410 mg/L, 2430 mg/L, and 2490 mg/L; the alkalinity of the electro-methanogenic reactor in R5 increased from 2380 mg/L to 2400 mg/L to 2460 mg/L. And in R6, alkalinity increased to 2350 mg/L, 2370 mg/L, and 2430 mg/L for 4, 6, and 8 hours of interval-based recirculation. The overall alkalinity of R2 was greater than in R3 and R4. The alkalinity in this investigation was kept consistent throughout the operation period, falling between a rational range of <2300 and >2500 mg/L, compared to the 2300–2500 mg/L alkalinity range that prior research indicates is ideal (Speece et al., 2006). For tracking the stability of digesters, alkalinity is a useful characteristic. It relies on the

concentration of VFAs and needs to be closely examined in conjunction with VFAs to ensure precise stability.

R1 had an VFAs accumulation of 1480 mg/L throughout the experiment. While optimizing the flow rate during upgradation, VFAs accumulation in the methanogenic reactor of R2, VFAs decreased from 1450 mg/L, 1430 mg/L, 1409 mg/L, and 1399 mg/L; in R3 accumulation of VFAs decreased from 1432 mg/L, 1420 mg/L, and 1406 mg/L to 1375 mg/L. And in R4, VFAs decreased by 1421 mg/L, 1399 mg/L, 1380 mg/L, and 1365 mg/L relative to at flow rates of 128, 96, 64, and 32 ml/min, respectively. VFAs accumulation decreased with the decrease in flow rate. While optimizing the time duration for biogas upgradation, in R2, where VFAs accumulation decreased by 1450 mg/L, 1440 mg/L, and 1424 mg/L; in R3 accumulation of VFAs decreased from 1438 mg/L to 1406 mg/L to 1377 mg/L. And in R4, VFAs accumulation decreased by 1426 mg/L, 1395 mg/L, and 1374 mg/L at 4, 6, and 8 hours of continuous recirculation. VFAs decreased with the increase in time. However, by optimizing the interval-based time for recirculation of gases, in R2, VFAs accumulation decreased by 1420 mg/L, 1390 mg/L, and 1374 mg/L, the VFAs accumulation in the methanogenic reactor in R3 decreased by 1398 mg/L, 1381 mg/L, and 1359 mg/L. And in R4, VFAs decreased by 1387 mg/L, 1360 mg/L, and 1341 mg/L, respectively, for 4, 6, and 8 hours of interval-based recirculation. The VFAs accumulation decreased with the increase in interval-based recirculation time. However, by optimizing the interval-based time for recirculation of gases with applied voltage, compared to R2, with the accumulation of VFAs decreasing by 1454 mg/L, 1436 mg/L, and 1424 mg/L; the VFAs accumulation in the electro-methanogenic reactor R5 decreased from 1415 mg/L to 1380 mg/L. And in R6, it decreased by 1395 mg/L, 1350 mg/L, and 1321 mg/L, respectively, for 4, 6, and 8 hours of interval-based recirculation. The VFAs accumulation decreased with the increase in interval-based recirculation time.

One of the primary drawbacks of simple in-situ biogas upgradation is an increase in the concentration of VFAs over hydrogen (Zhao et al., 2021). A VFAs concentration of less than 600 mg/L is required for increased biogas production. Even at greater VFAs concentrations, though, high digester alkalinity can support high rates of biogas production. But the

concentration of VFAs shouldn't go over 2000 mg/L since, because of their toxicity (Hendriks et al., 2018), these amounts can inhibit methanogens even at low alkalinity and ideal pH. However, the accumulation of VFAs in R5 and R6 does not rise in the hydrogen supply during interval-based recirculation; VFAs are processed to acetate and used by methanogens in their natural state in MEC-AD (applied voltage) mediated upgradation. And this could be because of the strong microbial activity brought on by the creation of biofilms. Because of the microorganisms' attachment to the surfaces, there is less accumulation of VFAs and a higher output of biogas in the methanogenic reactor since the microbes are kept from washing out with the wastewater. Another potential evidence is that the exogenous hydrogen injection may interfere with the digester's regular metabolic processes. In the study by Palu et al., it was found that stringent hydrogenotrophic methanogens gradually increased in abundance after H_2 injection, eventually taking up a dominant role in the microbial community for themselves. Moreover, the formation of VFAs is expected during methanogenesis, competition among microorganisms may result in a decrease or increase in the use of acetate or other VFAs as the substrate.

