ALGAE CULTIVATION IN PBR FROM FISHERY WASTEWATER AND INDUSTRIAL CO_2 FOR BIODIESEL AND FISH FEED PRODUCTION

FINAL YEAR DESIGN PROJECT (FYDP)

By

Fizzah Batool

Muhammad Umer Abbas

Irum Afzal

Supervisor

Dr. Zeshan

Institute of Environmental Sciences and Engineering (IESE)

School of Civil and Environmental Engineering (SCEE)

National University of Sciences and Technology (NUST)

APPROVAL SHEET

It is certified that contents and form of thesis entitled "Algae cultivation in PBR from fishery wastewater and industrial CO2 for biodiesel and fish feed production" submitted by Fizzah Batool, Muhammad Umer Abbas, Irum Afzal have been found satisfactory for the requirement of the degree.

28/08/23

Dr. Zeshan
Associate Professor/Supervisor
IESE, SCEE, NUST

For 28/08/2023

Dr. Zeshan Ali Khan
Associate Professor and Head of Department
IESE, SCEE, NUST

28/08/2023

Dr. Imran Hashmi
Professor and Associate Dean
IESE, SCEE,NUST

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Abstract

Fisheries in Pakistan need to be made more sustainable, due to improper wastewater management as well as lack of research in aquaculture, fish nutrition, & sustainable consumption. CO_2 emissions from combustion processes in industries can be utilized more efficiently instead of being discharged into the atmosphere causing climate change. Furthermore, there is a need to mitigate the energy crisis of diminishing fossil fuel reserves through clean energy technologies. The solution for all these targeted challenges is a photobioreactor (PBR). The study's methodology involved cultivating microalgae in 9 L capacity PBR under three distinct operating systems. The initial system employed aeration with air and fishery wastewater obtained from the Capital Biofloc fish farm in the capital. In the subsequent two systems, Industrial CO_2 and synthetic wastewater with and without organic carbon were introduced. Remarkably, the third operational system demonstrated exceptional efficiency in nutrient removal, specifically nitrate, phosphate, and ammonia. Additionally, this system exhibited higher algal growth and lipid production compared to the other setups. The microalgae generated from these systems were harnessed for lipid extraction. Subsequently, the residual algal biomass remaining after lipid extraction was examined to determine its protein content, essential to produce fish feed. The research analyses the feasibility of constructing a closed system which can produce microalgae for clean energy and fish feed using fish wastewater as well as treating wastewater simultaneously.

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CHAPTER 1

1. INTRODUCTION

The production of clean and sustainable energy has become a pressing global concern in recent times, given the adverse environmental impact of traditional energy sources. The search for alternative and renewable sources of energy has led to an increasing focus on the potential of algae-based biofuels. Algae have proven to be a promising feedstock to produce biofuels due to their high lipid content and fast growth rate. Additionally, algae can be cultivated in wastewater and can utilize carbon dioxide (CO_2) emissions from industrial processes, thus contributing to reducing greenhouse gas (GHG) emissions.

This research paper explores the potential of producing *Scenedesmus sp*. in a photobioreactor (PBR) using an integration of fishery wastewater and industrial CO_2 for biodiesel and fish feed production. The paper targets the challenges facing the clean energy sector, including industrial GHG emissions, wastewater treatment, and unsustainable fisheries. The study aims to address these challenges by proposing a sustainable and efficient approach for producing algae-based biofuels using bio-floc waste streams and reducing the environmental impact of industrial processes (Yong et al., 2021).

At its simplest, a photobioreactor is a closed system that uses algae to convert sunlight into biomass. But the potential applications of this technology go far beyond just energy production. By capturing and utilizing industrial carbon dioxide, photobioreactors can help mitigate the effects of climate change. And by growing algae that can be used as feed for fish and other aquatic creatures, this technology also has the potential to promote sustainable fisheries and help address food insecurity.

Prior research already supports use of wastewater media for algae growth, however little research has been done regarding an integration of bio-floc wastewater and industrial CO_2 and analyze its impacts on algae growth which can pave the path for commercialization of algal PBRs.

The findings of this research could have significant implications for the biofuel and aquaculture industries and could pave the way for the development of more sustainable and environmentally friendly practices. The objectives of this research are:

1. Design a PBR system for fishery wastewater treatment and CO_2 utilization.
2. Operate and monitor the PBR for algae cultivation.
3. Assess recycling potential of harvested algae for fish feed production.
4. Perform lipid extraction of algae to find biodiesel productivity; and
5. Construct a commercial design for the proposed PBR system.

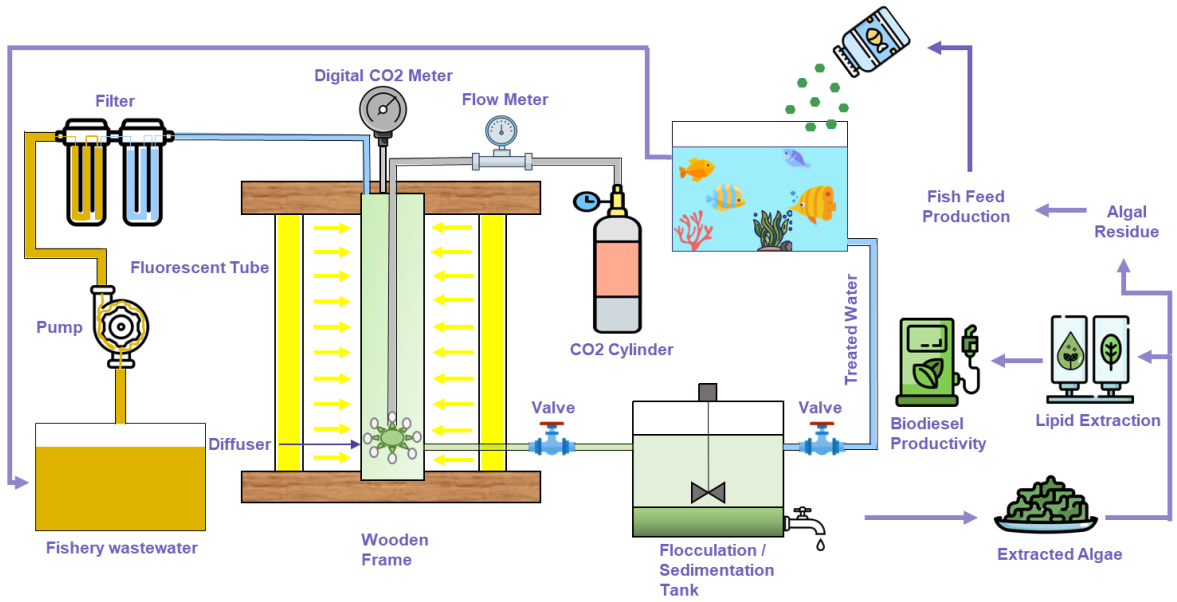

Figure 2.1: Comprehensive Research Project Schematic

A closed-system methodology for producing biodiesel and fish feed is shown in Figure 1.1 by combining algae culture with fishing wastewater and industrial CO_2

CHAPTER 2

2. LITERATURE REVIEW

Integration of bio floc effluent and industrial CO_2 for algal production in photobioreactors (PBR) for biodiesel production has emerged as a promising approach that addresses both wastewater treatment and CO_2 mitigation. Numerous research studies have focused on utilizing fish wastewater as a growth medium for algae, as well as investigating the use of industrial CO_2 as a carbon source. These studies provide valuable insights into the factors influencing algal growth, biomass productivity, lipid content, and biodiesel quality.

Bio floc technology, which involves the accumulation of microbial flocs in aquaculture systems, has been explored as a growth medium for algae. Fish wastewater, rich in organic matter and nutrients, serves as an ideal nutrient source for algal cultivation. Studies have shown that bio floc effluent supports robust algal growth and productivity, owing to the abundance of nitrogen, phosphorus, and other essential nutrients. Optimization of the bio floc effluent composition, including the carbon-to-nitrogen ratio and trace element supplementation, has been investigated to enhance algal biomass productivity. However, challenges such as microbial competition, fouling, and the need for efficient nutrient recycling systems remain to be addressed. Using fish farm effluent for algal production has been reported to achieve a desirable nutrient removal efficiency of 98.12, 52.02 and 33.19% for ammonia, nitrates and phosphates respectively but had lower biomass yield (Enwereuzoh & Low et al., 2021).With the development of PBR with wastewater treatment, it is possible to increase the biomass productivity from 5 to 8 g/L/day (Bošnjaković et al., 2020).

