PERFORMANCE EVALUATION OF PHOTOBIOREACTOR FOR MICROALGAE CULTIVATION AND ITS CODIGESTION FOR BIOGAS PRODUCTION

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BACHELORS OF ENGINEERING IN ENVIRONMENTAL ENGINEERING

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Approval Sheet

This is to certify that the contents and forms of thesis titled as "Performance Evaluation of Photobioreactor for Microalgae Cultivation and its Codigestion for Biogas Production" is the original work of author(s) and has been carried out under my direct supervision. I also certify that the thesis has been prepared under my supervision according to the prescribed format and I endorse its evaluation for the award of Bachelor of Engineering in Environmental Engineering Degree through the official procedure of the institute

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Abstract

Pakistan, being an under-developed country faces significant challenges in its energy sector with new power plant being constructed to address this issue. But the usage of fossil fuel in power plants is contributing to a significant emission of GHG gases into the atmosphere, specifically Carbon Dioxide (CO_2). The release of CO_2 is accelerating global warming and climate change that is adversely affecting the world-wide economic stability and social aspects. To mitigate these effects, research is being conducted to develop new solutions. One such solution lies through the intersection of technology and biology named algal Photobioreactor (PBR). The microalgae have extraordinary capabilities to sequester carbon dioxide (CO_2) in their cells. In this project, *Scenedesmus sp* was cultivated in bubble column type Photobioreactor supplied initially with 10% v/v CO_2 to replicate industrial flue gas concentration with supply period of 5 minutes at a flow rate of 5 L/min, 7 L/min, and 9 L/min. The flow rate of 5 L/min resulted in a significant increase in algal concentration by 305 mg/L in a few hours with the highest CO_2 fixation rate of 51.6 mg/L-hr. After harvesting from the PBR, the algae was co-digested with banana peels using cow manure as inoculum in an anaerobic digestor for biogas production. Cumulative production of biogas was 288 L/kgVS after just 21 days. The results of the study concluded that microalgae is a sustainable solution to sequester the CO_2 emitted from flue gas, mitigate its effect towards climate change, and be co-digested with suitable low nitrogen substrate as source of clean, green energy.

Keywords: CO_2 Fixation Rate, Sensor Integrated PBR, Anaerobic Codigestion, Climate Change, Greenhouse Gases

Chapter 1

Introduction

Pakistan, a southeast country is struggling with energy crisis and with time there is more demand of electricity and fossil fuels. This rising demand has disturbed Pakistan's energy infrastructure and framework which is leading towards power shortages. So, to fulfill the demand of citizens and industries, the government is increasing its dependency on fossil fuels such as coal. The burning of fossil fuels releases large amount of carbon dioxide (CO_2) along with other greenhouse gases GHGs into the atmosphere, leading towards climate change crisis in Pakistan. Pakistan is considered to be the 5^{th} most vulnerable country to climate change as a result it is facing changing weather patterns, rising temperatures, increased floodings. This urgent situation requires the country to adopt urgent sustainable and alternative solutions to mitigate climate change crisis. Furthermore, to mitigate this rising problem, the country needs to depend on renewable energy resources as they are present in abundance, produce minimal or no greenhouse gases, and enhance more economic growth creating more opportunities for the locals. The paper targets the challenges facing the clean energy sector, including industrial GHG emissions.

Renewable energy sources include solar energy, wind power, hydropower, bioenergy etc. Among these, bioenergy provides unique advantages over solar, wind and hydropower including continuous supply of energy (store biomass and use later phenomenon), making use of waste materials, and their ability to sequester carbon dioxide (CO_2). Bioenergy is further categorized into first generation bioenergy (originating from food crops), second generation bioenergy (originating from non-food crops), and third generation (originating from microalgae). The research focuses on third generation (microalgae based) as it stands out as the best option because of its sustainability. Microalgae comes under unicellular organisms, that have the capacity to convert the light energy coming from solar radiation or any other artificial source, to chemical energy.

There are two major microalgae cultivation techniques: Photobioreactor (PBR) and Open Ponds. Open ponds are exposed pools that are in direct contact with atmosphere conditions, but their efficiency is dependent upon environmental conditions which can hinder growth or exceed the

standards. The paper focused on the use of Photobioreactor as they are controlled growth systems, that have no interference from the environment, or direct exchange, which is beneficial in terms of zero contamination. PBR possesses proper regulation of pH, light supply, temperature, pressure, and nutrient supply which can optimize biomass growth and productivity. Photobioreactors are categorized into different geometries and shapes in accordance with their functions, for example tubular reactors, mainly for maximum space utilization, and light absorption. The paper focused on the potential use of Bubble column photobioreactor for microalgae cultivation. These are another type of vertical column photobioreactor in which microalgae is cultivated, the main advantage of this PBR is that it penetrates maximum light, enhanced mixing, efficient gas transfer, scalability and ease of operation. PBRs have the ability to operate in different modes, including batch mode and continuous mode. Batch mode involves fixed volume, no addition or removal during growth phase but for continuous mode it provides constant production.

Figure 1.1: Photobioreactor Based Microalgae Cultivation Technique

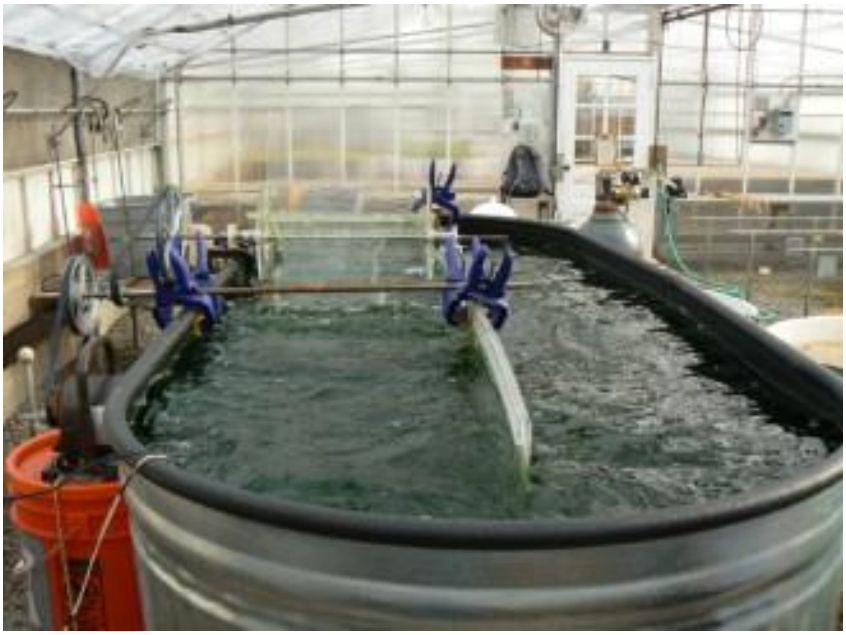

Figure 1.2: Open Pond Based Microalgae Cultivation

Cultivation of microalgae produces abundant biomass which can undergo different conversion techniques and produce a product that is valuable and sustainable alternative to the use of fossil fuels. The first conversion technique is thermochemical technique, that includes combustion, pyrolysis, gasification and liquification. This method can be used on big scale, results in reduced waste and multiple biproducts, but with its high capital and operational cost, emission of pollutants like carbon dioxide (CO_2), and energy requirement, they come at least priority for conversion technique as compared to other microalgal biomass conversion techniques. The second technique is physio-chemical technique, which includes mechanical extraction and esterification. Through this method, the understanding of complex systems is much better. This technique gives high precision and sensitivity but its equipment cost, usage of hazardous chemicals generate waste that is difficult to dispose. This makes it a less competitive technique because of these disadvantages that are not sustainable and environmentally friendly. This paper focuses on third technique: Biochemical microalgal biomass conversion technique which is further categorized fermentation and anaerobic digestion. This process converts organic matter present in microalgae to useful products like biogas and biofuels. It has high biomass productivity, low environmental impact as compared to other techniques.

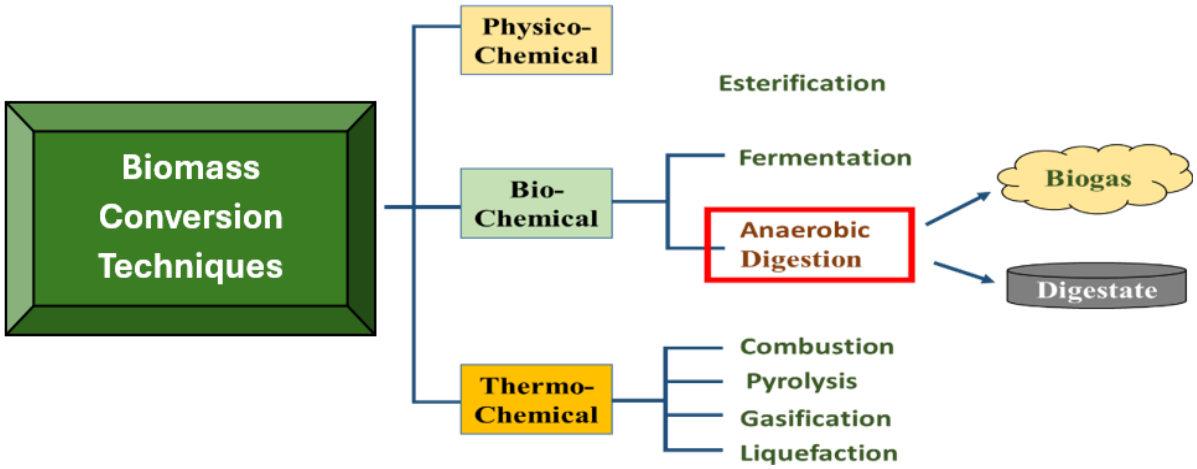

Figure 1.3: Biomass Conversion Techniques

Anerobic digestion is a biological method, that converts organic matter into microalgal biomass into biogas and digestate. Digestate is an organic rich nutrient that is left as a residue after anaerobic digestion process completion and can be used as a fertilizer. Biogas is a renewable energy source that has methane (CH_4) and carbon dioxide (CO_2) as its components. It can be used for electricity production, heat, and has a possibility to replace reliance upon fossil fuels. At homes, biogas can be beneficial for cooking, lightening, and be used for room heating. Anaerobic digestion offers scalability from household digestor to large industries. It provides large economic benefit if used properly, the process usage can cause decrease in the amount of food waste that is dumped into landfill.