It has been suggested that criteria for assessing digester stability be derived from VFAs and alkalinity interactions. A ratio in the range of 0.4 - 0.6 indicates successful AD process (Korres & Nizami, 2013). Since R1 retained a ratio of 0.6 for the entire course of the experiment, VFAs to alkalinity ratios varied from 0.4 to 0.6 for all phases in R2, R3, R4, R5, and R6. The overall trend for pH, alkalinity, VFAs accumulation, and the VFAs-to-alkalinity ratio was R2 > R3 > R4. However, the overall trend was identical—R2 > R5 > R6 —but the findings were significantly better when voltage was provided.

Chapter No. 6

Conclusions

Conclusions:

According to the study's conclusion, the ideal flow rate for both experimental setups was discovered to be 32 ml/min during flow rate optimization. The maximum methane content was achieved during time optimization in R3 and R4 after 8 hours of recirculation. The highest possible concentration of methane was achieved during interval-based recirculation thus reducing partial pressure in R3 and R4 after 8 hours of optimized interval-based time for recirculation. In order to evaluate the effect of MEC integrated with the biogas upgradation system during interval-based time optimization with applied voltage of 0.7V, the highest possible methane content recorded was 95% in R5 and 99% in R6 during 8 hours of interval-based recirculation. In a methanogenic reactor, the ideal range for methanogenic activities has been found for pH, alkalinity, VFAs accumulation, and the VFAs-to-alkalinity ratio without affecting the stability of the process. It was determined that the methane content and output-gas quality of two-stage anaerobic digestion can be improved without compromising process stability by recirculating gases and hydrogen supplied to the methanogenic reactor coupled with the microbial electrolysis system. Microbe-electrode interactions can improve biogas quality and bio-electrochemical performance by converting organic molecules into hydrogen or methane, thus increasing biogas upgradation efficiency and methane concentration and produces 99% methane content through interval-based recirculation, reducing the need for continuous recirculation with an increased surface area of electrodes.

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Future Aspects

Future Aspects:

In current development in biogas technology due to this innovative approach not only addresses environmental concerns but also offers several promising future aspects.

- Compare the effect of material of electrodes when changed from graphite to others. The inclusion of some less expensive conductive materials will boost electron transfer efficiency, resulting in increased methane generation from waste.
- Microbial profiling of electrodes.
- In the future, MEC research can focus on increasing the bio anode sensors' precision for self-sustaining, in-situ, real-time quality and biogas upgradation monitoring and developing advanced technology MEC designs for industrial upgradation of biogas.
- MEC has the potential to enable carbon capture during anaerobic digestion. This can be utilized to employ carbon dioxide in electrochemical reactions to lower greenhouse gas emissions.
- Interval-based recirculation of external hydrogen concentration while recirculation of gases during upgradation.

References

Adnan, A. I., Ong, M. Y., Nomanbhay, S., Chew, K. W., & Show, P. L. (2019). Technologies for biogas upgrading to biomethane: A review. *Bioengineering*, *6*(4), 92.

Agarry, S. E. (2017). Bioelectricity generation and treatment of petroleum refinery effluent by Bacillus cereus and Clostridium butyricum using microbial fuel cell technology. *Nigerian Journal of Technology*, *36*(2), 543–551.

Al Mamun, M. R., & Torii, S. (2015). Enhancement of production and upgradation of biogas using different techniques-a review. *International Journal of Earth Sciences and Engineering*, *8*(2), 877–892.

Ali, J., Rasheed, T., Afreen, M., Anwar, M. T., Nawaz, Z., Anwar, H., & Rizwan, K. (2020). Modalities for conversion of waste to energy—Challenges and perspectives. *Science of The Total Environment*, *727*, 138610.

Allegue, L. B., Hinge, J., & Allé, K. (2012). Biogas and bio-syngas upgrading. *Danish Technological Institute*, 5–97.

Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H., & Kougias, P. G. (2018). Biogas upgrading and utilization: Current status and perspectives. *Biotechnology Advances*, *36*(2), 452–466.

Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*, *34*(6), 755–781.

Arsova, L. (2010). Anaerobic digestion of food waste: Current status, problems and an alternative product. *Department of Earth and Environmental Engineering Foundation of Engineering and Applied Science Columbia University*.

Aryal, N., Zhang, Y., Bajracharya, S., Pant, D., & Chen, X. (2022). Microbial electrochemical approaches of carbon dioxide utilization for biogas upgrading. *Chemosphere*, *291*, 132843.

Augelletti, R., Conti, M., & Annesini, M. C. (2017). Pressure swing adsorption for biogas

upgrading. A new process configuration for the separation of biomethane and carbon dioxide. *Journal of Cleaner Production*, *140*, 1390–1398.

Azbar, N., & Speece, R. E. (2001). Two-phase, two-stage, and single-stage anaerobic process comparison. *Journal of Environmental Engineering*, *127*(3), 240–248.