In parallel, the integration of industrial CO_2 emissions with algal cultivation has gained attention as a means of mitigating greenhouse gas emissions. Algae can utilize CO_2 through photosynthesis, offering a potential solution to the problem of industrial carbon emissions. Various studies have investigated the effects of industrial CO_2 concentration on algal growth, productivity, and lipid accumulation. Researchers have explored strategies to enhance CO_2 utilization efficiency, including the use of flue gas from power plants and carbon capture and utilization technologies. Technological challenges, such as the supply and delivery of CO_2, as well as the economic feasibility of large-scale implementation, require further investigation. Using a PBR and a strain of Scenedesmus, average CO_2 capture efficiency of 44 % CAN BE achieved, also serving as a secondary scrubber for NOx and SOx, removing on average 41.5 % of the NOx and 100 % of the SOx from the flue gas, also achieving algae productivity of around 0.165 ± 0.057 g/(L day) (Wilson et al., 2016). Other sufficient studies have been conducted to confirm that the cultivation of algae using industrial CO_2 has a great environmental and economic potential for biodiesel production (Iglina et al., 2022).

The combination of bio floc effluent and industrial CO_2 integration presents a synergistic approach for algal production. The utilization of bio floc effluent as a growth medium enhances nutrient availability while simultaneously providing a means of wastewater treatment. The integration of industrial CO_2 further enhances carbon uptake by the algae, leading to increased biomass productivity. Very little research has been conducted on this phenomenon which has the potential to demonstrate a positive impact of this combined approach on algal growth, lipid content, and overall biodiesel quality. However, there are technological considerations, such as the design and optimization of integrated systems, to ensure efficient nutrient and CO_2 delivery, as well as

sustainable production. The environmental and economic sustainability of such integrated systems are critical factors to consider in their implementation.

Comparative analysis of the research studies reveals important findings and trends. The methodologies employed in the bio floc effluent studies include the characterization of the wastewater, determination of nutrient composition, and evaluation of algal growth parameters. Industrial CO_2 studies often involve laboratory-scale experiments utilizing flue gas or enriched CO_2 sources. Key factors influencing algal productivity and lipid content include nutrient availability, CO_2 concentration, light intensity, and temperature. Challenges identified across these studies include contamination control, scalability, and the development of cost-effective technologies (Juneja et al., 2013).

The integration of bio floc effluent and industrial CO_2 for algal production in PBRs holds great potential for sustainable biodiesel production, wastewater treatment, and CO_2 mitigation. The findings from this research study can provide valuable insights into the factors affecting algal growth, biomass productivity, lipid content, and biodiesel quality. However, there are challenges that need to be addressed, such as nutrient recycling, microbial competition, CO_2 delivery, and economic feasibility which will be addressed in this study. This research will also focus on developing efficient and scalable integrated systems, addressing knowledge gaps, and ensuring the environmental and economic sustainability of algal biofuel production.

CHAPTER 3

3. METHODOLOGY

3.1 Fabrication of PBR

A lab scale column photobioreactor was designed and fabricated with 1 valve for wastewater inflow, 1 valve for wastewater outflow, 2 valves for gas recycling, 1 inlet for gas, 1 sparger at bottom for bubbling (bubble size 2-4 mm), 1 air pump connected with air filter, and 3 fluorescent tubes for luminescence. Higher surface area to volume ratio was provided (H/D ⩭ 7) to allow maximum light penetration, with a height of 80 cm and inside diameter of 11.5 cm. 12/12 hours light and dark regime was set with a light intensity of around 2600 lx measured using a quantum flux meter.

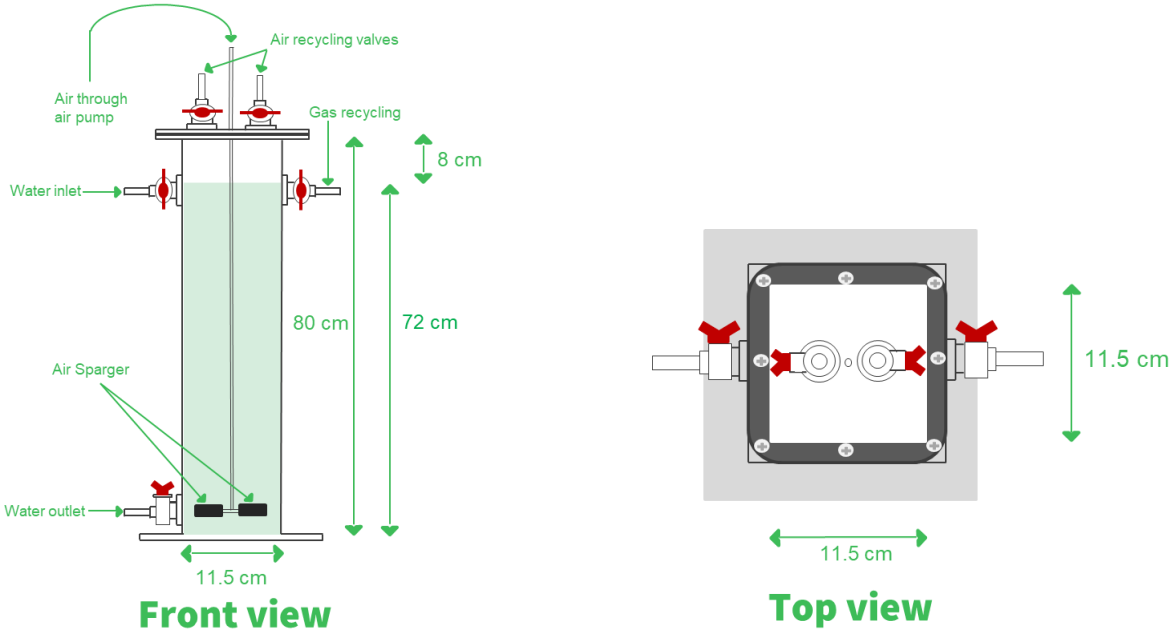

Figure 3.1: Auto CAD drawing showing the dimensions of PBR.

The Lab-scale photobioreactor (PBR) used in the project is shown in precise AutoCAD drawings in the figure 3.1, including front and side views.

3.2 Preparation of Algae Culture:

Figure 3.2: The algae culture media containing Scenedesmus Sp.

Scenedesmus sp. was chosen due to its fast growth rate in wastewater, efficient CO_2 utilization, ability to treat wastewater with higher nutrient removal efficiency, and ability to accumulate lipids for biodiesel. Using a PBR and a strain of Scenedesmus, average CO_2 capture efficiency of 44 % can be achieved during daylight hours. Z8 media was prepared and *Scenedesmus sp.* strain was transferred to the solution as shown in figure 3.2. Sterilization was done for 15-20 minutes to avoid contamination. Filters air was passed to the flask and algae was allowed to grow until dense algae of 9 g/L concentration was obtained. 500 ml of the algal solution was transferred to the PBR.

3.3 Collection and Pretreatment of bio-floc fish wastewater

Wastewater was collected from Capital Bio-floc in B-17, Islamabad. Sediments in wastewater were allowed to settle in sedimentation tank, followed by filtration of the wastewater before being pumped into the PBR. Wastewater characterization was done before and after pretreatment. For lab scale, one PBR contained 9L of wastewater with 500 mL of algae solution.

3.4 Experimental Design

Comparison was done for three different systems as mentioned in Figure. After 5 days of cultivation, microalgae were harvested, and separated from the growth medium using a sedimentation tank.

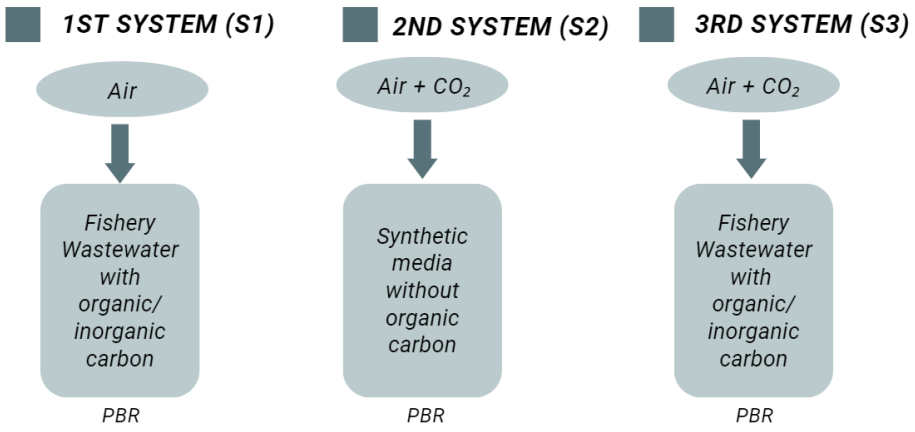

Figure3.3: Comparison between three systems under different operating conditions

Three different operating systems used in the experimental phase are compared in more detail in figure 3.3. Every system exhibits a distinct setup and inputs. Fishery wastewater and outside air were used in the first system. Moving to the second system, synthetic wastewater devoid of COD was combined with industrial CO2. Lastly, the third system involved synthetic wastewater containing COD alongside industrial CO2. The differences and results between various operating situations are captured in this graphic comparison.