The objectives of this research are:

1) To develop a Photobioreactor (PBR) for evaluation of microalgae growth and Carbon Dioxide (CO2) uptake
2) To produce biogas through Anaerobic Digestion (AD) of microalgae with suitable low Nitrogen (N) co-digestion material
3) To incorporate electronic sensors into PBR for evaluation of CO2 uptake rate in real time and analyze the removal efficiency of CO2 from industrial flue gas

The objectives of this research are straightforward in completing the goal of the study which included development of Photobioreactor which facilitates the growth of microalgae Scenedesmus Sp, and to measure the Carbon dioxide uptake rate. The second priority targeted

upon the completion of anaerobic digestion process through codigestion with banana peel to produce biogas that act as a clean renewable energy. The last was to install the electronic sensors like pH, temperature, pressure onto the photobioreactor to measure the carbon dioxide (CO_2) uptake rate by microalgae Scenedesmus Sp real time and to analyze how much of CO_2 can be removed from industrial flue gas, since majority of the industries in Pakistan emit CO_2 along with other gases into the atmosphere. The objectives align with the current and outgrowing problem of Pakistan that are climate change and energy crisis. With growing population and increasing day by day demand and the dependency on fossil fuels, it is necessary to shift to alternative sustainable solutions instead of relying on fossil fuels only. Relying on sustainable solutions not only gives environmental benefits but also economic and social benefits.

Chapter 2

Literature Review

According to (Makareviciene et al., 2013), *Scenedesmus sp*. microalgae is favorable for cultivation because of its usage in production of biomass, oil, growth under variable conditions. The focus is on production of biomass, in which microalgae was given optimum conditions for its growth like pH, temperature, pressure, sunlight and nutrient. Numerous research has also focused on the production of biofuels, under different cultivation methods, using autotrophic and mixotrophic growth. Cultivation of Scenedesmus microalgae offers other advantages like carbon dioxide mitigation in which *Scenedesmus sp* fix carbon dioxide (CO_2) from the atmosphere and reduce the overall level of carbon dioxide (CO_2) from the atmosphere, *Scenedesmus sp* can be used to treat wastewater, in which it absorbs nutrients and treat organic pollutants.

In (Egbo et al. (2018), photobioreactors are recognized as optimal system for the cultivation off microalgae, because they offer several advantages, they provide a controlled growth system providing optimal light supply, optimal temperature, optimal pressure and optimal pH leading to higher productivity, the design of Photobioreactors enhances the exposure of light into the walls of Photobioreactor. Studies have shown that controlled growth systems are the most efficient solution for microalgae growth since they lower the contamination chances. Their ability to provide conditions make them suitable for scalability, from small industries to big industries. PBRs have been shown to cultivate different strains of microalgae including microalgae.

In (Krishnamoorthy, R., Banat, F., & Show, P. L., 2022), it states that for cultivation of microalgae on large scale, it is necessary to monitor conditions like temperature, pH, frequently. There should be daily sampling to ensure consistent biomass production. This algae-water based biomass needs to be separated using different chemical, physical, electrical techniques which need to be cost effective as well.

In (Appl. Sci. 2022), it was proved that vertical column reactors like bubble column photoreactor provide advantages in microalgae cultivation. These vertical column photoreactor offer maximum light penetration. These vertical column photobioreactors produce biomass straight up to 6 months without any hindrances. This study's results concluded on a light intensity of 200 µmol photons m²/s, the optimum temperature maintained at a temperature of 25 C, pH was maintained at 7 which is considered an optimal. The vertical column PBR was able to produce biomass 0.6 g/L.

According to (Fettah, N., Derakhshandeh, M., Tezcan Un, U., & Mahmoudi, L., 2022), the growth of microalgae was observed under different light conditions including (100, 500, and 1000 $\mu mol.m^{-2}.s^{-1}$). Light intensity under 500 $\mu mol.m^{-2}.s^{-1}$was considered as the best optimal value for microalgae growth. Research shows that whenever light exceeded more than the optimum light conditions, in most cases, it caused inhibited growth and photodamage. A light cycle of 12h light and 12h dark was provided which kept a balance for microalgae growth.

According to (Difusa, A., Talukdar, J., Kalita, M. C., Mohanty, K., & Goud, V. V., 2015), pH played a vital role in in growth of microalgae Scenedesmus specie. Under this research it was concluded that pH below 5 and 6 did not increase cell density of microalgae and gave a lesser biomass productivity. A linear decline in density of cells of microalgae was seen in acidic environments. However, an alkaline condition over 9 were considered unfavorable, it showed a slow growth of microalgae. At alkaline range of pH 8, the growth of microalgae Scenedesmus gave the highest biomass productivity. Studies have shown that the optimum pH for microalgae growth is between 7 to 9.

Under (Zhang, Y., Ren, L., Chu, H., Zhou, X., Yao, T., & Zhang, Y., 2019), microalgae *Scenedesmus obliquus* acquired a maximum growth under light condition of 150 $\mu mol.m^{-2}.s^{-1}$, pH of 9, and temperature range of 25°C. Carbohydrate content fluctuated in accordance with the change in pH. At pH 5-6 the carbohydrate levels were low, but they increased with the increase in pH levels. Protein content shifted over 54% at a pH of 5-6, but it increased with the increase

in pH exponentially. Inexpensive and universal growth media for biomass production of microalgae. It focuses on comparing effects of different synthetic media on microalgal growth. It concluded that synthetic media proved to be a inexpensive medium for the growth of microalgae.

(Brettfeld, E.-G., Popa, D.-G., Moga, C.-I., Constantinescu-Aruxandei, D., & Oancea, F., 2023) supplied an elaborative comparison of 3 distinct freshwater cultivation media (BG-11, BBM, and Z8) for the growth of microalgae species. Z8 media proved to be an effective media for microalgae growth. Z8 medium appeared to be the most suitable for microalgae growth as it has a consistent performance, more biomass productivity and more versatility.

According to (Zeng, J., Wang, Z., & Chen, G., 2021), microalgae delve into different complex mechanisms to fix carbon dioxide (CO_2), it investigates the effect of light supply, temperature and carbon dioxide (CO_2) supply on microalgae growth, the research aims to optimize the carbon dioxide (CO_2) fixation rate by using optimum conditions. This study emphasizes the development of photoreactors for microalgae cultivation, and to reduce reuse by using the wastewater for microalgae growth instead of using large amount of water for microalgae cultivation. There should me a more detailed study on energy transformation in microalgae for understanding the fundamental laws of carbon fixation.

(Tamburic, B., Evenhuis, C. R., Crosswell, J. R., & Ralph, P. J., 2018) finds the use of empirical process and model to measure carbon fixation (CO_2) in photobioreactor, involving pH and dissolved oxygen (DO). The use of controlled conditions of temperature and light was provided to microalgae so there is maximum cell growth of microalgae. Some parameters were regularly measured that are dissolved oxygen (DO), pH, optical density (OD), total alkalinity (TA), and total carbon (TC) to assess biomass productivity and for carbon fixation rate. The findings that were published were that upon addition of carbon dioxide (CO_2) there was increase in the productivity of biomass while the pH and dissolved oxygen rates fluctuated upon addition of carbon dioxide (CO_2). Regularly carbon fixed was measured which shows an increase in cell density of microalgae.

(Gert-Jan Euverink june 5, 2024) aims to develop a system for microalgae cultivation and to measure carbon fixation (CO_2) rate using real time monitoring. The Arduino Mega system and Arduino based sensors are installed onto the photobioreactors to monitor the real time values of pH, pressure, temperature, and carbon dioxide (CO_2) gas concentration in the system. The carbon dioxide supply of 10% v/v showed an increase in biomass productivity from 15 grams of microalgae to 21.5 grams. The study found that there is a direct relation between carbon dioxide (CO_2) removal and algae growth rate.

According to Alarde et al. (2022), enhanced Biogas Production from the Anaerobic Batch Treatment of Banana Peels, this research provides an overview of using banana peel waste for sustainable energy approach by anaerobic digestion. The analysis highlights the effectiveness of co-digestion by examining the effects of organic loading and cow dung content on biogas production and VS removal.