Baek, G., Shi, L., Rossi, R., & Logan, B. E. (2021). The effect of high applied voltages on bioanodes of microbial electrolysis cells in the presence of chlorides. *Chemical Engineering Journal*, *405*, 126742.

Baena-Moreno, F. M., Rodr\'\iguez-Galán, M., Vega, F., Vilches, L. F., Navarrete, B., & Zhang, Z. (2019). Biogas upgrading by cryogenic techniques. *Environmental Chemistry Letters*, *17*, 1251–1261.

Batstone, D. J., Tait, S., & Starrenburg, D. (2009). Estimation of hydrolysis parameters in full-scale anerobic digesters. *Biotechnology and Bioengineering*, *102*(5), 1513–1520.

Bharathiraja, B., Sudharsana, T., Jayamuthunagai, J., Praveenkumar, R., Chozhavendhan, S., & Iyyappan, J. (2018). Biogas production--A review on composition, fuel properties, feed stock and principles of anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *90*(April), 570–582.

Biresselioglu, M. E., Demir, M. H., Rashid, A., Solak, B., & Ozyorulmaz, E. (2019). What are the preferences of household energy use in Pakistan?: Findings from a national survey. *Energy and Buildings*, *205*, 109538.

Bonse, N., & Beyene, A. (2021). Regression-based comparative analysis of pollutants in biogas and natural-gas-blend combustion outputs. *International Journal of Global Warming*, *25*(2), 150–167.

Bose, A., Lin, R., Rajendran, K., O'Shea, R., Xia, A., & Murphy, J. D. (2019). How to optimise photosynthetic biogas upgrading: a perspective on system design and microalgae selection. *Biotechnology Advances*, *37*(8), 107444.

Cai, W., Liu, W., Zhang, Z., Feng, K., Ren, G., Pu, C., Li, J., Deng, Y., & Wang, A. (2019).

Electro-driven methanogenic microbial community diversity and variability in the electron abundant niche. *Science of the Total Environment*, *661*, 178–186.

Colazo, A.-B., Sánchez, A., Font, X., & Colón, J. (2015). Environmental impact of rejected materials generated in organic fraction of municipal solid waste anaerobic digestion plants: Comparison of wet and dry process layout. *Waste Management*, *43*, 84–97.

Corbellini, V., Feng, C., Bellucci, M., Catenacci, A., Stella, T., Espinoza-Tofalos, A., & Malpei, F. (2021). Performance analysis and microbial community evolution of in situ biological biogas upgrading with increasing H2/CO2 ratio. *Archaea*, *2021*, 1–15.

Cord-Ruwisch, R., & others. (2019). Thermodynamics of anaerobic digestion: Mechanism of suppression on biogas production during acidogenesis. *INMATEH-Agricultural Engineering*, *57*(1), 287–301.

Cozma, P., Wukovits, W., M\uam\ualig\ua, I., Friedl, A., & Gavrilescu, M. (2015). Modeling and simulation of high pressure water scrubbing technology applied for biogas upgrading. *Clean Technologies and Environmental Policy*, *17*, 373–391.

Darmani, A., Arvidsson, N., Hidalgo, A., & Albors, J. (2014). What drives the development of renewable energy technologies? Toward a typology for the systemic drivers. *Renewable and Sustainable Energy Reviews*, *38*, 834–847.

Dennehy, C., Lawlor, P. G., McCabe, M. S., Cormican, P., Sheahan, J., Jiang, Y., Zhan, X., & Gardiner, G. E. (2018). Anaerobic co-digestion of pig manure and food waste; effects on digestate biosafety, dewaterability, and microbial community dynamics. *Waste Management*, *71*, 532–541.

Dhanya, B. S., Mishra, A., Chandel, A. K., & Verma, M. L. (2020). Development of sustainable approaches for converting the organic waste to bioenergy. *Science of the Total Environment*, *723*, 138109.

Fernández-Gómez, M. J., Nogales, R., Insam, H., Romero, E., & Goberna, M. (2010). Continuous-feeding vermicomposting as a recycling management method to revalue

tomato-fruit wastes from greenhouse crops. *Waste Management*, *30*(12), 2461–2468.

Fu, S., Angelidaki, I., & Zhang, Y. (2021). In situ biogas upgrading by CO2-to-CH4 bioconversion. *Trends in Biotechnology*, *39*(4), 336–347.

Fu, X.-Z., Li, J., Pan, X.-R., Huang, L., Li, C.-X., Cui, S., Liu, H.-Q., Tan, Z.-L., & Li, W.-W. (2020). A single microbial electrochemical system for CO2 reduction and simultaneous biogas purification, upgrading and sulfur recovery. *Bioresource Technology*, *297*, 122448.