3.4.1 Operating Conditions for S1

The purpose of this system was to run PBR without use of industrial carbon dioxide to measure nutrient removal efficiency using wastewater alone and its effects on algae production.

- Light Intensity: 35 μ mol/s/m^2 = 2600 Lx
- Air Provision: Through Air Pump & Sparger with 0.04% CO_2
- Light Duration: 12:12 light dark hour regime
- Algae Species: *Scenedesmus sp.*
- Growth Media: 9 L Fishery Wastewater

- Volume of Algae Culture: 500 mL of 9g/L concentration

3.4.2 Operating Conditions for S2

The purpose of this system was to run PBR in synthetic media without carbon content to analyze how much carbon from carbon dioxide provision is utilized by algae and how it affects productivity. 0.27 g/l/day CO_2 was provided.

- Light Intensity: 33 μ mol/s/m^2 = 2442 lx
- Air Provision: Through Air Pump & Sparger with 0.04% CO2 for 23 hours & 10% CO_2 at 200 ml/min for 1 hour/day ensured by using a flow meter.
- Light Duration: 12:12 light dark hour regime
- Algae Species: *Scenedesmus sp*.
- Growth Media: 9L of Synthetic media without COD
- Volume of Algae Culture: 500 mL of 9g/L concentration

Nutrients (nitrates, phosphates and ammonia) in synthetic media were added according to composition analyzed of fishery wastewater. *(Synthetic media contained 83.33 mg/L NH_4Cl, 14.44 mg/L KH_2PO_4, 53.89 mg/L $Mg(NO_3)_2$, 2.5 mg/L $Na_2MoO_4.2H_2O$, 15.56 mg/L NaCl, 10 mg/L $CaCl_2$, 2.44 $MgSO_4.7H_2O$, 3.33 mg/L Na_2EDTA, 0.83 mg/L Na_2MoO_4, 4.44 mg/L H_3BO_4, 0.25 mg/L $MnCl_2.4H_2O$, 1.1 mg/L $ZnSO_4.7H_2O$, and 4.44 mg/L $FeSO_4.7H_2O$.)* (Osorio, J.H., Pinto, & Pollio, 2019).

3.4.3 Operating Conditions for S3

The purpose of this system was to run PBR with an integration of CO_2 utilization and Bio-floc wastewater as growth media. 0.27 g/l/day CO_2 was provided.

- Light Intensity: 31 µmol/s/m2 = 2294 lx
- Air Provision: Through Air Pump & Sparger with 0.04% CO2 for 23 hours & 10% CO_2 at 200 ml/min for 1 hour/day ensured by using a flow meter.
- Light Duration: 12:12 light dark hour regime
- Algae Species: *Scenedesmus sp.*
- Growth Media: 9L of Fishery Wastewater
- Volume of Algae Culture: 500 mL of 9g/L concentration

3.5 Maintaining CO_2 Provision in Photobioreactor

10% CO_2 was added to the PBR at a constant rate of 200 ml/min for 1 hour/day followed by air containing 0.04% CO_2 for 23 hours, for the 5 day runs. It was important to optimize the CO_2 provision to the photobioreactor as an excess can lead to a number of negative effects on the growth and health of the algae culture, including carbon dioxide toxicity. High concentrations of CO_2 can become toxic to algae, inhibiting their growth and photosynthesis. This can lead to decreased biomass productivity, and in some cases, cell death. Furthermore, CO_2 dissolves in water to form carbonic acid, which can lower the pH of the culture medium. Excessive CO_2 can cause a drop in pH, which can stress the algae and affect their metabolic processes. While CO_2 is necessary for algae growth, providing excess CO_2 can lead to carbon limitation if other nutrients, such as nitrogen and phosphorus, are not provided in sufficient quantities. This can also negatively affect biomass productivity.

Therefore, it was important to maintain the optimal CO_2 concentration based on the specific requirements of the algae species being cultured, being *Scenedesmus sp.* in this study, as well as other environmental factors, to ensure healthy growth and high biomass productivity in a PBR.

3.6 Daily Growth and Biomass Analysis

Daily optical density readings were taken at 680 nm using spectrophotometer to determine the daily growth rate of the algae species. The algae biomass yield was gravimetrically estimated after 5 days. To determine biomass yield (in dry cell weight), 50 ml of culture samples were centrifuged at 4000 rpm for 5 mins, washed with distilled water, followed by the transfer of algae into filter papers of known weight, dried in the oven at 50 ◦C for 24 h, and weighed. Biomass productivity (mg/l/d) was gravimetrically estimated as the dry biomass at the end of cultivation. Biomass concentration can be found using the unitary method. Biomass productivity and specific growth rate were calculated using the following equations.

$$Biomass\ productivity = \frac{amount\ of\ biomass\ (\frac{mg}{L})}{number\ of\ days}$$

$$Specific\ growth\ rate = \frac{1}{t}\ln(\frac{Xf}{Xi})$$

Where Xf is biomass at last day of cultivation (mg/L), Xi is biomass on the first day and t is cultivation time in days which is taken as 5 in this study.

3.7 Nutrient Removal and Wastewater Treatment Analysis

Nutrient removal was determined at the end of microalgae cultivation. Algal slurry was taken from the bottom of the PBR to a sedimentation tank where algae was allowed to settle. Treated water was collected from the top of sedimentation tank for treatment analysis. Monitoring parameters for analysis included phosphates, nitrates, ammonia, TSS, TDS, COD, Turbidity, and pH.

A spectrophotometer was used to measure nitrate and phosphate content every odd day and Kjeldahl apparatus was used to measure ammnia every odd day of the run. Percentage nutrient removal was calculated using the following equation.

$$Percentage\ removal = (Xf - Xi)/(Xf) \times 100$$

Where Xf is initial concentration of nutrient (mg/ml), and Xi is final concentration of nutrient (mg/ml) (Hicks et al., 2022).

3.8 Determining lipid content

Total lipids were extracted using Bligh and Dyer method. To check equivalent dried algae, 1 ml of wet algae from the sample is taken and dried for 2 hours in oven at 105^{o}C. After weighing pre and post oven mass, quantity of wet algae equivalent of dried algae can be found. 100-500 mg of algal sample is weighed and added to a test tube. 2 mL of chloroform and 4 mL of methanol is added to the sample. The volume of solvent should be at least 10 times the weight of the sample. The sample and solvent were thoroughly mixed using a vortex mixer or sonication. 2 mL of chloroform and 2 mL of distilled water were added to the mixture and mixed again. The mixture was centrifuged at 3000 rpm for 10 minutes to separate the mixture into two phases, an upper aqueous phase and a lower organic phase containing lipids. The organic phase was carefully transferred to a clean centrifuge tube using a glass pipette. 2 mL of chloroform was added to the organic phase, mixed thoroughly, and centrifuged at 3000 rpm for 10 minutes. The lower organic phase was separated and dried under a stream of nitrogen gas until all the solvent had evaporated and a residue of lipids was left behind which was gravimetrically measured.

The extracted lipids can also be analyzed using techniques such as thin-layer chromatography, gas chromatography, or mass spectrometry to identify and quantify specific lipid species. The Bligh and Dyer method typically uses a ratio of 1:2:0.8 (algae: chloroform: methanol) for the initial extraction step and a ratio of 2:2:1 (organic phase: chloroform: distilled water) for the second extraction step. The following equations are used to find lipid content and lipid productivity (Shin et al., 2014).