(Ferreira, L. O., Astals, S., & Passos, F., 2022). Anaerobic co-digestion of food waste and microalgae in an integrated treatment plant. This study found that mono digestion of food waste alone produces low methane yields, however codigestion of microalgal biomass with food waste produces improved methane yields. In real life, more proportions of food waste are preferred due to their availability and less amount of microalgae is preferred to increase stability and increment in methane production.

Chapter 3

Methodology

3.1 Fabrication of Algal Photobioreactor

A lab scale acrylic algal photobioreactor of bubble column type was designed and fabricated. The reactor has a height (H) of 80 cm and internal diameter (I.D) of 11.5 cm with a total volume (V) of 12 L. This provides a high surface area to volume ratio (H/D = 7) which increases the penetration of light into the reactor. A reactor housing was developed with 3x fluorescent tube to provide luminescence. The reactor was air sealed to restrict leakage of carbon dioxide (CO_2) and provided with an air supply inlet with 2x bubble spargers at the bottom. The reactor also has a 1x liquid discharge valve, 1x gas outlet valve, 1x sampling valve, and 2x gas recycling valves.

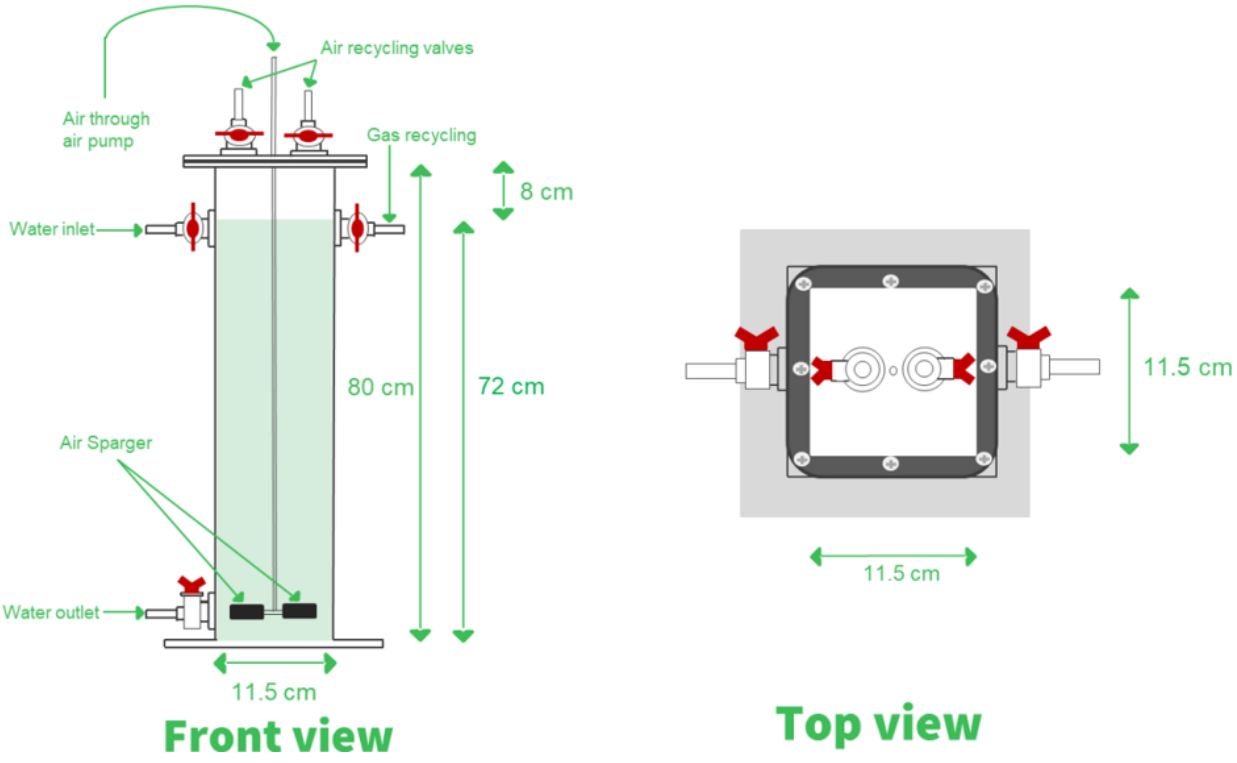

Figure 3.1: Engineering Drawing of Bubble Column Photobioreactor

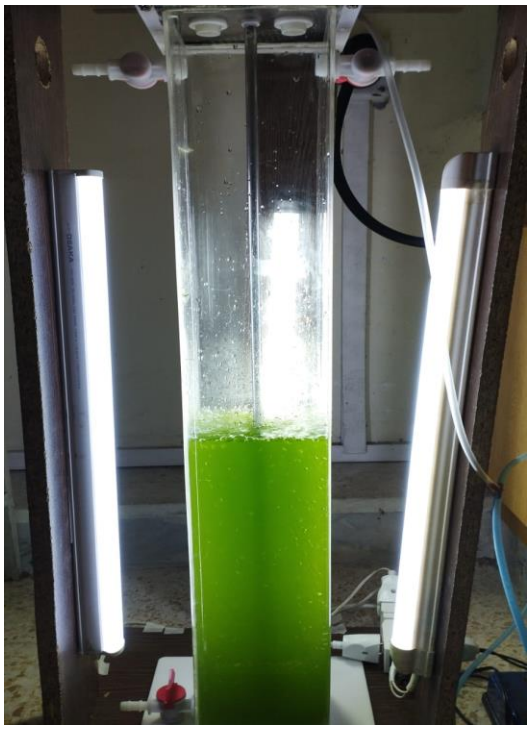

Figure 3.2: Bubble Column Algal Photobioreactor Setup

3.2 Fabrication of Anaerobic Digestor

A lab scale acrylic anerobic digestor was fabricated. The sphere shape was selected to reduce heat loss and improve mixing inside the digestor. The digestor has a total volume (V) of 4 L. The reactor was provided with a 100 mL syringe for gas measurements, and 1x ball valve for biogas collection. A positive pressure test was performed using a N_2 gas cylinder to ensure that the reactor was sealed, and no leakages were detected. The reactor was placed inside a water bath with a submersible water heater to maintain temperature throughout the experimental process.

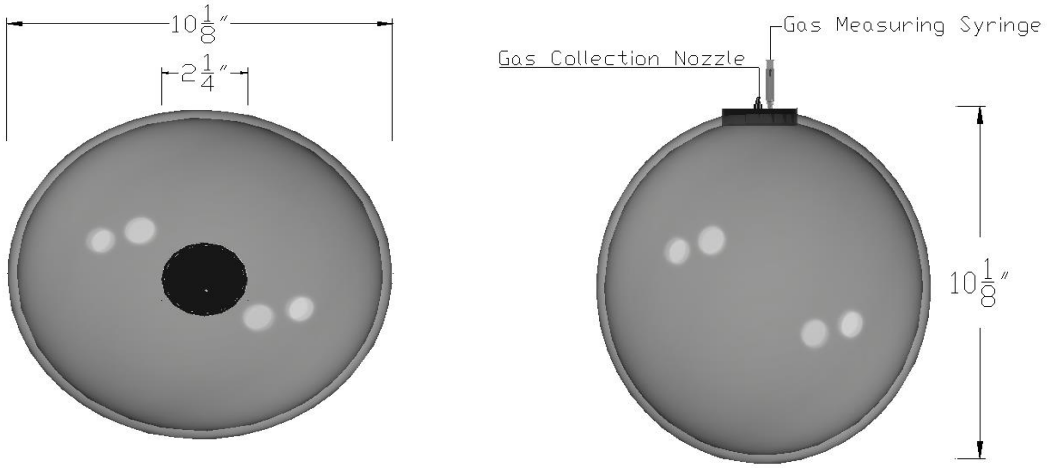

Figure 3.3: Engineering Drawing of Anaerobic Digestor

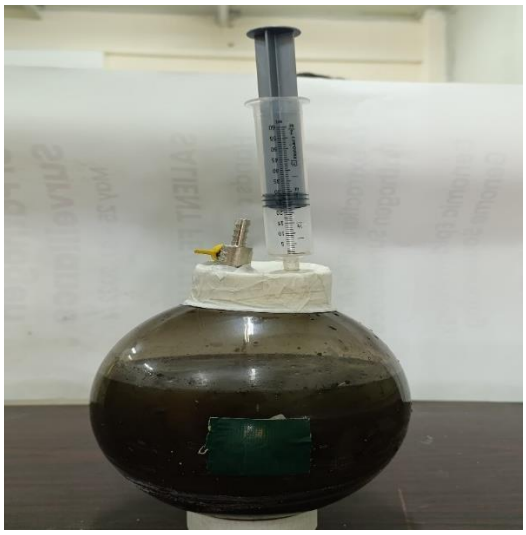

Figure 3.4: Anaerobic Digestor Setup

3.3 Media Preparation

Z8 media was selected and prepared to culture the microalgae. The media contained a mixture of 4 stocks solution i.e. stock solution 1, stock solution 2, stock Solution 3, and stock solution 4. Stock solution 1 was prepared by dissolving 46.7 g $NaNO_3$, 5.9 g $Ca(NO_2)_2.4H_2O$, 2.5g $MgSO_4.7H_2O$ in 1000 mL distilled water. Stock solution 2 was prepared by dissolving 3.1g K_2HPO_4, 2.1 g Na_2CO_3 in 1000 mL distilled water. Stock solution 3 was prepared by mixing 10 ml Fe solution and 9 ml EDTA solution in 1000 mL