Gahlawat, I. N., & Lakra, P. (2020). Global Climate change and its effects. *Integrated Journal of Social Sciences*, *7*(1), 14–23.

Gautam, R., Nayak, J. K., Ress, N. V, Steinberger-Wilckens, R., & Ghosh, U. K. (2023). Bio-hydrogen production through microbial electrolysis cell: Structural components and influencing factors. *Chemical Engineering Journal*, *455*, 140535.

Ghangrekar, M. M., Surampalli, R. Y., Zhang, T. C., & Duteanu, N. M. (2023). *Microbial Electrochemical Technologies: Fundamentals and Applications*. John Wiley \& Sons.

Gielen, D., Gorini, R., Wagner, N., Leme, R., Gutierrez, L., Prakash, G., Asmelash, E., Janeiro, L., Gallina, G., Vale, G., & others. (2019). *Global energy transformation: a roadmap to 2050*.

González, R., Peña, D. C., & Gómez, X. (2022). Anaerobic co-digestion of wastes: reviewing current status and approaches for enhancing biogas production. *Applied Sciences*, *12*(17), 8884.

Hasanuzzaman, M., Zubir, U. S., Ilham, N. I., & Seng Che, H. (2017). Global electricity demand, generation, grid system, and renewable energy polices: a review. *Wiley Interdisciplinary Reviews: Energy and Environment*, *6*(3), e222.

Hendriks, A., Van Lier, J. B., & De Kreuk, M. K. (2018). Growth media in anaerobic fermentative processes: The underestimated potential of thermophilic fermentation and anaerobic digestion. *Biotechnology Advances*, *36*(1), 1–13.

Herout, M., Malathacek~ ák, J., Kučera, L., & Dlabaja, T. (2012). *Biogas composition depending on the type of plant biomass used.*

Hua, T., Li, S., Li, F., Zhou, Q., & Ondon, B. S. (2019). Microbial electrolysis cell as an emerging versatile technology: a review on its potential application, advance and challenge. *Journal of Chemical Technology \& Biotechnology*, *94*(6), 1697–1711.

Jadhav, D. A., Chendake, A. D., Schievano, A., & Pant, D. (2019). Suppressing methanogens and enriching electrogens in bioelectrochemical systems. *Bioresource Technology*, *277*, 148–156.

Jaiswal, K. K., Chowdhury, C. R., Yadav, D., Verma, R., Dutta, S., Jaiswal, K. S., Karuppasamy, K. S. K., & others. (2022). Renewable and sustainable clean energy development and impact on social, economic, and environmental health. *Energy Nexus*, *7*, 100118.

Jensen, M. B., Ottosen, L. D. M., & Kofoed, M. V. W. (2021). H2 gas-liquid mass transfer: A key element in biological Power-to-Gas methanation. *Renewable and Sustainable Energy Reviews*, *147*, 111209.

Jiang, J., Zhang, Y., Li, K., Wang, Q., Gong, C., & Li, M. (2013). Volatile fatty acids production from food waste: effects of pH, temperature, and organic loading rate. *Bioresource Technology*, *143*, 525–530.

Kamran, M. (2018). Current status and future success of renewable energy in Pakistan. *Renewable and Sustainable Energy Reviews*, *82*, 609–617.

Kapoor, R., Ghosh, P., Kumar, M., & Vijay, V. K. (2019). Evaluation of biogas upgrading technologies and future perspectives: a review. *Environmental Science and Pollution Research*, *26*, 11631–11661.

Karthikeyan, O. P., Visvanathan, C., & others. (2012). Effect of C/N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester. *Bioresource Technology*, *113*, 294–302.

Khadka, A., Parajuli, A., Dangol, S., Thapa, B., Sapkota, L., Carmona-Mart\'\inez, A. A., & Ghimire, A. (2022). Effect of the substrate to inoculum ratios on the kinetics of biogas production during the mesophilic anaerobic digestion of food waste. *Energies*, *15*(3), 834.

Khan, A., Akbar, S., Okonkwo, V., Smith, C., Khan, S., Shah, A. A., Adnan, F., Ijaz, U. Z., Ahmed, S., & Badshah, M. (2022). Enrichment of the hydrogenotrophic methanogens for, in-situ biogas up-gradation by recirculation of gases and supply of hydrogen in methanogenic reactor. *Bioresource Technology*, *345*, 126219.

KHEMKA, P. K. (2023). *INVESTIGATIONS ON ABSORPTION TECHNIQUE FOR BIOGAS PURIFICATION*.