*% Lipid content = (mass of lipids extracted (mg) / mass of algae used (mg)) * 100*

*Lipid productivity = biomass productivity (mg/L/day) * (% lipid content/ 100)*

3.9 Protein Content for Fish Feed

Total Kjeldhal Assembly was used for the estimation of TKN in algal sample. For this, 0.1 g sample was digested in the heating device -providing the temperature of 375-380 ºC for 30 minutes. After digestion, the samples were distillated in the distillation unit and titrated. Following equation was used:

$$mg/g\ TKN = (A - B) \times 14 \times 0.02/\ g\ of\ sample$$

After that mg/g TKN of algal samples is converted to g/g and multiplied with conversion factor of 5.98 used for estimation of proteins and percentage protein content is obtained (Geada et al., 2021).

CHAPTER 4

4. RESULTS

4.1 Wastewater Characteristics

Sample was taken from each tank in the chosen bio floc fish farm and characterization was done on specified parameters. Characterization was done after pretreatment of the wastewater and results were compared as shown in Table 4.1.

Table 4.1: Comparing the parameters after and before the filtration of raw wastewater.

Parameter	Raw Wastewater	Range in Literature	Pretreated Wastewater	Range to Allow Algae Growth
pH	8.46	6-8.5	8.29	7-8.5
Conductivity	210 μS/cm	118-230 μS/cm	213 μS/cm	-
Temperature	23.5 ° C	20-30° C	20.1 ° C	15-30° C
Turbidity	110 NTU	100-159 NTU	3.52 NTU	Less preferred
DO	8.3 mg/L	> 4.0 mg/L	8.3 mg/L	-
Nitrates	44.1 mg/L	<50 mg/L	42.3 mg/L	1.5 -100 mg/L
Phosphates	13.95 mg/L	0.5-20 mg/L	4.1 mg/L	1-150 mg/L
Ammonia	29.4 mg/L	<100 mg/L	28 mg/L	<100 mg/L
COD	2800 mg/L	<3500 mg/L	400 mg/L	High C content preferred
TSS	260 mg/L	<500 mg/L	92.1 mg/L	Less TSS preferred
TDS	997 ppm	50-1000 ppm	920 ppm	-

pH, conductivity, DO, and TDS remained constant after filtration as dissolved solids pass through the filter. Turbidity is reduced along with TSS and COD due to removal of suspended and insoluble impurities from pre-treatment. Nitrates and ammonia were almost the same as they are soluble and pass through the filter. Phosphate concentration greatly decreased due to presence of insoluble phosphate salts in the raw wastewater which were removed during filtration. The characteristics of pretreated wastewater lied in the ranges suitable for algae growth. 9 L of this pretreated wastewater was added to the PBR to allow micro algal cultivation.

4.2 Wastewater Treatment Comparison

Results for treated wastewater obtained at the end of 5 day run are given in the following tables.

Table 4.2: Results and Analysis for three systems after and before filtration

Parameter	**System 1**		**System 2**		**System 3**	
	Before	**After**	**Before**	**After**	**Before**	**After**
Phosphates (mg/L)	8.3	2.44	10	2.8	10	2.37
TSS (mg/L)	92.1	18.2	115.3	36.57	123.3	17.9
TDS (ppm)	1020	880	272	151	1190	205
Nitrates (mg/L)	42.3	19.3	45	11.67	45	8.78
Ammonia (mg/L)	28	2.8	28	0	28	1.1
COD (mg/L)	400	80	0	0	400	80
Turbidity (NTU)	3.52	2.4	2.62	2.23	5.23	2.17
pH	8.29	7.44	7	7.23	7	7.26

Table 4.2 compares the wastewater parameter values for the three different systems before and after filtering. This analysis shows how well each system's filtering process worked by showing how treated parameter values changed.

System 3 with integration of both CO_2 utilization and wastewater as growth media showed better results as compared to the other two systems. The ability of the three runs to treat water would depend on the ability of the algae to remove nutrients and contaminants from the water. Scenedesmus sp. has been shown to remove nutrients such as nitrogen and phosphorus from wastewater and to potentially remove contaminants such as heavy metals.

COD can be removed in a PBR through a combination of physical, chemical, and biological processes. Biological removal of COD is achieved through algae that consume the organic matter in the wastewater as a food source. Algae can consume organic matter in wastewater through a process called "photosynthetic assimilation". This process involves the use of sunlight, carbon dioxide, and nutrients by algae to produce energy and organic compounds such as sugars, proteins, and lipids. In the presence of light and nutrients, algae can break down organic matter in wastewater through the following mechanisms:

(i) Extracellular enzymes: Algae secrete enzymes that can break down complex organic compounds in wastewater into simpler compounds such as amino acids and sugars, which can be more easily assimilated by the algae.

(ii) Phagocytosis: Algae can also consume organic matter through a process called phagocytosis, in which they engulf and digest solid particles in the wastewater.

(iii) Adsorption: Algae can adsorb dissolved organic matter onto their cell surfaces and transport it into their cells for assimilation.

(iv) Biofilm formation: Algae can form biofilms on surfaces in the wastewater, which can help to break down organic matter and promote nutrient cycling.

Scenedesmus sp. are efficient at consuming organic matter in wastewater due to their ability to utilize a range of organic compounds as a food source, and their capacity to rapidly grow and reproduce under the right conditions.

4.3 Nutrient Removal

Removal of ammonia, nitrates and phosphates was analyzed with respect to time throughout the 5-day run and the results were compared. Graphs show the removal of each nutrient from each system.

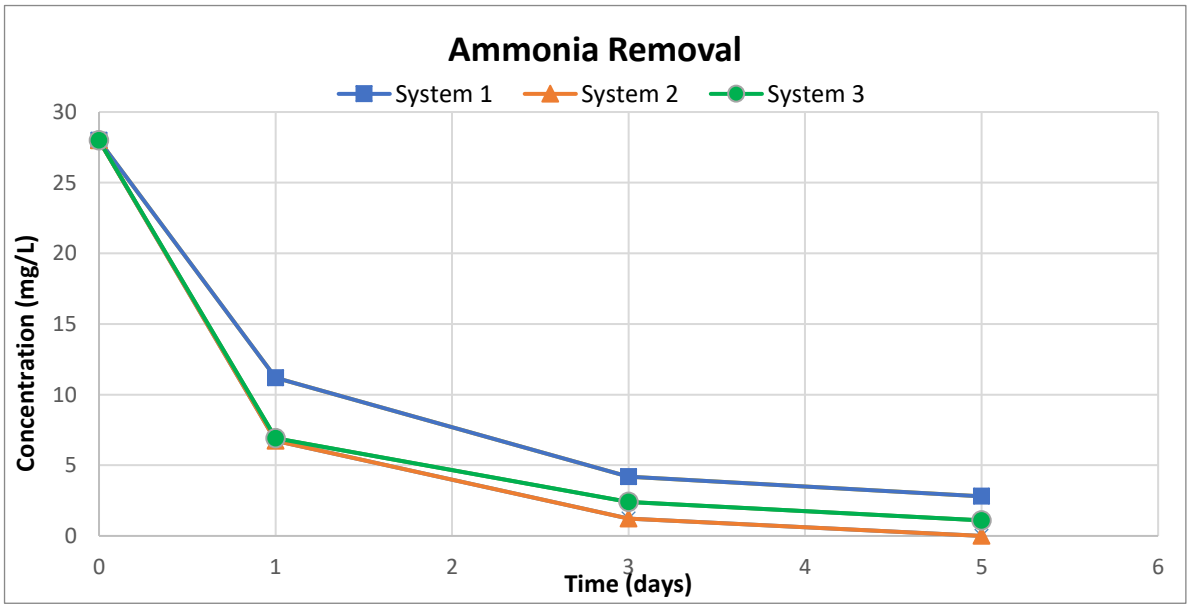

Figure 4.1: Removal concentration of ammonia with time in days

The figure 4.1 depicts the temporal progression of ammonia removal. Ammonia concentrations were monitored on the 1st, 3rd, and 5th days for all three systems. The second system is notably more effective in terms of ammonia removal.