distilled water. Fe solution was prepared by dissolving 2.6 $FeCl_3.6H_2O$ in 100 mL 0.1N HCL and EDTA solution by dissolving 3.9 g $Na_2EDTA.2H_2O$ in 100 mL 0.1 N NaOH. Stock solution 4 was prepared by mixing 0.330g $Na_2WO_4.2H_2O$, 0.880g $(NH_4)_6Mo_7O_{24}.4H_2O$, 1.2 g KBr, 0.830 g KI, 2.87g $ZnSO_4.7H_2O$, 1.54g $Cd(NO_3)_2.4H_2O$, 1.46g $Co(NO_3)_2.6H_2O$, 1.25g $CuSO_4.5H_2$, 1.98g $NiSO_4(NH_4)_2SO_4.6H_20$, 0.410g $Cr(NO_3)_3.9H_2O$, 0.089g V_2O_5, 4.74g $Al_2(SO_4)_3K_2SO_4.24H_2O$, 3.1 g H_3BO_3, and 2.23g $MnSO_4.4H_2O$ in 1000 mL distilled water. In 1000 mL distilled water, 10 mL stock solution 1, 10 mL stock solution 2, 10 mL stock solution 3, and 1 mL stock solution was dissolved to prepare the Z8 media. The media was sterilized in an autoclave for 3 hours to remove any contaminants.

3.4 Preparation of Microalgae Culture

The microalgae specie *Scenedesmus sp.* was selected for the study because of its high CO_2 fixation rate, increased growth rate, tolerance to high CO_2 concentrations, and biodegradability. The algae was transferred in an Erlenmeyer flask with 1 L Z8 media and was provided with filtered CO_2 air supply. It was provided with 12H : 12H light dark cycle and allowed to reach a high concentration of 10 g/L before being transferred to the bubble column photobioreactor.

Figure 3.5: Initial Culturing of Scendesmus Sp. in Z8 Media

3.5 Development of Sensor Circuit

The photobioreactor was integrated with multiple sensors to monitor the culturing conditions and growth of microalgae inside the reactor. The sensor provided real time data to analyze the reactor conditions, study the CO_2 fixation rate of microalgae, and make appropriate changes to the culturing conditions to optimize the microalgae growth. The reactor was integrated with pH probe (E 201 C pH sensor with BNC connector), water temperature sensor (DS18B20 waterproof probe), pressure sensor (BMP180 module), and NDIR CO_2 sensor (MH-Z16 10% v/v sensor). The pH sensor, pressure sensor, and temperature were installed inside the reactor. The CO_2 sensor was installed to the gas outlet valve of the reactor. The sensors were linked with microcontroller board (Arduino Mega 2560) to collect, analyze, and display the reading of the sensor in real time mode. The sensor system was powered using a 12 V DC power supply. A feedback system was also installed consisting of 5V mini submersible water pump placed in an acid solution to adjust the reactor pH to optimal range of 6.5-8.5. The pump was connected to using a DC relay (5V 1 Channel 240VAC Relay Module). The code was for the system was developed on C/C++ language in Arduino IDE and the sensor data was collected by serial communication software CoolTerm.

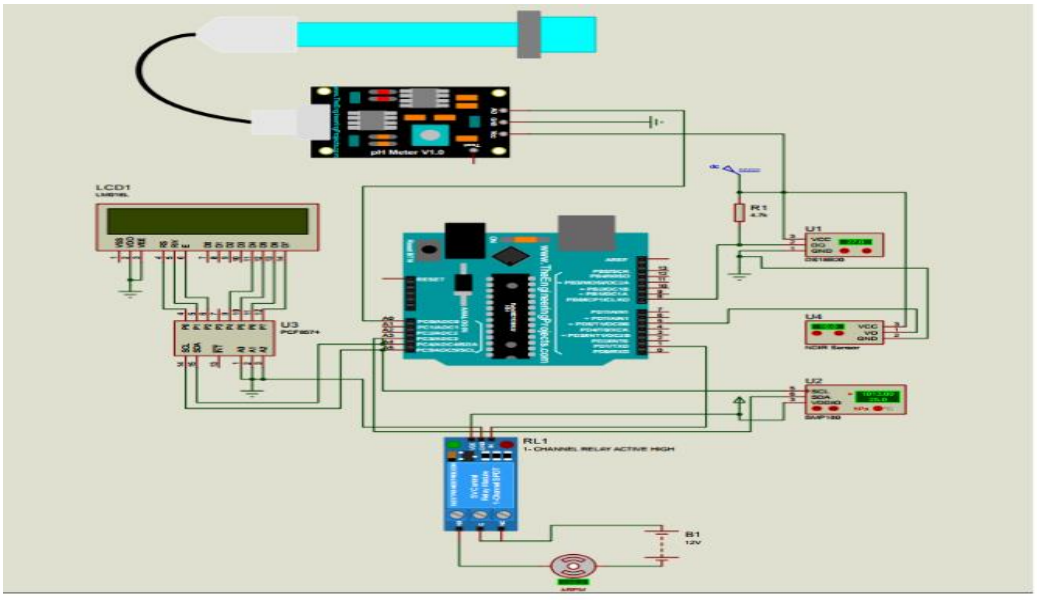

Figure 3.6: PBR Sensor Circuit Diagram

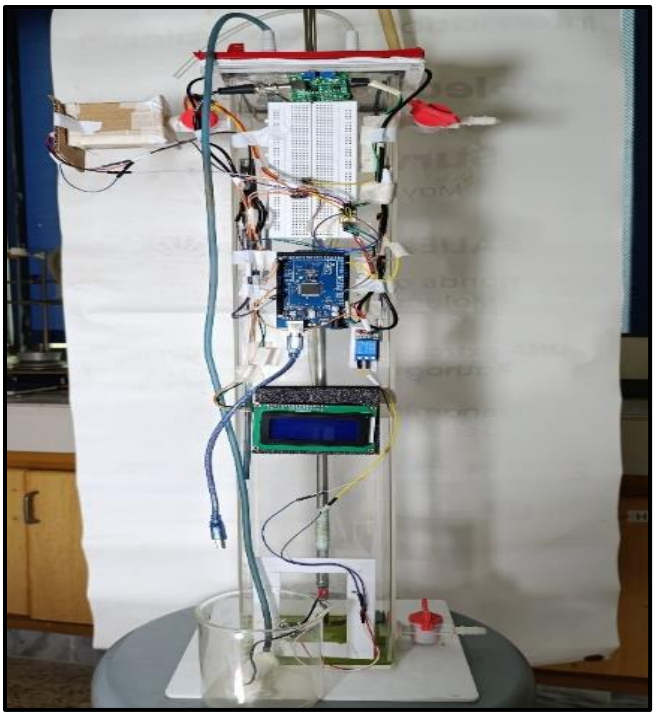

Figure 3.7: Sensor Integrated PBR for Real Time Monitoring

3.6 Photobioreactor Experimental Design

The following experimental design was developed to study the effect of various carbon dioxide (CO_2) flow rates on microalgae growth and culturing conditions.

Experimental Condition 1	Experimental Condition 2	Experimental Condition 3
CO_2 Flow Rate = 5 L/min	**CO_2 Flow Rate =** 7 L/min	**CO_2 Flow Rate =** 9 L/min
Supply Time = 5 mins	**Supply Time =** 5 mins	**Supply Time =** 5 mins

Figure 3.8: PBR Experimental Conditions

3.6.1 Condition 1 Operation Configuration

- CO_2 Flow Rate = 5 L/minute
- Supply Time = 5 minutes
- Gas Supply Method = 10% v/v CO_2 Cylinder
- Operation Mode = Batch Mode
- Initial pH = 7.5

- Initial Algal Concentration = 2.32 g/L
- Light Intensity = 100 $\mu mol.m^{-2}.s^{-1}$
- Light Cycle = 12H Light:12H Dark

3.6.2 Condition 2 Operation Configuration

- CO_2 Flow Rate = 7 L/minute
- Supply Time = 5 minutes
- Gas Supply Method = 10% v/v CO_2 Cylinder
- Operation Mode = Batch Mode
- Initial pH = 7.5
- Initial Algal Concentration = 2.32 g/L
- Light Intensity = 100 $\mu mol.m^{-2}.s^{-1}$
- Light Cycle = 12H Light:12H Dark

3.6.3 Condition 3 Operation Configuration

- CO_2 Flow Rate = 9 L/minute
- Supply Time = 5 minutes
- Gas Supply Method = 10% v/v CO_2 Cylinder
- Operation Mode = Batch Mode
- Initial pH = 7.5
- Initial Algal Concentration = 2.32 g/L
- Light Intensity = 100 $\mu mol.m^{-2}.s^{-1}$
- Light Cycle = 12H Light:12H Dark

3.7 Microalgae Growth Monitoring and Characterization:

The microalgae growth was monitored through spectrophotometric analysis using UV-VIS spectrophotometer (Thermo Scientific Helios Omega UV-VIS Spectrophotometer). A calibration curve was developed by preparing known concentrations of microalgae and measuring the absorption values. A linear equation was developed to estimate unknown concentrations of microalgae using absorption values. The values were taken at 680 nm wavelength using distilled water as reference. Characterization of microalgae was also done through analysis of its total solids (TS), volatile solids (VS), moisture content (MC),

and total carbon (TC). The total solids (TS) and moisture content (MC) were measured through the standard method 2450 B, volatile solids (VS) and total carbon (TC) through standard method 2540 E.