Kim, M., Gomec, C. Y., Ahn, Y., & Speece, R. E. (2003). Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion. *Environmental Technology*, *24*(9), 1183–1190.

Korai, M. S., Mahar, R. B., & Uqaili, M. A. (2016). Optimization of waste to energy routes through biochemical and thermochemical treatment options of municipal solid waste in Hyderabad, Pakistan. *Energy Conversion and Management*, *124*, 333–343.

Korres, N. E., & Nizami, A. S. (2013). Variation in anaerobic digestion: need for process monitoring. In *Bioenergy production by anaerobic digestion* (pp. 194–230). Routledge.

Kougias, P. G., Treu, L., Benavente, D. P., Boe, K., Campanaro, S., & Angelidaki, I. (2017). Ex-situ biogas upgrading and enhancement in different reactor systems. *Bioresource Technology*, *225*, 429–437.

Li, Y., Han, Y., Zhang, Y., Luo, W., & Li, G. (2020). Anaerobic digestion of different agricultural wastes: A techno-economic assessment. *Bioresource Technology*, *315*, 123836.

Liebetrau, J., Sträuber, H., Kretzschmar, J., Denysenko, V., & Nelles, M. (2019). Anaerobic digestion. *Biorefineries*, 281–299.

Lindmark, J., Thorin, E., Fdhila, R. B., & Dahlquist, E. (2014). Effects of mixing on the result

of anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *40*, 1030–1047.

Lisowyj, M., & Wright, M. M. (2020). A review of biogas and an assessment of its economic impact and future role as a renewable energy source. *Reviews in Chemical Engineering*, *36*(3), 401–421.

Lohani, S. P., & Havukainen, J. (2018). Anaerobic digestion: factors affecting anaerobic digestion process. *Waste Bioremediation*, 343–359.

Lóránt, B., & Tardy, G. M. (2022). Current Status of Biological Biogas Upgrading Technologies. *Periodica Polytechnica Chemical Engineering*, *66*(3), 465–481.

Mahmoodi, P., Farmanbordar, S., & Karimi, K. (2018). Analytical methods in biogas production. *Biogas: Fundamentals, Process, and Operation*, 221–238.

Mao, C., Feng, Y., Wang, X., & Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *45*, 540–555.

Mart\'\in-Pascual, J., Reboleiro-Rivas, P., López-López, C., González-López, J., Hontoria, E., & Poyatos, J. M. (2014). Influence of hydraulic retention time on heterotrophic biomass in a wastewater moving bed membrane bioreactor treatment plant. *International Journal of Environmental Science and Technology*, *11*, 1449–1458.

Martins, G., Salvador, A. F., Pereira, L., & Alves, M. M. (2018). Methane production and conductive materials: a critical review. *Environmental Science \& Technology*, *52*(18), 10241–10253.

Meegoda, J. N., Li, B., Patel, K., & Wang, L. B. (2018). A review of the processes, parameters, and optimization of anaerobic digestion. *International Journal of Environmental Research and Public Health*, *15*(10), 2224.

Menon, A., Wang, J.-Y., & Giannis, A. (2017). Optimization of micronutrient supplement for enhancing biogas production from food waste in two-phase thermophilic anaerobic digestion. *Waste Management*, *59*, 465–475.

Menzel, T., Neubauer, P., & Junne, S. (2020). Role of microbial hydrolysis in anaerobic digestion. *Energies*, *13*(21), 5555.

Mishra, A., Kumar, M., Bolan, N. S., Kapley, A., Kumar, R., & Singh, L. (2021). Multidimensional approaches of biogas production and up-gradation: Opportunities and challenges. *Bioresource Technology*, *338*, 125514.

Mkhize, N. T., Msagati, T. A. M., Mamba, B. B., & Momba, M. (2014). Determination of volatile fatty acids in wastewater by solvent extraction and gas chromatography. *Physics and Chemistry of the Earth, Parts A/B/C*, *67*, 86–92.

Mlinar, S., Weig, A. R., & Freitag, R. (2022). Influence of NH3 and NH4+ on anaerobic digestion and microbial population structure at increasing total ammonia nitrogen concentrations. *Bioresource Technology*, *361*, 127638.

Montag, D., & Schink, B. (2016). Biogas process parameters—energetics and kinetics of secondary fermentations in methanogenic biomass degradation. *Applied Microbiology and Biotechnology*, *100*(2), 1019–1026.

Muñoz, R., Meier, L., Diaz, I., & Jeison, D. (2015). A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading. *Reviews in Environmental Science and Bio/Technology*, *14*, 727–759.