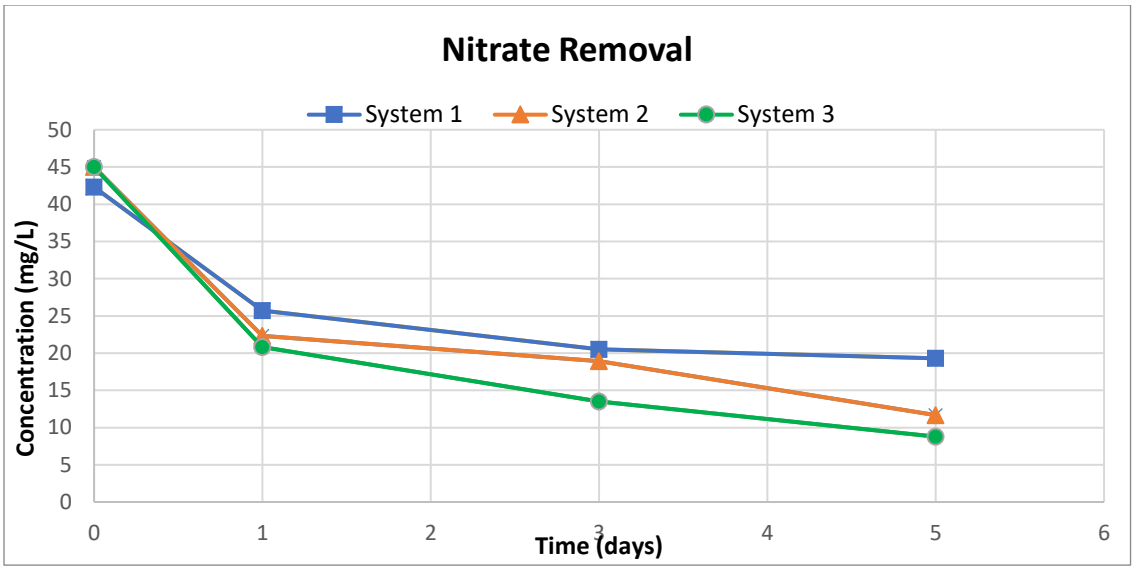

Figure 4.2: Removal concentration of nitrate with time in days

The figure 4.2 above depicts the temporal progression of nitrate removal. Nitrate concentrations were monitored on the 1st, 3rd, and 5th days for all three systems. The third system is notably more effective in terms of nitrate removal.

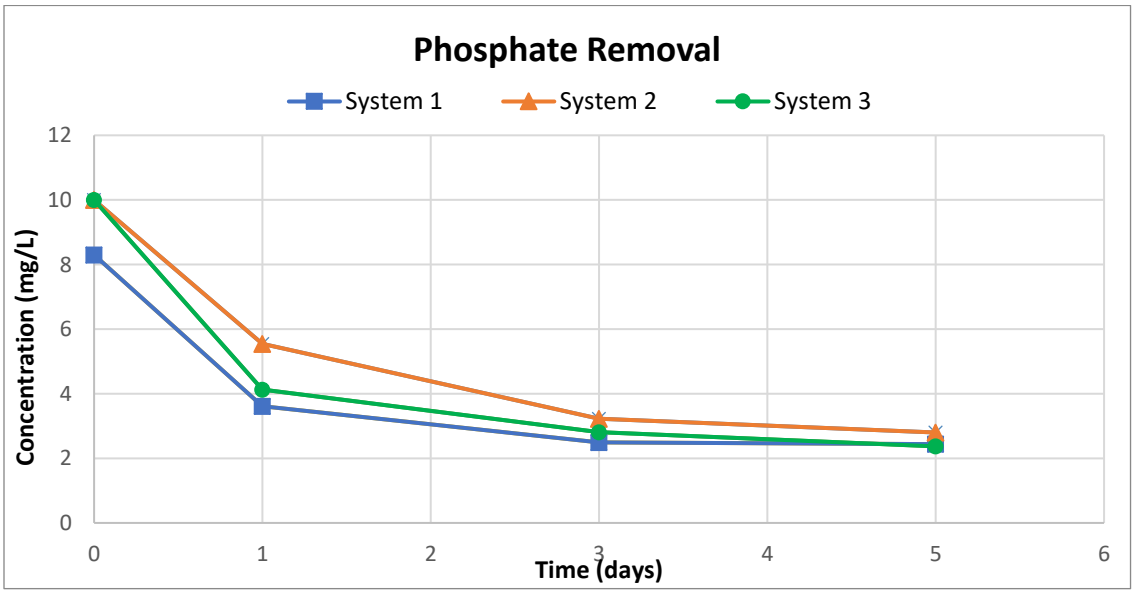

Figure 4.3: Removal concentration of phosphate with time in days

The figure 4.3 above depicts the temporal progression of phosphate removal. Phosphate concentrations were monitored on the 1st, 3rd, and 5th days for all three systems. The third system is notably more effective in terms of phosphate removal.

System 3 showed the fastest nutrient consumption as compared to the other two systems. This can be linked to better algal growth leading to better nutrient removal from the wastewater. Overall nutrient removal from the three systems was also compared.

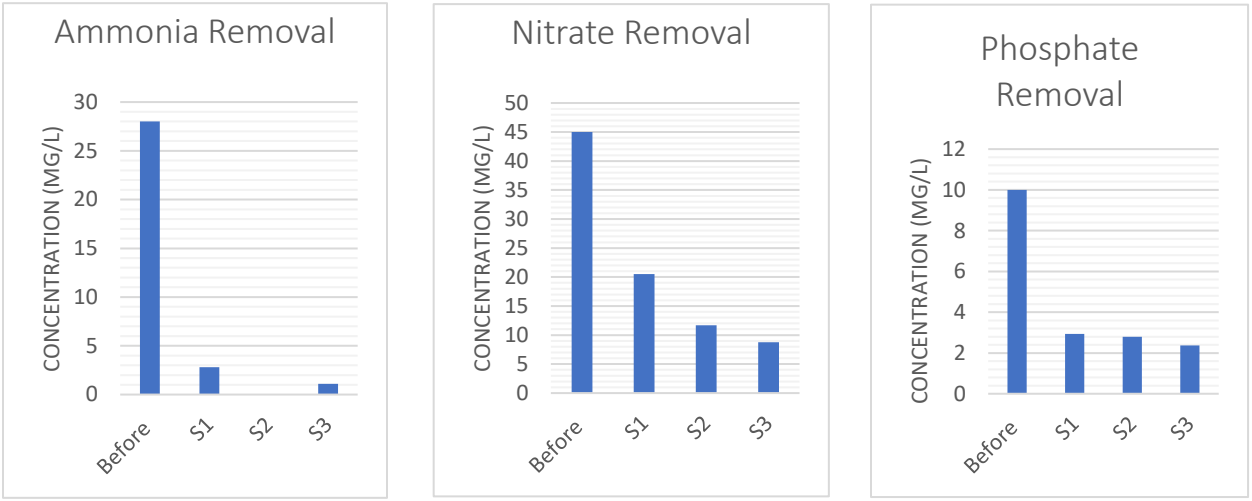

Figure 4.4: Removal concentration (mg/L) of nutrients in three systems

The figure 4.4 is depicting three graphs demonstrate how the three systems' ammonia, nitrate, and phosphate levels adapt to various operating conditions. These figures make it quite evident how effectively each method eliminates these materials in various settings.

Table 4.5: Percentage removal of the nutrients in three systems

Parameter	**S1 Removal %**	**S2 Removal %**	**S3 Removal %**
Phosphate	70.6	72	73.3
Nitrate	54.37	74.1	80.4
Ammonia	90	100	96.1

The table 4.5 is showing percentage removal of the nutrients in three systems. As, Phosphate and nitrate removal considerably improved to 73.3% and 80.4% respectively with integration of CO_2 utilization and bio floc wastewater. Ammonia removal remained almost constant in all three systems ranging from an average of 90-100%. It is not always possible to achieve 100% removal of ammonia using algae in a photobioreactor. However, algae are capable of consuming ammonia as a nutrient, and under the right conditions provided, they can remove a significant amount of ammonia from wastewater; for example, a higher light intensity during daylight hours can enhance their ability to remove nutrients from wastewater. Moreover, if any of the nutrients, including nitrogen, phosphorus, and micronutrients become limiting in the PBR, the removal efficiency of available nutrients may be reduced. Furthermore, Algae have specific pH and temperature requirements for optimal growth and metabolic activity. pH varies during the day due to acidic conditions created by CO_2 provision which can affect nutrient removal efficiency.

The removal efficiency of nutrients in the systems under analysis can vary depending on several factors, but with careful management of environmental conditions as provided in the runs. a significant reduction in pollutant/nutrient levels in wastewater can be achieved. Results acquired from system 3 were favorable in terms of nutrient uptake, wastewater treatment as well as algal growth.

4.4 Algal Growth & Productivity

Algal growth readings using spectrophotometric and gravimetric analysis for each system were plotted and compared. Algal growth showed greater variance due to fluctuating external conditions. However, consistent difference in growth was observed in all three systems and the

trends were analyzed.