3.8 Microalgae Harvesting

The microalgae was allowed to sequester CO_2 supplied through the CO_2 cylinder through its growth period. Once the growth cycle was completed, the algae was harvested from the reactor using two different methods i.e. gravity-based filtration through cotton cloth and water pump filtration. The microalgae was collected onto the cotton cloth and clear water passed through the cloth. The algae was removed from the cloth and placed inside a container store at 4^{o}C in a refrigerator for further use.

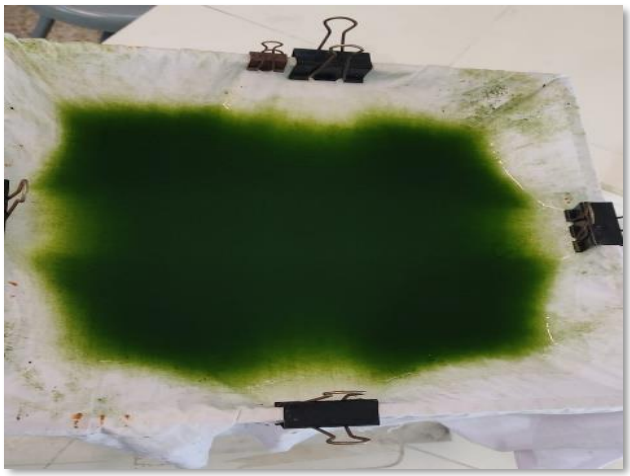

Figure 3.9: Harvesting of Microalgae through Filtration

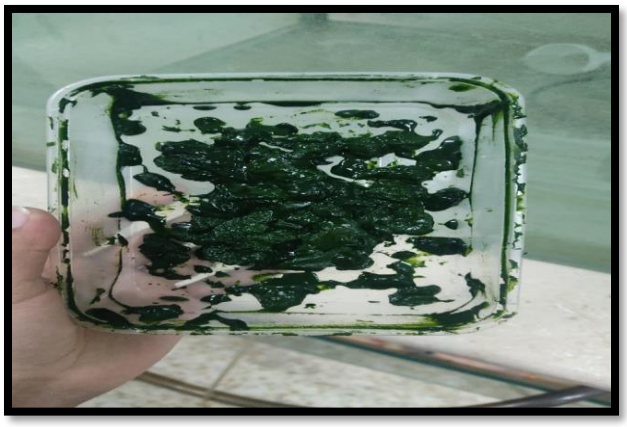

Figure 3.10: Harvest Microalgae Stored in Container

3.9 Substrate-Inoculum Characterization

The microalgae have a low carbon and high nitrogen content (C:N ratio = 7) for anaerobic digestion. The high nitrogen content indicates that ammonia (NH_3) inhibition can affect anaerobic digestion and biogas production. Banana peels were chosen as co-substrate to adjust the CN ratio to a range of 25-30 for anaerobic digestion. The banana peels typically have C:N of 31 translating to high carbon content in their structure. The banana peel underwent physical pretreatment and reduced their size (<1 cm) for improve degradation. Then peels were tested for their total solid (TS), and moisture content (MC) through standard method 2450B while the volatile solids (VS) were analyzed through standard method 2540E.

Figure 3.11: Microalgae after Total Solids (TS), and Volatile Solids (VS) Assessment

Figure 3.12: Homogenous Mixture of Banana Peels

Figure 3.13: Banana Peels after Total Solids (TS), and Volatile Solids (VS) Assessment

The inoculum that was selected for the study was cow manure as it is easily available and contains the necessary microbes required for anaerobic digestion. The cow manure was degassed over a period of 30 days to degrade its organic matter and reduce the production of biogas. The manure was characterized through total solids (TS), volatile solids (VS), total alkalinity (TA), Volatile Fatty Acids (VFA), VFA to Alkalinity ratio, and pH measurements.

Figure 3.14: Digested Anaerobic Digestion Inoculum

Figure 3.15: Inoculum after Total Solids (TS), and Volatile Solids (VS) Assessment

3.10 Conditions for Anaerobic Digestion

The anaerobic digestion was performed using a combination of 50% microalgae and 50% banana peel on volatile solids (VS) basis. This combination adjusted the C:N ratio to range between 25-30 required for anerobic digestion. The anaerobic reactor was operated with an organic loading rate (OLR) of 2gVS/L. The substrate to inoculum (S:1) ratio of 1:2 was selected to increase the rate of digestion and biogas production. The reaction was performed in mesophilic temperature condition at 35^{o}C using water bath heating system. This temperature was selected for efficient degradation process and increased biogas production rate. The reaction was performed for a period of 21 days. The digestion mixture was injected into the reactor, and it was sealed to ensure no oxygen (O_2) leaked into the reactor to maintain anaerobic conditions. The reactor was purged with nitrogen (N_2) gas

to remove oxygen molecules from the sealed reactor and increase the rate of anerobic conditions development.

3.11 Collection and Measurement of Biogas

The degradation of microalgae and banana peels by the anaerobic microbes resulted in production of biogas inside the anaerobic reactor. The biogas measurements were taken regularly after 24-hour intervals. The gas production was measured through the 100 mL syringe installed on the reactor. The reactor was thoroughly mixed through manual shaking to ensure homogenous regime inside.

Chapter 4

Results and Discussion

4.1 Results

4.1.1 Effect of CO_2 on pH and Algal Concentration

The effects of CO_2 towards algal concentration, solution pH, and outlet CO_2 were analyzed at the flow rate of 5 L/min, 7 L/min, and 9 L/min with respect to time over the 15-hour experimental period. The results of these experiments are provided in the table below

Table 4.1: PBR Experimental Condition 1 and effect on Algal Concentration, pH Value, and Outlet CO_2 Concentration

Photobioreactor Condition 1			
Run Time (Hr)	**Algal Concentration (mg/L)**	**pH Value**	**Outlet CO2 Concentration (ppm)**
0	2320	6.1	100,000
3	2360	6.4	81,297
6	2440	6.76	65,901
9	2492	6.98	48,300
12	2554	7.25	31,251
15	2625	7.45	14,201

Table 4.2: PBR Experimental Condition 2 and effect on Algal Concentration, pH Value, and Outlet CO_2 Concentration

Photobioreactor Condition 2			
Run Time (Hr)	**Algal Concentration (mg/L)**	**pH Value**	**Outlet CO2 Concentration (ppm)**
0	2405	5.9	100,000
3	2503	6.2	86,306
6	2523	6.43	68,604
9	2595	6.91	53,574
12	2653	7.3	37,876
15	2712	7.51	22,178

Table 4.3: PBR Experimental Condition 3 and effect on Algal Concentration, pH Value, and Outlet CO_2 Concentration

Photobioreactor Condition 3			
Run Time (Hr)	**Algal Concentration (mg/L)**	**pH Value**	**Outlet CO2 Concentration (ppm)**
0	2381	5.8	100,000
3	2409	5.95	88,753
6	2420	6.0	80,123
9	2428	6.38	76,783
12	2433	6.75	72,576
15	2438	6.94	70,756

Table 4.4: Comparison of Algal Concentration Increase and CO_2 Concentration Reduction between PBR Experimental Conditions

Parameter	**Condition 1**	**Condition 2**	**Condition 3**
Algal Concentration Gain (mg/L)	305	307	57
Percentage Increase in Algal Concentration (%)	13.14	12.7	3.29
Absorbed CO_2 Concentration (ppm)	85,799	77,822	29,244
Percentage Decrease in CO_2 Concentration (%)	-85.8	-77.8	-29.2

The comparison between Table 4.5, Table 4.6, and Table 4.7 suggests that experimental condition 2 at 7 L/min demonstrated an increase in microalgae concentration from 2405 mg/L at the beginning of the experiment to almost 2712 mg/L just after 15 hours resulting in a net gain of almost 307 mg/L. This indicates presence of ideal growth conditions likely due to optimal flow of CO_2 into the photobioreactor, nutrient rich environment, and good lighting conditions. The algal concentration for experimental condition 2 showed an increase from an initial 2320 mg/L to almost 2625 mg/L during the same time period of

15 hours. This resulted in a net gain of almost 305 mg/L which is equivalent to the algal concentration increase observed in condition 2. For experimental condition 3, the algal concentration at the start of the experiment was 2381 mg/L but displayed a small biomass increase of almost 2438 mg/L at the end of the experiment. This equaled a net gain of just 57 mg/L in 15 hours, extremely low as compared to the net gain observed in condition 1 and 2. Condition 2 demonstrated the highest biomass increase followed by condition 1 and condition 3.