Ndayisenga, F., Yu, Z., Zheng, J., Wang, B., Liang, H., Phulpoto, I. A., Habiyakare, T., & Zhou, D. (2021). Microbial electrohydrogenesis cell and dark fermentation integrated system enhances biohydrogen production from lignocellulosic agricultural wastes: Substrate pretreatment towards optimization. *Renewable and Sustainable Energy Reviews*, *145*, 111078.

Newell, R., Raimi, D., & Aldana, G. (2019). Global energy outlook 2019: the next generation of energy. *Resources for the Future*, *1*, 8–19.

Nie, E., He, P., Zhang, H., Hao, L., Shao, L., & Lü, F. (2021). How does temperature regulate anaerobic digestion? *Renewable and Sustainable Energy Reviews*, *150*, 111453.

Nsair, A., Cinar, S. Ö., Qdais, H. A., & Kuchta, K. (2019). Optimizing the performance of a large scale biogas plant by controlling stirring process: A case study. *Energy Conversion and Management*, *198*, 111931.

Obaideen, K., Abdelkareem, M. A., Wilberforce, T., Elsaid, K., Sayed, E. T., Maghrabie, H. M., & Olabi, A. G. (2022). Biogas role in achievement of the sustainable development goals: Evaluation, Challenges, and Guidelines. *Journal of the Taiwan Institute of Chemical Engineers*, *131*, 104207.

Omar, B., El-Gammal, M., Abou-Shanab, R., Fotidis, I. A., Angelidaki, I., & Zhang, Y. (2019). Biogas upgrading and biochemical production from gas fermentation: Impact of microbial community and gas composition. *Bioresource Technology*, *286*, 121413.

Ometto, F., Karlsson, A., Ejlertsson, J., Björn, A. V., & Shakeri, S. Y. (2019). Anaerobic digestion: an engineered biological process. In *Substitute Natural Gas from Waste* (pp. 63–74). Elsevier.

Paolini, V., Petracchini, F., Segreto, M., Tomassetti, L., Naja, N., & Cecinato, A. (2018). Environmental impact of biogas: A short review of current knowledge. *Journal of Environmental Science and Health, Part A*, *53*(10), 899–906.

Parawira, W., Murto, M., Read, J. S., & Mattiasson, B. (2007). A study of two-stage anaerobic digestion of solid potato waste using reactors under mesophilic and thermophilic conditions. *Environmental Technology*, *28*(11), 1205–1216.

Park, J.-G., Heo, T.-Y., Kwon, H.-J., Shi, W.-Q., & Jun, H.-B. (2020). Effects of voltage supply on the methane production rates and pathways in an anaerobic digestion reactor using different electron donors. *International Journal of Hydrogen Energy*, *45*(16), 9459–9468.

PASSARIS, I., Smets, I., & Vankelecom, I. (2018). *Development and Validation of Biological Monitoring Tools for Anaerobic (Membrane) Bioreactors*.

Prado, J., Ribeiro, H., Alvarenga, P., & Fangueiro, D. (2022). A step towards the production of manure-based fertilizers: Disclosing the effects of animal species and slurry treatment

on their nutrients content and availability. *Journal of Cleaner Production*, *337*, 130369.

Purahong, W., Wubet, T., Lentendu, G., Schloter, M., Pecyna, M. J., Kapturska, D., Hofrichter, M., Krüger, D., & Buscot, F. (2016). Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular Ecology*, *25*(16), 4059–4074.

Ram, C., Kumar, A., & Rani, P. (2021). Municipal solid waste management: a review of waste to energy (WtE) approaches. *Bioresources*, *16*(2), 4275.

Rasheed, T., Anwar, M. T., Ahmad, N., Sher, F., Khan, S. U.-D., Ahmad, A., Khan, R., & Wazeer, I. (2021). Valorisation and emerging perspective of biomass based waste-to-energy technologies and their socio-environmental impact: A review. *Journal of Environmental Management*, *287*, 112257.

Rauf, O., Wang, S., Yuan, P., & Tan, J. (2015). An overview of energy status and development in Pakistan. *Renewable and Sustainable Energy Reviews*, *48*, 892–931.

Rawoof, S. A. A., Kumar, P. S., Vo, D.-V. N., & Subramanian, S. (2021). Sequential production of hydrogen and methane by anaerobic digestion of organic wastes: a review. *Environmental Chemistry Letters*, *19*, 1043–1063.

Rocha-Meneses, L., Zannerni, R., Inayat, A., Abdallah, M., Shanableh, A., Ghenai, C., Kamil, M., & Kikas, T. (2022). Current progress in anaerobic digestion reactors and parameters optimization. *Biomass Conversion and Biorefinery*, 1–24.