Table 4.6: Average concentration and standard deviation of three system

Time (days)	SYSTEM 1		SYSTEM 2		SYSTEM 3	
	Avg Concentration (mg/L)	Standard Deviation	Avg Concentration (mg/L)	Standard Deviation	Avg Concentration (mg/L)	Standard Deviation
0	630	22.913	660	22.912	635	26.457
1	1255	181.865	1508	93.050	1658	190.416
2	1295	147.564	1928	85.781	2237	165.401
3	1635	118.216	2222	193.476	2448	236.449
4	1860	57.663	2515	232.54	2970	159.765
5	2390	249.650	3258	283.12	3687	163.579

The table 4.6 displays algae concentrations (mg/L) observed across three different systems, each tested through three cultivation runs. To present a unified trend for each system, standard deviations were calculated from the values obtained in the three runs.

Table 4.7: Showing the biomass productivity, percent lipid content and lipid productivity.

Parameter	**SYSTEM 1**	**SYSTEM 2**	**SYSTEM 3**
Biomass Productivity (mg/L/day)	478	651.6	737.4
Lipid Content (%)	11.92%	12.09%	12.03%
Lipid Productivity (mg/L/day)	56.98	77.98	88.7

A thorough explanation of biomass productivity, lipid content percentage, and lipid productivity is given in Table 4.7. The superiority of System 3, which exhibits outstanding efficacy in both biomass and lipid synthesis, is particularly notable. This emphasizes its importance in the study's broader context.

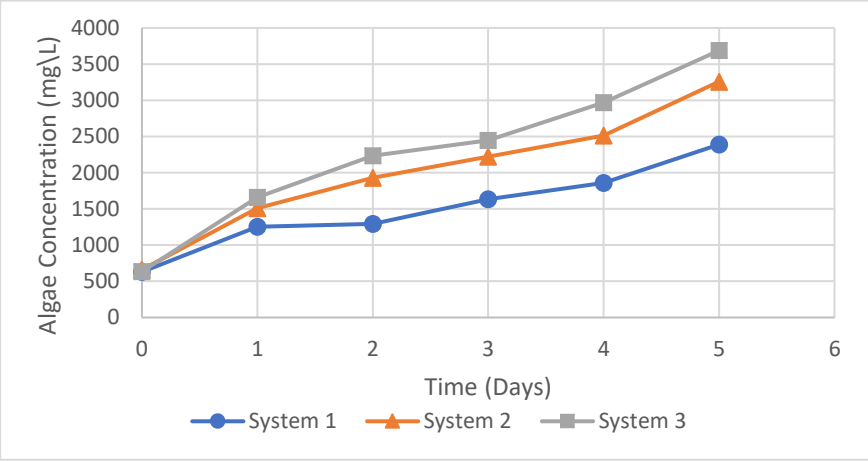

Figure 4.5: Comparison of Algal Growth in all 3 system

Figure 4.8 shows the algal concentration for the three systems through time in days using daily measurements of growth patterns. System 3 stands out with much stronger algal growth while beginning with the same starting concentration.

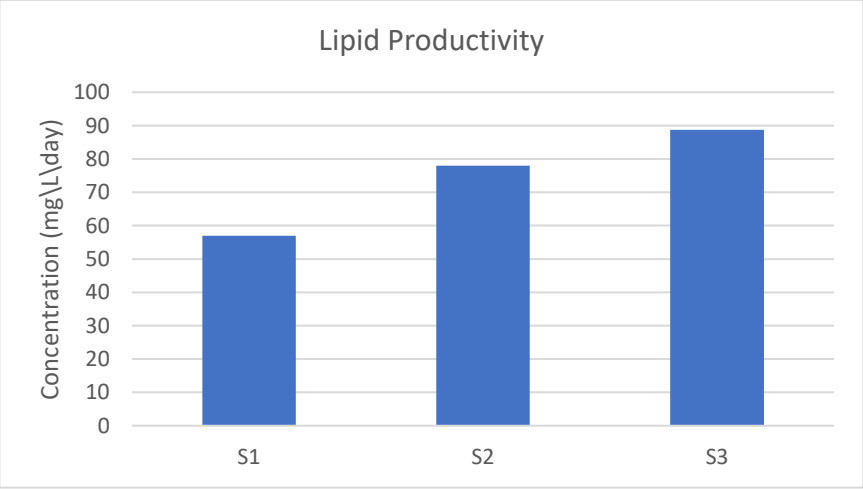

Figure 4.6: Comparison of Lipid Productivity in all 3 systems

The figure 4.9 is graphically displays lipid productivity for each of the three systems, highlighting System 3's exceptional lipid production, which significantly outperforms the yields of the other systems.

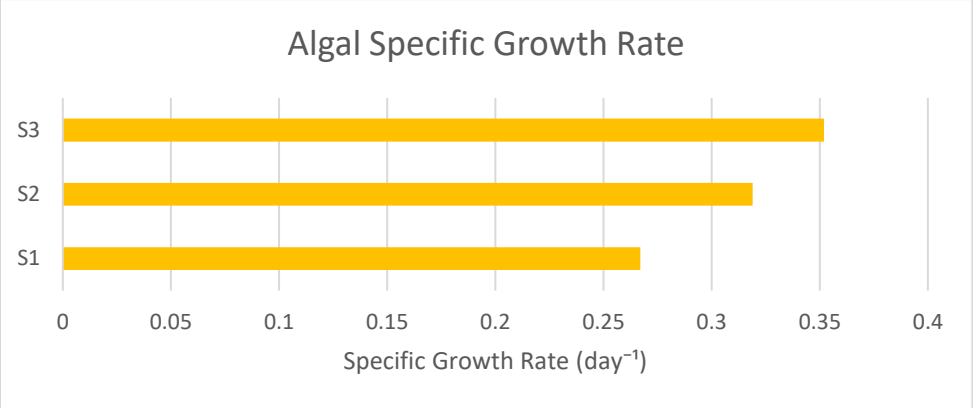

Figure 4.7: Comparison of specific growth rate of all 3 systems

System 3 showed the highest biomass productivity of 737.4 mg/L/day. Though the lipid content remained consistent to around 12%, lipid productivity of system 3 was highest; around 88.7 mg/L/day. System 3 also showed the fastest algal growth as daily algal concentrations recorded showed the steepest growth curve following a faster trend. System 3 was then followed by system 2 which used industrial CO_2 alone and system 1 was the slowest in terms of algal growth and productivity, implying that bio-floc wastewater alone may not be a suitable growth media for mass production of biodiesel. Highest specific growth rate was shown by S3 of 0.352/day followed by S2 with 0.319/day and lastly S1 with specific growth rate of 0.267/day as shown in figure 4.10. Since system 3 showed more optimized results, S3 algal samples were taken for analysis of protein content for fish feed production. S3 results were further used to make the commercialized upscaled design for a PBR system which aimed to support mass production.

4.5 Protein Content

Calculations:

mg/g TKN = (A−B) ×14×0.02) /(g of sample)

mg/g TKN = (50−39) ×14×0.02)/ (0.1)

mg/g TKN = 30.8

Converting the TKN content in mg/g to g/g:

g/g TKN = 30.8 1000 = 0.0308

Multiplying by the conversion factor of 5.98 to estimate the protein content:

= 0.0308 x 5.98 = 0.184

Obtaining the percentage of protein content: = 0.184 x 100 = 18.4%

CHAPTER 5

5. DISCUSSION

a) System 3 (PBR run with wastewater and 10% CO_2): The presence of wastewater as a nutrient source in this scenario could provide a range of nutrients that are required for growth, including both organic and inorganic compounds, which could support the growth of Scenedesmus sp. and its ability to remove nutrients and contaminants from the water. Additionally, the addition of 10% CO_2 could potentially stimulate growth and increase nutrient uptake by the algae. Therefore, this scenario has shown the highest water treatment ability, algal growth, and lipid productivity among the three runs.

b) System 2 (PBR runs with water without organic content and 10% CO_2): Providing water without organic content in this scenario could help eliminate potential toxins or contaminants that could limit algae growth and water treatment ability. However, the absence of organic nutrients could limit the ability of the algae to remove nutrients from the water. The addition of 10% CO_2 could potentially stimulate growth and increase nutrient uptake by the algae, but the absence of organic nutrients may still limit the water treatment ability and algal growth of this scenario. Therefore, this scenario has intermediate results among the three runs.

c) System 1 (PBR run with only using wastewater and air): In this scenario, the algae would have access to organic nutrients in the wastewater, but not a source of carbon, which could limit growth and the ability of the algae to remove nutrients from the water. Additionally, the presence of toxic compounds or competition from other microorganisms in the wastewater could limit algal growth of this scenario. Therefore, this scenario has the lowest desired results among the three runs.