PBR operating at CO_2 flowrate of 5 L/min initially experienced a pH drop to almost 6.1 (acidic) from set point of 7.5 (neutral) due to supply of CO_2 into the reactor as represented in Figure 4.1. As the experimental process proceeded, the microalgae began to consume and assimilate the CO_2 supplied to it into its cell during its growth phase releasing oxygen (O_2). The pH began to increase and showed a positive trend from 6.1 to almost 7.45 returning to its original set point. For the flow rate of 7 L/min, the pH dropped to almost 5.9 (acidic) when CO_2 was supplied, this drop is much higher than observed at condition 1 as represented in Figure 4.2. The pH returned to its set point 7.51 (neutral) at the end of experiment as the microalgae observed the CO_2. For the experimental condition 3, the pH dropped to 5.8, much lower than observed in condition 1 and 2 but only increased to 6.94 failing to return to the original set point of 7.5. This suggests that PBR operating at a flow rate of 7 L/min has greater buffering ability as compared to other conditions while according to Figure 4.3, conditions 3 displayed least buffering capability indicating that microalgae was unable to adapt the conditions inside the reactor.

The outlet CO_2 concentration for experimental condition 1, 2, and 3 dropped from 100,000 ppm at the beginning of the PBR operation to 14,201 ppm, 22178 ppm, and 29,244 ppm respectively at the end of the experiment. PBR condition 1 demonstrated the highest CO_2 absorption efficiency, displaying a CO_2 reduction of 85,799 ppm in 15 hours. This was followed by condition 2, where the microalgae absorbed almost 77,822 ppm CO_2 during its experimental run. The lowest CO_2 absorption efficiency was observed in experimental condition 3 with a CO_2 reduction of just 29,244 ppm in 15 hours. This suggests that

microalgae at low flow rate has of has a higher capability to absorb and reduce CO_2 concentration and as flow rate increase this capability is highly affected due to large flow conditions.

Table 4.4: Comparison of Algal Concentration Increase and CO_2 Concentration Reduction between PBR Experimental Conditions demonstrates that the microalgae cultivation under condition 1 provided the highest percentage increase in algal concentrations and maximum percentage reduction in CO_2 concentrations of 13.14% and 85.8% respectively. This was followed by condition 2 with an increase of 12.7% and 77.8% reduction in CO_2 concentrations. Conditions 3 demonstrated lowest performance in both parameters.

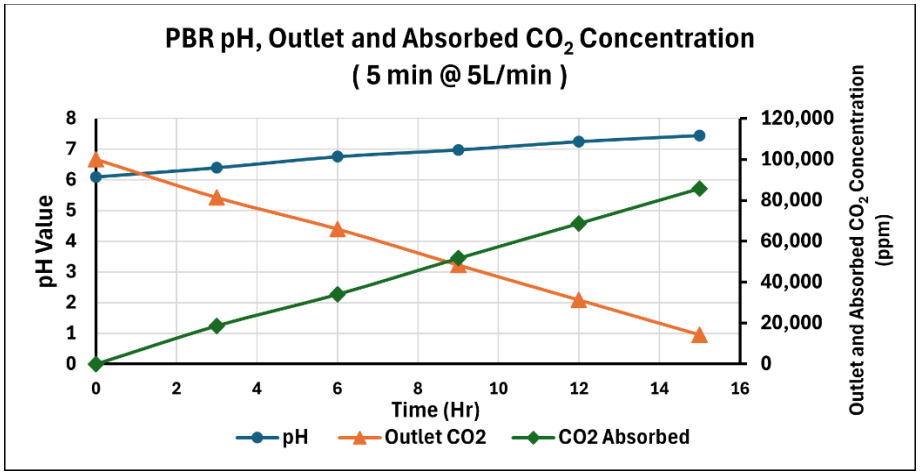

Figure 4.1: Effect of CO_2 on pH, Outlet, Absorbed CO_2 at Condition 1

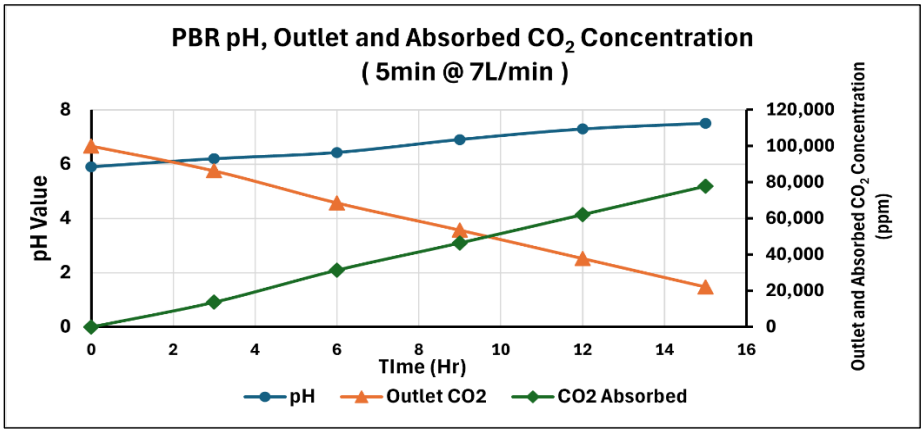

Figure 4.2: Effect of CO_2 on pH, Outlet, Absorbed CO_2 at Condition 2

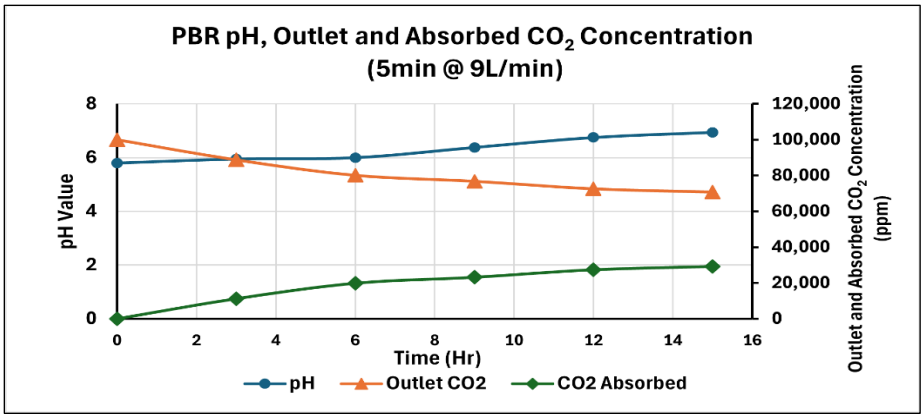

Figure 4.3: Effect of CO_2 on pH, Outlet, Absorbed CO_2 at Condition 3

4.1.2 Effect of CO_2 on Carbon Dioxide (CO_2) Fixation Rate

The effects of CO_2 towards algal concentration, and CO_2 fixation rate were also analyzed at the flow rate of 5 L/min, 7 L/min, and 9 L/min with respect to time over the 15-hour experimental period. The results of these experiments are provided in the table below

Table 4.5: CO_2 Fixation Rates and Algal Concentrations for PBR Condition 1

Photobioreactor Condition 1		
Run Time (Hr)	**Algal Concentration (mg/L)**	**CO2 Fixation Rate (mg/L-hr)**
0	2320	0
3	2360	11.2
6	2440	20.5
9	2492	31.1
12	2554	41.3
15	2625	51.6

Table 4.6: CO_2 Fixation Rates and Algal Concentrations for PBR Condition 2

Photobioreactor Condition 2		
Run Time (Hr)	**Algal Concentration (mg/L)**	**CO2 Fixation Rate (mg/L-hr)**
0	2405	0
3	2503	8.2
6	2523	18.9
9	2595	27.9
12	2653	37.4
15	2712	46.8

Table 4.7: CO_2 Fixation Rates and Algal Concentrations for PBR Condition 3

Photobioreactor Condition 3		
Run Time (Hr)	**Algal Concentration (mg/L)**	**CO2 Fixation Rate (mg/L-hr)**
0	2381	0
3	2409	6.8
6	2420	12.0
9	2428	14.0
12	2433	16.5
15	2438	17.6

The Table 4.5 summarizes the experimental condition 1 at 5 L/min and demonstrated a higher fixation ability resulting in an increase in CO_2 fixation rate to almost 51.6 mg/L/hr. This higher fixation rate is due to the higher concentration of CO_2 that was absorbed by the microalgae in experimental condition 1 where the microalgae absorbed 85,799 ppm CO_2. Condition 1 was followed by experimental condition 2 demonstrating a CO_2 fixation rate of 46.8 mg/L/hr at the end of the experimental run as provided in Table 4.6. In Table 4.7 summarizing experimental condition 3, it` demonstrated the lowest CO_2 fixation rate of 17.6 mg/L/hr. This low fixation rate is due to the fact that at the higher flow rate of 9 L/min, the microalgae was unable to absorb a large quantity of CO_2 supplied into the reactor, absorbing only 29,244 ppm CO_2. The higher fixation rate observed at flow rates

of 5 L/min and 7 L/min allowed microalgae to assimilate more CO_2 in its cell and res ulted in higher biomass concentration increase as compared to high flow rate of 9 L/min. Figure 4.4, Figure 4.5, and Figure 4.6 provides visual representation regarding the changes in measured parameters over the experimental time frame. Figure 4.1 represents nearly linear relation between algal concentrations increase and CO_2 fixation rate increasing over time. Figure 4.5 show an initial rapid increase in algal concentrations with linear increase in CO_2 fixation rate while Figure 4.6 suggests that after 6 hours, the increase in algal concentrations and CO_2 fixation rate are negligible.