Rousseau, R., Etcheverry, L., Roubaud, E., Basséguy, R., Délia, M.-L., & Bergel, A. (2020). Microbial electrolysis cell (MEC): Strengths, weaknesses and research needs from electrochemical engineering standpoint. *Applied Energy*, *257*, 113938.

Ruggeri, B., Tommasi, T., & Sanfilippo, S. (2015). *BioH2 \& BioCH4 through anaerobic digestion: from research to full-scale applications*. Springer.

Sarker, S., Lamb, J. J., Hjelme, D. R., & Lien, K. M. (2018). Overview of recent progress towards in-situ biogas upgradation techniques. *Fuel*, *226*, 686–697.

Sawyerr, N., Trois, C., Workneh, T., & Okudoh, V. I. (2019). An overview of biogas production: Fundamentals, applications and future research. *International Journal of Energy Economics and Policy*.

Schnürer, A. (2016). Biogas production: microbiology and technology. *Anaerobes in Biotechnology*, 195–234.

Shamurad, B., Sallis, P., Petropoulos, E., Tabraiz, S., Ospina, C., Leary, P., Dolfing, J., & Gray, N. (2020). Stable biogas production from single-stage anaerobic digestion of food waste. *Applied Energy*, *263*, 114609.

Soltani, A., Rajabi, M. H., Zeinali, E., & Soltani, E. (2013). Energy inputs and greenhouse gases emissions in wheat production in Gorgan, Iran. *Energy*, *50*(1), 54–61. https://doi.org/10.1016/J.ENERGY.2012.12.022

Sovacool, B. K., Sidortsov, R. V, & Jones, B. R. (2013). *Energy security, equality and justice*. Routledge.

Speece, R. E., Boonyakitsombut, S., Kim, M., Azbar, N., & Ursillo, P. (2006). Overview of Anaerobic Treatment: Thermophilic and Propionate Implications-Keynote Address—Association of Environmental Engineering and Science Professors—78th Annual Water Environment Federation Technical Exposition and Conference, Washington, DC, Oct. 29--Nov. 2, 2005. *Water Environment Research*, *78*(5), 460–473.

Sterling Jr, M. C., Lacey, R. E., Engler, C. R., & Ricke, S. C. (2001). Effects of ammonia nitrogen on H2 and CH4 production during anaerobic digestion of dairy cattle manure. *Bioresource Technology*, *77*(1), 9–18.

Subramanian, K. A., Mathad, V. C., Vijay, V. K., & Subbarao, P. M. V. (2013). Comparative evaluation of emission and fuel economy of an automotive spark ignition vehicle fuelled with methane enriched biogas and CNG using chassis dynamometer. *Applied Energy*, *105*, 17–29.

Sun, J., Rene, E. R., He, Y., Ma, W., Hu, Q., & Qiu, B. (2023). Carbon, iron, and polymer-

based conductive materials for improving methane production in anaerobic wastewater treatment systems: A review on their direct interspecific electron transfer mechanism. *Fuel*, *342*, 127703.

Tang, S., Wang, Z., Lu, H., Si, B., Wang, C., & Jiang, W. (2023). Design of stage-separated anaerobic digestion: Principles, applications, and prospects. *Renewable and Sustainable Energy Reviews*, *187*, 113702.

Tareen, W. U. K., Anjum, Z., Yasin, N., Siddiqui, L., Farhat, I., Malik, S. A., Mekhilef, S., Seyedmahmoudian, M., Horan, B., Darwish, M., & others. (2018). The prospective non-conventional alternate and renewable energy sources in Pakistan—A focus on biomass energy for power generation, transportation, and industrial fuel. *Energies*, *11*(9), 2431.

Tartakovsky, B., Lebrun, F., Guiot, S. R., & Bock, C. (2021). A comparison of microbial and bioelectrochemical approaches for biogas upgrade through carbon dioxide conversion to methane. *Sustainable Energy Technologies and Assessments*, *45*, 101158.

Thapa, A., Jo, H., Han, U., & Cho, S.-K. (2023). Ex-situ biomethanation for CO2 valorization: State of the art, recent advances, challenges, and future prospective. *Biotechnology Advances*, 108218.

Thiruselvi, D., Kumar, P. S., Kumar, M. A., Lay, C.-H., Aathika, S., Mani, Y., Jagadiswary, D., Dhanasekaran, A., Shanmugam, P., Sivanesan, S., & others. (2021). A critical review on global trends in biogas scenario with its up-gradation techniques for fuel cell and future perspectives. *International Journal of Hydrogen Energy*, *46*(31), 16734–16750.