5.1 Lipid Productivity:

It is possible to achieve higher lipid productivity by providing stress to the system, however, providing a stress adds to the cost due to energy and chemical consumption, making it harder for the system to be economically viable (Song et al., 2022). By judging prior research done in normal conditions, without provision of stress using waste as growth media, lower lipid productivity has been reported. Cultivation of micr0-algae using municipal wastewater as a growth media reported a lipid productivity of 10.71 mg/L/day (Mohamed et al., 2018) which is quite less. Lower lipid productivity could be a result of fluctuating conditions of wastewater as it is totally dependent on municipal use. Municipal wastewater contains toxic chemicals from washing, detergents etc. as well as biological inhibitors such as bacteria from fecal matter and other organics which can hinder algal growth (Mamta et al., 2021). Algal growth from fish farm effluent reported a lipid productivity of 27.64 mg/L/day (Enwereuzoh et al., 2021). The reason can be lower organic content in normal fish farms. The conducted study uses bio floc wastewater as growth media which contains a much higher organic content due to being a highly concentrated effluent due to its working conditions (García-Martínez et al., 2022). This aids in algal growth through sufficient provision of required carbon. Using pharmaceutical wastewater as a growth media reported a lipid productivity of 17.15 mg/L/day (Amit et al., 2020). Pharmaceutical wastewater contains persistent pollutants as well as highly toxic chemicals for medical waste which can hinder algal growth. Thus, through an integration of bio floc wastewater and industrial CO_2, higher lipid productivity of around 88.7 mg/L/day without any applied stress, which is higher than similar systems analyzed. Further comparison needs to be made with more systems to achieve a better understanding of the added benefits of the system under study.

5.2 Wastewater Recycling

Recycling of bio floc fish farm wastewater using a photobioreactor can help reduce freshwater resource consumption. Bio floc fish farming systems generate a significant amount of wastewater, which traditionally needs to be discharged and replaced with fresh water. However, by implementing a photobioreactor, the wastewater can be treated and recycled, allowing it to be reused within the fish farming system. This eliminates the need for continuous freshwater intake, thus reducing the overall consumption of freshwater resources. Algae in the PBR can be used to treat and purify wastewater. Through the conducted treatment analysis, it was found that algae were highly efficient in purifying water and removing nutrients such as nitrogen and phosphorus from the water through a process called assimilation. This treated water can then be reused for the fish farming system, reducing the reliance on freshwater sources. Recycling and reusing the wastewater from the fish farm significantly reduces the need for continuous freshwater replenishment. This conservation of water resources becomes especially valuable in regions where water scarcity is a concern. By reducing the dependence on fresh water sources, the strain on local water supplies is alleviated, leading to a more sustainable and efficient use of water resources.

Thus, using the photobioreactor to treat and recycle fish farm wastewater can have positive environmental implications. Discharging untreated or partially treated wastewater can contribute to water pollution, leading to the degradation of aquatic ecosystems. By implementing a complete recycling system through PBR, the release of pollutants into the environment is minimized, promoting cleaner and healthier water bodies. The efficiency and effectiveness of the photobioreactor system depends on various factors, including the size of the fish farm, the type and density of fish being farmed, and the design and operation of the photobioreactor. However, when implemented properly, complete recycling of bio floc fish farm wastewater using a

photobioreactor can significantly reduce freshwater resource consumption and promote sustainable aquaculture practices (Tom et al., 2021).

CHAPTHER 6

6. COMMERCIALIZATION

Results from system 3 offer a unique advantage in that they can produce biomass at a much faster rate with a biomass productivity of 737.4 mg/L/day and lipid productivity of 88.7 mg/L/day. This means that they can produce more biomass per unit area of cultivation, which can make them comparatively an efficient source of biomass for bio-diesel production as well as co-production of fish feed.

In the context of photobioreactors, high biomass productivity can be a game-changer in terms of commercialization. The ability to produce more biomass per unit area of cultivation means that PBRs can be smaller and more compact, which can significantly reduce their cost and improve their scalability (Piccinno et al., 2016).

Moreover, high biomass productivity in algae can also help to increase the yield of high-value products that can be derived from algae, focusing on biodiesel and fish feed in this study. By maximizing the amount of biomass that can be produced per unit area of cultivation, the cost of producing these products can be reduced, making them more economically viable. By harnessing the full potential of integrating bio-floc fish farm wastewater and industrial CO_2 utilization, we can create a sustainable and economically viable source of biomass that can meet the growing demand for renewable energy, food, and other high-value products.

6.1 Commercial Design of Upscaled PBR

Upscaling the PBR system allows for the cultivation of larger volumes of algae. This translates to higher production yields, which can be particularly advantageous for industries that want to shift towards biofuel production, food supplements, or wastewater treatment. As the PBR system

is scaled up, there is potential for achieving economies of scale. Larger PBR systems often result in improved efficiency and reduced production costs per unit of algae produced. This can make algal biofuels more cost-competitive in the market.

Algae cultivation can be made commercially viable by increasing the production capacity. Market demands can be met, stable revenue streams can be ensured. This scalability can also attract investors and partners who are interested in supporting larger-scale biofuel production. Larger PBR systems provide more opportunities for research and development activities. Scientists and engineers can conduct experiments, optimize growth conditions, and test new technologies at a larger scale. This can lead to improved understanding of algae growth dynamics, cultivation techniques, and strain selection.

Advanced monitoring and control systems can be implemented on upscaled PBR systems. These systems allow for better control over parameters such as temperature, light intensity, nutrient supply, and pH levels. Enhanced process control ensures optimal growth conditions, leading to improved productivity and consistency in algae production. This can drive technological advancements in the field of algae cultivation. As the demand for larger systems increases, there is a need for innovations in PBR design, materials, harvesting techniques, and algae cultivation methodologies. This can lead to the development of more efficient, cost-effective, and sustainable PBR solutions.

Upscaling the PBR system can further allow for larger-scale carbon capture from industrial emissions and the utilization of waste nutrients for algae growth. This can contribute to mitigating greenhouse gas emissions and reducing nutrient pollution in water bodies. However, upscaling requires careful planning, adequate infrastructure, and expertise to ensure successful operation. Factors such as light distribution, mixing, and contamination control become more critical as the

system size increases. Additionally, optimization of growth conditions are essential to achieve the desired productivity in an upscaled PBR system. In this study, the lab scale system was also upscaled to a capacity of 100,000 L capacity PBR and AutoCAD drawings were constructed. The figure 6.1 illustrates a top view of a commercial-scale photobioreactor (PBR) system designed using AutoCAD, featuring a parallel installation setup.

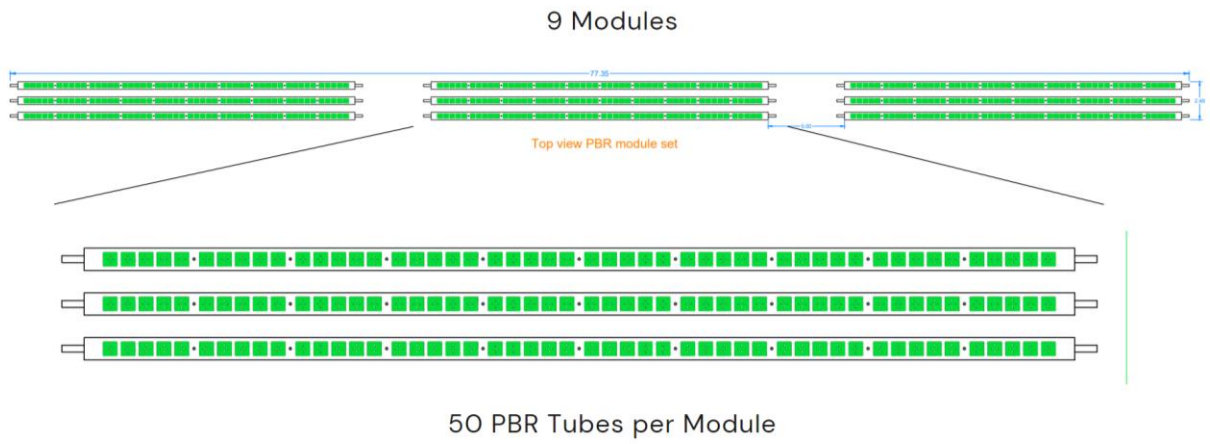

Figure 6.1: Top view of upscaled PBR drawing by using AutoCAD.