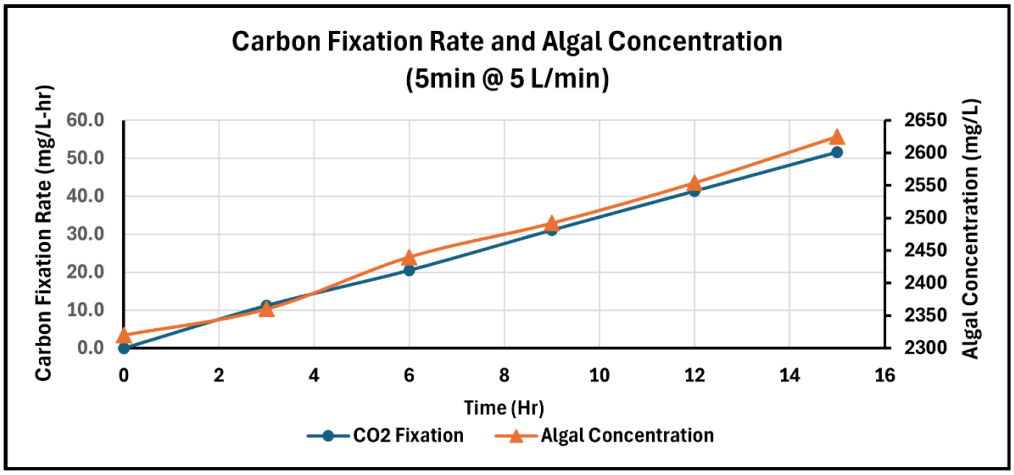

Figure 4.4: PBR Condition 1 CO_2 Fixation Rate

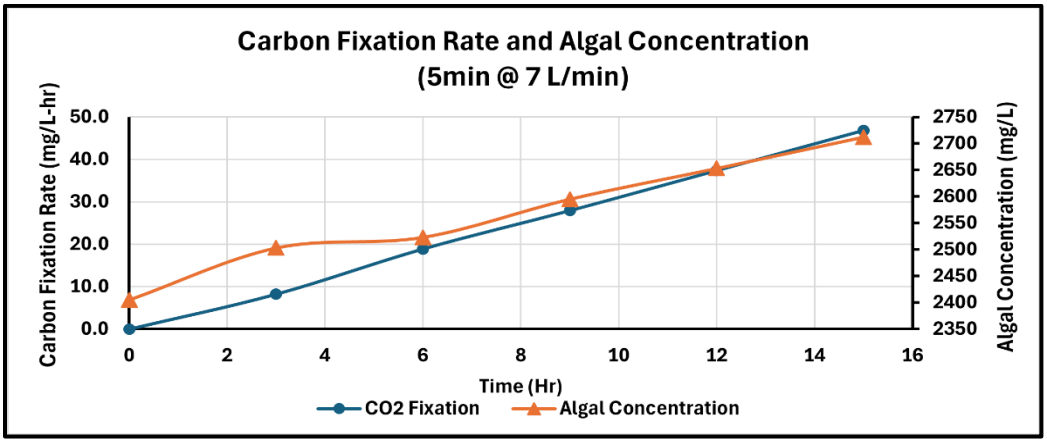

Figure 4.5: PBR Condition 2 CO_2 Fixation Rate

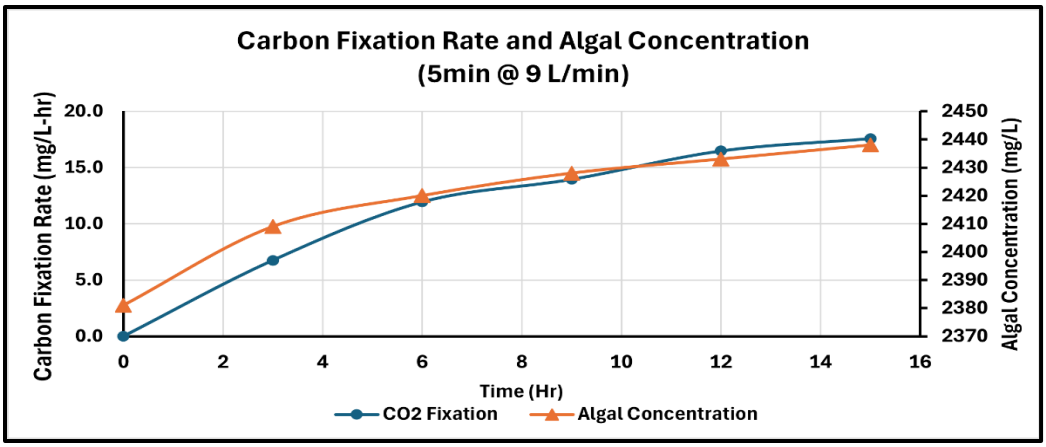

Figure 4.6: PBR Condition 3 CO_2 Fixation Rate

4.1.3 Substrate and Inoculum Characterization

The microalgae, banana peel, and cow manure were characterized based on their total solids (TS), Volatile Solids (VS), Moisture Content (MC), pH, Total Alkalinity (TA), and Volatile Fatty Acids (VFA). The results of these experiments have been provided below

Table 4.8: Microalgae Parameter Characterization

Microalgae Characterization	
Parameter	**Value**
Total Solids (TS) (%)	20.33
Volatile Solids (VS) (%TS)	81.41
Total Carbon (TC) (%TS) (%)	45.23
Moisture Content (MC) (%)	79.60

Table 4.9: Banana Peel Parameter Characterization

Banana Peels Characterization	
Parameter	**Value**
Total Solids (TS) (%)	31.08
Volatile Solids (VS) (%TS)	55.70
Moisture Content (MC) (%)	68.92

Table 4.10: Inoculum Parameter Characterization

Cow Manure Characterization	
Parameter	**Value**
Total Solids (TS) (%)	22.78
Volatile Solids (VS) (%TS)	87.70
Moisture Content (MC) (%)	79.60
Volatile Fatty Acid (VFA) (mg/L)	1098
Total Alkalinity (TA) (mg/L)	4650
VFA/Alkalinity Ratio	0.236
pH	7.5

Table 4.1 summarizes that the microalgae total solids content is almost 20.33% which is much lower than banana peels and cow manure, indicating high moisture content relative to its dry matter. In contrast in Table 4.9, the banana peels have a significantly high total solids (TS) content at 31.08% which is significantly higher than cow manure and microalgae. This indicates that banana peels contain relatively low moisture than microalgae. Cow manure has a total solids content at 22.78% shown in Table 4.10 which is in between that of microalgae and banana peel. In terms of volatile solids (VS), cow manure has the highest percentage at 87.7% of its total solids (TS) concentration indicating a higher level of solids that can be utilized for anaerobic digestion. Microalgae and banana peels have volatile solids (VS) content of almost 81.41% and 55.7% respectively. This indicates that the total amount of solids in microalgae are volatile in nature as compared to those of banana peels which has the least amount of volatile solids (VS) composition in its structure. Furthermore, the moisture content (MC) of microalgae, banana peel, and cow manure are 79.6%, 68.92%, and 79.6% respectively. This indicates that banana peels are drier in nature than microalgae and cow manure. The cow manure was further characterized to assess its potential as an inoculum for anaerobic digestion. The pH of cow manure is 7.5 indicating that it is neutral and neither acidic nor alkaline in nature. The volatile fatty acids (VFA) and total alkalinity (TA) were also calculated to be 1098 mg/L and 4650 mg/L respectively. This indicates that the VFA-Alkalinity ratio was 0.236 which is less than 0.4 necessary for optimal anaerobic digestion. This would allow the digestion to buffer pH changes and remain stable.

4.1.4 Production of Biogas through Anaerobic Digestion

The daily biogas production had been measured in the anerobic digestor along with the calculation of cumulative and specific cumulative biogas production. The results of these measurements have been provided below.

Table 4.11: Production of Daily, Cumulative, and Specific Cumulative Biogas Production from Anaerobic Digestion

Run Day (d)	Daily Biogas Production (mL)	Cumulative Biogas Production (mL)	Specific Cumulative Biogas Production (mL/gVS)
1	82	82	14
2	93	175	29
3	95	270	45
4	75	345	58
5	99	444	74
6	97	541	90
7	100	641	107
8	96	737	123
9	94	831	139
10	90	921	154
11	92	1013	169
12	89	1102	184
13	82	1184	197
14	84	1268	211
15	77	1345	224
16	75	1420	237
17	71	1491	249
18	63	1554	259
19	60	1614	269
20	57	1671	279
21	58	1729	288