Van, D. P., Fujiwara, T., Tho, B. L., Toan, P. P. S., & Minh, G. H. (2020). A review of anaerobic digestion systems for biodegradable waste: Configurations, operating parameters, and current trends. *Environmental Engineering Research*, *25*(1), 1–17.

Voelklein, M. A., Rusmanis, D., & Murphy, J. D. (2019). Biological methanation: Strategies for in-situ and ex-situ upgrading in anaerobic digestion. *Applied Energy*, *235*, 1061–1071.

Wahid, R., Mulat, D. G., Gaby, J. C., & Horn, S. J. (2019). Effects of H 2: CO 2 ratio and H 2

supply fluctuation on methane content and microbial community composition during in-situ biological biogas upgrading. *Biotechnology for Biofuels*, *12*, 1–15.

Wang, H., Liu, Y., Du, H., Zhu, J., Peng, L., Yang, C., & Luo, F. (2021). Exploring the effect of voltage on biogas production performance and the methanogenic pathway of microbial electrosynthesis. *Biochemical Engineering Journal*, *171*, 108028.

Wang, W., Lee, D.-J., & Lei, Z. (2022). Integrating anaerobic digestion with microbial electrolysis cell for performance enhancement: A review. *Bioresource Technology*, *344*, 126321.

Wang, X., Yang, G., Feng, Y., Ren, G., & Han, X. (2012). Optimizing feeding composition and carbon--nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *Bioresource Technology*, *120*, 78–83.

Yang, J., Liu, K., Yi, W., Si, B., Tian, C., & Yang, G. (2024). Effects of biochar, granular activated carbon, and magnetite on the electron transfer of microbials during the anaerobic digestion process: Insights into nitrogen heterocyclic compounds degradation. *Fuel*, *358*, 130079.

Yaqoob, H., Teoh, Y. H., Din, Z. U., Sabah, N. U., Jamil, M. A., Mujtaba, M. A., & Abid, A. (2021). The potential of sustainable biogas production from biomass waste for power generation in Pakistan. *Journal of Cleaner Production*, *307*, 127250.

Yin, C., Shen, Y., Yu, Y., Yuan, H., Lou, Z., & Zhu, N. (2019). In-situ biogas upgrading by a stepwise addition of ash additives: Methanogen adaption and CO2 sequestration. *Bioresource Technology*, *282*, 1–8.

Yu, Z., Leng, X., Zhao, S., Ji, J., Zhou, T., Khan, A., Kakde, A., Liu, P., & Li, X. (2018). A review on the applications of microbial electrolysis cells in anaerobic digestion. *Bioresource Technology*, *255*, 340–348.

Zabed, H. M., Qi, X., Yun, J., & Zhang, H. (2019). Anaerobic digestion of microalgae biomass for methane production. *Microalgae Biotechnology for Development of Biofuel and*

Wastewater Treatment, 397–421.

Zakaria, B. S., Lin, L., Chung, T., & Dhar, B. R. (2020). An overview of complementary microbial electrochemical technologies for advancing anaerobic digestion. *Advances in Bioenergy*, *5*, 129–167.

Zeppilli, M., Cristiani, L., Dell'Armi, E., & Majone, M. (2020). Bioelectromethanogenesis reaction in a tubular Microbial Electrolysis Cell (MEC) for biogas upgrading. *Renewable Energy*, *158*, 23–31.

Zhang, Y., & Banks, C. J. (2013). Impact of different particle size distributions on anaerobic digestion of the organic fraction of municipal solid waste. *Waste Management*, *33*(2), 297–307.

Zhang, Y., Li, C., Yuan, Z., Wang, R., Angelidaki, I., & Zhu, G. (2023). Syntrophy mechanism, microbial population, and process optimization for volatile fatty acids metabolism in anaerobic digestion. *Chemical Engineering Journal*, *452*, 139137.

Zhao, J., Hou, T., Lei, Z., Shimizu, K., & Zhang, Z. (2020). Effect of biogas recirculation strategy on biogas upgrading and process stability of anaerobic digestion of sewage sludge under slightly alkaline condition. *Bioresource Technology*, *308*, 123293.

Zhao, J., Li, Y., & Dong, R. (2021). Recent progress towards in-situ biogas upgrading technologies. *Science of The Total Environment*, *800*, 149667.

Zhu, G., Feng, Q., Wang, K., Song, Y.-C., Zhou, Y., & Zhou, Q. (2024). Investigating the performance of different applied voltages on lignite biomethanation in microbial electrolytic cell coupled anaerobic digestion. *International Journal of Hydrogen Energy*, *52*, 147–159.

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