Designing the PBR for 100000 L Capacity.

For 1 PBR tube:

Dimensions of occupied vol = 0.3m x 0.3m x 2.465m = 0.22185 m^3

1 m^3 = 1000 L

(0.2218 x 1000) L = 221.85 L

Volume of free board = 0.3m x 0.3m x 0.135m = 0.01215 m^3

= 0.01215 x 1000 = 12.15 L

Total height of PBR tube = height of occupied vol + height of free board

= 2.465m + 0.135m

= 2.60m

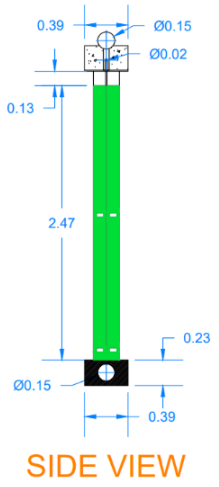

Figure 6.2: Side view of upscaled PBR by using AutoCAD.

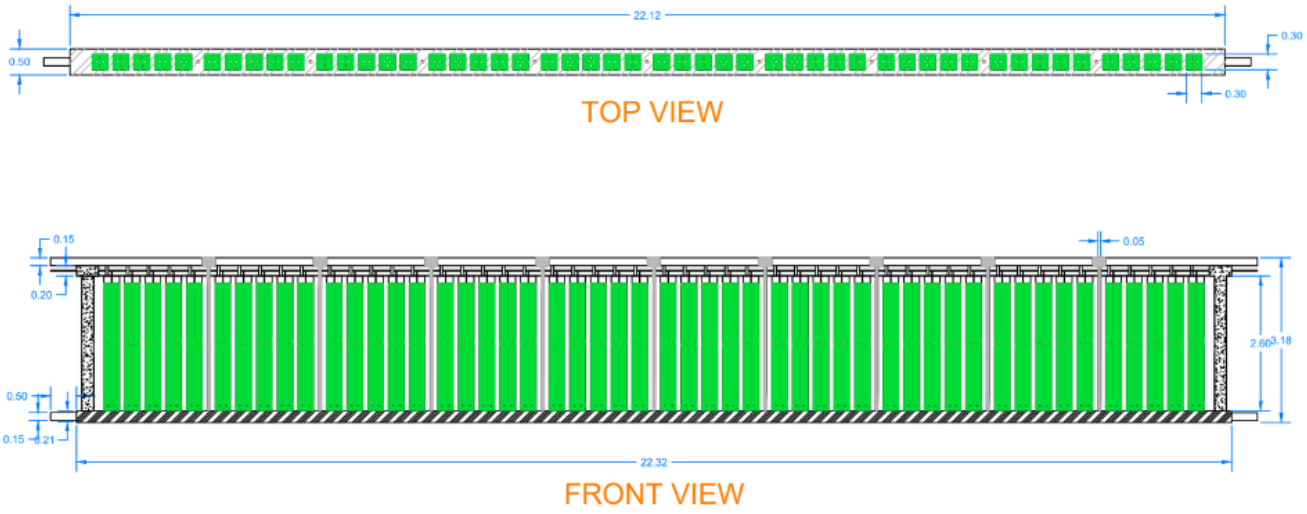

Figure 6.3: AutoCAD drawing of front and side views of upscaled PBR.

Figures 6.2 and 6.3 depict detailed AutoCAD drawings of the commercial-scale photo bioreactor (PBR) from side and front views, respectively. These illustrations offer a closer examination of the PBR system, which measures 3.18 meters in height and 22.32 meters in length for a single module.

Number of PBR tubes = $\frac{100000}{221.85}$ =450

1 module = 50 PBR tube

9 module = 9 x 50 = 450 PBR tube

For 100000L capacity, we need 9 modules for the cultivation of algae.

PBR tubes and modules cost depend upon the type of material used for this purpose.

6.2 Total area required for PBR

1 module length = 20.0m

1 module height = 2.60m

There will be 3 sets. Each set contains 3 modules which will be placed parallel to the next set. Each module will be in a line with 5 m space between each set.

Total area required: 77.35m x 2.49m = 192.6 m^2 = 7.6147 Marla

Thus, larger scale PBR system can be constructed in a more compact space. The primary advantage of a compact PBR system is its ability to maximize space utilization. By occupying a smaller footprint, it allows for the cultivation of algae in limited or confined areas, such as urban environments, rooftops, or indoor facilities. This makes it suitable for businesses or research facilities with space constraints. It can be integrated into existing infrastructure, such as buildings or industrial facilities, without requiring extensive land or construction. This versatility enables algae cultivation to be carried out in non-traditional locations.

The modular system is easily scalable. They can be easily expanded by adding more units or modules, allowing for flexible growth according to demand. This scalability feature enables

businesses to start with a smaller setup and gradually increase production capacity as needed. This also makes the system to be portable or easily relocatable. This mobility allows for greater flexibility in deployment and experimentation. It can be advantageous for research purposes, pilot projects, or operations that require temporary or mobile algae cultivation. Moreover, this affordability makes them more accessible to smaller industries. The compact size also translates to easier maintenance. Cleaning, sterilization, and inspection tasks can be performed more efficiently and quickly due to the reduced surface area and simplified access to components. This can save time, effort, and maintenance costs.

6.3 Biodiesel Production

Algae concentration acquired = 3.68 g/L

1L = 3.68 g

100000L = 368000 g or 368 kg

Lipid content is 12% observed at lab scale then extracted lipid will be 44.16 L.

About 80% of lipid content converts into biodiesel: = 0.80 x 44.16 (Wu et al., 2017).

= 35.328 L of biodiesel / run (5 days)

Numbers of run can perform in a year = $\frac{365}{5}$ = 73

Total biodiesel production in a year = 73 x 35.328 L = 2578.9 L

6.4 For fish feed

Total algae concentration for fish feed= (368 – 44.16) kg = 323.84 kg ⊐ 300 kg

Bio floc fish farms use 30 CP (30% protein content) for mature fish.

Protein content of *Scenedesmus sp.* at lab scale = 18.4%

On the lab scale, we find out 18.4% of protein content present in Scenedesmus species of algae.

Fish feed is prepared by mixing 50% powdered 40 CP fish feed and 50% algae obtained after lipid extraction. The fish feed prepared will be 30 CP.

Amount of fish feed in 1 bag = 50 kg

Replacing capability of algae to fish feed = $\frac{300}{50}$ = 6 bags

Total 50 kg bags in a year = 6 * 73 = 438 bags

Total mass of fish feed obtained per year = 21,900 kg

CHAPTER 7

7. CONCLUSION

The research showed successful fabrication of a PBR with integration of wastewater treatment and industrial carbon dioxide utilization with biomass productivity of 738 mg/L/day and lipid productivity of 88.7 mg/L/day. Nutrient removal observed for phosphate, nitrate and ammonia were 76.3%, 80.4%, 96.1% respectively. Water treatment results for other parameters such as TSS, TDS, COD, pH and Turbidity were also found to be suitable for bio floc farm reuse. Moreover, upscaling the PBR to the commercial design suggested has a potential to reduce biomass production cost with the aim of making the entire system economically viable for commercialization. Conclusively, System 3 with the integration gave the best results as compared to the other two systems which analyzed growth using bio floc effluent and CO_2 utilization separately. It gave higher lipid productivity which can potentially allow mass production of biodiesel.

CHAPTER 8

8. FUTURE RECOMMENDATIONS

Further research can be done on the following:

- More testing needs to be done to confirm reliability and consistency of results.
- Elemental carbon of grown algae needs to be analyzed to determine its specific carbon content.
- Produced energy from the algae needs to be studied to determine its specific energy content with respect to its mass which needs to be compared with other fuel sources to conduct a thorough cost benefit analysis.
- Specific CO_2 reduction emissions with respect to its mass as well as per unit energy also need to be analyzed and compared to determine its environmental impact in terms of its contribution to greenhouse gas emissions.
- A detailed cost model needs to be constructed of the entire system to analyze its potential for commercialization.
- Further improvement to the PBR and system can be studied for more optimization of biodiesel production from algae.

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