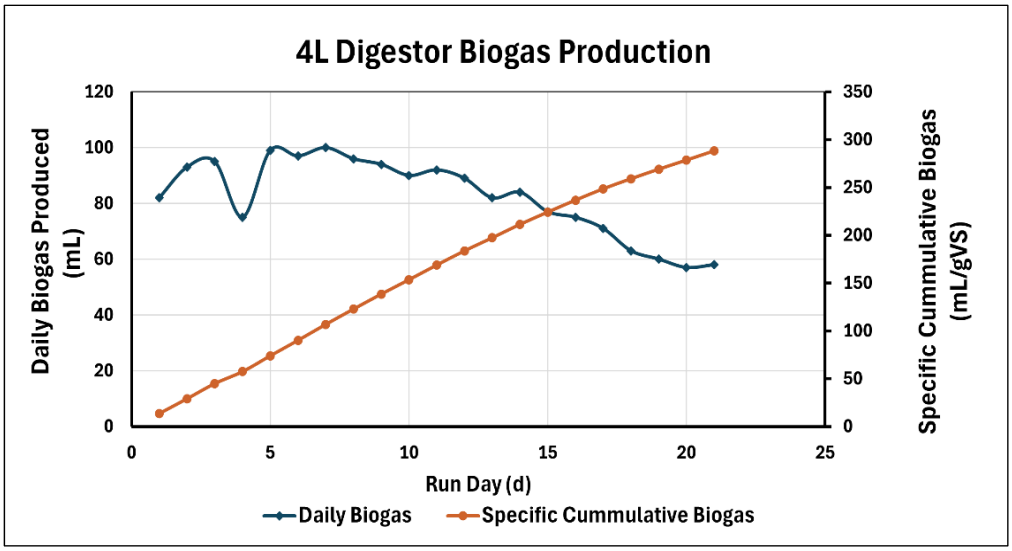

Figure 4.7: Production of Biogas from Anaerobic Digestion

According to Table 4.11, initially, the daily biogas production due to anaerobic digestion was measured to be 82 mL on the first day. The specific cumulative biogas production was calculated from the daily biogas production and was measured to 14 mL/gVS on day 1. The production of biogas began to gradually increase, reaching almost 100 mL by day 7 as show in Figure 4.7. This is the highest biogas production that was measured over the degradation experiment. The specific cumulative biogas production was measured to be almost 107 mL/gVS. As the process continued and the anaerobic bacteria further degraded the microalgae and the banana peels, the daily biogas production began to reduce. The biogas production each day was higher as compared to previous day until the peak production was reached. After that the biogas production was lower each day due to less amount of available material to be degraded. The daily biogas production was almost 58 mL by the experiment day 21 much lower compared to the initial experimental days. At the end of the degradation process, the cumulative biogas was calculated to be almost 1729 mL while the specific cumulative biogas production was measured to be almost 288 mL/gVS as provided in Table 4.11. Since it is estimated that biogas production contains more than 50% methane gas (CH_4), it is estimated that almost 865 mL of

methane (CH_4) was produced from the degradation of microalgae and banana peel with cow manure.

4.2 Discussion

During photosynthesis, algae incorporate CO_2 as they utilize sunlight to combine CO_2 and water into new chemical compounds from a green colored substance referred to as chlorophyll as well as other pigments. When the energy from the sunlight is being absorbed in the water the water molecule is split into oxygen, protons, and electrons. This process takes place at the thylakoid membrane of the chloroplasts. In the carbon fixation process, these electrons and protons help in fixing CO2 into glucose through the process Callvin cycle which occurs in the stroma of chloroplast.

$$6CO2+6H2O+light\ energy \rightarrow C6H12O6 +6O2$$

Here there is reduction of the CO_2 into organic carbon compounds which serves as the foundation of the algae body.

In Water Absorption, the uptake of the water occurs through a process that involves the cell membrane of the algae cells. Besides that, it is involved in photosynthesis, setting the pressure of cells, as well as distribution of nutrients in the cells.

Carbon fixation efficiency was evaluated at three different CO_2 flow rates. The flow rate is set at 5 L/min, 7 L/min, and 9 L/min. Another advantage of algae is in the matter of carbon fixing, which is a key advantage in developing renewable energy and fostering sustainable environment. During photosynthesis, algae use CO_2 to produce their body tissues, and since the process involves the use of light energy, it makes the plant lightweight. When with regard to the results obtained in the present study, it was observed that optimum biomass yield of Scenedesmus sp. Was used for determination of the fixation efficiency of CO2 for all flow rates applied in the experiments but exhibited distinctions depending on the rate of flow.

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for determination of the fixation efficiency of CO_2 for all flow rates applied in the experiments but exhibited distinctions depending on the rate of flow.

(a) At 5 L/min: The fixation rate was at the highest at 51 when the money was set flowing rate of CO_2 in L/min equal to 5. At a concentration of 6 mg/L-hr, the rate of ammonia removal was the highest among all the provided rates. This, in a way, suggests enhanced capability of the algae to absorb and use CO_2 in its photosynthetic processes. This flow rate enabled the stable regulation of pH and CO_2 uptake in the system, which resulted in optimal biomass and carbon fixation rate as noted in the study.

(b) At 7 L/min: At a flow rate of 7 L/min, the flow rate of CO_2 was 46, while the carbon fixation rate was calculated to be 46 g/m 2 /hr. 8 mg/L-hr. Though obtained density rate was slightly lower than the calculated 5 L/min rate, the results indicated that it proved useful. Nonetheless, there are several reasons for such tendencies, like the partial CO2 saturation and changes of pH in water affected by higher CO2 flow. This indicates a way that elevated CO 2 levels may enhance growth, but where efficiency starts to decline they appear to be at, or close to optimal levels.

(c) At 9 L/min: The fixation rate at 9 L/min reduced to 17 to fixate at the base rates. 6 mg/L-hr. The significant reduction of absorption efficiency to a figure far below the initial rate points to CO2 oversaturation, lower and higher flows of the CO2, as well as imbalance of pH level. The high levels of CO2 that are possibly present point at suboptimal use or even waste, which are both counterproductive and signal the need to control flow rates on both conduits.

From the observations made, 5L/min of CO_2-flow rates is ideal for enhancing carbon fixation and biomass accumulation. This flow rate gave a optimal supply of CO_2 removal without leading to high saturation levels or altering the pH levels. These findings confirm the significance of regulating the source rates of CO_2 within microalgae production systems to improve the overall production efficiency and yields

Production of biogas was determined from the co-digestion of microalgae and banana peels through the anaerobic digestion process. This co-digestion has been found to increase biogas

production as opposed to digestion of microalgae in isolation. Co-digestion of algae provided higher respective methane yields of 145 and 101 mL CH4/g VS than the yield from algae alone of 61 and 82 mL CH4/g VS (Bilassé Zongo, 2023). The produced microalgal biomass was mixed with banana peels at a ratio of 1:1 and co-digested in a 4-liter mesophilic anaerobic digester maintained at 35℃ for 3 weeks. This study attempted at comparing the profile of biogas produced from the digesting of a mixture of microalgae and banana peel in a 4L anaerobic digester. The organic loading rate (OLR) was maintained at 2 kgVS/m3, with an inoculum-to-substrate (I:S) ratio of 2:1. When subjected to biochemical methane potential test, 15 g dry microalgae with 15 g dry banana peels yielded almost 950 ml methane in 21 days. This result proves the viable possibility of co-digesting algae with organic waste for increased biogas production.

The percentage composition shows that much of the biomass has been converted to methane gas, which is present in high concentrations (55%) in the biogas, hence the renewable nature of this energy source. Co-digestion process involves mixing of two or more substrates into digesters to enhance the microbial activities hence increased yield of biogas. This increases biogas yield, but it also has an additional advantage since it affords a way to manage waste by using organic waste materials like banana peels.

Also, added to that, the economic and environmental gains of employing co-digestion in biogas manufacture. This decreases feedstock costs of biogas and promotes proper waste management within the same locality. These characteristics justify the interest in its use as a source of renewable energy; it has the potential to be an independent energy source and a solution to energy insecurity resulting from dependence on fossil fuels.

Chapter 5
Conclusions and Recommendations

5.1 Conclusions

The study effectively applied the principle of microalgae cultivation in photobioreactors for biogas generation and carbon capture. The CO2 fixation rate in the PBR was high and attained a maximum value of 51. showing a strain rate of 6 mg/L-hr at a flow rate of 5 L/min, this means that Scenedesmus sp. Hence, for reducing greenhouse gas emissions, As shown, at an indicated CO2 flow rate of 9L/min we observed greater flow rate led to reduction of algal growth and wastage of CO2 resource since it is not assimilated by algae. Also, it was found that the anaerobic co-digestion of microalgae biomass with banana peels produced a total of 950 mL of methane which further spices up the idea of using this approach in the production of renewable energy.

It was found that approaches of proper CO2 flow rates and placing of monitoring sensors play a vital role to achieve maximum algal growth and efficient CO2 use. The process of co-digesting microalgae with organic waste like banana peels increases the yields of biogas thus offering an opportunity in production of sustainable energy. The economic potential of the process is rather great, stemming from potential applications in waste disposal and a decrease in greenhouse gas emissions.

5.2 Recommendations

Further research can be done on the following:

- More testing needs to be done to confirm reliability and consistency of results.
- Study effects of CO2 on microalgae growth by varying carbon dioxide supply time into the Photobioreactor and shape of the reactor.
- Perform an experimental analysis of the powdered form of the substrates to compare the Biochemical Methane Potential (BMP) with the results from the regular particle size to assess the feasibility of reducing the size of the substrates for enhanced anaerobic digestion and biogas production.

- Explore other microalgal biomass conversion techniques, such as lipid extraction, pyrolysis, or gasification, to produce valuable products like biofuels, chemicals, or fertilizers, enhancing the overall economic viability of the system.
- A detailed cost model needs to be constructed of the entire system to analyze its potential for commercialization.
- Conduct further studies on co-digestion with different substrates to identify the most effective combinations for biogas production.
- Advocate for supportive policies and regulations to facilitate the commercialization and adoption of algae-based bioenergy solutions.

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